A STUDY OF THE LIFE-HISTORIES OF CERTAIN TRYPANOSOMES.

By

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Introduction.

The following paper is a study of the life-histories of certain trypanosome species. The work is the outcome of several years of investigation undertaken under varying conditions and was carried out in Glasgow University, in the Govt. Museum in Colombo, in the Lister Institute at Chelsea and at Elstree, and in Uganda. The problems of trypanosomiasis presented themselves under a variety of aspects and the different chapters make no attempt at a uniform treatment of the various species. Each section is merely an account of the points of interest revealed by the successive opportunities for studying the genus afforded by the nature of the individual form and the local conditions.

The work has , with the exception of Chapter III, already been published from time to time in various scientific journals and in the Sleeping Sickness Reports of the Royal Society.

Methods.

The methods used throughout all the work discussed in this paper may be set out briefly in this place.

As in all work dealing with Protozoa it was found that direct observation of the living organism under conditions approximating as nearly as possible to those of its natural habitat yielded the most valuable results. The opportunity of direct microscopic examination afforded by the size and transparency of the organisms is one of the great advantages of this branch of biology and full use was made of the privilege.

A number of different methods of fixation and staining were used. Fixation by drying or by exposure to osmic acid vapour and immersion in alcohol absolute followed by Giemsa's stain was adopted extensively in the earlier work. The errors produced by this proceedure were gradually revealed during the progress of the research and it was abandomed for detailed cytological observation, though it has always been used for certain aspects of the work, notably for the measurement and enumeration of the blood types in the case of the trypanosomes of mammals.

The fixation by drying in air with or without exposure to osmic acid vapour destroys the structure of the trophonucleus, but preserves the general shape of the body and has the great advantage of retaining all the organisms present in the blood spread upon the film. The orgaisms are flattened into two dimensions, and while this is an arbitrary proceeding the actual result ,especially by the osmic vapour method is sufficiently uniform to afford very valuable data. These dried films can be used for the measurement and enumeration of the different types , for the detection of variation in the

relative position of the nuclei, for the enumeration of the individuals in in division and for the rough observation of the sequence of forms in the life-cycle. Dried films are useless for the study of the finer details of the structure and do not present a correct view of the cytology of the organism.

The other methods used comprise the wet fixation of rapidly spread films so generally adopted in the study of protozoa, followed by washing in alcohol, staining and final mounting in balsam. Many fixatives were used, the sublimate mixtures being found to give the best results, notably Schaudinn's fluid; Flemming's fluid was found to be unsuitable as it caused considerable distortion. Bles's formaline acetic mixture was very serviceable in the fixation of trypanosomes in the blood vessels and tissues for study in sections. It may however be remarked that films are much more suitable than sections for the detailed study of the actual trypanosomes themselves. Nevertheless results of great interest as regards their distribution in the host can be obtained by this method. Häidenhein's Iron haematoxylin (or one of the modifications of this stain) produces undoubtedly the best picture of the finer structures of trypanosomes, but Delafield's haematoxylin, Mayer's haemalum, Twort's lichtgrun and neutral red, and acid fuchsin are all useful and apply a basis of criticism of the Häidenhein's stain.

The final criterion of fixation and staining always lies in the relation of the fixed specimen to the living object. Fortunately a good illumination and high power lenses permit of a clear view of the living nucleus in nearly all trypanosomes. This happy state of affairs is very marked in the case of the larger types such as T. raiae and T. vittatae. Nevertheless even in T. gambiense and the trypanosomes of mammalia generally a patient observer can make himself acquainted with the more obvious features of the nuclear structure in the living object.

Cultivation of the trypanosomes upon artificial media was not practised, as in nearly all the cases dealt with the natural alternation between the vertebrate and invertebrate hosts could be studied at first hand and the sequence of stages could be obtained directly without any alteration of the natural conditions. A certain amount of in vitro observation of the continuous development of trypanosomes under the coverslip was undertaken, but this was rather an attempt to transfer the normal development to the slide under the microscope for purposes of observation, rather than an endeavour to cultivate the flagellates outside the body of the host.

Experimental methods of various kinds were used to elucidate the life-histories, these are described in the course of the work.

I would like to mention one other method that yields an extraordinary amount of insight into the conditions of infection in the case of the pathogenic trypanosomes of mammals, namely the measurement and classification of the blood forms. This biometric method has, it must be admitted, been used not infrequently in a somewhat uncritical spirit, nevertheless it affords the the key to a great deal of data of first class importance which can be obtained by no other means. It has too often been assumed that the blood of an animal infected with numerous trypanosomes affords a constant and homogeneous mixture of blood elements and parasites, and it is further frequently assumed that today's sample and yesterday's sample etc. are for practical purposes of equal value. A few days spent in analysing the distribution of the different blodd types and the percentages of dividing individuals etc. from a series of samples from a single animal will show the worker what a variable and fluctuating population he really has to deal with. So much so that a film fixed in osmic acid vapour and stained by Giemsa's method should be filed for reference in correlation with every experiment dealing with transmission or with the

effect of drugs or sera upon the parasites in vitro or in vivo. Many of the discrepancies and contradictions in this kind of work resolve themselves into a difference in the conditions of the experiment rather than a real disharmony in the facts. This method when used on a sufficiently broad basis affords a differential diagnosis between certain species but the diagnosis does not depend upon the shape of the curve but upon its position on the scale. Thus T. evansi may be distinguished from T. brucei in infections for instance derived from camels by the fact that the minimum measurement of T. evansi is 18 μ whereas the minimum measurement of T. brucei is as low as 10 to 12 μ . The value of some of the data to be obtained by methods of measrement will be made clear in a later part of the work.

I. LIFE CYCLES OF THE TRYPANOSOMES OF CERTAIN FISH AND REPTILES.

A. T. raiae.

(i) Notes on the Relations and Natural History of the Hosts.

All the trypanosome species so far as is at present known are parasitic, and a very large proportion of them are parasitic in two hosts. The life-cycle of the trypanosomes is therefore not only adapted to the conditions of the individual hosts but is dependent on their mutual relations. As a result these life-cycles present a biological group of great interest and the natural history of the metazoan hosts must be taken into consideration.

T. raiae is found inhabiting the blood stream of various species of skate, it is never present in very large numbers in any individual and apparently causes no inconvenience to its host nor amy appreciable loss of condition. The infection is very common in the skate caught in the Clyde area, more than 80% of these fishes show the parasite in the peripheral blood in sufficient numbers to be found in th course of the microscopical examination of two or three coverslip preparations of the freshly drawn blood. The actual percentage of fish infected must be much higher.

The leech Pontobdella muricata is frequently found in the Clyde estuary and is apparently common also at Naples. It feeds upon the various species of skate and can be captured most conveniently by searching the skate caught on night-lines. The leeches are almost always knocked off the fish caught in nets during the struggling incidental to this method of capture. The great bulk of the leeches used in this investigation were got through the courtesy of Messers John and Thomas Thorburn of Rosthesay from the skate caught upon

their night-lines in the neighbourhood of the island of Bute. Pontobdella is I believe also found upon the angler (Lophius) but I have had no opportunity of examining this creature.

The leech grows to a considerable size, I have seen specimens that measured more than six inches when extended. They are of various shades of olive green and are marked with rows of wart-like protruberences along the body. Nearly every leech is infected with trypanosomes thus in a certain series of "wild " leeches out of 60 only one was found to be quite free from these flagellates.

From the observations I have been able to make upon freshly captured leeches it appears to me that Pontobdella probably fills its crop at one meal and then proceeds to digest the contents at leisure without seeking food until the blood is pretty well digested. This is however only a matter of conjecture.

Pontobdella lays very characteristic eggs in the form of cocoons which are found firmly attached to shells or stones by means of a short stalk. They are greenish brown and leathery , oval in shape and about the size of a pea. It is interesting to note in passing that the egg-cases were formerly considered to be a young state of Fucus loreus and it was Darwin who as quite a young man discovered their true nature while studying in Edinburgh in 1826. In the Spring of 1907 I received a number of Pontobdella cocoons from the Marine Station at Plymouth ; they had been deposited upon clam-shells. I put them into a glass jar filled with clean sea water which had been kept in the dark for six or seven weeks, covered it with a loosely fitting glass lid and put it on a shelf in the laboratory in a subdued light. I went to Ceylon in the course of the summer and was later informed that the leeches had hatched out about the beginning of Oct. 1907 , but the exact date was not noted. On my return in the autumn of 1908 they were still alive in the

original jar and in the same water. In November I took them to the Marine Station at Millport on the Clyde and fed them on infected skate to obtain the first stages of the parasite in the leech.

The leeches ,it was observed , showed the greatest excitement waving about actively in all directions when a shadow was made to fall upon the jar containing them . This reaction to the appearance of a shadow is very marked indeed and may be of service to the creature in its natural state. The following is an account of the habits of feeding observed in these young leeches.

The young leeches attached themselves readily enough to the skate, except in one or two cases where they required a good deal of coaxing; ultimately , however they all fed. The Pontobdella apparently does not feed at once, possibly on account of the difficulty in first piercing the skin. The attitude of the leech when attaching itself with the intention of feeding is very characteristic. It takes firm hold with its posterior sucker , and then bends its anterior sucker in so that it is touching its own body. It then slides the anterior sucker down along the body, finally attaching it to the skate at a spot close beside the posterior sucker, these leeches made quite a large wound for such small creatures.

In some cases, after feeding for a time, the leech detaches the anterior sucker, but does not as a rule , remove the posterior one. Finally , after a greater or less interval of time has elapsed the feeding is resumed.
On one occasion I was able to observe the process very clearly. A leech was put on to a skate at 2.30 p.m. on the 14th Nov. ; at 10 a.m. on the 15th it was attached by the posterior sucker only. The leech had fed , but had not taken very much(the leech is,of course ,rather transparent when young, and it is possible to tell at a glance if it has fed or not). Three quarters of an hour later it was still in the same position with the anterior sucker

free. I then held the skate under water in the tank in such an attitude that I could see the leech which had begun to make searching movements with the anterior sucker. It presently slid the anterior sucker down its body and onto the skate, but moved it about until it passed over the old wound when it buried the sucker in it. The leech seems to force its proboscis in pretty deeply, judging from the wound and its attitude while sucking. At 7p.m. on the 15th the leech was again only attached by its posterior sucker, but it was much swollen, and had blood visible right up to the anterior end. It was left attached to the skate till the morning of the 16th , but did not seem to feed again.

Anyone who has reviewd a large number of leeches of a given species will have observed that there is a good deal of individual variation in the process of digestion. The broad lines are of course the same, but there is always a certain amount of individual idiosincrasy. This in Pontobdella is chiefly expressed in the greater or less fluidity of the blood in the crop and the nature of the bacterial flora, moulds, schizomycetes, etc., present. THese complex circumstances no doubt react upon the trypanosomes and may explain a certain slight variability in the detail of some of the developments.

The blood in the crop of Pontobdella has a tendency to coagulate. It forms a rather dry mass with fluid in the intertices. The time factor in this stiffening of the blood is rather variable. It always occurs, but the time at which it happens and the length to which it goes differs a good deal in individual specimens. Late in digestion it, the mass in the crop, tends to become fluid again.

In Pontobdella the crop is a single rather thin-walled sac passing back from the oesophagus right to the posterior end of the body. The opening from the crop into the intestine is placed at the a point about two thirds

of the way from the anterior end and it passes back as a narrow tube lying on top of the crop.

(ii) Life-cyle of T. raiae.

T. raiae was first described by MM. Laveran & Mesnil (Trypanosomes et Trypanosomiasis. Paris 1904) It is a very large form measuring 75 - 80 μ it moves rather deliberately and is an extremely good object upon which to study the features which characterise the whole genus. The large trophonucleus lies about one third of the distance from the anterior (flagellar) end of the body. It is in the form of a vesicle containing a softly refractile sphere, in optical section it appears as a disc surrounded by a halo. The posterior end of the body is frequently drawn out into a slender process. Near the posterior end lies the kinetonucleus , a dense compact structure which can be distinguished in favourable specimens as a greyish softly refractile object at the posterior end of the undulating membrane. The undulating membrane is wide and well developed, it stands out from the body like a pliable fin. The flagellum , which forms its outer margin arises from a small basal granule in close proximity to the kinetonucleus. The anterior end of the flagellum projects out beyond the body. Fine longitudinal striations may sometimes be observed in the periphery of the protoplasmic body, these are myonemeta and are concerned in the contractile movements of the body. The myonemeta are probably present in most trypanosomes but they are very conspicuous features in certain species notably those parasitic in freshwater fishes and in T. vittatae from the "milk turtle" of Ceylon.

As in the case of all trypanosomes parasitic in fish very little is known about that part of the cycle which is passed in the vertebrate host. T. raiae is never numerous in the blood of the skate and while the individ-

individual parasites vary in size, the method of multiplication is so far not known and I have never observed forms in process of division.

It will be convenient to give a general account of the life-cycle in the leech Phobdella muricata and then to record some of the experimental data and conclude with a description of the stained specimens.

The trypanosomes when taken into the crop of the leech along with the blood round themselves off and discard the locomotor apparatus but retain the kinetonucleus. These forms were first described by Brumpt. (C.R. SOC. BIOL. T. 60 pp.160 -166, 27th Jan 1906-) . These individuals which are usually dividing fairly actively gradually disappear from the crop and are passed into the intestine. This occurs at a relatively early stage of digestion. In the intestine they become motile but continue for a considerable period in the form of a Leptomonas-like creature, with a short straight flagellum but without an undulating membrane. These stages are in active division , they vary considerably in size and are often very small .(Figs. 1 & 6, p.15-)

The leptomonas forms are very persistent and are found throughout the whole period of infection in greater or less numbers.. As time goes on the leptomonad forms lengthen and develop gradually into trypanosomes which show the greatest variation in size and shape during this period of growth. At about the middle period of digestion the intestine of a well infected leech presents a quite bewildering range of forms. Rounded resting individuals & leptomonad stages in every phase of development and trypanosomes varying in type from broad slow moving creatures to long slender individuals with a rapid flickering motion, are all present together at this time. Division is in active progress in all these forms with the exception of the very long slender tryoanosomes. It is these long slender types which

migrate forwards towards the end of digestion into the proboscis.

At the close of digestion, that is to say ,when the crop is quite empty of blood , and when the intestine shows none of the dark fluid formed by the breaking down of the blood by the digestive fluids of the leech, there may be very large numbers of the long slender trypanosomes pretty well throughout the whole alimentary tract of the leech, but very often they are still most numerous in the intestine. Besides these, there are present the little resistent creatures in the rounded off or very early leptomonad stage; these may sometimes be quite minute, and seem to be able to persist through a period of starvation and may preseve the infection without any external aid. Thus in a freshly captured leech which showed the blood in the crop in a perfectly unaltered condition, and in which I could find no large newly ingested types, there were a great number of these very minute rounded-off forms and also the regular cycle of derivatives i.e. the leptomonas stages and trypanosomes all in miniature and showing signs of division. These forms apparently proceed to grow and may sometimes give rather confusing pictures. Thus there may be a persistent infection and a fresh one at the same time.
The slender proboscis forms appear to degenerate and die if they remain indefinitely in the leech though it is difficult to be perfectly certain of this point. It will be seen that these elongated forms play a very important role in all trypanosome life-histories.

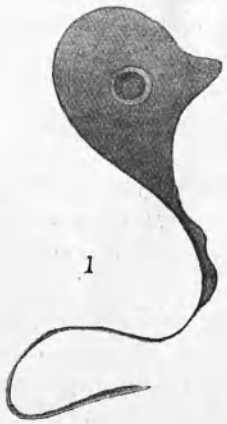
The following observations were made upon the young laboratory hatched leeches whose history has already been given above (page 8). They had never been fed at all since first hatching out and had lived rather more than a year without any food before these experiments were undertaken.

A leech (5a) was put onto an infected skate at 9.30 p.m. one evening; twelve hours later it was feeding ,and had already ingested a good deal of

blood; two and a half hours later it had ceased feeding . The leech was opened seveteen and a half hours after it had been first put onto the slate and three and a half hours after it had finished feeding. So that the earliest ingested trypamosomes had been in the leech about sixteen to seventeen hours and the last ingested ones about three to four hours.

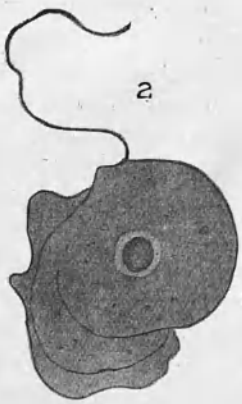
The blood was very fresh -looking and no obvious changes had taken place in the blood-corpuscles. The trypanosomes showed very variable appearances. A good number still showed the flagellum,but were no longer in the typical trypaniform condition. For the most part they were somewhat pyriform with an imensely long thick flagellum protruding from one end. (text-fig. 3) Some very fantastic appearances were seen where the body of the trypanosome had assumed an irregular shape with curious rounded bulges,and where the flagellum had broken loose from the membrane and had become tangled round the body, the end usually was free and still motile. In other cases detached flagella still actively motile were seen; this has often enough been observed with trypanosomes, but in this case the flagellum does not take the kinetonucleus with it. Uninucleate stages of this parasite have never been seen. One of these free motile flagella was seen to become secondarily attached to a resting individual. This animal was watched for many hours in case the process might prove to be of more than merely casual significance, but no development took place. Besides these flagellate creatures others were present which had already discarded the flagellum (text-fig. 4) These were rounded egg-or pear-shaped individuals with a very clearly visible nucleus. It is composed of a softly refractile spherical body surrounded by a bright hyaline halo. The nucleus lies towards the broader end of the body. These animals present a very characteristic appearance but nevertheless they may easily be overlooked in the mass of leucocytes and blood-corpuscles.

TEXT-FIG. 1.

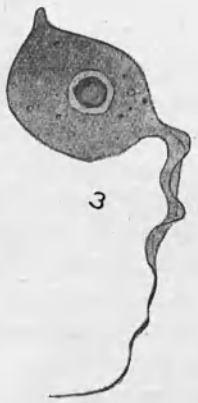


Drawing of live Trypanosome from the crop of Pontobdella. The animal, which has been recently ingested with the blood, is in process of rounding off.

TEXT-FIG. 2.



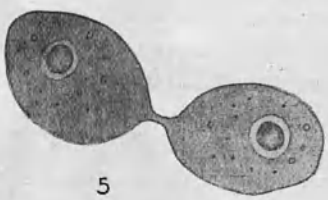
TEXT-FIG. 3.



Trypanosomes from the blood of the skate rounding off on a sealed slide. The drawings are from live specimens.

TEXT-FIG. 4.

TEXT-FIG. 5.



TEXT-FIG. 4.—Rounded off Trypanosome.
TEXT-FIG. 5.—Division of resting Trypanosome.
Drawings made from live specimens in a sealed slide of skate's blood.

These non-flagellate organisms were already in a few instances undergoing division but no sign of the new flagellum was as yet forthcoming.

Another leech (7a) opened forty-eight hours after it began to feed showed only resting forms. The blood in the crop had coagulated into a rather dry mass but the corpuscles showed no signs of degeneration. .

These resting stages of the trypanosome are identical in appearance with those so frequently seen in Pontobdella found infected in nature. A resting pear-shaped individual was chosen as a subject for investigation at 4.30 p.m. When it was first observed the trophonucleus was clearly visible and had its usual appearance of a sphere surrounded by a halo. Half an hour later the animal was more rounded, the nucleus was less distinct and a slight groove had appeared at the broad end. By 5.30 the nucleus as such had disappeared , but a large clear oval space had appeared in its stead. At about 5.50 two nuclei began to appear, joined by a clear area. At 6.15 the two nuclei were quite clearly defined but the clear are joining them remained visible till about 9 o'clock after which it was no longer to be detected. This clear band is the remains of the division spindle; it is a very characteristic feature in the stained specimens. During the division of the nucleus the body had gradually become flattened in an antero-posterior direction and correspondingly widened laterally. Grooves also began to appear in the antero-post-

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I am indebted to the late Mr. C.H. Martin for kind assistance in carrying out some of these continuous observations upon live specimens..

antero-posterior direction. It is also to be noted that these grooves arose both at the anterior and the posterior end _ the anterior end being the broad end at which the nucleus lies when the animal is in the pear-shaped condition and at which the flagellum is later developed.

The grooves altered a good deal in appearance during the next few hours and towards 4 a.m. had deepened till the animal presented the picture of two pears stuck together in the middle, with however, the two broad ends and the two narrow pointed ends free. This is a point of some sloght importance. When in the trypaniform state division of the protoplasm usually begins froom the anterior end and proceeds to the posterior end. This is likewise the rule in the leptomonad and crithidial stage. The question of the grooves arising at both ends of the parasite is not in itself deserving of much remark, but it explains some curious appearances where division of the protoplasm goes from the posterior to the anterior end ,to be mentioned in a later part of the work. These appearances are apt to be interpreted as conjugation stages but the evidence does not in my opinion warrant any such assumption. They are certainly division phenomena in the instances here described where the actual sequense of changed could be continuosly observed upon the same individual.

This specimen was watched for another two hours and one of the two daughter individuals developed a short clearly defined flagellum which, however showed no signs of movement. At 6.30 a.m. the animal was finally abandoned, although complete separation of the protopaslm had not yet occurred.

The flagellum seems to appear for the first time somewhere between the second and third day after the ingestion of the blood. It is a very characteristic feature that it generally arises at a division stage . The flagellum is at first a stiff and relatively thick little rod which sticks straight out from the anterior end of the organism. A very considerable time seems to elapse before it becomes motile, I cannot say exactly how long, but it seems

to be more than twelve hours.

A leech(6a) opened six days after feeding upon the blood of an infected skate, showed a typical infection of the varied type so characteristc of Pontobdella .

True trypanosomes had already appeared, some of these were broad individuals and others were much more slender, but not of the elongated type which appears at a much later period of digestion. The broad and slender types were joined up by innumerable intermediate forms, besides these, crithidial forms were also present. I call crithidial forms those in which an undulating membrane has been developed ,but which have not the typical arrangement of the kinetonucleus and the trophonucleus. Leptomonad forms with the flagellum sticking straight out from the broad end of the body and with as yet no undulating membrane were also to be seen, and finally many rounded forms , some in process of division and some showing the development of the flagellum , were likewise present . Some of the trypaniform individuals were also undergoing division.

Conjugation was very carefully searched for as it seemed possible that it might occur at this stage of the life-cycle, but no signs of such a process were detected. Two individuals were found joined by their posterior ends, one slightly broader in shape than the other. They were watched continuously from 6 p.m. when they were first seen till 3.15 a.m. the protoplasmic junction between the two was seem to become much more slender and pulled out suggesting that the individuals were dividing. (Text-fig. 5.)

An interesting corroberation of the stages above described was obtained from blood drawn from a skate and sealed up between a coverslip and slide. A trypanosome was continuously watched from 2.45 p.m. when the slide was made.

At 4.30 the animal had come to rest. The flagellum which when it breaks free from the membrane, is seen to be relatively of immense length was tangled up round the animal. The slide was watched for some hours longer , but as the trypanosome had come to rest it was left and the observation was continued next morning when it was found to have divided into two.

The behaviour of the trypanosomes from the blood of a skate upon a s sealed slide is interesting, a number do not alter at all, others very soon after the slide is made begin to react to the altered conditions . They adopt a dumpy spiral shape, or the posterior end may become much thickened at the expense of the rest of the body. (text-figs.2,3) . Sometimes the most fantastic shapes are seen , finally the flagellum breaks free but may remain attached to the trypanosome by its posterior end. It may then become tangled round the body and stick out in stiff loops. The trypanosomes in these phases on the sealed slide made from the skate's blood are identical in appearance with the ~~xxxxxx~~x forms described in leech 5a(cf.text-fig. 4,5) The interval of time after which the trypanosomes come to rest varies considerably.

On another accasion a rounded non-motile trypanosome which had discarded its flagellum was chosen for continuous observation on a slide of skate's blood which had been mounted for fourteen hours. The animal was seen to divide into two at about 11 o'clock in the forenoon. During the afternoon , at about 4 o'clock, these two individuals each divided thus forming four little rounded animals lying more or less in contact. By 7 o'clock on the same evening they had become more oval and were identical in appearance with tge resting phases in the leech. By 9.30 p.m. short projections were seen at the broad end of two out of the four creatures under observation. The slide was left about 10 o'clock that night as the animals were not motile. Next morning at 9,30 , thirty-six hours after mounting the slide , observation was again resumed and it was found that the creatures had each divided. The resulting

eight individuals were still closely apposed but not connected with each other. Unfortunately these creatures were lost owing to a careless movement. The slide was however in the following condition; unaltered trypanosomes , still actively moving, were to be seen , non-motile groups of four and also a few groups of six and eight individuals were present.

I had often noticed that the trypanosomes on a sealed slide of skates blood altered their shape ,but, thinking this was merely a pathological manifestation , had not persevered with the observation. The process is easily enough passed over unless the observation is continuous , as the infections are generally slight,and, once the trypanosome has come to rest , it is not quite a simple matter to see it among the large number of leucocytes and red-corpuscles. Moreover, the curious fact that all the trypanosomes on a slide do not round off leads one to imagine that no development has taken place.

A low temperature seems to favour the process . This work on the live skate's blood was carried out at the Millport Marine Station , and the most successful set of observations was obtained in very cold weather, when the temperature of the laboratory was much lower than usual.

I now wish to give a brief account of the parasite as seen in stained films, fixed for the most part ,in Schaudinn's fluid (alcohol-acetic corrosive sublimate), The stains used were Delafield's haematoxylin, Heidenhain's iron haematoxylin, Twort's licht-grun and neutral red combination and acid fuchsin.

Delafield's haematoxylin gives an excellent result, staining the nuclear structures with precision; the flagellar apparatus takes the stain only faintly.

Iron haematoxylin gives a very clear picture, staining the nuclear parts coal black, and bringing into good relief flagellar and cytoplasmic detail. Great care ,however must be taken in the interpretaion of this stain,as it leads one into much the same errors as Giemsa's method ,in so far as it stains chromatic and achromatic structures alike. Therefore while Heidenhain's method gives a really splendid pixture, it is necessary to check the results by Delafield's haematoxylin which is a much safer stain.

Twort's combination of neutral red and licht-grun was also used. This is a clear, transparent stain, giving a red reaction for chromatin, and a green reaction for cytoplasmic and achromatic structures . The drawbacks to this stain are the uncertainty of action which seems to attach to all delicate double stains, and the fact that there is another loophole for uncertainty in the process of washing out the stain , so that it remains doubtful in some cases whether the nuclear colour is absent from a structure owing to the absence of chromatin or through the stain having been washed out.

Fuchsin gave quite good nuclear pictures but did not bring up the flagellar apparatus sufficiently well.

The drawings in the plate are made from two well infected leeches found infected in nature. They were at the early part of what I have called the middle period of digestion. For convenience sake the periods of infection may be divided into three, corresponding to the stage of digestion.

(i) The early stage when the blood is just coagulating. and when the first

signs of the dark green-brown fluid is visible in the upper part of the intestine. The parasite at this time is in the condition of throwing off the original flagellum and adopting the resting state ,during which division begins to take place. The parasites are for the most part still in the crop.

(2) The middle period of digestion when the intestine is full of the green-brown fluid and when breaking down of the blood is going on actively . The parasite is now in the intestine in large numbers. It shows the whole range of forms from the spherical non-motile creature to the typical trypanosome. Great variation in size and thickness of the parasites is to be observed . Very slender long forms are only occasionally to be seen. This middle period is of very long duration.

(3) Final period of digestion ,when the crop is empty (or almost so) of blood, and the intestine nearly,or completely , free from the characteristic green-brown fluid. The trypanosomes are now long, slender forms, with the kinetonucleus in the typical trypanosome position. The forms now begin to remount the crop, and are also to be found in a still more slender condition in the proboscis.

The drawings in the plate, being made from leeches in the earlier phase of the period (2) , do not show the long slender trypanosomes developed during period (3). This final stage is however illustrated in fig. .

It will be convenient first to give an account of the trypanosome phase as found in the intestine of the leech (figs. 7 - 17), and then to describe the points of interest in its development from the resting form.

The protoplasm is finely alveolar and evenly granular without vacuoles, protopasmic inclusions are only occasionally present.

The trophonucleus is composed of a large central karyosome surrounded by a wide halo, which is in turn surrounded by a membrane. Fine, but perfectly

distinct rays pass from the karyosome to the outer membrane. The karyosome is clearly made up of two substances , namely ,the chromatin and an achromatic substance in which the chromatin lies embedded. This achromatic substance frequently receives the name of plastin , and ,while this does not convey any very clear idea ,it is nevertheless a convenient and useful term . With Delafields haematoxylin the plastin stains a greyish blue,in iron haematoxylin preparations it is brownish,and it takes on a green colour when stained with Twort's combination. The nature of the rays is a little obscure, they stain as a rule , rather faintly with Delafield's haematoxylin ,but in some cases take the colour more deeply ; Heidhain's stain shows them up black ,but they wash out easily. I am inclined to consider that they are composed of the plastin substance ,but they seem at times to carry chromatin. The membrane very often shows little condensations of chromatin-staining material at the points where the rays meet it. The membrane stains well with Delafields stain and also with fuchsin, likewise by Heidenhain's method ,but the colour is retained much less tenaciously than in the karyosome. The condensations on the membrane appear to me to be chromatin, but with Twort's stain they do not take up the red colour. I do not lay very much stress on this point as it is just in a question of this kind that I think such a stain as Twort's is rather unreliable. There seems to be in the membrane ,as in other parts of the nucleus , an underlying substance of an achromatic mature ,in or on which which the chromatin is deposited.

The nucleus is exceedingly constant in all the stages of the parasite as found in the leech, the only variation lying in slight differences in the condensation of the chromatin in the karyosome and the membrane.

The pictures presented in the dried preparations made according to Giemsa's method differ greatly from this account. The most curious feature

about this is that some of the appearances obtained with Giemsa's stain give a very tolerably accurate representation while others depart widely from the type shown by the wet method of fixation. The "chromosomes" chromosomes so often seen in the nuclei of specimens stained with Giemsa's method are not to be detected at all in the haematoxylin films. The rays and the condensations on the membrane are, I have no doubt, the manner in which these appear in the wet films. The number of the rays cannot be made out as they are excessively fine nor do the condensations on the membrane stand out sufficiently clearly to be considered as individual structures. Giemsa's stain always increases the apparent size of any nuclear element into which it penetrates. This circumstance and the fixation by drying which is usually, though not necessarily, adopted when Giemsa's stain is used, accounts for most of the discrepancies observed in the results of the two methods.

The kinetonucleus takes on all the stains mentioned with great intensity; it is relatively large and rod-shaped. In close proximity , and apparently attached to it lies the blepharoplast (Minchin, Quart. Journ. Micr. Sci. May ,1908, vol 52,) (figs. 9-11)
This structure will be more fully considered when the development of the flagellum is discussed. The blepharoplast stains with iron haematoxylin, but the stain is washed out more readily than from the nuclear structures; it appears grey-blue and stains faintly with Delafield's haematoxylin and is difficult to detect at all in preparations made according to Tworts's method. The flagellum runs forward from the blepharoplast, ending as usual in a free whip. It stains green with Twort's combination and is picked out faintly by Delafield's haematoxylin , Iron haematoxylin stains it more deeply. The undul ating membrane is developed to a varying degree but is never much frilled.

Two other structures remain to be described. The first is a small granule

which takes on Heidenhain's stain . It lies posterior to the kinetonucleus near the posterior end of the body. Sometimes it appears to be connected to the kinetonucleus by a delicate strand. This granule is also found in the preparations made according to Giemsa's method and is clearest in the trypanosome phase. It has possibly something to do with the anchoring of the kinetonucleus. (figs. 9 & 10) The second is an element which I have never seen in the dried preparations at all, but which is a pretty constant feature in the films fixed by the wet method and appears equaly in with all the four stains used. Just anterior to the trophonucleus a small condensation is to be observed in the protoplasm surrounded by quite a definite little halo. In rather dark iron haematoxylin preparations it stains almost black (figs,19) with delafield's haematoxylin it looks grey-blue with soft outlines and is usually only slightly darker than the surrounding protoplasm (figs.11&9) . It stands out clearly in these films more by reason of the halo than on account of its greater depth of colour. In fuchsin films it is very clear ;it is also visible in preparations made according to Twort's method but it does not take on the red colour. It sometimes appears to be double. The nature and function of this structure is quite obscure ; it is most clearly visible in the trypanosome stage and its position in the body is constant,it is however also present in the leptomonad phases. The position of the kinetonucleus often obscures it at this period and makes it difficult to see.

The trypanosome just described arises as has been said , from a rounded resting form which develops a flagellum. (figs, 12 &) . The body gradually elongates and the kinetonucleus migrates backwards until it is well behind the trophonucleus. An undulating membrane develops during this process and the creature takes on the typical trypanosome facies. The only point about this that calls for special attention is the development of the flagellum.

It may be noted in passing that, owing to the flattening of the trypanosomes prepared by the dry method , certain details may be more distinctly visible in such specimens than in those prepared by the wet mwthod.

The earliest stage in the development of the flagellum of which one can be quite certain is shown in fig. 2 . Here it will be seen that two little projections have grown out from the neighbourhood of the kinetonucleus which is itself in process of division. These little structures sometimes stain rather deeply with iron haematoxylin; they are not,however , very easy to make out as any obliquity in the position of the kinetonucleus is apt to obscure them . Later stages are shown in figs. 3,4,5/34. Here the flagellum appears as a thick strand arising from a granule with contours which are not very definite ,which is in turn attached to the kinetonucleus. This granule at the origin of the flagellum is the blepharoplast. The minute detail is not very clear ,but as far as can be seen the blepharoplast appears to be attached to the kinetonucleus by a double thread. This may be seen in much later stages. (figs 5,12-14). The blepharoplast seems to arise from the kinetonucleus ,but I do not think in the light of its behaviour with the various stains that it is a chromatic body. It is true that it often stains a sharp black with iron-haematoxylin, but that is no test for chromatin. It takes on a pale grey-blue colour with Delafield's stain and shows up only dimly when at all with fuchsin. By Twort's method it stains green like the flagellum and is not very precise or clear. I am therefore inclined to regard the blepharoplast and the flagellar apparatus which grows out from it as achromatic.

Giemsa's stain presents a greatly exaggerated picture of the above development. The fixation by drying pulls the blepharoplast away from the kinetonucleus and makes the thread joining them stand out very clearly; it also greatly enlarges the apparent size of the blepharoplast, and markedly increases the dimensions of the flagellar rudiments ,which are always thick

at this stage.

Division. The figs. 12 & 22 give pictures of various division stages, and it is perhaps in this point that Giemsa's method has, generally speaking been the least misleading. The division starts as a rule bu the tramnsverse division of the elongated kinetonucleus, but this point is open to slight variation.

The trophonucleus shows first an arranging of the chromatin into two masses within the karyosome. A well-developed spindle subsequently arises, but the chromatin is divided without the formation of an equatorial plate. (figs. 13 & 14). The wet method of fixation shows the spindle very well. Centrosomal functions seem to be exercised by the condensations at the extreme points of the spindle, and the chromatin passes from the two main central masses in such a stage as that shown in fig. 13 , to either pole of the spindle. At a slightly later stage (figs. 14 & 15) there is often a curious double appearance in the nuclei. Figures 16 & 17 show the final stages. The spindle persists for some time after the nuclei are reformed, and can be clearly seen as a bright line in the protoplasm in the living specimens.

Division occurs at all the different stages of development, and there is an occasional tendency to mutiple division. Equal division is the rule, but unequal division is sometimes met with. Considerable variation in the division of the protoplasm is seen, as is shown in figs. 9, 21 & 22. It will be observed that in figs. the division of the protoplasm has started at the posterior end instead of at the anterior end, as is more usual. Specimens of this type were watched for many hours in the live state in the hope that they might be individuals in conjugation, but no evidence of this was forthcoming. Some very curious appearances were observed where the protoplasm had split into several rod-like processes. This is shown in fig. , and , while not a common appearance, is still too frequently seen to be dismissed as a casual

abnormality. The figures ,I may say , hardly do justice to all the varying sizes and shapes to be seen at this stage of development.

FIG. 1.—Resting phase, showing longitudinal division of the kinetonucleus.

FIG. 2.—Early stage in development of flagellum.

FIGS. 3, 4 AND 5.—Stages showing newly-formed flagellum.

FIGS. 6 AND 7.—Crithidial stages.

FIG. 8.—Trypanosome phase.

FIG. 9.—Trypanosome showing blepharoplast, granule at posterior end, and the structure just anterior to the trophonucleus.

FIGS. 10 AND 11.—Trypanosome phase.

FIG. 12.—Early division phase. Note condition of trophonucleus, kinetonucleus and blepharoplast.

FIG. 13.—Division stage showing spindle.

FIG. 14.—Later division stage.

FIG. 15.—Division stage showing trophonucleus spindle and also second division of the kinetonucleus. This is a rather unusual appearance.

FIGS. 16 AND 17.—Later division stages.

FIG. 18.—Division of Trypanosome.

FIG. 19.—Division of Trypanosome.

FIGS. 20–22.—Division stages where the protoplasm divides from the posterior end.

FIG. 23.—Rather unusual appearance where the posterior part of a dividing Trypanosome has formed a large rounded mass.

FIG. 24.—Division stage showing irregular splitting of the protoplasm.

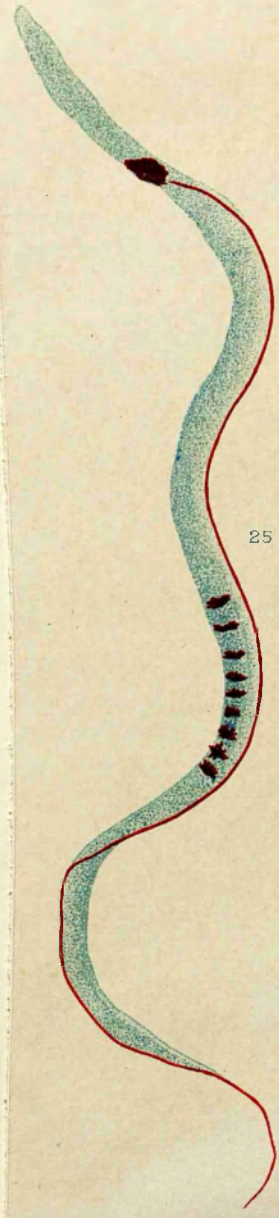
FIG. 25.—T. raiæ from the skate's blood. Note this is drawn at a much smaller magnification (× 1600) than remaining figures.

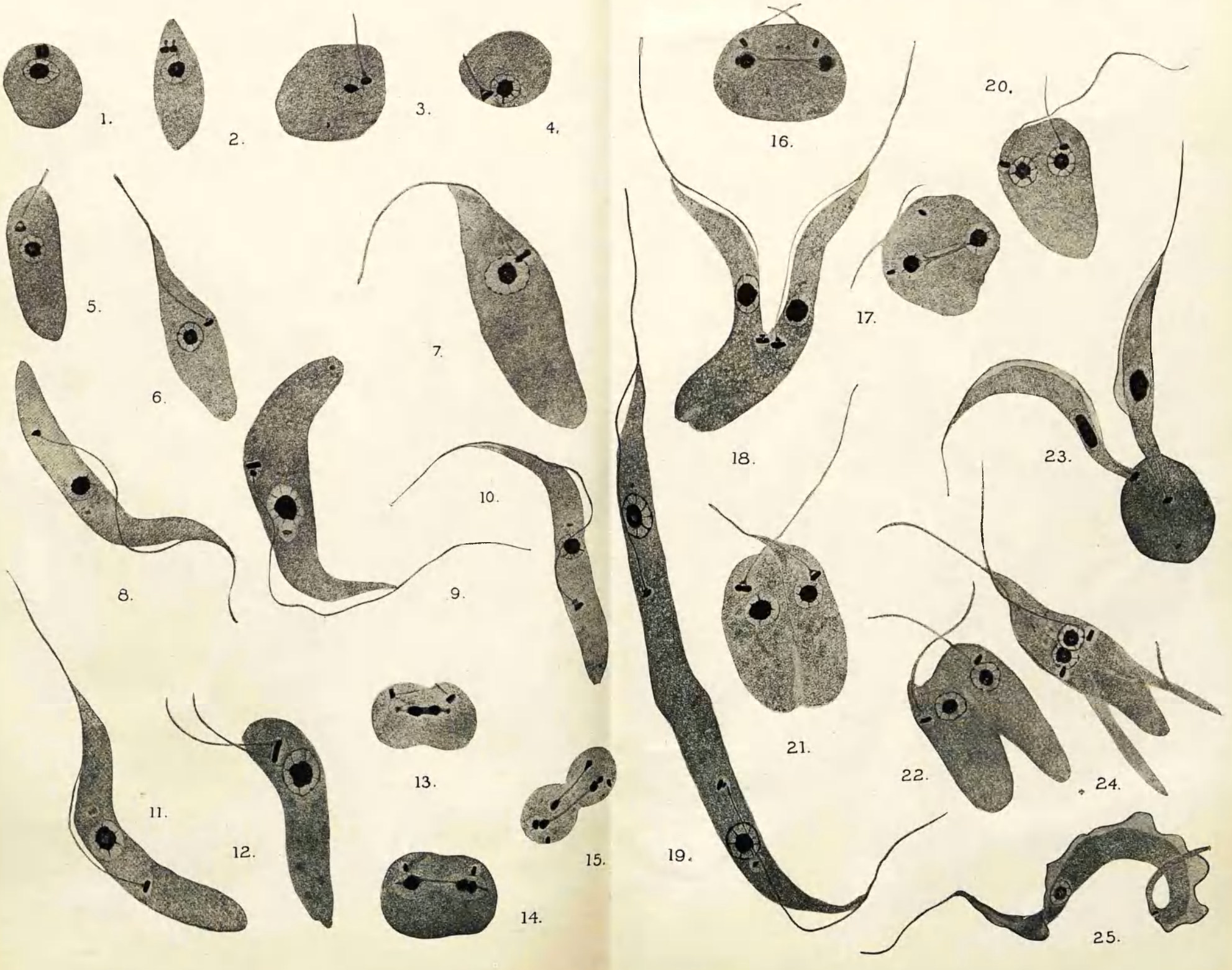
Figs. 1—24 are drawn with 2 mm. apochr. immersion lens by Ziess, 1·40 N.A. long tube and oc. No. 12, with the assistance of the camera lucida. The magnification is approximately 4500 diameters.

Fig. 25 is drawn with the No. 2 eyepiece; the magnification is approximately 1600 diameters.

Figs. 1, 2, 3, 4, 8, 9, 10, 13, 15, 17, 18, 19, 21 and 23 are from Heidenhain's hæmatox. preparations. The remaining figures are from Delafield preparations.

All the figures, with the exception of 25, are from the leech Pontobdella.

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B. Trypanosoma vittatae.

(i) Notes on the relations of the hosts.

The" milk turtle" of Ceylon, Emyda vittata, is a water tortoise covered all over with a thick ,smooth skin. It is black above and pure white underneath and has flaps on the underside so arranged that all four limbs and the head can be completely withdrawn.

Like most of this group it is more or less nocturnal in its habits ,it is said to leave the water very rarely but I have myself xxxxx while watching a pool in jungle country seen one come out of the water shortly after sunset and start prowling about at the edge. Emyda vittata is found all through the low country in Ceylon and in the Kandyan distict which is at an elevation of 1500 ft, but I never xxxxx saw it nor heard of its being observed in the higher mountain country , although Nicoria trijuga the common water tortoise which shares the low country pools with Emyda is found all throughout the whole Island.

Emyda vittata is a true aquatic form and dies if kept out of water for more than a few hours. Nearly every specimen examined was found to be infected both with a large trypanosome and with a haemogregarine.

A little water leech belonging to the genus Glossiphonia is parasitic on Emyda vittata , it was not found upon the other inhabitants of these pools such as Nicoria trijuga the common water tortoise, nor upon the fish Saccobranchus and appears to be confined to the one host.
This leech was almost invariably found to harbour trypanosomes in its alimentary canal . It was assumed that these were derived from the blood of the "milk turtle " but experimental proof was not obtained owing to the difficulty of getting uninfected specimens of Emyda upon which to demonstrate

the transmission.

The common water leech of Ceylon , Limnatis granulosa, a large creature which seems to be indifferent as to the nature of its host, being quite ready to draw blood from a warm blooded creature or from a reptile within a few minutes of each other ,was investigated. None of the freshly caught individuals exanined were found to contain flagellates . The leech was put onto infected Emyda and fed readily enough, the flagellates underwent developmental changes and multiplied ,but the work was abandoned owing to the leech never being found infected under natural conditions nor was there any reason to suppose that it played any part in the natural transmission of the trypanosome. Limnatis granulosa was never found on any of the specimens of Emyda when they were captured.

(ii) Life-cycle of T. vittatae.

Owing to the absence of experimental proof that the flagellates in the Glossiphonia form part of the life-cycle of T. vittatae ,I do not intend to give the history in detail. There is strong presumptive evidence of this identity but as the experimental side of the history was obtained very fully in the case of another trypanosome to be dealt with later,there is no need to dwell upon this aspect of the work in connection with T. vittatae.

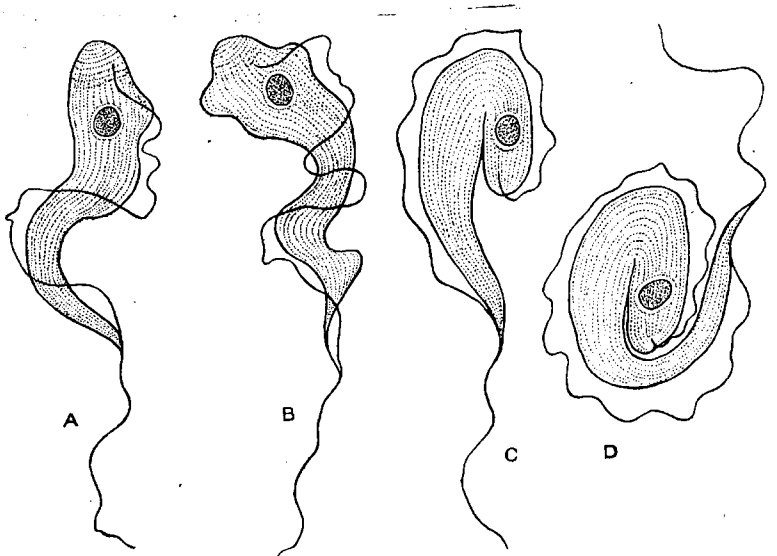
The cycle runs much the same course as in T. raiae, the portion of the history that could be well studied deals with the earliest changes in the trypanosome upon leaving the vertebrate and these have an interest in that they confirm with slight modifications the observations made upon T. raiae and upon other trypanosomes.

In the vertebrate the trypanosomes vary in size (figs. / 2 7) the origin of the smaller forms is obscure; they may either arise from the

large forms by division or they may possibly be the young forms derived from the leech. Division stages in the large trypanosomes in the blood of the tortoise must be exceedingly rare ,as although a great number of well infected fims have been searched ,I have never come accross any of the full grown forms in this condition. Among the forms intermediate between the large and the small specimens , however, individuals with two nuclei and dividing kinetonuclei are to be found ; they are never numerous and I can say nothing about the details of the process.

The following development was observed upon a number of occasions in blood drawn from an infected Emyda, placed upon a slide covered with a coverslip and sealed round the edges with vaseline. These observations were made upon blood drawn from the foot of the tortoise and no particular care was taken to ensure that there was no admixture of moisture. The foot was washed in clean water and wiped but the skin of the "milk turtle " is thick and contains deep pores and it is almost impossible to dry it thouroughly. That moisture could have any close bearing (cf. infra pag 60)upon the behaviour of the trypanosomes did not occur to me at the time and in the very moist atmosphere of Colombo particular precautions would have been necessary to avoid the presence of some moisture in the preparation.

Some time after making the preparation the trypanosomes begin to show various modification in the external appearance. The length of time which elapses before the creatures begin to yield to the altered conditions is remarkably variable, the time factor throughout the whole process is in fact very inconstant. Generally speaking the organisms remain unaltered for about an hour and a half. The alterations in appearance culminate in the complete loss of the trypanosome shape and the rounding off of the organism but this condition is arrived at in various ways. Some of the trypanosomes simply

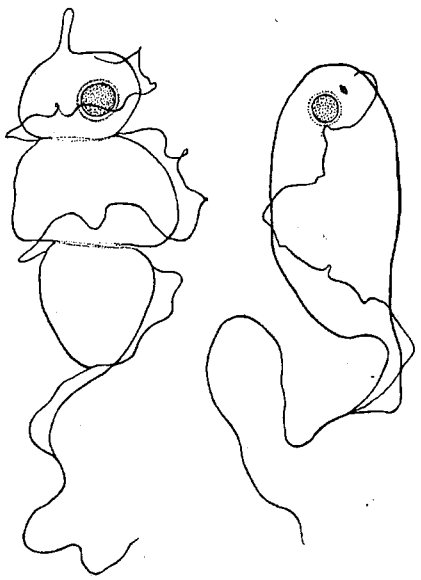


TEXT-FIG. 1.—Sketches of live Trypanosomes to show early phases in the rounding off of the parasites. A and B are one individual, so also C and D.

become much thickened at the non-flagellar end. Many become bent upon themselves, and the two limbs of the bend then fuse together (text-figs. 1.). The text-figures illustrate these appearances. The myonemeta become much more evident in most cases during these early phases. In some cases the animal broadens considerably and adopts the spiral shape, the turns of the spiral fuse together, and the most grotesque dumpy creature is produced, which keeps up a slow corkscrew or revolving motion (text-fig 2.).

Another appearance of rather a curious character is that in which the screw movement backwards and forwards is kept up but very slowly, and the body no longer preserves its regular fusiform shape ,but bulges now in one direction now in another(Text-fig. 1. A α β) The movement is difficult to convey in words , but is best described as a very metabolic euglenoid movement associated with a slow screwing backwards and forwards. During this movement the myonemeta can still be seen very clearly, and besides these ,circumferential lines can be seen running round the creature at the non-flagellate end, especially during the screw forward movement. These appearances seem on the surface to show a curious amount of variation, but this is easily explained if it is remembered that at this stage there is and obvious decrease in the firmness of the peripheral protoplasm; it fact it becomes relatively soft and viscid. This in correlation with the various methods of movement found in the normal unaltered trypanosome produces all the figures noted above. Thus, for example the trypanosome in text-fig 1 D. has obviously been executing the wheel motion when its protoplasm begab to soften and fusion occurred, and so on.

Finally ,whatever the method adopted the trypanosome comes to rest, and the flagellum breaks loose from the membrane while retaining its attachment at the kinetonucleus. It lashes about for a time. All trace of the myoneneta completely disappear, and the animal appears as an irregular mass



TEXT-FIG. 2.—Two different methods of rounding off.

See over for Text fig 3. →

instance, in text-fig. 1, C and D the Trypanosome has obviously

of protoplasm (text-fig. 3.) Occasionally part of the body at the extreme anterior end (flagellate end) projects ,this is , as far as I could make out, not withdrawn into the body,but seems like the membrane and the flagellum to disintegrate. After a time the nucleus becomes indistinct, and I noticed that after this I was never able to get a very clear view of the nucleus until just before the flagellated condition was again adopted when it showed with customary distinctness. Furrows now begin to appear in the animal, and in divides into two (text-fig. 3.). Another division follows this, and four irregular rounded or pear-shaped creatures are thus formed, lying generally more or less connected with each other. They now each put out a flagellum at one end. The flagellum is at first simply a short thick process. It lengthens and begins to lash slowly from side to side,but as as yet not capable of moving the body. Presently a slight oscillation of the body of the parasite is to be observed, and ultimately as the flagellum lengthens the creature becomes motile.

On one occasion I observed the whole process under a high power lens in an already pear-shaped individual . The flagellum can only be said suddenly to have appeared as a short, relatively thick process at the blunt end of the organism. This lengthened and became motile, after a time its origin from the body appeared to lie more laterally and a slight ridge became visible at that point. I am inclined to think that the ridge is the first appearance of the undulating membrane.

At this stage again, both as observed upon a sealed slide made from the blood of the tortoise and when the process takes place in the leech, much variation in minute detail is to be remarked, especially in relation to the relative times at which the different processes occur. Thus in the present case the preparation for the second division may , and very often does, take

place before any preparation for the completion of the first. Or, on the other hand , the two products of the first division may be come quite separate before any preparation for the second division can be detected. In the appearance of the flagellum there is also much variation. Sometimes all the four flagella are developmed before the first division of the protoplasm occurs or this may not take place until the completion of the second division. Generally speaking, the development of the flagellum lags behind when the process occurs on the seadled slide from the blood of the Emyda, while in preparations from the leech the flagella are developed as a rule very early.

The typical pear shape, which ultimately becomes fusiform,may be adopted very early; in fact , sometimes at the second division the protoplasmic body will assume the form of a longitudinally-furrwed cone rounded at the broad end. These furrows are rather curious, as there may be a number of tham giving the animal a ridged appearance. The deepest furrow is where the ultimate line of division occurs. The other furrows disappear. The length of the flagellum is in some cases considerable before the body of the organism begins to lengthen at all,and rounded little creatures with quite long flagella ,may not uncommonly be seen in blood from the crop of the leech.

The digestion in the Glossiphonia completes itself in about two to six days according to the size of the leech,but I do not know exactly what period of time must elapse before the animal feeds again under natural conditions. An apparently empty leech will sometimes quite refuse to not only to feed, but to remain on the tortoise. Nevertheless from observations upon captive leeches ,it does not appear to me to be more than a few days. The Glossiphonia show a marked tendency to get into the less-exposed corners of the body, such as the folds of skin at the back of the neck, round the bases of the limbs,and under the tail. They were actually seen to enter the cloacal chamber which is a relatively large cavity in these tortoises.

Glossiphonia shows the trypanosome very frequently in nature ,in fact, the majority of the specimens are infected, and the parasite persists in the empty leeches when no coloured matter is to be detected in the alimentary tract. I was never able to find the very earliest stages of the parsite in this leech owing to the difficulties of manipulation. It was not easy to get the leech in a condition willing to feed nor to catch it at exactly the right moment after feeding. This was ,of course ,due to its small size and wandering habits correlated with the exceeding rapidity of the early changes in the trypanosome.

In the most recently-fed animals at my disposal the parasite was already in the shape of a rather broad flagellate approaching the crithidial condition -- the first two divisions had ,in most cases already occurred. Thus a Glossiphonia which had fed on infected blood at some time between 8 a.m. on April 6th and 7a.m. on April 7th was opened just after the latter hour. The trypanosomes were already mostly in the shape of crithidia, but a few were still in the rounded state just completing division. Some long slender forms ,very narrow ,with pointed posterior end and the flagellum only reaching back to a little more than the middle of the body were already present. These long forms were not, I think, left over from the previous meal as they were of a type not usually found at the end of digestion.

The course of the infection in the Glossiphonia appears to be in brief as follows: -- The trypanosomes ingested with the blood develop in a few hours into flagellates,rather rounded and broad in shape . They may grow very considerably in size, and adopt the trypanomorphic condition,i.e. with the kinetonucleus posterior to the trophonucleus. Division still proceeds. Great variation in shape and size occurs in this middle period of digestion, and the relative position of the two nuclei varies very much even in the two products of one division. All stages from the round, rather

dumpy early crithidia to immensely long and very slender forms moving with great rapidity darting accross the field in a flash, are to be seen in the crop at the same time.

Towards the end of digestion the type becomes much more uniform, and slender forms with little protoplasm and with flagella hardly exceeding the length of the body seem to dominate to the exclusion almost of all other stages. These creatures very often have the kinetonucleus just anterior to and almost embedded in the trophonucleus. They seem at this stage ,moreover, to have reached the limit of division as dividing forms were not found. It appears probable that death would now ultimately supervene unless unless the flagellates are passed into the blood of the vertebrate host.

Conjugation was carefully watched for, but no sign of it was found.

EXPLANATION OF PLATES 16 AND 17.

All figures were drawn with the Abbé camera under a 2 mm. Zeiss apochromatic immersion objective. A No. 12 compensating ocular and tube length of 250 mm., giving a magnification of approximately 3600 diameters. This has been reduced by the lithographer to approximately 2400 diameters.

Figs. 1–7.—Trypanosoma vittatæ from the blood of Emyda vittata.

Fig. 1.—Trypanosome from blood of tortoise stained with Heidenhain's iron hæmatoxylin after fixation with corrosive and acetic, wet method throughout.

Fig. 2.—As above, showing a characteristic attitude.

Fig. 3.—Dried Giemsa film of Trypanosome showing protoplasmic halo and red ring round karyosome.

Fig. 4.—Trypanosome as above, showing myonemata and line along undulating membrane.

Figs. 5 and 6.—Small specimens from blood of tortoise.

Fig. 7.—Dividing stage from blood of tortoise.

Figs. 8–12A.—Early stages in crop of leech. These are from the water leech, Limnatis granulosa.

Fig. 8.—Division stage; the four new flagella are already developed; kinetonuclei are dividing by longitudinal splitting.

Fig. 9.—Division stage showing nuclear spindle.

Fig. 10.—Second division occurring before the completion of the first as regards the protoplasm. Both kinetonuclei in act of division, only two flagella so far developed.

Fig. 11.—Rounded flagellate—the product of the divisions of the rounded off Trypanosome.

Fig. 12.—Early flagellate stage; note the lengthening of the body.

Fig. 12A.—Another early flagellate stage.

All the remaining figures are from the Glossiphonia, with the exception of 16 and 17.

Figs. 13–15.—Osmic fixed film from leech just about the beginning of the middle stage of digestion.

Fig. 13.—Flagellate stage showing elongated body.

Fig. 14.—Flagellate, with broad posterior end.

Fig. 15.—Very long flagellate kinetonucleus at same level as trophonucleus.

Figs. 16, 17.—Early flagellate stages from horse leech to show secondary increase in size and preparation for division. Note the condition of the flagella showing outgrowth from the kinetonucleus.

Figs. 18–27 from the Glossiphonia at middle stage of digestion.

Fig. 18.—Division stage. Note relative position of the kinetonuclei and the condition of the flagella. The unequal character of the division is obvious.

Fig. 19.—Another division stage. The features are much as in Fig. 18.

Fig. 20.—Early division stage to show condition of kinetonucleus and flagella.

Fig. 21.—Trypaniform individual with broad posterior end and many red-staining granules in the protoplasm.

Fig. 22.—Small broad form.

Figs. 23 and 24.—Short, rather broad, trypaniform individuals.

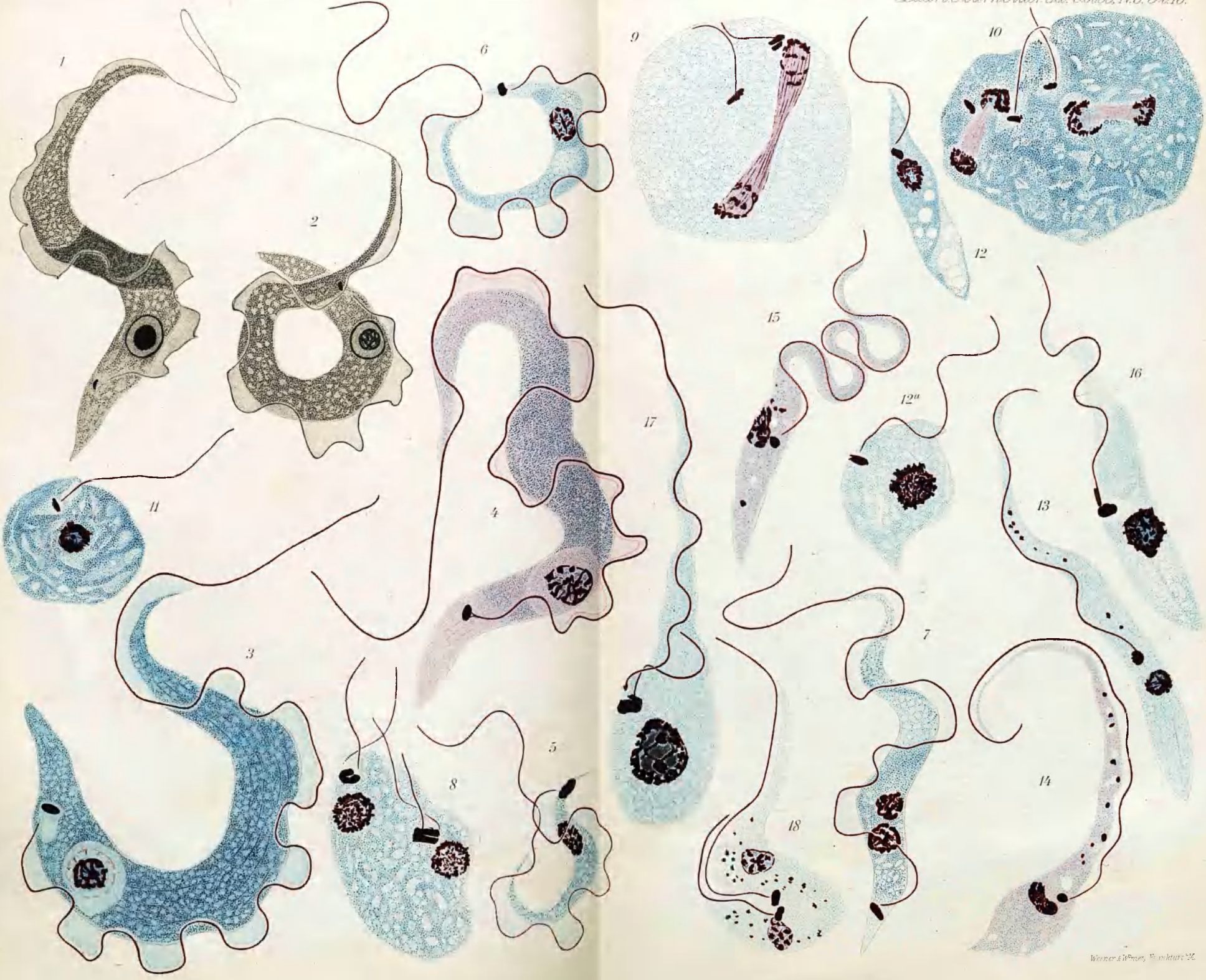
Figs. 25 and 26.—Long slender forms.

Fig. 27.—Isolated forms of this type are just appearing in this leech which is at the middle stage of digestion. This is very like the final type developed at the close of digestion.

Fig. 28.—Very long slender form.

Figs. 29 and 30.—From a leech whose digestion is still more advanced. Note the difference in the type of the organism.

Figs. 31–36.—Flagellates from leech at end of digestion, this type alone is present with very few exceptions.



Werner & Winter, Frankfurt a/M.

Trypanosomes of Certain Freshwater Fishes.

(i) Notes on the relations and natural history of the hosts.

In 1905 , Dr. Petrie (22)- had observed that the goldfish in the pond t Queensberry Lodge, in the garden of the Lister Institute at Elstree , were lmost invariably infected with a trypanosome. Three years later, Dr. J.D. homson (28) again found the flagellate in question ,and gave an account f the appearances presented in cultures grown upon blood-agar. The trypano- ome infection still persists (1911) in the blood of the fish in this pond nd, in addition, a trypanoplasm has appeared since 1908 when Dr. Thomson arried out his work.

It was obvious that the problem of the transmission of the flagel- ates presenting itself in so restricted an area was a particularly favour- ble subject of study. The pond is quite isolated , and is fed by surface rainage. Wild duck visit occasionally .

During the early part of the summer (1910- the pond was full of weed elonging to the genus Potamogeton. On dredging with a net, Nepa, Notonecta, orixa, various hydrometrids and Dytiscus were found, as well as a number of mall larval forms; none of these were found to play any part in the trans- ission of the fish-parasites. Several specimens of Argulus from a pond at iston, in Cambridgeshire, where many of the fish showed trypanosomes were xamined; they contained, however , no protozoan parasites and did not appear to feed upon blood. I never found Argulus in any of the Elstree ponds.

Nepa cinerea from the Quennsberry Lodge pond showed infection with a long slender Crithidia, presenting quite a different appearance from the herpeto- monas (H. jaculum) described by Leger (14) and by Miss Porter.(25)

The form described by these authors was subsequently obtained in Nepa sent me by Messrs. Bolton ,of Birmingham, so that the parasites could be compared with each other. The origin of the crithidia in Nepa is not clear; it may be a natural flagellate of the Nepa , or possibly derived from one or other of the animals it preys upon. I found that Nepa will attack small leeches. This part of the work was, however, not carried far enough to warrant any conclusions being drawn.

Finally, a number of leeches belonging to the species Hemiclepsis marginata were found , and these proved to be infected both with trypanosomes and trypanoplasms. Curiously enough, no other leeches were met with in this pond , except a single specimen of Glossiphonia complanata, which showed an infection with a trypanoplasm. This leech is said to feed exclusively on molluscs, acquatic annelids and small insect-larvae; I have not so far succeeded in getting it to feed upon fish. It is, on the whole ,very probable that the trypanoplasm of G. complanata is derived from one or other of the molluscs preyed upon. A parasite of this type has been described from the common Helix by Friedrich ( ). That author seems, however, to be of the opinion that the whole life-cycle of the flagellate takes place in the mollusc. Brumpt also mentions the occurrence of trypanoplasms in G. complanata and is inclined to consider that they are transmitted from pareht to offspring. ( ).

Hemiclepsis marginata from the Queensberry Lodge pond attacked the goldfish very readily . Fasting individuals were put on clean (non-infected) fish, with the result that, in one case ,a very good trypanoplasm-infection appeared ,while in the other case a slight infection with both trypanosomes and trypanoplasmswas produced. This gave a basis for more precise experiments.

In order to obtain really convincing evidence in work of this type , the quisite factors are clean vertebrates and clean specimens of the transmittimg ent. As regards the fish, circumstances were particularly favourable. . Riches very kindly placed goldfish from an artificial pond in his garden my disposal. These creatures had been examined very carefully some years o by Mr. Riches, Prof. Minchin and Dr. Thomson, and found to contain no rsites; they still showed no flagellates when examined this summer (1910-. is practically impossible to obtain a reliable source of uninfected speci- ns in nature, more especially when ,as in fish ,the infections are of a ight and chronic type. I found, as a matter of practice, that, as will be en later ,clean leeches could be used to test the blood of fish which do t show parasites in the course of ordinary microscopic examination. If a tch of five to ten small, clean leeches have fed on the blood of the fish question and do not any of them, when dissected , show flagellates, it y be concluded that the fish is not infected.

The supply of clean leeches was obtained as follows. About half a le from the Queensberry Lodge pond there is a reservoir which supplies the and Junction Canal. It is well stocked with fish, bream ,pike,perch ,rudd d roach being all fairly abundant. Wild duck and water-hens come in good mbers and nest in the reeds at the water's edge. These reservoir fish very nerally show trypanosomes and trypanoplasms in their blood.

Upon hunting carefully among the broad-leafed rushes, I found that eches of almost every British species were to be found in good numbers. e following is a list of those collected: --

(1) Hellobdella stagnalis.

(2) Glossiphonia heteroclita.

(3) Glossphonia complanata.

(4)

(4) Herpobdella atomaria.

(5) Herpobdella octoculata.

(6) Haemopis sanguisuga.

(7) Protoclepsis tessellata.

(8) Hemiclepsis marginata.

A few remarks on the habits of these leeches are necessary to make clear the conditions obtaining in the reservoir. The facts recorded are derived partly from personal observation partly from Mr. W.A. Harding's excellent account of the British leeches. (8).

(1) Helobdella stagnalis is a very common form in the reservoir and also in the neighbouring ponds. It broods its eggs and feeds upon the juices of Gasteropods, and also upon the larvae of Chironomus. I have observed that it attacks the quite young leech embryos, still showing yolk, of the genus Hemiclepsis, in cases where these have become detached from their parents Flagellates were never found in Helobdella , but a ciliate parasite belonging to the genus Anoplophrya, and closely allied to the species A. paranidis, Pierantoni (29) parasitic in the alimentary tract of a Paranais from the Gulf of Naples, was found pretty frequently in these leeches , both in specimens from Elstree and also from Histon in Cambridgeshire.

(2) Glossiphonia heteroclita broods its eggs and feeds upon the juices of Gasteropods. Anoplophrya is the only protozoan parasite found by me in this leech , which is a common species in the reservoir and in the Elstree ponds generally.

(3) Glossiphonia complanata is chiefly parasitic on Limnaea, Planorbis , and other freshwater molluscs. It lays its eggs (according to various authorities) on weed or some foreign body and broods them. Protozoan parasites were not observed, except in one individual already mentioned from the Queensberry

pond, where trypanoplasms were found in the proboscis and anterior part of the crop. It may be observed in passing that these trypanoplasms were examined in tap-water and were actively motile, while the trypanoplasm found in the proboscis-sheath of Hemiclepsis died when set free in tap-water. This leech was fairly common in the reservoir, but was not found in any of the neighbouring ponds, with the exception of the single specimen cited above.

(4) and (5) Herpobdella atomaria and H. octoculata. These leeches are very much alike in appearance and are closely allied species. They lay their eggs in transparent capsules on water-weed. They chiefly feed upon small Oligochaetes. I found that these two species were present in large numbers in the reservoir, but not elsewhere in the other ponds investigated. Orcheobius herpobdellae , a Coccidian described by Schuberg and Kunze (27) was obtained from these leeches in specimens from Histon and Elstree.

(6) Haemopis sanguisuga was also found in the reservoir ,but not in large numbers, this leech is not a blood-sucker ,notwithstanding its bloodthirsty title. It is a carnivourous creature of catholic tastes, devouring worms, several species of leeches, tadpoles, molluscs, and insect larvae; it is even said to attack small fish and newts. Protozoan parasites were never observed in this leech.

(7) Protoclepsis tessallata is a true blood-sucker . It is a very active leech and is said to feed upon the blood of wild-fowl. A number of newly-fed specimens were examined and found to contain nucleated blood-corpuscles smaller in size than those found in fishes or amphibians, and most probably derived from the numerous wild-duck which live upon the reservoir. This leech is said to be very rare in England; it is, however abundant in the Elstree

reservoir. I have never found it in other places . It did not show any protozoan parasites.

(8) Hemiclepsis marginata, a common form in the Elstree reservoir, is also a true blood-sucker and attacks fish. I have seen it feed on goldfish ,perch, bream, roach ,rudd,tench,pike and eels, and I have no doubt that it would probably attack many more species. Hemiclepsis, like Protoclepsis ,is said to be of rare occurrence ,but is, I expect, a much more common parasite than has hitherto been supposed. It is a difficult leech to find ,but from my own experience and that of Mr. Harding, I am much inclined to think that it has been overlooked. This leech has of course a number of natural enemies. I found that Nepa and Dytiscus will attack pretty big specimens. Trocheta subviridis, a large semi-terrestrial leech, devours them eagerly; fish also attack them very readily, but do not seem to become infected with trypanosomes by this channel. The young specimens of Hemiclepsis are, I have noticed,preyed upon by insect-larvae and by leeches of the species Helobdella. I have no doubt that many other creatures may ayyack Hemiclepsis, but these are the only cases I happen to have observed.

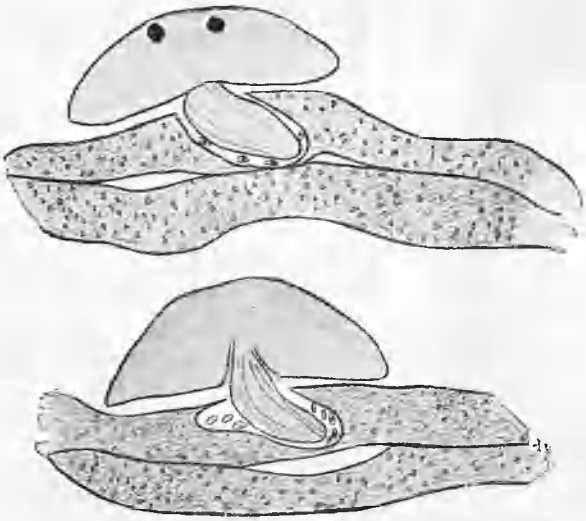
During the summer and autumn months I found in the reservoir a number of specimens of Hemiclepsis with broods. These individuals were isolated in beakers with some water-weed, and the young grew up successfully in the majority of cases,. The weed is rather important,as the leeches seem to thrive much better when it is present. The water in the beakers requires only to be changed very occasionally. It was noticed that eggs deserted by the mother died, but quite young embryos not infrequently developed by themselves. If a leech is harassed it usually deserts its brood . The young creatures have at first a good deal of yolk, which may be of a bright apple-green,a bright yellow-green or an opaque white colour. By the time the yolk is absorbed the young leeches

usually begin the desert their mother ,and are ready for their first feed of blood. They can ,however ,persist for months without any food at all.

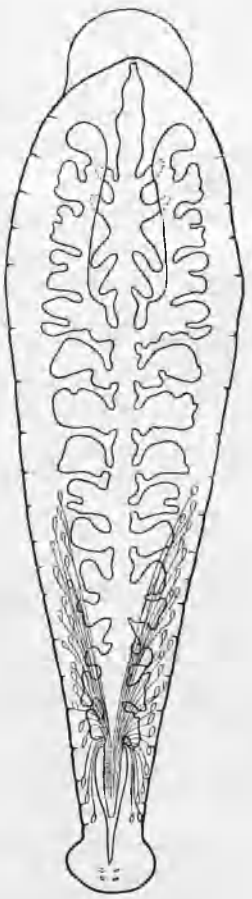
Some individuals from each brood were allowed to feed on clean fish. The small individuals take hold of their host very readily; they force their way in between the scales and seem to have no difficulty in piercing the skin. A favourite method of attack is for the leech to penetrate into the external nares. During the act of sucking the proboscis is extruded and pierces the tissues of the fish (see text-fig. /.) ,while slow and more or less rythmic contractions pass backwards along the body of the leech; these are more marked during the earlier stages of feeding. Hemiclepsis does not possess jaws or teeth.

More than 70 young leeches from 12 different broods were fed on clean godfish. and none of them developed flagellates. With the exception of four individuals, which were examined before they had fed, all these leeches were allowed to remain for a varying number of days after their first feed , in order to permit the flagellates , if any were present , to develop and multiply; some specimens were kept till after their second clean feed. Neither trypanosomes nor trypanoplasms ever appeared in any of these control leeches. This result,taken in conjunction with the precisely similar experience of Brumpt (/.) ,who carried out analogous experiments with over a hundred young individuals of this species, seems to establish the fact that ,in the case of Hemiclepsis marginata, trypanosomes and trypanoplasms are not transmitted from parent to offspring. It may be mentioned in passing that every "wild" Hemiclepsis hitherto examined has shown a greater or less infection with either trypanoplasms or trypanosomes or both.

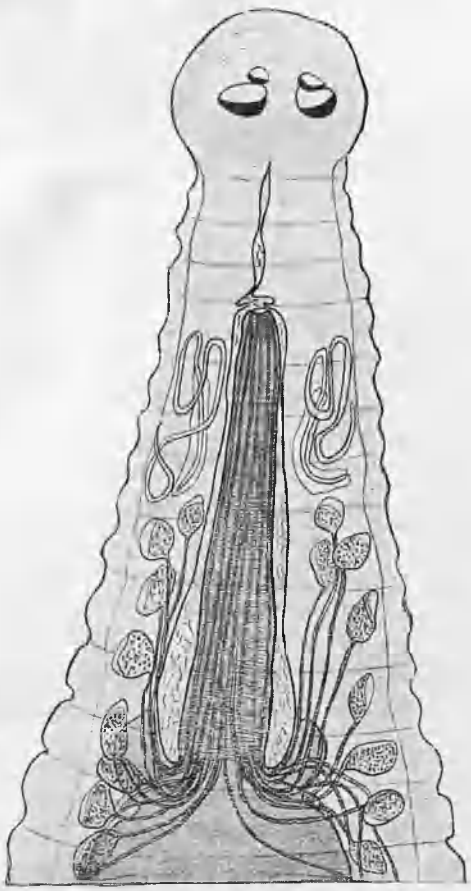
Having established that the young leeches were clean, it remained to use them as the vehicle of transmission and to observe the features of the process.



TEXT-FIG. 1.
Two Consecutive Sections of the Head of a Leech in the act of Sucking Blood from the Tail of a Goldfish.



TEXT-FIG. 2.—Diagram of Alimentary Tract of *Hemiclepsis marginata*. (After W. A. HARDING, slightly modified.)*



TEXT-FIG. 3.—Anterior end of *H. marginata* drawn from transparent living specimen. The flagellates may be seen as very minute threads in the proboscis-sheath.

* I am indebted to the editors of "Parasitology" for permission to use this figure.

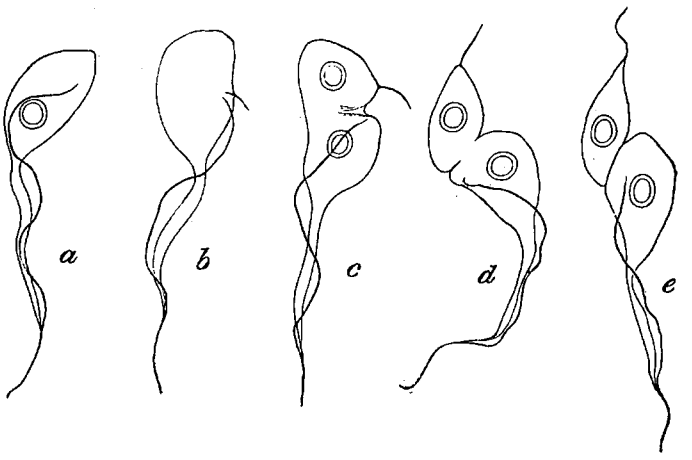
Anatomy of the leech Hemiclepsis marginata.

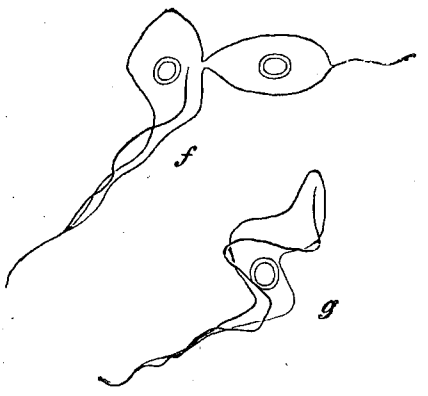
I t is necessary to give a brief account of certain points in the anatomy of the leech, more especially those concerning the alimentary tract (see text-fig. 2 & 3).

The mouth lies roughly in the centre of the anterior sucker, and leads into a somewhat thin-walled collapsible sac in the centre of which is the muscular proboscis (see text-fig 2) . This thin-walled sac is the proboscis sheath , it ends blindly at the posterior end where it becomes confluent with the proboscis. The latter which can be extruded at will leads into the large lobed crop. From the crop there opens the stomach, with four diverticula on either side; from the stomach arises the intestine, a simple coiled tube leading to the exterior by means of the short straight rectum. Just at the base of the proboscis ,at the point where it joins with the crop, there enter the many ducts of the salivary glands, which run forward in the wall of theprobascis and open at its extremity.

The salivary gland is not a compact organ , but is simply a number of single-celled elements , each with its own duct; they are very numerous, and stretch through a considerable part of the length of the body. They secrete a fluid containing many bright refractile particles, which in the live specimen can be seen to pass down the ducts to the tip of the proboscis (see text-fi 3.). The proboscis is only extruded while the leech is feeding, and in th long period during which it is retracted the salivary fluid pours out into the sheath surrounding it; it is thus always more or less bathed in the secretion from the glands.

The above description of the relation of the salivary glands applies also in the case of another British fish-leech which I had the opportunity of examining , namely Piscicola. I may remark that the points noted above





TEXT-FIG. 4 (*a*—*f*).—Sketches of one living Trypanosome, showing different moments in the process of division. ×2000. The trypanosome is from the blood of a goldfish, and is dividing in response to the admixture of water with the blood. This method of division is identical with the first divisions in the crop of the leech; *g* shows the short spiral shape which is usually the first reaction to the presence of the water.

are very clearly visible in living specimens investigated under the lower powers of the microscope. I have not seen any account of these glands which mentions the prolongation of the ducts down the proboscis, little attention has, however been paid to the anatomy of the species in question.

(2) Life-cycle of Fish-Trypansomes .

It was found that trypanosomes derived from goldfish in the Queensberry Lodge pond, from bream and perch out of the Elstree reservoir and from rudd from Histon, would all complete their cycle in clean Hemiclepsis. I use the word cycle to denote the very regular and marked succession of stages which the trypanosome passes through in the crop of the leech, and which culminate in the definite slender inoculative type found in the proboscis-sheath.

Not only did the trypanosomes from all these different sources invariably succeed in arriving in due course of time in the proboscis-sheath of the Hemiclepsis, but, in the case of the goldfish , bream and perch they could be transmitted to cleam goldfish by allowing the leeches to feed upon them. There seems to be no valid reason for considering the trypanosomes from thses different sources to be other than one species. So far as can be judged , the cycle gone through in the leech appears to be identical in the case of each of the trypanosomes mentioned . The description given here is drawn from observations on newly-hatched leeches receiving their first feed.

The trypanosomes are taken into the crop of the Hemiclepsis along with the blood. About six to nine hours after being ingested the flagellates begin to divide; in cold weather this time is somewhat increased. The division is of a very characteristic type, and consists in the budding off of small, broad Herpetomonas-like form from the posterior end of the original trypanosome, whcih remains motile throughout the process. The daughter-individual presently

develops an undulating membrane , assumes a somewhat broad crithidial condition and proceeds to divide in turn after a few hours. The details of the first division will be gone into more fully later on (see Plate and text-fig. 4) ; it suffices for the present , to say that, both parent and daughter-individuals go on dividing , so that, in two to three days , the crop is peopled with numbers of broad, somewhat spear-headed or tadpole-shaped flagellates, with the kinetonucleus anterior or just posterior to the tropho-nucleus. (figs 10-14, 19-23) That is to say , they are in a crithidial condition as regards their nuclei , but the body is much broader in shape.

Muptiplication proceeds , and it must be borne in mind that there is a very striking amount of variation in the method of division in respect to the protoplasmic body of the parsite and a general tendency to unequal fission. I have over and over again spent hours continuously watching specimens of unusual appearance in the hope that they might be conjugating individuals, and have in every case found that division and not fusion was in progress. The multiplication that goes on during this period is positively amazing, and the crop becomes filled with the flagellates even when only a very few have been originally ingested. Presently some of the individuals begin to lengthen out, and there is a general increase in size. (figs 14 - 23) . As a rule this does not take place till the fifth to the seventh day; from the eighth day onwards very slender elongated trypanoform individuals arise by division from the broader long forms (figs. 16) These very slender creatures are the inoculative type (figs 17,18); they appear at first in quit small numbers, but predominate greatly as time goes on, and end by being almost but never quite exclusively the only form present.

The final stages arise gradually from the broader creatures, and every inter-

ntermediate type is for a time represented in large numbers.

From about the tenth day onwards these very slender trypanosomes continue to pass forward into the proboscis in increasing numbers, and are to be seen lying in the sheath. They can be seen moving actively in the sheath, but have a tendency to get crowded together at the posterior end. Trypanosomes may appear in the sheath as early as the ninth day , and in one experiment three leeches feeding on a clean goldfish on the tenth day after an infected feed, produced an infection in the clean individual. This is, however, unusually early; the leeches were quite newly hatched, and the weather was warm.

I wish to emphasise particularly that the time-factor in the whole of this development is subject to great variation; trypanosomes may not appear in the proboscis-sheath for more than thirty to thirty-five days or even longer. (This refers to young leeches having their first feed -.) The point is one which seems to depend entirely on the rapidity of digestion in the individual leech.

The appearance of the inoculative type and its migration into the proboscis are, so far as I can see, the response to some chemical or physical stimulus. If the leech is larger the digestion goes on much more slowly, and the middle period of , during which there is a great range of form in the trypanosomes, is of very long duration (several months), and the appearance of the inoculative type in the proboscis-sheath is correspondingly delayed. The sheath contains no trypanosomes as a general rule until some time after the leech has absorbed all the red blood in its crop. One may often find the crop crammed with slender forms of the inoculative type ,or in stages nearly approximating to it, while the sheath is still quite clear from flagellates. The trypanosomes get firmly established throughout the crop ; it is no

infrequent occurrence to find the whole crop simply seething with amazing numbers of the creatures. I have never found the stomach or intesetine to be infested with them.

The leech is not infective until the slender forms are in the sheath, even though the rest of the leech is full of trypanosomes. Occasionally the leech is willing to feed before the flagellates reach the proboscis, but this seems to be unusual in the young individuals; it is not so rare in adult leeches. I have not up to the present observed division in the inoculative slender type of trypanosome. If the leech is not allowed to feed when digestion is quite complete and the sheath is full of trypanosomes, there seems to occur a certain amount of degeneration and death among the parasites, but new forms keep coming up from the crop to supply the deficiency. In a young leech ,after its first feed, I have found trypanosomes to persist in the sheath for more than 61 days from the time of their first appearance in that situation. Of course in nature a leech may very probably have to wait a long time between successive feeds, and the very large numbers of flagellates produced seem to be adapted to meet this circumstance.

A rather interesting point in reference to the digestion of the leech was observed accidentally. Some clean leeches, which had fed on the blood of an infected pike, were put into an incubator at a temperature of 25° C. in order to hasten the digestion of the meal. Another batch of leeches fed on the same fish at the same time were left at room-temperature. The leeches in the incubator seemed quite happy , and digestion proceeded more rapidly. After about ten days some of these leeches were examined, but to my surprise contained no parasites at all. I then examined some of the specimens which had not been incubated, and found them to be literally crammed with trypanosomes, At the same time I had put into the incubator two leeches containing

trypanosomes from and infected perch; in these specimens the red blood was all digested before they were put into the incubator. The specimens were very transparent, and I had observed very numerous trypanosomes in the crop through the body wall under a low power of the microscope, before putting them into the incubator. My purpose in subjecting then to the greater heat was to hasten the appearance of the inoculative type in the proboscis-sheath. In these specimens the trypanosomes remained alive, therefore their disappearance in the other case was not a question of absolute temperature. The explanation seems to be that, at the higher temperature, the leech is able, during the active period of secretion , simply to digest the trypanosomes. The same thing happened with some leeches at the active period of digestion which contained trypanosomes from a rudd, but in this case I had unfortunately put the whole batch into the incubator.

It may be mentioned in passing that leeches sometimes suck lymph instead of blood. The trypanosomes in an infected leech seem to be as numerous in the lymph as in the blood. This circumstance is rather fortunate , as young leeches which have fed on lymph remain very transparent.

It is interesting to note the affect on a leech infected with thypanosomes, of a single feed of blood from a clean leech. The proboscis-sheath in a trypanosome infection is invariably cleaned entirely of the flagellates, but I have never up to the present found that a single clean feed causes the parasites to disappear from the whole leech.

It is a point of some interest to determine which type of trypanosome carries on the infection in the intermediate host in the absence of fresh infection from the fish. The forms found in the crop of infected leeches immediately after a clean feed are rather small, broad trypaneform and crithidial creatures(see figs(1-4) ; sometimes they are of a curious

squat type with a very broad undulating membrane. Rare proboscis forms, very slender, may also be present but these usually show clear sihns of degeneration

After 36 to 72 hors the trypanosomes show the stages typical of the early period of digeation in the leech, and it is quite impossible to tell by inspection whether the infection is a primary one, derived directly from an infected ~~xxxxx~~ fish, or a secondary infection dating from the last feed but one (see figs 23 - 26). Numerically, these secondary infections are soon just as heavy as a good primary infection, and the inoculative type of trypanosome does not appear in the proboeis-sheath any sooner than in a primary infection, that is to say ,not until some time after all the red blood has been digested.

It is a point of some importance how closely adapted the inoculative form of these trypanosomes is to the condition obtaining at the end of digestion. Although the infection of trypanosomes as a whole is not dislodged by feeding on clean blood (certainly not by one such feed, and probably not by several-, nevertheless the inoculative type ,as such , comes and goes in direct correlation with the digestive phases of the leech, and probably in response to some quite definite environmental stimulus.

The forms from which these secondary infections originate are certainly for the most part derived from the ever-present residue of broad and sometimes nearly spherical individuals,which remain when the large majority develop into the inoculative type. I have not been able to determine quite definitely if they are the only source. It is important to discover if any of the slender proboscis-forms are capable of reverting to the broad phase. Up to the present the evidence is against this ,and degeneration seems to overtake forms ofthis kind left over in the crop from a previous meal.

If an infected leech is given a feed of blood containing trypanosomes

of the same kind, i.e. derived originally from the same species of fish (the bream was used for one experiment-), the newly-ingested flagellates can only be distinguished from the forms already there for about 24 to 36 hours, that is to say ,only so long as they still retain certain features characteristic of the parasite in the blood of the fish. Conjugation was most carefully wathched for in the leeches of this last experiment ,as it was though that it was just possible that the persisting trypanosomes might fuse with the newly ingested forms. This expectation was, however ,not fulfilled. Up to the present all attempts to find the moment of conjudation in these fagellates have been unsuccessful. This does not necessarily invalidate the theoretical expectation that it may occur at some period.

Behaviour of the trypanosomes in the fish.

Generally speaking ,trypanosome-infections in fish are of a slight and c chronic type, the number of parasites in the blood being relatively low. In my experience small-sized perch have shown the best natural infections. The fish do not as a rule , show any pathogenic symptoms, though occasionally there is quite a marked anaemia; the gills become exceedingly pale in colour,and the blood is watery. Occasional deaths occur but it is difficult to be sure that the trypanosomes are the cause.

I have obtained no information whatsoever as to how the parasite multiplies within the vertebrate host,having never come accross division stages. There is often a great variation in size in the trypanosomes of one infection, and an increase in the number of parasites seems to synchronise with the appearance of small forms, but I am quite in the dark as regards their origin. These occasional exacerbations of the infections are never very marked, and are of rare occurrence;I have not come accross them sufficiently often to

to have obtained a really satisfactory insight into their nature.

The usual course of an infection is as follows. (it must be mentioned that the clean fish were infected in the late autumn, and have not been under observation for more than a few months. I do not know if the season of the year exercises any influence on the course of the infection, but should not expect this to be an important factor.) If leeches in the right condition are allowed to feed on a clean goldfish, trypanosomes may be found by ordinary microscopic examination of the blood as early as the 5th day; they may not appear however till much later. The early finding of trypanosomes in the usual way by direct examination depends, of course, on the number of individuals injected by the leech, as well as on the subsequent multiplication in the fish. The flagellates which appear early are of a type quite markedly smaller and more slender than those seen at a later pericd. The time elapsing before the appearance of the normal form varies within a few days. Although I have never seen trypanosomes in live films before the fifth day, nevertheless a fish may already be infective for clean young leeches as early as 48 hours after the infected ones have been allaowed to feed upon it. This time is also subject to variation.

It is interesting from the point of view of the biology of the trypanosomes to find that they can resume the multiplicative condition in the leech once more after so short a sojourn in the blood of the fish. Increase in the numbers of the newly injected parasites occurs in the blood of the fish during the early days of the infection, but this soon ceases, and the numbers become stationary. Much attention cannot be paid to slight variations in the number of trypanosomes counted in a film at the different examinations, as questions of chance enter to some extent. Often there is a gradual decrease, and periods when no flagellates appear in the blood super-

vene; the blood is, nevertheless, infective to clean leeches. Slight recrudescences occur from time to time.

One infection ,even when it has become so slight as to ~~appear~~ be apparently latent,does not protect against a subsequent infection from a second leech. This was shown by the following experiment; a rather large goldfish (fish 22) from the Queensberry Lodge pond showed a slight natural infection with trypanosomes. After being in captivity for some time, flagellates were no longer to be found in the blood . Two "wild " Hemiclepsis from the reservoir were allowed to feed upon this fish,and 10 days later a number of trypanosomes were observed in its blood,as many as eight being found in a wet film under ~~a~~ a $\frac{3}{4}$by $\frac{3}{4}$ inch coverslip. The numbers decreased again rather rapidly and on the 20th day after the leeches had fed only one trypanosome was found in a film of blood. This point however needs further elaboration, as it would be of interest to see if a fish would reinfect with its own strain of trypanosomes, that is to say,by a leech whose parasites were derived from that same fishes blood while still infective.

(iii) Effect of Reagents on the Trypanosome in the Blood of the Fish.

In the early part of the investigation attempts were made to see in what way the trypanosome reacted to the presence of water. It was found to behave in a very characteristic manner. If a drop of blood from a fish showing a the usual type of infection with trypanosomes is mounted on a slide with an approximately equal amount of either tap-water or distilled water and sealed , the flagellates undergo a number of changes which culminate in division.

To quote an individual experimant, a slide was prepared with

I am indebted to Dr. Henderson Smith, of the Lister Institute, for kindly relieving me at the microscope from time to time ,so that these observations could be carried out continuously.

tap-water,as described above at 3.45 p.m. on August 4th. At 9.45. a trypanosome was selected for observation, and was watched continuously till 2,50 p.m. on August 5th. This creature when first observed already showed the first alterations, that is to say ,the body had become very much broader at its non-flagellate end. Individuals at this stage are very often in the shape of a dumpy spiral (see text-fig. 4. pag. 51.). The whole creature had become somewhat shorter, but the anterior (flagellate) end tapered out in the characteristic way. The posterior end became still more thickened and presently somewhat club-shaped, and the flagellum no longer had its origin at the posterior extremity of the body, but arose now from a point considerably further forward. The trophonucleus was very clearly visible just at this time; after a little while it disappeared ,and presently about twenty minutes later,two nuclei were to be distinguished.

There now grew out from a point quite close to the origin of the flagellum a little stiff process which gradually lengthened out and became motile; this was the flagellum of the daughter-individual. A constriction began to appear in the club-shaped thickened end, and there was gradually split off an actively motile pear-shaped creature. It had as yet no undulating membrane, the flagellum striking straight out as in a Herpetomonas. In many cases however ,the creature had already approached the crithidial phase by the time it was set free. The parent had never ceased to move all through this process, and preseved its original locomotor apparatus intact. The young animal is simply budded off from from the thickened end,

In the trypanosome under observation, the daughter form had got completely free by 1.25 a.m. on August 5th. The parent and daughter remained in the same field, and were kept under observation. The former was still somewhat broad and club-shaped, and did not resume the elongated condition

found in the undiluted blood. By 5.a.m. the nucleus in the parent had disappeared ; by 5.20. a second flagellum had grown out ,and at 6.50 a.m. this second daughter -individual was thrown off. During this same period the first daughter, which had now begun to develop an undulating membrane,divided also, but did so by longitudinal fission. At the beginning of this division it appeared as though the grand-daughter would be smaller than the daughter, but the inequality disappeared, and the two creatures were practically the same size when separation took place.

The original parent had thus split off daughter (I) at 1.25 a. m.,and had then split off daughter (II) at 6.30 a.m.; daughter (I) had in turn given rise to grand-daughter (I), the separation being complete at 7.30.a.m. At 11 a.m. daughter (II) began to divide, and at 11.5. the original parent started to divide for the third time, that is to say to throw off daughter (III) . Grand-daughter(I) began to divide at 1.50 p.m. Unfortunately at 2.50.p.m. on August 5, the trypanosomes ceased to move,their death being due to the development of bacteria on the slide.

To put the result of the foregoing observations in brief, the trypanosomes under the condition of dilution of the blood given above, divides after about six to nine hours, and the products of division in turn divide after a similar period, a third division ocurring again in six to nine hours. These ~~dixxxixxxx~~ observations have been repeated over and over again as far as the first division and somewhat less frequently to the second. The time-factor in these divisions varies somewhat, especially in relation to temperature.

It has been observed that sometimes a few individual trypanosomes react very tardily, or not at all ,to the stimulus of the water, and occasionally a whole infection is found where none of them react on a given occasion. These are generally ,so far as my observation goes, very slight infections,

where the flagellates are very slender. The bearing of this was not very clear at the time when these experiments were made. Subsequent consideration and further work however, showed that here as in nearly all protozoan life-cycles the organism must have reached a certain stage before it is ripe for the next process of development. In other words it will not react to the external stimulus until the requisite internal condition has arisen. Distilled water which has been boiled and allowed to cool is just as effective as tap-water in producing division. In blood quite unmixed with water no multiplication occurs, nor in blood mixed with 1 per cent. salt solution.

The trypanosomes will live for a couple of days and remain quite active and unaltered without multiplying on a slide of blood mixed with 1 % potassium chloride, likewise in 1 % ammonium nitrate. This last was tried to see if the laking of the corpuscles had anything to do with bringing about division, since the tap-water or distilled water ,of course, lakes the fishes blood-corpuscles at once. L 1% salt solution with ½ per cent ammonium nitrate caused no alteration in the tryapnosomes ,which lived for quite the normal length of time. In ½ per cent. sodium phosphate, division of the trypanosomes took place in the case of a perch ,but was much delayed. This was used in order to see if any alteration in the behaviour of the flagellates was to be observed in the presence of phosphorous, as this substance seems to influence the production of microgametes in Saprolegnia, (Klebs //.). Extract of medicinal leech made with distilled water brought about division, whereas extract made with 0.85 per cent salt solution produced no division. Eel-serum was used to see if it caused any laking of fish blood, and also to find out if it in any way affected the trypanosomes. The blood-corpuscles showed only the very slightest signs of laking ,and the parasites seemed utterly unaffected and lived for quite the usual length of time.

In these experiments , the infected blood , which when diluted with water showed the divisions, and which when quite pure or when treated with such solutions as 1 ½ NaCl, etc showed no alteration, was often drawn from an individual fish on the same day and at the same time, so that the possibility of having struck a chance outburst of natural division of the parasite in the fish may be discounted,. Such outbursts were never found at any time. Great caution is required in analysing the results of experiments where such complicated factors are involved; the one constant feature,however, in the cases above mentioned is that, where the salt content of the blood was lowered by the fluid added, division of the trypanosomes occurred,and not under other conditions.

The osmotic pressure of the blood is about 7 atmospheres, that is to say it is isotonic with a salt solution of 0.936 per cent. This estimation is that given by Hamburger (7) for the blood of the tench, and by Hoeber (9) for the blood of the barbel. The lowering of the osmotic pressure of the blood and the probable absorption of water by the trypanosome consequent upon this seems to be the stimulus which sets off the divisions. These divisions in the diluted blood are identical with the first divisions in the crop of the leech, and it is highly probable that the lowering of the osmotic pressure of the fluid in which the trypanosomes find themselves is at least one of the factors at work in bringing about the extraordinary burst of multiplication in the intermediate host. It may be said that by adding the water I have simply started a cultivation similar to what might occur in a blood-agar tube. That is doubtless the case. The only point of the experiments in question is that they give an indication of at least one of the factors, and probably one of the chief factors conditioning the said cultivation.

I have come across divisions of this kind as noted above on slides of

blood infected with T. vittatae from the milk-tortoise and with T. raiae from the skate. In both cases I was in the habit of taking the blood from the peripheral circulation without killing the animal, and no care was taken to exclude all moisture.

This question of the absorption of water by the trypanosome is of course a similar phenomenon to that observed by Loeb (15) and others in the artificial parthenogenesis of sea-urchins eggs. It is possible that the very stimulating change from the blood of the fish to the alimentary canal of the leech has taken the place of conjugation. The possibility of a chemical stimulus taking the place of fertilisation is clearly demonstrated in Loeb's work, and there is no good reason why such a process should not occur in nature, more especially with such a relatively simple organism as a flagellate. a The point is at present one of pure speculation, but might yield some result when more work has been done.along this line.

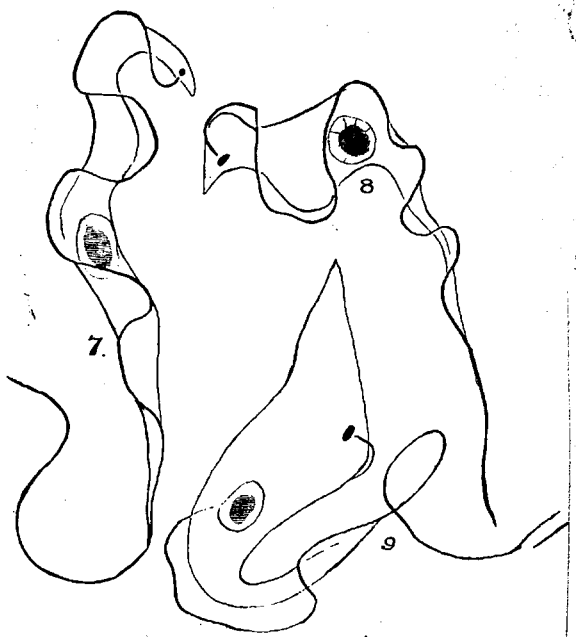


PLATE 1.

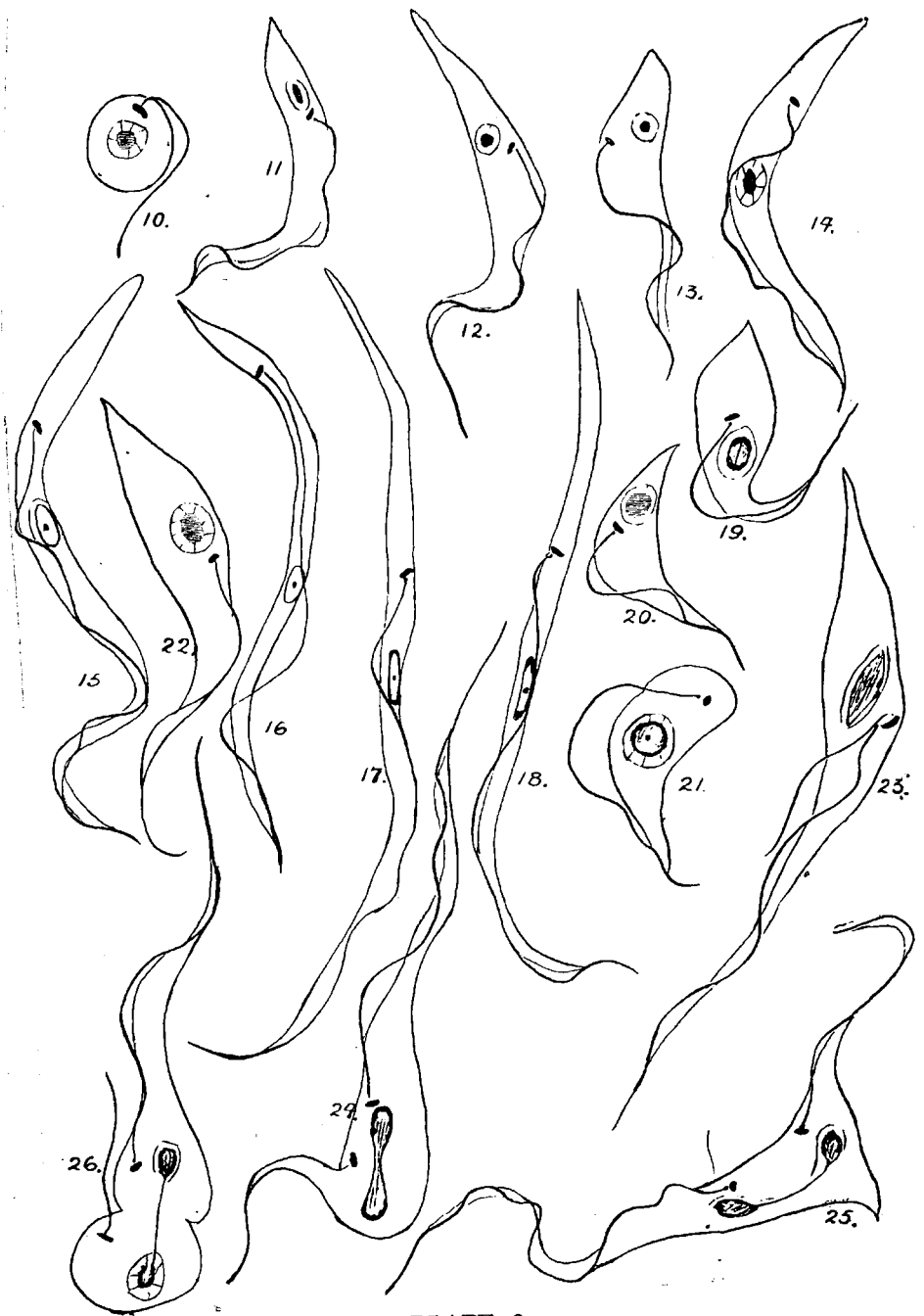


PLATE 2.

Fig. 7. Trypanosome from the perch newly ingested by a young Hemiclepsis, and still quite unaltered.

Fig. 8. Trypanosome showing earliest change in the crop of Hemiclepsis which had been feeding for 3½ hours.

Fig. 9. Trypanosome from crop of young Hemiclepsis which had been feeding for 8½ hours.

PLATE 2.

Figs. 10-13. Developmental phases from the crop of a young Hemiclepsis on 8th day after feeding.

Fig. 14. Early trypaniform phase.

Fig. 15. Long, somewhat slender form from the crop of a young Hemiclepsis on the 10th day after feeding.

Fig. 16. Slender form from the crop of a young Hemiclepsis on 9th day after feeding.

Figs. 17 and 18. Slender inoculative type from the proboscis of an adult Hemiclepsis.

Figs. 19-21. Trypanosomes from the crop of a young Hemiclepsis well infected with trypanosomes from the bream, 24 hours after a feed of clean blood.

Fig. 22. Trypanosome from the crop of a young Hemiclepsis, well infected with trypanosomes from the bream, 5 days after a feed of clean blood.

Figs. 23-26. Division-stages from crop of the same leech as in fig. 22.

II. LIFE-CYCLES OF TRYPANOSOMA GAMBIENSE AND CERTAIN OTHER TRYPANOSOMES PARSITIC IN MAMMALS.

The conditions presented by the pathogenic trypanosomes infecting man and domestic animals in Africa reveal when viewed from a broad standpoint a biological problem of very important proportions. This great question has been studied by a large number of workers and the most varied aspects have been dealt with from time to time.

In the present paper I have brought together certain results obtained during a period of two and a half years work in the Uganda Protectorate. THe conditions of work were extremely favourable and I had the great advantage of th the experience of local opportunities and the laboratory organisiation left behind on Mpumu by the Royal Societies Commission of 1908 -10 . (Sir David & Lady Bruce, Messers Hammerton ,Bateman ,Fraser etc.)

The aspect of the problem I have dealt with concerns the actual behaviour of the trypanosomes in so far as its various relations could be studied.

(i) Notes on Certain Aspects of the Development of T. gambiemse in Glossina palpalis.

In the course of an attempt to obtain an insight into the details of the life-cycle of Trypanosoma gambiense in Glossina palpalis, certain experiments were undertaken involving the feeding of a relatively large number of flies under closely observed conditions. Although primarily undertaken with a view to the morphology and development of the parsite , they have a bearing on the general relation between the trypanosome and the glossina that is of some interest.

The present account deals with the infections produced in the flies as a whole . I must point out that the experiments in question are not concerned with actual transmissions of T. gambiense from an infected to a clean host, but with the number of flies in which trypanosomes will develop. That flies harbouring trypanosomes are infective from about the 26th day onwards has been shown over and over again; it was therefore considered to be a wanton waste of life to allow every cage kept beyond the 30th day to infect a clean animal. Late cages were usually fed on cock's blood after the 24th day. A small proportion were actually tested and an infection was invariably produced if the box contained flies showing trypanosomes.

There is no evidence to show that a trypanosome infection once established in a fly is ever got rid of subsequently. T. gambiense may be held to be established if the gut shows trypanosomes after the 5th day in flies which have had at least one feed of clean blood subsequent to the infecting feed.

This last statement bears on a point of some importance; it has been found during the course of these experiments that flies allowed to feed on infected blood and then starved absolutely, when dissected between the 6th and 12th day, show an extraordinary number of individuals in which trypanosomes are to be found. Flies starved in this way rarely live beyond the 12th day or 13th day. These experiments will be referred to as starvation experiments.

Of 103 flies so treated and fed (for one infecting feed only) in groups on different monkeys infected with T. gambiense, 22 showed trypanosomes between 6th and 12th day, that is to say 21.3 $\frac{1}{2}$ of the flies harboured trypanosomes. Six flies of the total 22 showed trypanosomes only in the sucking stomach ,or crop, as this organ should perhaps be more appropriately called, 13 showed a well-established infection in the gut and three showed trypanosomes in both

situations. It is perhaps advisable to neglect the six flies in which the parasite was only present in the crop, although a certain amount of development may go on in this organ; the percentage thus obtained is still very high, namely 15.5 .

Monkeys infected with T. gambiense, and probably most other animals with trypanosomes in their blood, have negative periods, that is to say periods during which they do not infect flies. A number of experiments have shown that trypanosomes may be found by microscopic examination, although the blood is not infective to flies. It is interesting to note that such negative periods appear to be negative in the starved as well as in the flies which are subsequently fed on clean blood. In starvation experiments the microscopic appearances do not ,so far as I have yet seen , show any distinction from those to be observed in established infections from fed flies of the corresponding ages,.

The crucial moment in the cycle appears to be the first feed of clean blood subsequent to the infecting feed. It is not evident if this clearing out of the trypanosomes by the clean blood is a purely mechanicam actiondue to the flooding of the gut or is a result of the general chemical and physical changed of condition thus brought about.

In any case the number of flies containing trypanosomes obtained in starvation experiments during periods when the vertebrate is in the infective condition would give the maximum register of the potential infectivity of that individual strain to fly. The actual number of flies containing trypanosomes from parallel experiments where the flies were however, subsequently fed, would give an indication of the additional inhibiting power of the fly under ordinary conditions whatever the the cause to which the inhibition may be due. Experiments of this type were undertaken but gave no result, as the whole

series proved negative.

The total number of flies used in the whole group of experiments under consideration in this section is 1,411 males and 1,322 females of which 42 males and 39 females show trypanosomes. Irrespective of sex, the total number is 2,733, of which 81 gave positive results.

From this total must be deducted the starvation experiments and a small group which are not strictly comparable, owing to the feeding having included toad's blood ,and also those flies dissected before the 5th day.

Consisely ,the figures stand thus:

Total	2,733 flies ;	infected flies 81
Toad and starvation experiments	234 flies ;	infected flies 17
Flies dissected between days 1--5	84 flies;	infected flies 9

Deducting the last two batches from the total there remain 2,415 flies ,of which 55 were infected ;that is 2.27 per cent of the flies harbour trypanosomes.

This percentage i.e. ,2.27, is naturally not the measure of the infectivity to the fly of any strain (or strains) of trypanosomes . It is the percetage of infected indiviuals produced by allowing 2,415 flies to feed at random ,in groups, through a period of two and a half months,on a population of nine infected monkeys. Each group receives ,of course ,only one or two feeds on the infecting monkey ,and is then fed on clean animals.

Certain obscuring features , habitually neglected in dealing with trypanosome infections , must be pointed out in figures handled in this way. The nature of the individual strain must be considered,and the occurrence of negative periods(i.e. periods when the vertebrate is not infective to fly) must be duly taken into account. As they syand the figures cited above have no real meaning. The number of infected individuals obtained by feeding flies at random upon an infected animal is neither an index of the if the infectivity of the strain mor of the potential danger of such an animal at large in

a fly area. The percentage ,however, of infected individuals produced among flies fed during periods when the blood is infective ,gives the index of the virulence of the strain as regards fly. If on the other hand, batches of ,say ,50 or 100 flies were fed on an infected monkey for every day of its life during the course of the disease, the infected glossinae produced would give an index of the infective power of the monkey as a whole.

It is obvious that these are two quite different aspects of the question, and calculations in which they are treated as one must naturally be misleading. In practice it seems usual to neglect this distinction,with the result that there has been a tendency to underestimate the potential transmitting capacity of the fly, and to overrate its individual idiosincrasy.. Given reasonably favourable conditions of temperature and moisture, it is the strain of trypanosomes and not the fly that within a relatively wide range plays the deciding role in limiting the number of infected glossina. There is of course ,as has already been mentioned ,a serious difficulty in the way of the trypanosome in its attempt to establish itself at all in the glossina, but that must be very nearly constant in all cases.

To consider some of the experiments in detail; Monkey 113, infected by wild flies from the lake-shore, first showed trypanosomes in its blood on July 25th,1911.

On August 23rd ,Monkey 113 showed trypanosomes in its blood; 137 flies were fed in groups , 36 of these were treated as a starvation experiment; the whole series proved negative with the exception of one starved cage, which showed one infected fly on the 12th day.

On August 24th , 45 flies were fed on the same monkey ,and there resulted five infected flies , that is,a percentage of 11.1 showed trypanosomes.

On August 25th ,53 flies were fed, of which two became infected which is

equal to a percentage of 3.7.

On August 26th, however, 89 flies were fed in three groups on this same mokey, and produced no infected flies at all. The experiments ran concurrently ,and shared the same weather and other external conditions, andwrre similarly fed, after the infecting feed. Here, as all through this paper ,results of flies dissected before the 5 th day are excluded. In the experiments just mentioned the flies were dissected at different periods but all after the 20th day. One is forced to the conclusion that, although the monkey showed trypanosomes on all the four days in question at the time of feeding the flies, the blood was only very slightlu infective to flies on the 23rd probably not at all to fed flies; that on the 24th and 25 th it was infective producing (adding the results for the two days) seven infected individuals out of 98 flies ,i.e. 7.1 $\frac{1}{2}$; andthat it was once more non-infective on the 26th. Under conditions so similar it is impossible to consider the difference of 7.1 $\frac{1}{2}$ to be due to the ibdividual differences of the two batches of laboratory-hatched flies. The onus of this discrepancy must it seems to me be borne by the trypanosomes derived from the monkey. In this experiment the blood on August 26th showed very numerous trypanosomes ,the cage of 89 flies produced negative results, on the following day there were very few trypanosomes in the blood. The swarming period on the 26th which was not infective corresponded therefore to a moment of physiological depression of thetrypanosomes population when the hostile mechanism which in the next 24 hours produced the very great reduction in numbers was probably already beginning to operate. It is interesting to note that the infectivity for the fly was already less on the 25th than on the 24th.

Another experiment carried out later exemplifies a similar negative state of the blood as regards infectivity to fly,but here the absolute numbers of the trypanosomes were low. On the 18th of February there were a fair number

( ) of trypanosomes present in the blood of monkey 597 , 143 flies fed on that day yield 8 infections that is, 5.6% -- on the 19th a cage of 65 flies produced 2 infections i.e. 3 % of the flies were positive. The next say however marks a reduction period overtaking a moderate infection instead of a swarming infection as in the experiment cited above,on this day Feb,20th, a cage of 65 flies are fed wiht negative results ,the absolute numbers of the the tryapnosomes in the blood are few. On the 21st the trypanosomes population is reduced to very scarce individuals in the peripheral blood, but their condition is nevertheless viable in the fly and 5 % of infected flies are produced in a cage of 98. The 22 nd is again negative to fly but the test is not so good as only 46 flies were fed , the absolute numbers of the parasites in the blood are still very low.

for the sake of completeness I add the percentage of infected flies produced over all the infected boxes containing infected flies obtained from monkey 113. Starvation results and flies dissected before the fifth day are as usual excluded.

experiment	25	69	flies fed	2	infected.
"	29	60	" "	1	"
"	32	71	" "	1	"
"	43	32	" "	3	"
"	45	13	" "	2	"
"	46	53	" "	2	"
"	54	107	" "	2	"
"	64	104	" "	2	"
"	68	70	" "	1	"
"	75	20	" "	2	"
Total		599		18	

The percentage of infected flies is equal to 3 percent. I do not add the percentage obtained by including all the boxes in which the result was negative . The number so obtained would have little value as, it is purely a matter of chance so far as our present knowledge goes how often the experimenter lights upon a non-infective period. It is obvious that an uncontrolled

factor is introduced if the figures are so handled. It becomes clear that although a trypanosome-infection is in a continual state of flux the percentage of positive flies produced over infective periods gives a measure of the virulence of the strain to fly and forms a basis of comparison between different strains.

Another set of experiments bears uppn this point. Monkey 199 was infected by Dr. Duke by direct injection of the blood from a bush-buck which had been infected with T. gambiense by laboratory -infected flies. The bush-buck had harboured ~~fixas~~ T. ganbiense for 15 months. This monkey showed infective and non-infective periods in exactly the same way as other infections, but the infective periods gave quite an unusual number of flies harbouring trypanosomes. Thus ---

Experiment	71	13/9/11,	54 flies fed,	gave	4	positive	.... 7.4%.
"	70,	14/9/11,	50 "	"	7	"	.....14.0%.
"	74,	15/9/11,	46 "	"	5	"	..... 10.8%.

Considering all the figures together, out of 150 flies, 16 showed trypanosomes, that is a percentage of 10.6% .

This relatively very high percentage was also borne out by experiments of Dr. Duke's in which he made use of this monkey and which he kindly permits me to quote. Thus of 188 flies from two experiments the conditions of which admit of comparison with those of experiments 71,70, and 74 just cited, 11 were infected that is 5.8 % .

Taking this set of figures with those quoted above, of 388 flies, 27 were infected, which is equal to a percentage of 8% . This is ~~double~~ more than double the the normal percentage of infected flies produced by the Uganda atrain of T. gambiense in monkeys. All things being considered,it is impossible to attribute this difference under conditions so similar to anything but the strain of trypanosomes.

Besides having a virulent character as regards the production of in-

fected flies as a whole, an individual strain has often a recognisable type or method of development in the glossina. For instance , all flies fed on monkey 199 gave very numerous and rapidly developing infections; the trypanosomes reached the proventiculus earlier than usual,and were established in the salivary glands much more promptly than in the case of ordinary cycles. One cage was infective on the 24th day. This difference of character appeared in the flies from Dr. Duke's experiments as well as in those cited above, and I am indebted to him for the opportinity of examining them. The monkeys infected by fly fed on monkey 199 showed good onfections in the blood (monkeys 330, 390 and 391), but flies fed on these monkeys gave only the average number of infected flies ,i.e. /3%, which, however showed rather sluggish and very slowly developing infections. Thus, one of the cages fed on one of these monkeys showed an infected fly in which the infection had not yet reached forward beyond the mid-gut on the 22nd day, and another on the 56th day,in which the salivary glands were not yet infected. There was no possible chance of a "pick up " infection in either case. A stray fly, showing a very backward infection ,is generally due to having allowed a cage to stay too long on the test animal , that is until after it has produced an infection. A new cycle may then be started in a fly which had escaped on the previous occasion. This point is I may mention in passing, another argument in favour of the failure if the trypanosomes to establish themselves in the fly being due rather to the flagellates than to any absolute inhibiting quality or condition in the recalcitrant glossina.

Monkey 199 illustrates some particularly important points in regard to the cycle of T. gambiense as a whole. It has been shown ,by many experiments carried out on Mpumu, that infected buck produce a high percentage of positive flies, but that monkeys infected by means of these flies give in turn only

the usual low percentages characteristic of cycles started from monkeys. The important features are --

1. The long period during which the trypanosomes had been in the buck, namely, 15 months.
2. The infection of monkey 199 by direct injection of the blood from the bush-buck.
3. The large percentage of infected flies yielded by 199.
4. The loss of this last character when the strain is transmitted by flies to other clean monkeys.

A great deal of biological work during recent years has suggested that the function of syngamy or nuclear fusion is not reproduction but the preservation of the charcters of the species as a whole, the effect upon variation being looked upon as the most important result arising from this process. In the case of trypanosomes, the individuals rum through a relatively very large number of generations in the vertebrate, and in consequence , as is well known, are capable of developing very well-marked strains that might almost be called varieties. [illegible] One of the functions of the fly, as is suggested from these experiments appears to be to sift out these cariations of the individual strains ,and to produce a fairly even type. There is at present no sound evidence of conjugation in any trypanosome life-cycle so far worked out, and the [illegible] question must be left unprejudiced. It is a very plausible suggestion that the great and undoubtedly stimulating change of environment that occurs in the alternation of hosts has gradually led to the suppression, and finally taken the place of conjugation. This hypothesis would explain the labile characters and the extraordinary merging of species in the trypanosome group, but it is obviously open to much criticism on the score of its speculative nature, .Further ,such a conception only stands so

long as no sound evidence of conjugation in any trypanosome -cycle is at hand.

In any case it seems clear that the cyle in the fly as a whole ,whether conjugation actually occurs or not , has much of the biological significance of that process. This conception is of some importance to workers dealing with laboratory strains passed directly for long periods without reference to the intermediate host.

(ii) Notes on the Polymorphism of Trypanosoma gambiense in the blood and its Relation to the Exogenous cycle in Glossina palpalis .

The following section deals with the well-known phenomena of the fluctuaction in the numbers of trypanosomes present in the blood of an animal infected with T. gambiense ,and with the equally familiar question of the polymorphism of the parsites.

The relation of these factors to the production of infected G. palpalis is also discussed, and evidence is brought forward to show that a certain type of trypanosome is apparently responsible for the carrying on of the cycle in thetransmitting hast. The actual details of the structure of the trypanosomes and the sequence of developmental stages in the fly are not touched upon in this account . they will be dealt with in a subsequent section.

General condition of a T. gambiense infection.

It is advisable to consider first the fluctuation in numbers of the parsites. It is important to note here that a close study of any given infection brings out very clearly that the multiplication occurs very largely in the circulating blood-stream. Search has been made for any type of multiplication in the cells of the lung, liver and spleen ,so far entirely without success. Moreover, the correspondence betwwen a rise in the number of trypanosomes and a rise in the percentage of dividing forms renders such a development very improbable. The factors controlling the numbers of flagellates present in the blood at any given time fall naturally into two categories;

1. Those conditioned by the vertebrate host.
2. Those conditioned by the parasite.

Phagocytosis, alterations in temperature and the liberation in the serum of protective substances constitute the more important reactions on the part of the host likely to influence the condition of the trypanosomes, to act as inhibiting circumstances to multiplication and to cause reduction in the number of the parsites.

The important factors in regard to the trypanosome itself are the self-conditioned processes within the body of the parasite and the production of toxic substances due to its metabolism.

By self-conditioned processes I mean such manifestations as the inherent capacity for division and nutrition and the general vigour of the parasitic organism. The actual reduction in numbers of the trypanosome sets in as soon as the nugatory conditions, whatever their origin, get the upper hand. It is obvious that a continual state of tension exists between the capacity of the host to destroy the parasite and the capacity of the parasite to maintain itself. If these facts are borne in mind the nature and the details of the fluctuations are more easily comprehended, though it may not be clear exactly which set of factors is at work.

The two sets of factors here mentioned form, merely two general groups of conditions; each group represents a very complex aggregation of inter-acting circumstances concerning which we have at present very little knowledge.

Thus so far from knowing what inhibits the multiplication of an organism, we have still, in spite of the very valuable work of Hertwig, Loeb and Klebs, very little knowledge as to what are the conditions that determine the normal division of any cell. It is not therefore to be supposed that the unravelling of the conditions obtaining in an infected monkey does more than afford a certain clearness and precision to our view of the picture presented. It does not offer any actual explanation of the phenomena.

The drop in the number of trypanosomes is often sudden, but the completeness of the clearance varies within wide limits, and the duration of the depressed period is quite inconstant.

The complex factors at work producing such a reduction may supervene at any period as regards the absolute number of trypanosomes present, but the first indication that these factors have begun to come into operation is most often a reduction in the percentage of the dividing forms. The actual disappearance of the trypanosomes is, moreover, generally preceded by a shorter or longer period ,during which the numbers are relatively stable and the divisions few. There are ,however, occaional exceptions to this rule ,and the numbers may suddenly be checked and reduced in the middle of a rise.

The mechanism by which the trypanosomes are destroyed has not been studied, but the work of other observers all points at present as far as I am aware ,to phagocytosis. One must, however,consider in passing the possibility of the trypanosomes entering into the cells of the host ,and undergoing some form of development at this stage period. All the evidence is against this assumption on account of the important fact that the trypanosomes reappear relatively gradually, and their appearance is accompanied by active multiplication in the blood stream. This fact is also of interest in showing that the disappearance is a genuine destruction and not merely a withdrawal of the parasites from the peripheral blood. Lysis in the blood stream is also a possible explanation of the method of reduction in numbers,. There is a certain amount of evidence in favour of this to be drwan from the consideration of the endogenous cycle. It will be discussed later on.

The essentials of the fluctuation just sketched are repeated interminably with monotonous similarity, the only features liable to variation being the absolute numbers of trypanosomes involved and the time coefficient.

That is to say, the drop may leave a fair number of parasites in the blood , and the rise may occur without further reduction, or the drop may be very complete; it may be sudden or more gradual. The depressed period may be long or short. Thus also the rise may be more or less rapid; the exalted period msy be longer or shorter; the whole process may take place with low absolute numbers that is to say thatthe exalted period may not involve very high numbers of the parasite ---- this last is very often characteristic of the later months of an infection. A stable or relatively level period may proceed to a rise instead of to a drop, owing probably to the removal of some temporary inhibition. The extreme variability of these two elements ,namely , the duration of time of the various periods and the absolute number of the trypanosomes involved, produces a great but quite fictitious appearance of confusion. The essentials of the process are in reality extremely constant.

As a matter of practical technique these points are more easily demonstrated in the earlier periods of the infection when the absolute numbers are relatively high. I have worked exclusively with monkeys ,but no doubt the process is very similarin other animals infected with T. gambiense.
The coming and going of the trypanosomes described above may be considered to be the endogenous cycle in the blood of the vertebrate.

To consider now the polymorphism of the trypanosome and its relation (1) to the endogenous cycle, (2) to the production of infected flies,i.e. to the exogenous cycle in the fly.

Method.

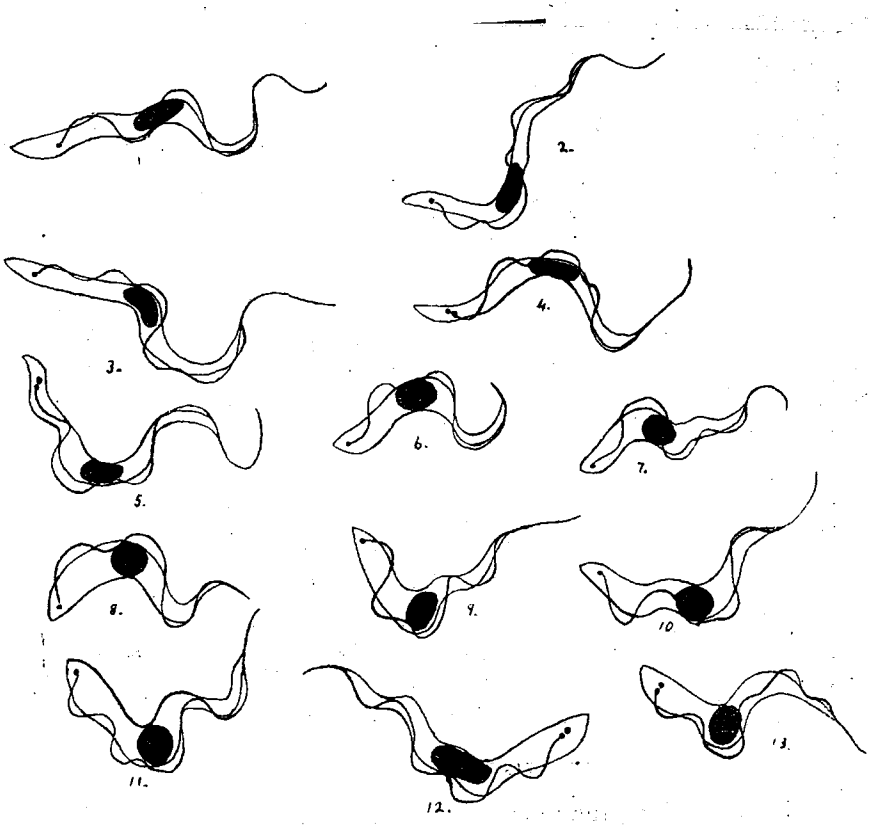
The method employed in studying the trypanosome is as follows. Blood-films are taken daily from a monkey infected with T. gambiense at the same

at the same hour (9 a.m.) ; are fixed by exposure to osmic acid vapour for half a minute; are immediately plunged into absolute alcohol and left for 10 to 20 minutes. They are then dried in air and stained with Giemsa's fluid.

This method while it gives the worst possible picture of the nuclear detail has nevertheless excellent qualities in regard to the type of result at present required. To begin with ,it fixes all the trypanosomes present in the drop of blood ,which no true "wet method " of fixation does with absolute certainty; it flattens the creatures into one plane so that they may be more easily drawn. it is rapid. the errors are apparently very uniform, and it agrees with the method used by others workers.

Whenever possible 100 trypanosomes were drawn with the Abbe drawing apparatus at a magnification of 2000.diameters. They were measured by means of a compass set at a distance corresponding to one micron, as in Bruce's method. The results were tabulated, and finally plotted on squared paper and a curve drawn. In cases where the trypanosomes were very scarce 25 or 20 , and in one instance 10 trypanosomes had to suffice; it is however ,obvious that in these cases the smaller numbers are quite as good a sample of the total forms present as 100 individuals on days when the parasites were more numerous. All possible care has been taken with the drawings, and it is hoped that the inevitable coefficient of human error will be compensated for and evened out by the relatively large number of individuals involved.

By comparing the daily curves, the distribution of the different types of trypanosomes in relation to the endogenous cycle can be observed with considerable precision. Cages of newly hatched laboratory-bred flies were fed daily when possible upon the selected monkey, and the nature of the trypanosome infection



Camera drawing. × 2000.

FIGS. 1–3, long slender types. FIGS. 4–5, typical early division stages. FIGS. 6–8, short forms. FIGS. 9–11, intermediate forms. FIGS. 12–13, rare individuals of intermediate type in division.

at the time of feeding was further studied in relation to the number of flies infected with the trypanosome produced. Each cage was fed only once on the infected monkey and every possible care was taken to see that the flies had actually fed. To obviate individuals idiosincrasies in the blood of the clean monkeys used to nourish the flies during the experiment, the monkeys were pooled, and every cage was fed on each of the group in turn during the first 15 days. The nature of the food does not seem to affect the production of infected flies after the first days.

Owing to some other experiments being carried on at the same time in the laboratory, most of the cages were actually tested as regards infectivity, and produced typical infections in the test animals in every case in which flies showing the flaggelate were present.

Endogenous cycle.

Before considering the experiments in detail , it is best to give a brief account of the general results obtained.

T. gambiense varies in length from 10 microns to 34 microns. The minimum and maximum lengths are rarely reached, the bulk of the forms varying between 15 and 32 . There is really no sharp distinction into separated types, though the range of variation is relatively wide. The trypanosomes may ,nevertheless, be divided up readily enough into short, long and intermediate forms, but the intermediate individuals insensibly join up the short and the intermediate specimens. There is a continued transition from one type to another , but the duration of the different states seems to vary considerably, and it is this variability of the time factor ,coupled with the striking appearance of the individuals at either end of the scale, that has led to the belief that the species is really dimorphic. Such figures as (text-fig p. 85) ,showing respectively short and long forms, have fostered this idea , and seem further

to have suggested that the difference is an expression of sex. Neither of these hypotheses has howver ,been sufficiently tested.

If one infection is carefully followed through a typical revolution of the endogenous cycle, the following conditions are found to obtain. It is convenient to choose the period immediately after a marked drop in the numbers, as the starting point. At such a time there are very few trypanosomes present in the peripheral blood, and they are short types. As a matter of fact, the shortest trypanosomes of the cycle are to be found at this period. They have a short free flagellum ,or may show practically no free flagellum, though it is never easy to be perfectly certain of the exact point ~~where~~ at which the body may be said to end and the flagellum alone to exist. The breadth varies, but the forms are usually rather broad, measuring about 2 to 2.5 microns. In the course of the next few days there may be a level depressed period,with little or no increase in number,and there is correspondingly little change in type. As already said, the depressed period may be very short ,or may last a number of days. The time factor, it must be emphasised,is open to the most capricious variation at all periods of the cycle. At the time when the first signs of a rise in numbers have occurred , it is found that with these there appears an immediate alteration in type, and long ,slender and intermediate forms are to be found, in addition to the short forms already present.

The sequence of events can be seen very clearly in cases where the depressed period shows a fair number of trypanosomes and where the rise is relatively slow; this is quite a frequent occurrence and the conditions are as follows :--

The first sign that a rise is about to take place is an increase in the size of a number of the trypanosomes; they become lomger and there is a general increase in the length of the free flagellum. The width does not alter at first

,and there is thus produced a state of affairs showing only short and intermediate forms ,or if the increase in size is very general,intermediate forms may exist for the time being almost alone. This is an important moment in the cycle as will be seen hereafter; some deeper physiological change in the trypanosomes accompanies the increase in size,or, rather, sets in at some period during the increase.

It is to be noted here that the increase in the numbers of intermediate forms at this stage occurs at the expense of the short forms, and one is it appears justified in assuming that they are derived one from the other. There now occurs a marked drawing out of the body in a proportion of the parasites, and there are thus produced long slender individuals such as that shown in text fig. /t 5 p 55 and immediately upon this the first burst of division occurs.

Naturally, if this process just sketched takes place with very low numbers ,if it is passed through very rapidly or very gradually,it may be somewhat obscure,as the consecutive stages do not stand out with sufficient clearness.

As soon as the divisions actually take place there is a reappearance or an increase, as the case may be ,of the short forms. So that there are present long, short and intermediate forms and dividing individuals. As the rise proceeds the relative numbers of these types vary, but there is always ,as a glance at the tables given in the appendix will show, a relative decrease in the numbers of the long and intermediate individuals as soon as there is a lull in the number of the divisions. At the height the divisions cease or drop to a very low percentage,and correlated with this there is a reduction in the numbers of the two types just mentioned. This disappearance of the long and intermediate forms is so marked that, just before the large drop in absolute numbers sets in , there is a state of affairs in which once more the shorter range of forms is almost the only one rneresented. (Chart 16

It is rather important to note that the main drop in numbers is usually preceeded by a slight but quite definite numerical reduction which seems to set in after the divisions begin to slacken, so that it appears as though the inhibition of the divisions were already accompanied by a certain amount of actual destruction of the trypanosomes, though this is not yet serious in extent. This check in the divisions before the large drop in the numbers suugests that the serum is exerting a harmful influence upon the parasites.
The vacuolated appearance of the trypanosomes to be noted in some cases just before or during the drop seems to lend further weight to this view.

One of the most striking points in the foregoing account is that the forms about to divide are the long slender ones, The disappearance of this form when the divisions cease is also most striking in this connection, so that one is led to the conclusion that the long types are the forms in preparation for division.

Briefly therefore my interpretation of the endogenous cycle is as follows:

The short forms (15 - 20 μ) constitute the normal adult blood type ; this expression which is admittedly not altogether suitable, is merely used to indicate the form which has the longest duration in time in the cycle and which is the most stable. These increase in size and bulk and form that sliding range of individuals which may be called the intermediate forms; these in turn lengthen out into the long, more slender types which proceed to divide, giving rise once more to the short forms. The products of division are often unequal to a varying extent. One individual is often much shorter, has a very short free flagellum or none at all, while the other pertner may be of considerable length.

Early dividing individuals are found more nearly approximating to the inter-

mediated type, but they are not numerous (text-fig 12 & 13) and appear to be simply the representatives of the lower range of size at which division occurs. In very numerous swarming periods or during a very rapid rise they appear to be somewhat more frequent and are probably due to a tendency to telescope the consecutive stages. It is to be observed that the kinetonucleus in these types not infrequently divides at right angles to the long axis of the trypanosome instead of prallel to it as is usual in the typical Gambiense division. I am inclined to think that the general tendency to drawing out in length has been suppressed in some way in these individuals. These divisions form a very small part of the total numbers.

I am unable to suggest any reasom why the trypanosomes should increase in length before division. We are at present without any clear knowledge of the internal stimuli and general factors producing division, although Hertwig's work upon the general nature of the physiological tension existing between the nucleus and cytoplasm of certain organisms would possibly find an application in the present instance; but it is highly doubtful if that would afford any explanation of the alteration in the actual body form.

We know nothing as to what intensely complex factors really determine the body form of such an organism as a trypanosome. To maintain in the face of the facts brought to light by the study of the endogenous cycle that the long forms are males and the short forms females seems obviously unreasonable. We have at present no basis upon which to distribute the sex labels.

The point now arises as to whether there is any observable difference which might be attributed to sex among what I have termed the "adult types". There is a certain variation in length and breadth among these ,forms , but but it is not marked and there is no evidence so far as I have seen for attributing these trifling differences to sex. This leaves of course the actual ques-

tion of conjugation quite unprejudiced for two very important reasons ;

(1) Flagellates both may and do conjugate without any external visible difference in the gametes.

(2) If differentiation of gametes does take place iy may not occur until the forms are ingested by the transmitting host.

It may be pointed out in passing that a clear view of the endogenous cycle reveals a wide range of difference in the physiological state of the trypanosomes at different times and it is probable that this is worthy of consideration from the therapeutical aspect of the trypanosome problem. The introduction of a drug would probably produce somewhat different effects ,according to the moment of the cycle chosen for its application.

Relation of the Endogenous cycle to the Production of the Infected fly.

The chief point brought forward by this aspect of the question may briefly be stated as follows: --

Is there any definite condition either of the individual parasite or of the infection as a whole requisite for the production of infected glossina? In a previous section it has been shown from experiments treated in a different manner, that negative periods in relation to fly occured, although trypanosomes might be present in the blood at the time of feeding. Moreover, these experiments pointed towards the trypanosomes rather than the fly as being responsible for the negative result. The present work has confirmed this general observation.

Failure to infect fly must depend on some one or other of the following factors: --

(1) Failure of the flies to feed: this may be called an adventitious negative,and can be obviated by keeping the flies for at least 24 hours

emerged, and by exercising patience and care at the time of feeding.

(2) Absence of trypanosomes from the blood imbibed by the flies in the experiment.

(3) Absence of sufficient numbers of a given type (or types) of trypanosome capable of surviving in the fly.

(4) Presence of the requisite type, but in a physiological state unsuitable for survival in the fly.

(5) The capacity on the part of all the flies in the experiment of digesting all the trypanosomes imbibed.

(6) External conditions of temperature or moisture unsuitable to the evolution of the trypanosome in the fly .

It must be mentioned that all fly results are obtained in spite of a certain general tendency on the part of the flies to digest their parasites. All the experiments go to show that this negative factor may be taken as being very fairly constant. Any transmitting host whose digestion is rapid tends to show a relatively low percentage of carriers ---- thus mosquitoes, fleas and tsetse flies, quite apart from the widely divergent nature of the protozoan parasites involved, all produce relatively few carriers,. Leeches and ticks, for instance ,whose digestion is very slow in comparison, give on the other hand ,practically 90 to 100 % of carriers.

It is advisable to take the same psrt of the cyclen as that selected in the last description for the starting point ,namely immediately after a drop in the numbers. Such a period which shows very few trypanosomes and all of the shorter type is an infective period and generally produces about the average number of infected flies. This is a result of considerable importance There is at this time a population of trypanosomes which have just suffered a process of elimination ,they have just passed through some set of conditions

that has proved fatal to the vast majority. There are two things to consider (1) the type of trypanosome which has survived, and (2) its condition. The type of trypanosome is very clearly the shorter individuals (see Charts 5,17,&18 in the appendix., and they must moreover ,be those capable of resisting the particular adverse circumstances to which the majority have succumbed.

There is therefor at these periods a given resistant type of trypanosome which has been shown by experiment to be ,capable of infecting flies. It can therefor be concluded that not only is it from among the short forms that the resistant type is produced but also that this type is by itself capable of inaugurating the infection in the flies.

The production of resistant strains by the use of drugs and sera has no doubt some relation to these probably temporary states of resistance occurring naturally in the untreatd host. And it is not without significance to an understanding of the general biology of trypanosomes that these resistant indiviuals are also those capable of carrying on the cycle in the transmitting host. This has a bearing too on the interesting discovery of the Sleeping Sickness Commision of 1908, that trypanosomes persisting in antelope are rare in the blood but afford a relatively high percentage of infected flies. The low numbers and the absence of pathological symptoms on thepart of the vertebrate imply that the conditions in the latter exercise a high degree of control over the parsite ,and correlated with this , the trypanosomes must in all probability be in a much more active state of resistance to their environment while sojourning in such a host. That this should also be the condition of affairs producing a large number of infected flies is of obvious importance and the connection with one another of the various instances just cited hardly needs to be further emphasised.

It has now been shown that the short forms are definitely capable of surviving in the transmitting host,but that in itself does not exclude the inte

intermediate and long forms from a similar development . This apparently does not occur.

The days of the depressed period continue to be infective to fly until just before the rise, that is until the appearance of the intermediate forms in sufficient numbers to cause a serious diminution in the short forms . This period before the rise is one in which the production of positive flies sinks so low that none are found in the experiments ---- the numer of flies ranges from about 45 to 100 in each experiment. In some cases this is probably only a relative negative, and if several hundred flies were fed probably an infected individual would be obtained.

The positive and negative periods in regard to the infectivity to fly shade insensibly into one another, thus there is a moment when the drawing out in length of the trypanosomes has begun which is still positive although the negative period supervenes in a short time. When divisions are actually taking place ,the blood is infective to fly just in proportion to the number of short forms present. A study of the charts and the analysis of the actual experiments given in the appendix make this point clear. Such a period of multiplication may have a negative phase as soon as the proportion of intermetdiate and long forms preponderates unduly.

At the height the infectivity has a tendency to diminish although the requisite form is present in large numbers. This is very marked in a swarming infection showing very numerous parsites, here the swarming period is often negative -- it has been a common experience to feed cages even for two consecutive days on a monkey in this state without producing a single infected fly. The reason is pretty clearly that the trypanosomes are in an exhausted state physiologically, and force is added to this when it is noted that the actual period

of a drop is negative.

This last fact is also an argument in favour of the surviving trypanosomes found in the depressed period having developed their resistance during the unfavourable time. That is to say these survivng trypanosomes found immediately after a drop are not, as it were, a separate type of trypanosome of a resistant character , but are a certain number of the ordinary adult type which have been capable of adapting themselves to the unfavourable conditions at the time of their occurrence.

The very first day on which trypanosomes are to be found in the blood of a newly infected monkey falls into exactly the same category as any other active period of multiplication, and is positive or negative to fly according to whether the adult type is present in sufficient numbers or not.

While in the actual experiments set out in the appendix the negative periods do not deem to be of very long duration, neverteless in feeding many cages on a population of monkeys at random these negative states have a profound influence on the results. Thus in a previous grpup of experiments, out of 62 cages of flies each of them containing from 45 to 100 flies only 29 cages showed infected flies.

The above results are it is clear , entirely opposed to a sex interpretation of the polymorphism. If the long forms were males and essential to the development in the fly then the depressed periods would not be infective. The question of sexual phases does not seem to me to require further discussion in relation to the endogenous cycle.

5. There are definite periods when the blood is not infective to fly although trypanosomes are present. Such periods are (*a*) just before an outburst of multiplication; (*b*) during the destruction of trypanosomes preceding a depressed period; (*c*) the summit of an exalted period involving very numerous trypanosomes—at such a time the parasites very frequently show signs of exhaustion; (*d*) certain periods of rapid multiplication when both the absolute and relative numbers of the shorter forms are low.

Appendix.

CHARTS AND ANALYSIS OF EXPERIMENTS.

The series A consists of observations of the blood of Monkey 597 for nineteen consecutive days. Monkey 597 was infected by wild flies from Kibanga. This strain was in general and over a long period found to produce both greater numbers of plus flies and more rapidly developing cycles in the fly than is usual with the Uganda strains.

The infection was tested in the customary ways, and the organism is undoubtedly the trypanosome which has always been considered to be *T. gambiense* by the Commission and workers in the Mpumu laboratory.

The monkey first showed trypanosomes on February 9, 1912. The series A was started on February 17.

The symbols used to give a rough estimate of the comparative numbers of parasites present upon the different days are as follows:—

+	indicates that trypanosomes are present in the blood, but very few in number.
+ to ++	= trypanosomes present, but not numerous.
++	= trypanosomes present in fair numbers.
++ to +++	= trypanosomes present in good numbers.
+++	= many trypanosomes.
++++	= blood swarming with trypanosomes.
−	= indicates that no trypanosomes were seen either on live or on stained films after a reasonable period of search. In the case of a stained film, if 30 to 45 minutes with a low power failed to reveal any trypanosomes, the film was abandoned and no drawings made.

Where not otherwise stated, each table consists of the percentage of trypanosomes of varying lengths found in the process of drawing 100 individuals. Where there is a red and a black curve, the red curve corresponds to the actual number drawn, the black to the percentages which are obviously only approximations in these cases. This has only been done, of course, in those instances in which numbers less than 100 have been drawn.

Chart I. shows few divisions—3·6 per cent.—and a curve, whose highest point coincides with the length of trypanosome equal to 19 m. Altogether the individuals, measuring between 14-20 m.

inclusive, comprise 65 per cent. of the total. The curve is remarkable in registering the shortest trypanosome found, namely, a specimen of 10 m. in length. No cage of flies was fed on this day.

Chart II. shows a typical curve of a stable period with relatively low percentages of divisions (5 per cent.) and with the highest point coinciding with the length of 19 m. 74 per cent. of the trypanosomes are between 14-20 m. in length. The cage fed on that day yielded 5 and 6 per cent. of plus flies; this is a pretty typical positive state. A fair number (+ +) of trypanosomes were present in the blood. This day, as will be seen from the following tables, is the top of this period of increase, although the absolute numbers involved are not large.

Chart III.—Here there is a diminution in the number of divisions (2 per cent.), but the most numerous trypanosomes are of 20 and 21 m. in length, and there is a drop in the percentage of plus flies (3 per cent.) produced. This curve suggests that the trypanosomes are beginning to draw out for another burst of division, which, however, does not occur, as Chart IV. appears to correspond to an actual period of reduction; there are fewer trypanosomes present, there are no divisions; the trypanosomes are in many cases vacuolated and suggestive of degeneration. A cage of 64 flies fed at this time produced no plus individuals.

Chart V. of the following day shows very few trypanosomes, only 20 being drawn, of which one is in division. The cage 68, which contained 78 flies, produced 5 per cent. of plus individuals. This curve is characteristic of the period immediately after a drop in numbers, though the depressed period is not to be of long duration, as the trypanosomes are already drawing out for a fresh rise. The highest point of the curve corresponds to a length of 19 m. The proportion of trypanosomes below 20 m. in length is large in comparison with the whole.

Chart VI. is a typical negative blood period. Here the trypanosomes are still very few in number, but they have drawn out in length, and intermediate and long forms preponderate. One individual out of the 25 drawn was in division. A cage of 46 flies yielded negative results.

The next day's Chart VII. is of interest. In the twenty-four hours between the two examinations there has been a relatively great numerical rise, so that now trypanosomes are present in good numbers (+ + to + + +); the actual divisions have, however, slacked off, and now number 4 per cent. This proves, as the next tables show, to be the highest point reached, and a short level period is being established. The forms between 14-20 m. inclusive equal only 21 per cent. of the total, but as the trypanosomes are numerous they are present in sufficiently large absolute numbers to produce a large percentage of plus flies, namely, 8·9 per cent.

Chart VIII. shows a slight drop in absolute numbers, although the actual percentage of divisions has increased from 4 per cent. to 12 per cent. The infectivity has dropped to 2 per cent., and from

the disappearance of the vast majority of trypanosomes during the following twenty-four hours one may conclude that the parasites are already in Chart VIII. struggling against the conditions which are again to check the rise. It is interesting to note that there is here a cutting down of the numbers in the middle of an obvious attempt at further multiplication.

Chart IX. marks once more the time immediately after the destruction of the trypanosomes, and once more the preponderating individuals are between 14-20 m. in length; the infectivity to fly is high—namely, 8·9 per cent. of plus flies were produced. Very few trypanosomes are present; but here again the depressed period is soon to be over, as the divisions are again setting in.

Chart X. shows the drawing out of the forms preparatory to the next rise; the relative and absolute numbers of the short forms are low; divisions number 16 per cent. of the whole, although trypanosomes are still scarce. This is, as Chart VI., a typical negative period; it is the non-infective phase of the rise. Fifty flies fed at this time produced no plus individuals.

Up to Chart IX. the infection has been fluttering backwards and forwards; there have been constant attempts at a rise always cut down by some set of contrary conditions. The absolute numbers have never been high, and the infectivity has swung between very wide limits in relatively short periods of time.

From Chart X. on there is a steady rise for some days, and the parasite definitely gets the upper hand, and the conditions are more clearly outlined.

Chart XI. shows an increase in numbers; 6 per cent. are in division. The most numerous form measures 20 m. There was no cage of flies available that day.

Chart XII. shows a fair number of trypanosomes present; an increase upon those present on the previous day; divisions equal 8 per cent. The height of the curve corresponds to trypanosomes of the length of 22 m. (*i.e.*, intermediate forms), and the shorter forms, though present, are not relatively numerous, nor are the absolute numbers yet sufficiently high to neutralise this discrepancy. Correlated with this, one finds a rather low percentage of plus flies, namely 2 per cent.

On the following day (Chart XIII.) the steady rise still continues; the trypanosomes are now present in good numbers (++ to +++). Divisions number 9 per cent., but the short forms greatly preponderate, and the summit of the curve corresponds to the length of 19 m. With the increase in the short forms, we find the infectivity rising once more, and 8·5 per cent. of plus flies were produced from the cage fed that day.

Chart XIV. shows many trypanosomes (+++) and 12 per cent. of divisions; the short forms still predominate, and the infectivity is still pretty high—5·8 per cent. of plus individuals being produced from the flies fed on that day. 19 m. is still the most numerous length of trypanosome, and the individuals of this

measurement have risen from 18 per cent. (as in Chart XIII.) to 21 per cent. of the total. In the next Chart (XV.) this length of trypanosome forms 24 per cent. of the total. The numbers have risen still further, and the parasites are now very numerous (+++ to ++++), divisions have fallen to 7 per cent., and there are signs of exhaustion beginning to set in. The infectivity drops to 1·3 per cent.—a cage with 75 flies producing only 1 plus individual.

Chart XVI. is of some importance. The divisions drop to 2 per cent., and with this one gets a marked closing of the curve which becomes tall and narrow; the length of 19 m. is now represented by 32 per cent. of the total. This is very significant in connection with the view expressed earlier in the paper that the multiplicative forms are the long types.

It is hardly necessary to point out that all the long types are not merely forms actually *in* the process of dividing, as a glance at the charts show that the percentage of individuals in division is markedly less than the percentage of long forms. Table XXVII. also disposes of this question.

There has been a reduction in the number of parasites in the twenty-four hours intervening between XV. and XVI., but there are still a fair number (++) of trypanosomes present, and this is a good example of the smaller destruction which often precedes the main clearing off of the parasites. On the following day (Chart XVII) the destruction of the trypanosomes had been so sweeping that only ten specimens could be got after prolonged searching of a 3 × 1 film. The chart gives the actual numbers, not the percentages. All the trypanosomes are below 20 m. in length, and it is of particular interest to note that, in spite of the very low absolute numbers present, a cage of 50 flies produced 2 plus flies, which equals a percentage of 4 per cent. The significance of such a result has already been discussed.

In Chart XVIII. the trypanosomes are still very scarce, but more numerous than on the previous day; 20 specimens were drawn, of which 1 was in division. Eighteen out of the 20 individuals measured between 13 and 20 m., and the cage produced 5·2 per cent. of flies.

The last chart of Series A (XIX.) shows the beginning of the next rise; the intermediate and long forms predominate. Divisions equal 20 per cent., and the cage fed on that day was negative. This is a quite typical negative picture, but the cage only contained 31 flies, which is not quite an adequate number.

Series C.

A shorter series of experiments were carried out with Monkey 642.

Monkey 642 was infected by flies from Monkey 199, which had been infected by the injection of the blood of a reedbuck. This

reedbuck had been infected with a human strain of *T. gambiense*, and had harboured it for fifteen months at the time of injection into 199. 199 produced an unusual number of plus flies, and showed virulent rapid cycles in the flies. Monkeys infected by flies carrying the 199 strain were only normally infective, producing, on the average, the 3·4 per cent. of plus flies characteristic of the usual Uganda strains. The flies, however, had infections which were markedly backward in developing and never showed very high numbers; they were, nevertheless, capable of infecting monkeys in the normal way when once the cycle was complete.

642 agreed in every way with the other strains of a similar origin. This monkey showed trypanosomes for the first time on February 15, 1912. Series C was started on March 12, 1912.

Chart XX.—Trypanosomes were very scarce. Twenty specimens drawn; 9 of these measured between 14-20 m. in length. A cage of 79 flies afforded 5 plus individuals, which is equal to 6·3 per cent. of plus flies.

Chart XXI.—Here the parasites were still rather few in number (+), though much more numerous than on the previous day. 43 per cent. of the total number measured between 13 and 20 m. The divisions equalled 16 per cent., and the plus flies amounted to 17·8 per cent. of the total. The curve is interesting as suggesting a trimorphic division of the trypanosomes; it is a transitory state; but both transient dimorphism and trimorphism are to be seen at times. The conditions already discussed seem to afford ample explanation of these phenomena.

Chart XXII. is very like XXI., and requires no further comment. The great spread of the curve may be noted in correlation with the high percentage of division, namely, 26 per cent.

Chart XXIII.—The numbers are still rising (+ + to + + +), and the curve is still very much spread. Infectivity is high, as is also characteristic of the two preceding charts. Divisions 2 to 4 per cent.

Chart XXIV. shows a sudden drop in number of divisions, which now equal only 4 per cent., and there is a slight diminution in numbers (+ +). The curve closes with corresponding suddenness, and the summit coincides with the length of 19 m. Trypanosomes of this measurement equal 26 per cent. of the total.

The absolute numbers of the parasite have never been very high, and there is no appearance of exhaustion, and in correlation with this the infectivity remains high, 8·6 per cent. of flies being produced.

By the following day there was a complete and sudden clearance, so marked that ordinary searching revealed no trypanosomes. The cage fed was negative, but was not a test, as, unfortunately, some ants broke into the cage and ate a large number of the flies.

Some further experiments were carried out, but as the results were in no way divergent they are not quoted.

Two rather interesting charts are, however, picked out; both are from the first day on which trypanosomes were observed in the blood of two monkeys newly infected by fly.

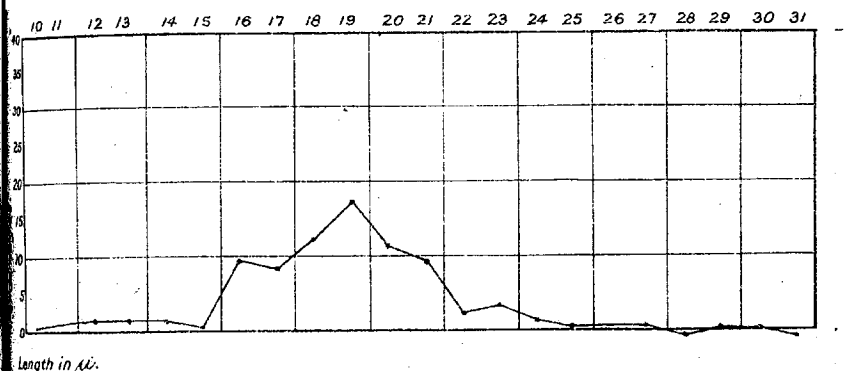
Chart XXV.—Here the trypanosomes are not numerous (+ to + +); divisions amount to 15 per cent.; the curve is wide, and the intermediate and long forms preponderate greatly. The blood is not infective to fly; 95 flies were fed, but no plus individuals resulted.

Chart XXVI.—The trypanosomes in this case are numerous (+ + to + + +); division figures amount to 30 per cent., and the curve is very wide. The percentage of short forms is curiously enough exactly the same as in XXV., namely, 17 per cent. of the whole, but the absolute numbers are here much higher, and correlated with this one finds that the blood is infective to fly, producing 4 plus individuals out of 129 flies, which is equal to 3·1 per cent.

Chart XXVII. gives the percentages of the trypanosomes of various lengths derived from drawing 2,155 trypanosomes (none of which were in division). These specimens are all the non-dividing individuals from the various series of experiments, and represent a population of trypanosomes derived from five monkeys in groups taken upon different days. The chart shows a steep curve, and supports the view that the species is polymorphic with a continuous range of variation not truly dimorphic. Sir David Bruce, in a recent paper (Proc. Roy. Soc., B. vol. 84, 1911), gives the measurement curves for *T. gambiense* and *T. brucei*; he describes the former (with which I am alone concerned) as markedly dimorphic—a statement which the curve drawn hardly bears out. I am in substantial agreement with Sir David Bruce as to the general measurements and the types figured, but do not consider that there is any evidence of marked dimorphism. The difference in contour between the curve in Chart XXVII. and that of Sir David Bruce's paper is easily explained by the much more varied population from which I have drawn my specimens. It is not stated in Sir David Bruce's paper, but probably the curve there depicted is derived from one or two slides.

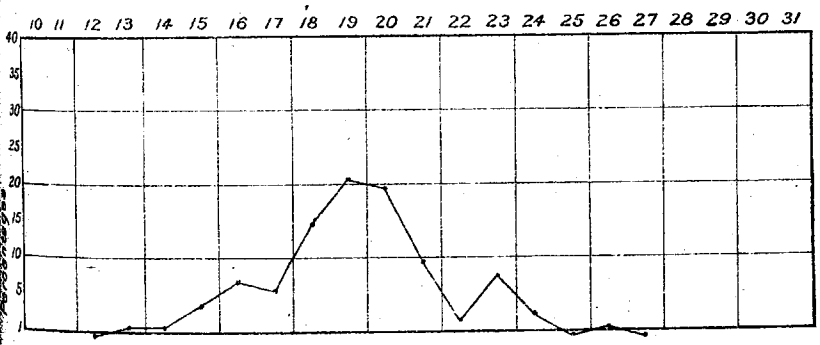
I should like to point out that the results here recorded offer an obvious criticism of the biometric method of identifying trypanosomes. A slide taken at random is clearly of no value, as a glance at the very different curves shown in the charts will at once reveal. Moreover, the number of days after infection is not a sufficiently good control of the conditions; the percentage of dividing forms would afford some basis of comparison, but even that is not entirely above reproach.

Chart I.



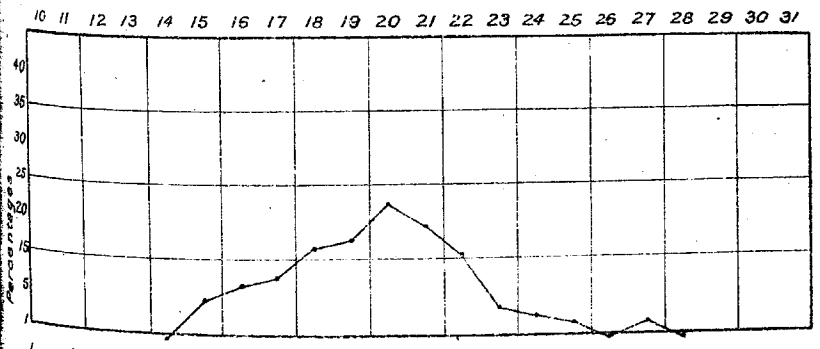
Monkey 597. 111 Tr. drawn. N° of div. = 4 = 3·6 % No cage fed.
17/2/12.

II.



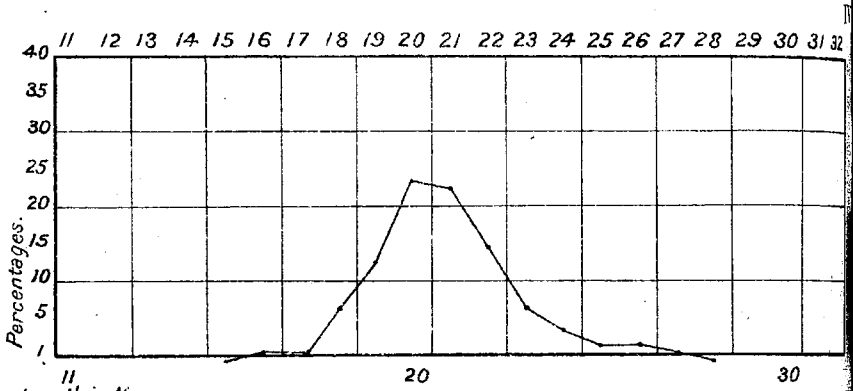
597. 100 Trypanosomes fair N° ++. div. 5%
18/2/12. Cage 95, 143 flies 8+ = 5·6 %.

III.



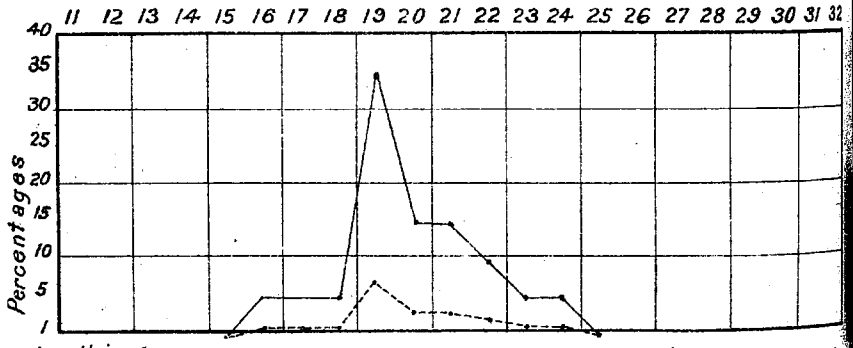
Monkey 597 2% divisions cage 96, 65 flies fed 2 + = 3% of + flies.
19/2/12

106/0 95. W. B & L. 3. 13.



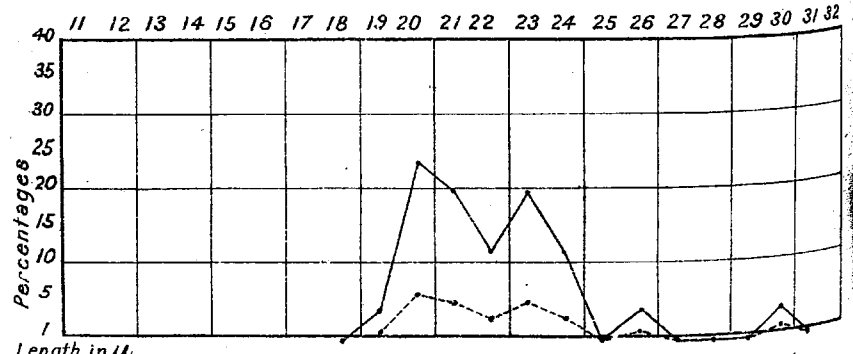
Length in μ.
Monkey 597
20/2/12

Few Ts. divisions 0%. Cage 97 fed 64 flies all negative.



Length in μ.
Monkey 597
21/2/12.

Ts very scarce 20 drawn 1 ind. in div. = 5%. Cage 98 fed 78 flies 44

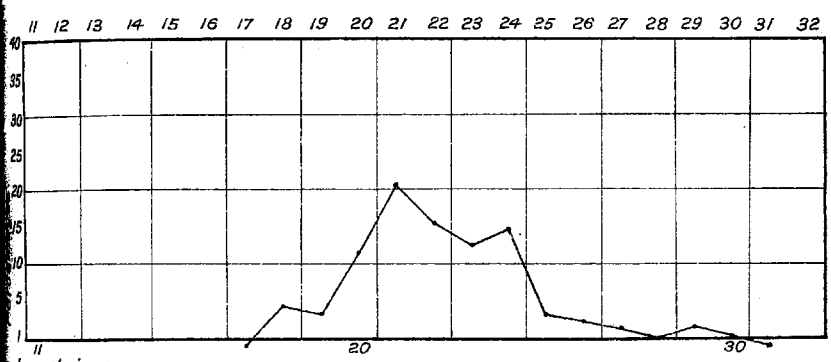


Length in μ.
597
22/2/12.

Ts very scarce 25 drawn. 1 individual in division = 4%.
Cage 101 containing 46 flies all negative.

44706/0/95. W.B.&L.-3 13.

VII.

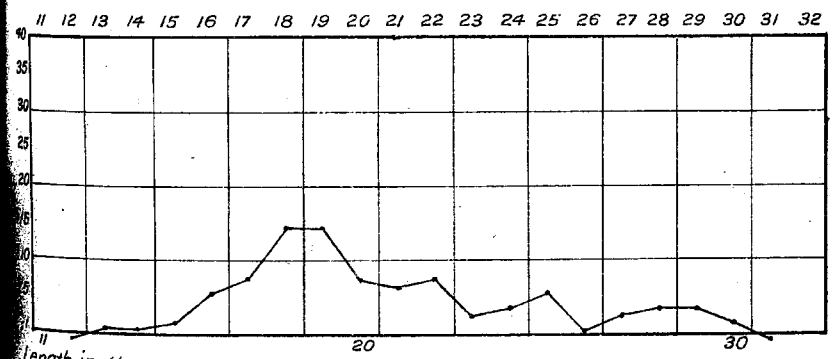


Length in μ.
Monkey 597.
23/2/12.

division 4%.
good No. of Ts. present ++ to +++.

Cage 103 fed 56 flies, 5+ = 8·9% +

VIII.

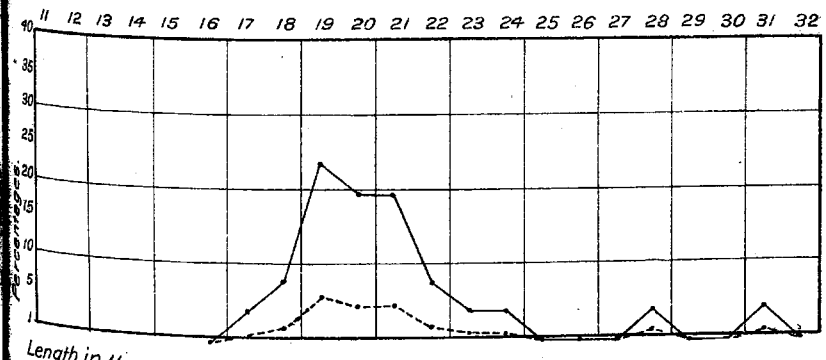


Length in μ.
Monkey 597.
24/2/12.

divisions 12%
Fair No. of Ts. ++

Cage 106, 45 flies 1+ = 2·2% of + flies.

IX.

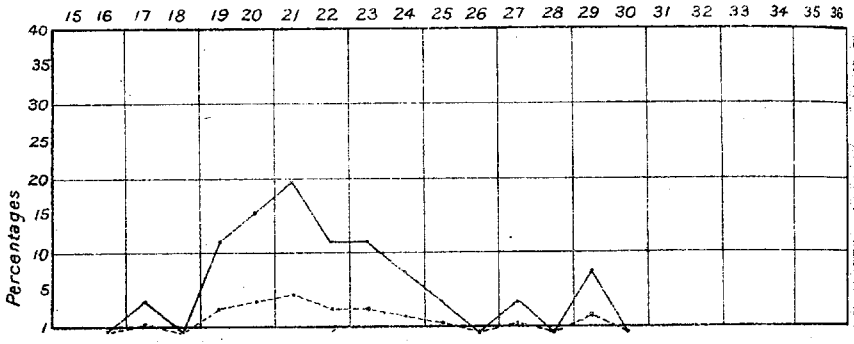


Length in μ.
Monkey 597.
25/2/12.

Ts. very scarce 25 drawn 2 div. = 8%.

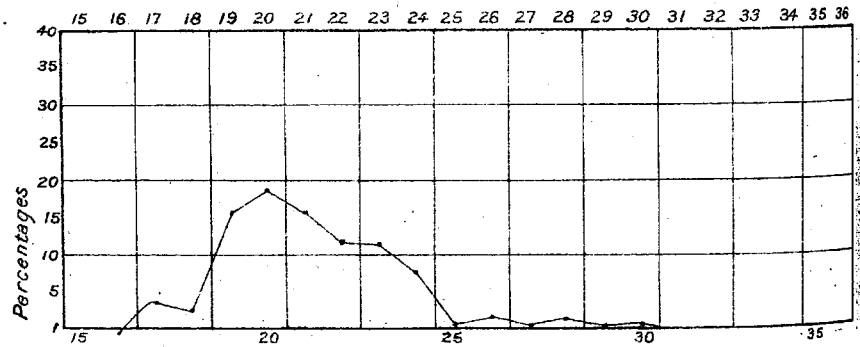
Cage 107 fed. 78 flies 7+ 8·9% of + flies.

X



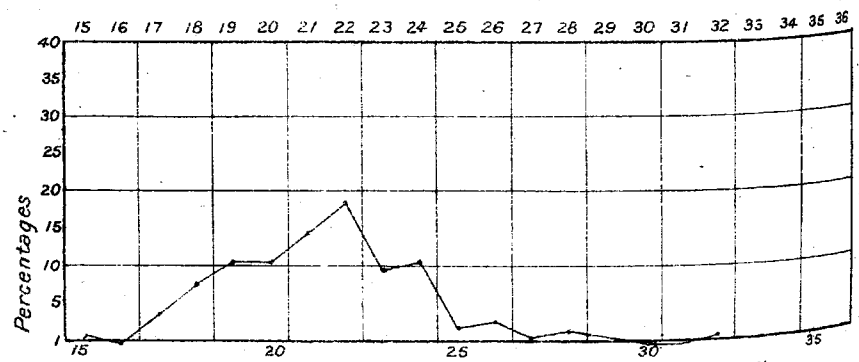
Length in μ
597 Ts. very scarce. 25 drawn. 4 in div. = 16%. (110) cage of 50 flies fed all neg.
26/2/12.

XI



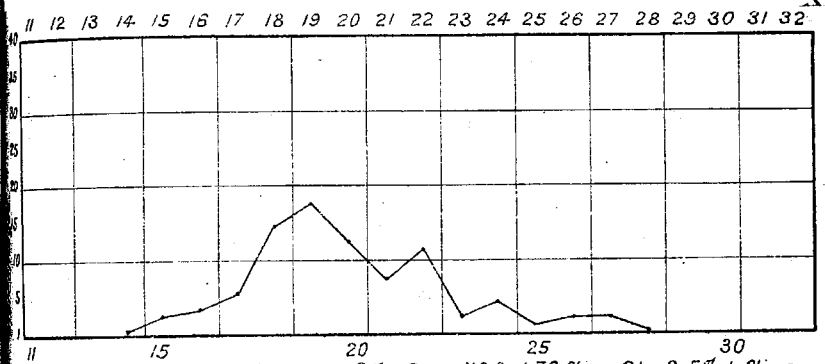
Length of Ts. in Microns. Monkey 597 date 27/2/12. Divisions 6%.
no cage fed. Ts. + to ++

XII



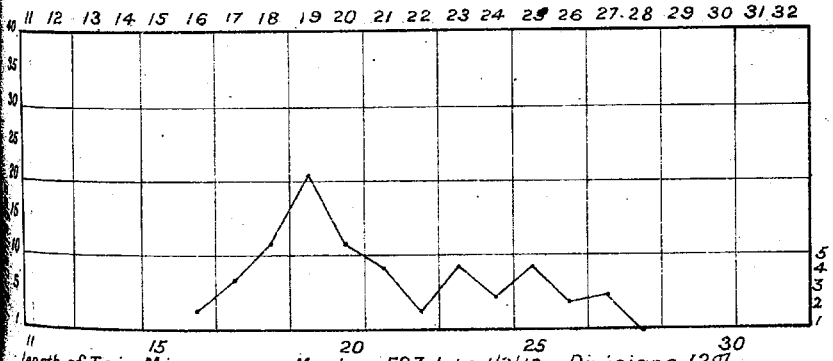
Length of Ts. in Microns. Monkey 597 date 28/2/12. Division 8%
Ts. ++ Cage (112) fed 41 flies 1 + = 2·4%.

XIII



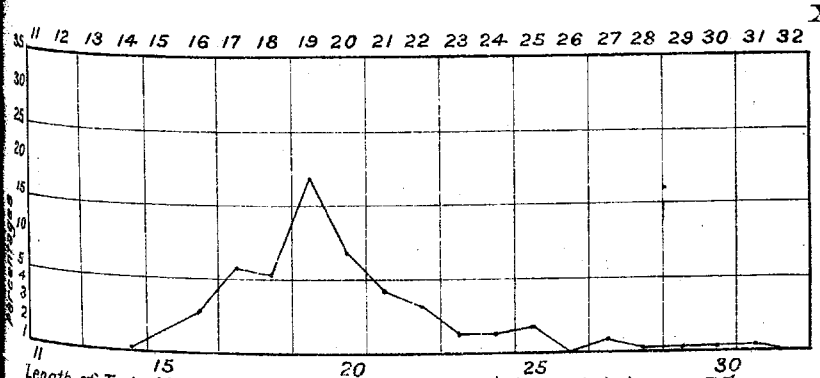
Length of Ts in Microns divisions 9%. Cage 113 fed 70 flies 6+=8·5%+flies.
Monkey 597 date 29/2/12 Ts++ to +++.

XIV



Length of Ts in Microns. Monkey 597 date 1/3/12. Divisions 12%.
Many Ts +++ Cage 115 fed 68 flies 4* = 5·8%

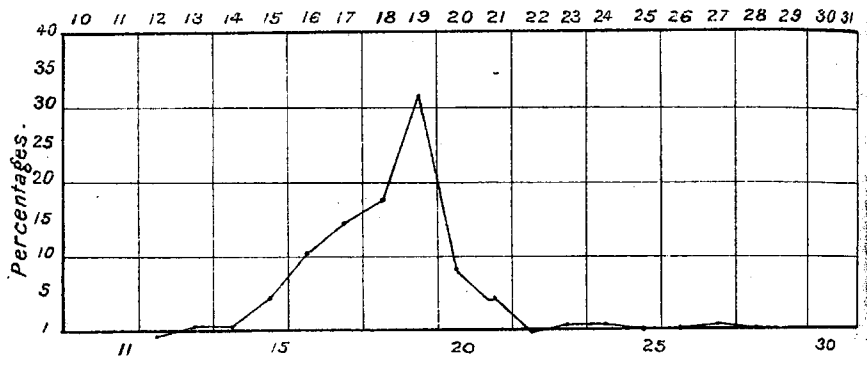
XV



Length of Ts in Microns. Monkey 597 date 2/3/12. divisions = 7%
Ts+++ to ++++. Cage 119 fed 75 flies 1* = 1·3% plus flies.

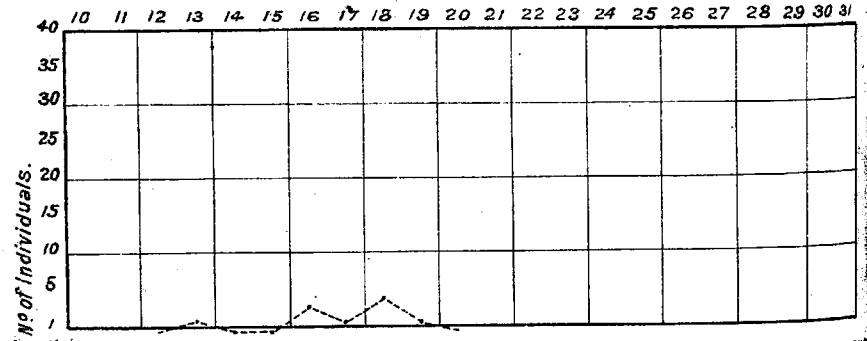
47061035. W.B.&L.3.13.

XVI



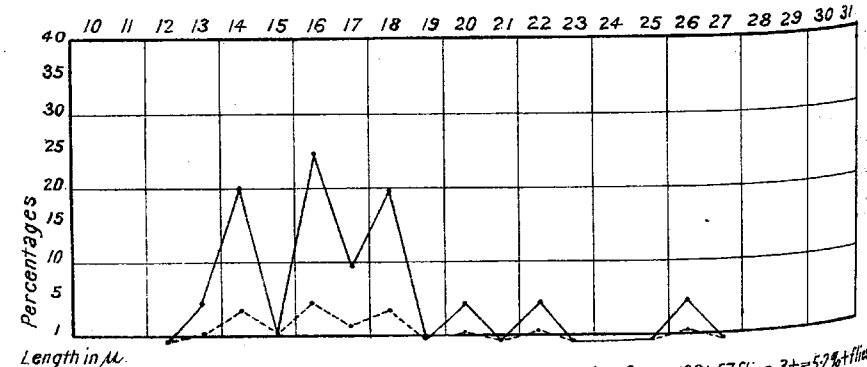
Length of Ts in Microns. Monkey 597 date 3/3/12. divisions 2%.
Ts ** much fewer than on 2nd
Cage 120 fed 72 flies 2*=2.7%.

XVII



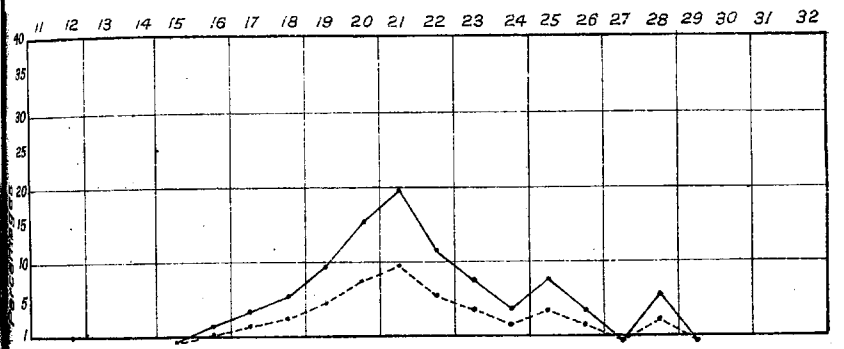
Length in μ. 597. 4/3/12. Very few Ts indeed, 10 got with great difficulty. Cage(121) fed 51 flies 2+=4% of flies.

XVIII



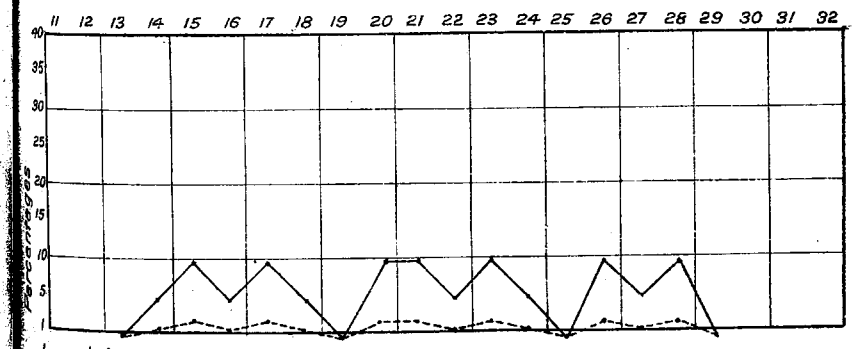
Length in μ. Monkey 597 5/3/12. Ts. very scarce 20 drawn 1 div. =5%. Cage(122) 57 flies 3+=5.2% flies.

XIX.



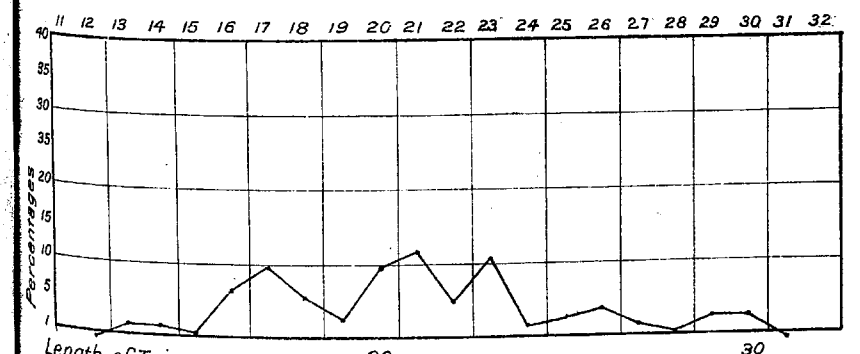
Length in μ.
597 Ts. scarce + more numerous than on 5th 50 drawn 10 div = 20%
6/3/12. Cage (126) fed 31 flies neg.

XX.

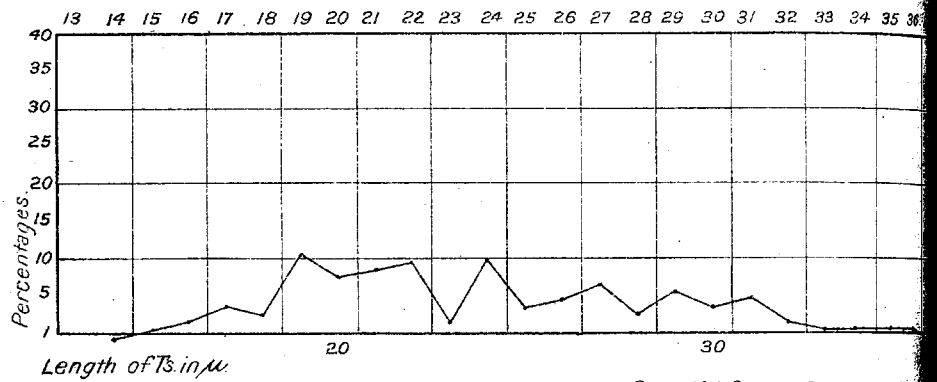


Length in μ.
Monkey 642 Ts. very scarce 20 drawn. 5 in division = 25%
12/3/12. Cage 131 fed 79 flies 5 + = 6·3% plus flies.

XXI.



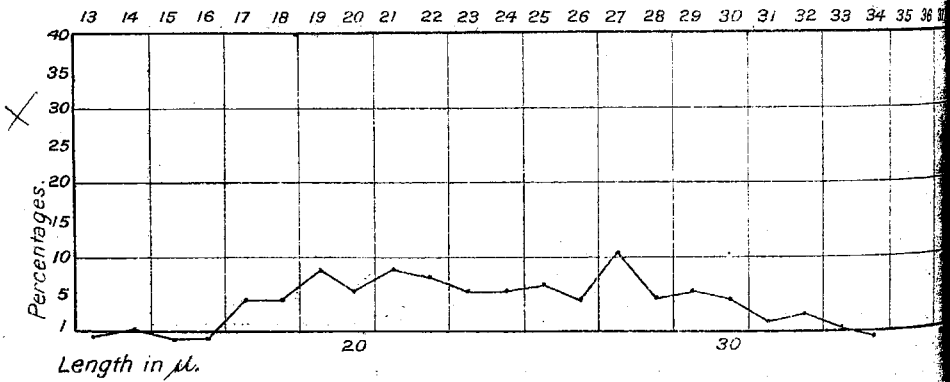
Length of Ts. in μ.
Monkey 642
13/3/12. divisions 16%. Few Ts. + Cage 132 of 64 flies, 5 plus = 7·8% plus flies.



Length of Ts. in μ.

Monkey 642 divisions 26%. fair N° of Ts. ++. Cage 134 fed, 78 flies, 6+=7·6

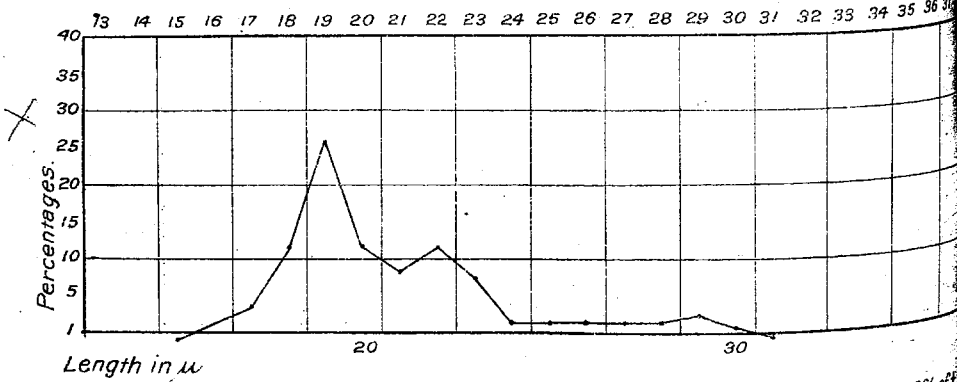
14/3/12.



Length in μ.

Monkey 642 divisions 24% Good numbers of Ts. ++ to +++.

15/3/12. Cage 135 fed. 75 flies, 6+=8

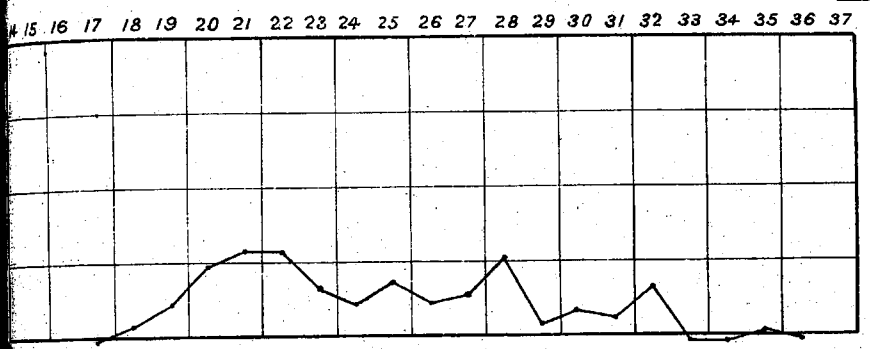


Length in μ

Monkey 642 fair N° of Ts. ++. Cage 136 fed. 58 flies, 5 plus = 8·6%

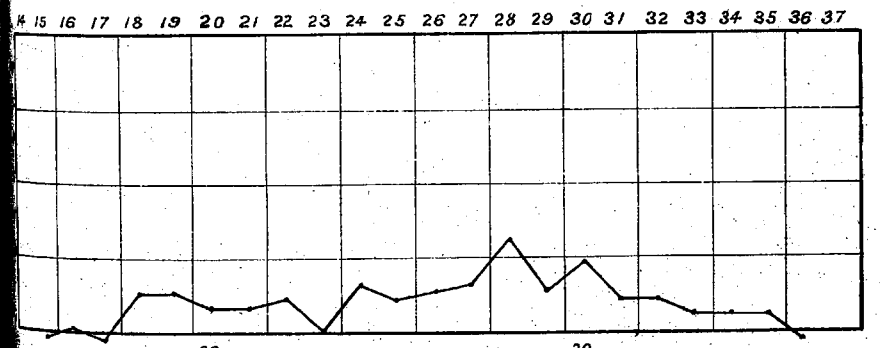
16/3/12. divisions 4%

XXV.

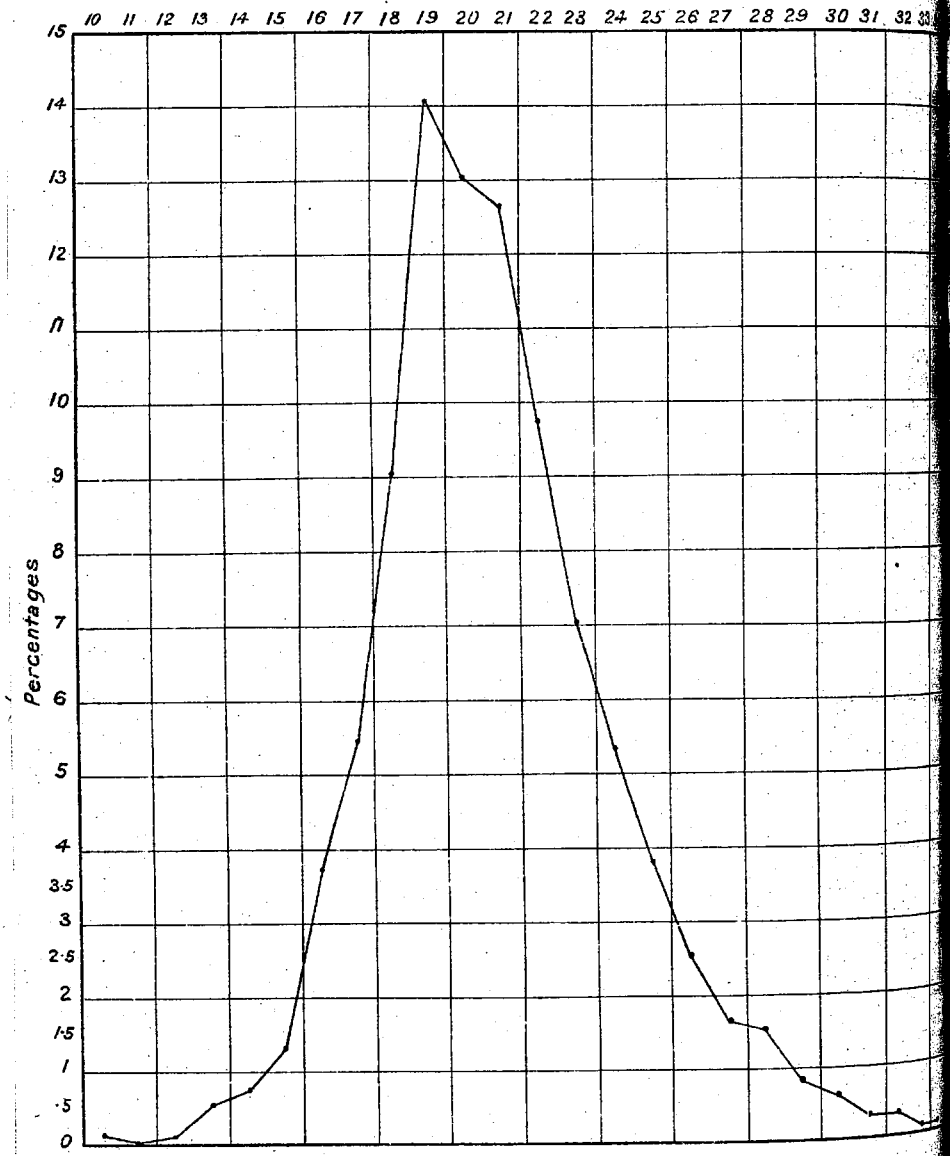


...μ. 1st day of inf. div. 15% Ts.+ to ++ Cage 148 fed 95 flies all negative.
...by 631
...12.

XXVI.



1st day of infection divisions = 30% Cage 151 fed 129 flies, 4✱ = 3.1%.
Ts. numerous, ++ to +++



Percentages	0·04	0·03	0·13	0·51	0·7	1·3	3·7	5·4	9·0	14·0	13·0	12·6	9·7	7·9	5·3	3·8	2·5	1·6	1·5	0·8	0·6	0·3	0·3	0·04
Actual Nº	1	0	3	11	17	29	81	118	198	305	283	272	210	154	115	82	56	36	34	18	13	7	7	1
Length in μ.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33

Chart of all non-dividing forms in Experiments. Nº of Ts = 2155.

14706/095. W. B & L. 3.13.

(iii) Notes on the behaviour of a polymorphic trypanosome in the blood-stream of the mammalian host.

In a previous section the conditions obtaining in the peripheral blood of monkeys infected with Trypanosoma gambiense were treated in some detail, and it was found that a certain sequence of forms recurred at irregular intervals, but that the nature of the sequence was extraordinarily uniform. This recurrence of certain types was called the endogenous cycle, and it was pointed out that this development and the duration of the different phases were dependent on the inter-relation of two complex groups of factors, namely those involved in the reaction and general metabolism of the host and those involved in the similar processes of the parasites.

The main result of this work was to show that the well known polymorphism of T. gambiense was a growth and division phenomenon the short forms, from their greater duration in time and their importance in the intermediate host were termed the adult individuals; the intermediate types were shown to be growth forms and the long slender individuals were shown to be those about to divide. The products of division directly or indirectly produce the short forms.

As this interpretation was quite unsupported by the views of other workers the earliest opportunity was naturally siezed to test its value in the case of another polymorphic trypanosome. The trypanosome here discussed was forwarded to Mpumu by Mr. Montgomery of the British East Africa Veterinary Department.

The species, which is markedly polymorphic is morphologically very like T. brucei and is well suited to test the question under consideration. .ore-

Moreover, it was readily transmitted by G. palpalis so that the strain had the opportunity of experiencing the normal passages through a suitable insect host.

As in T. gambiense the numbers in the blood fluctuate but there are two slight differences in that the depressed periods are often very short---- 12 to 24 hours, and the tendency to temporary checks in the numbers during a general increase is more marked than in T. gambiense. The drop also may be more gradual and less dramatically sudden than in the species already investigated. Nevertheless depressed periods of 3 to 4 days occur at times just as in T. gambiense and perfectly smoot rises to a high numerical climax followed by a sharp drop are also not by any means rare occurrences. Briefly while as will be seen hereafter the morphological evidences of the cycle are perhaps even clearer than in T. gambiense the duration of the physiological phases are shorter and somewhat less sharply defined.

Method.

The method adopted in carrying on these observations is as follows. The films were taken from an infected monkey on consecutive days fixed by exposure to osmic acid vapour and immediately immersed in alcohol absolute. They were then dried in air and stained by Giemsa's routine method. The image of the trypanosomes was thrown on to a sheet of paper ,by means of Abbe's drawing apparatus, a line was carefully drawn down the centre of the image to the tip of the flagellum this line being subsequently measured. The first hundred trypanosomes met with on the slide were drawn in this way including the dividing individuals. This gives the percentage of dividing forms for the film in question. These dividing specimens were then neglected and the hundred non-dividing specimens were made up by drawing the requisite number. The tables are all made up of the measurements of non-dividing specimens with the excep-

tion of table V which deals solely with division forms.

One point is made very clear by the results to be described in this part of the work ,namely that the relative percentages of long , short and intermediate forms are of absolutely no diagnostic value whatever, these proportions change from day to day in direct correlation with the phases of the endogenous cycle. See Tables III and IV.

I should like to mention in passing that one hundred non-dividing specimens happens to be a very satisfactory number as a sample of the daily condition. This is shown by the fact that two such sampes taken by the same observer on two different occasions from the films taken on one day , gave in two instances averages differing only within .5 of a micron. A similar correspondence occurred on two other occasions when 100 individuals from two other series of homologous slides were drawn by two different observers. From the four independent cases of correspondence it seems reasonable to conclude that a 100 non-dividing individuals will give a fairly accurate account of the average length of the trypanosomes for that day and a fairly reliable picture of the general conditions. It is however clear that the fluctuations in the distributions of lengths will not always be detected from the averages The weakest part of the method used is the absence of really precise estimates of the numbers present on each day.

The following table (table I) gives the distribution of length of 100 non-dividing slecimens from each of 6 consecutive days, the monkey (830- was infected by laboratory-bred flies carrying the trypanosome in question and Sept. 27th was the first day upon which trypanosomes were to be seen in the peripheral bloos. The percentages of dividing specimens found on each day and the numerical symbol of the infection are added in the right hand columns.

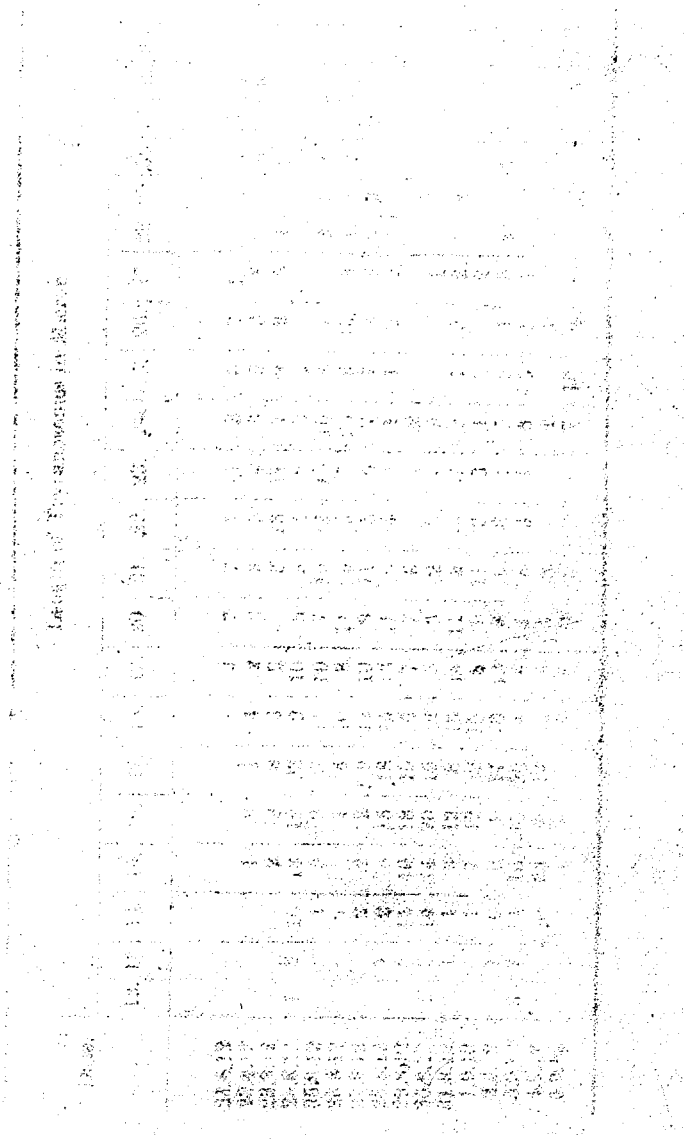
Dividing specimens are not included in the measurements of the tables I.,II., III., and LV.

Table I. Distribution of Trypanosomes of various lengths.

Monkey 830.

Date	Length of Trypanosomes in Microns.																							Percentage of division forms in blood.	Numerical formula
	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35			
27.9.12					2	3	7	1	9	9	11	10	14	6	4	6	7	5	3	2		1		27	+++
28.9.12	1		1	2	5	3	7	11	18	14	12	5	10	4	3	2		2						18	++
29.9.12	1	3	10	19	18	13	4	8	7	7	4	1	3	2										4	+
30.9.12	7	25	18	23	15	6	3	1	2			6												4	+
2.10.12	1	3	10	10	13	16	10	10	8	10	2	2	3	1		1								10	++
3.10.12	1	1	6	8	18	6	10	8	11	11	1	3	3	1	4	3	1		4					9	+++

Table LL is the corresponding account of the distribution of lengths of 100 non-dividing specimens taken daily from monkey 821 throughout 16 consecutive days. the monkey was inoculated on Aug.15th and first showed trypanosomes four days later. The table starts on Aug 21st .



Monkey 821.

Table II.—Distribution of Trypanosomes of various lengths.

Date.	Length of Trypanosomes in Microns																								Per cent. of divisions on date.	Numerical symbol.
	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.	27.	28.	29.	30.	31.	32.	33.	34.	35.	36.	37		
21.8.12								4	7	7	1	7	3	8	7	12	5	9	7	10	4	5	3	1			Per cent.	
22.8.12				1	3	1	4	2	2	3	5	4	6	5	6	8	9	7	9	14	8	1	1			1	20	++
23.8.12				2	9	8	6	9		3	13	11	7	9	9	5	4	1	3						1		24	++ to +++
24.8.12	1	2	16	13	12	23	9	10	5	3	1	2	2	1													22	+
25.8.12			1	3	9	7	11	10	7	10	12	10	10	4	1		2	2	1								6	±
26.8.12			1		1	4	10	8	11	9	5	7	20	6	10	3	1	1	2		1						29	+
27.8.12			2	2	2	9	12	10	9	11	5	6	7	7	8	3	2	2		2				1			16	++
28.8.12		1	2	6	15	18	15	13	11	8	4	1	1	1	2	2											9	+++
29.8.12		6	11	10	18	15	16	7	7	6		1	2						1								8	++ to +++
30.8.12	1	3	14	14	20	15	12	5	3	2	3	4	3			1											3	+ to ++
31.8.12		1	1	3	12	8	13	14	11	8	4	7	3	7	3	2	1	2									12	++
1.9.12			1	7	12	19	15	8	9	11	9	3	1	3		2											20	+++
2.9.12		1	6	8	9	14	10	13	8	6	6	1	5	2	4	3	2		1					1			12	+++
3.9.12	3	7	14	15	15	20	4	6	1	3	3	1	3	3	1	1											16	++
4.9.12			13	23	28	14	5	8	4	2	1		1		1												10	++ to +++
5.9.12				1	6	4	4	8	6	5		6	7	10	6	14	7	4			3						6	++ to +++
																											37	+

Tables III and IV give the same data as tables I and II, but considered from a different aspect, the percentages of long, short and intermediate trypanosomes are given for each day, the average length and the percentages of dividing individuals. The division into the three types is of course arbitrary, a specimen of 15 microns is obviously short and one of 27 microns is equally obviously long, but the actual length chosen as the dividing points are merely convenient signposts in the series. These tables show at once the manifest uselessness of the relative percentages of types as any sort of a guide in diagnosis.

Table V. gives the distribution of length for 250 dividing individuals drawn from the infection of 821 studied above. The results for all the days are combined.

Table V. Distribution of length of Dividing Trypanosomes.

in M.	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	Total number
Trypan- of given		1	2	3	11	20	36	38	23	33	30	11	16	9	8	1	5	3	250.

Monkey 830.

Table III.

Date.	Per cent. of specimens between 12 and 18 m. inclusive.	Per cent. of specimens between 19 and 23 m. inclusive.	Per cent. of specimens above 23 m.	Average length.	Per cent. of divisions on date.	Numerical symbol.
	Per cent.	Per cent.	Per cent.	m.	Per cent.	
27.9.12	2	29	69	25·4	27	+ to ++
28.9.12	9	53	38	22·7	18	++
29.9.12	51	39	10	19·4	4	+
30.9.12	88	12	—	16·3	4	±
1.10.12	37	54	9	19·7	10	++
2.10.12	34	46	20	20·9	9	+++

Monkey 821.

Table IV.

Date.	Per cent. of specimens between 12 and 18 m. inclusive.	Per cent. of specimens between 19 and 23 m. inclusive.	Per cent. of specimens above 23 m.	Average length.	Per cent. of divisions on date.	Numerical symbol.
	Per cent.	Per cent.	Per cent.	m.	Per cent.	
21.8.12	0	26	74	26·7	20	++
22.8.12	9	16	75	26·8	24	++ to +++
23.8.12	25	36	39	22·1	22	+
24.8.12	76	21	3	16·9	6	±
25.8.12	31	49	20	19·3	29	+
26.8.12	16	40	44	22·3	16	++
27.8.12	27	41	32	21·5	9	+++
28.8.12	57	37	6	18·5	8	++ to +++
29.8.12	76	21	3	17·1	3	+ to ++
30.8.12	79	17	4	17·0	12	++
31.8.12	38	44	18	20·0	20	+++
1.9.12	54	40	6	18·0	12	+++
2.9.12	48	34	18	19·5	16	++
3.9.12	78	14	8	16·8	10	++ to +++
4.9.12	83	15	2	16·5	6	++ to +++
5.9.12	15	34	51	23·3	37	+

Two points must be borne in mind in considering these tables.-----

(1) The trypanosomes are living in an active and at times even a definitely hostile environment and therefore do not have it all their own way.

(2) The percentage of forms about to divide will be greatest at the beginning of a rapid rise in numbers and will naturally diminish in frequency as the numerical height for any individual revolution of the cycle is reached, and this relative diminution in the forms about to divide will of course precede somewhat in time the actual diminution in the forms in division. Equally the percentages of forms about to divide will be lower in comparison to the forms in division in a slow rise. To put this briefly, the time factor in the process of multiplication is bound to affect the relative balance of the different types.

From tables II and III it is clear that there is a general correlation between a high percentage of long forms and a high percentage of divisions, thus to pick out a few typical cases: In monkey 830 on September 27th , there were 69 per cent of long forms and 27 per cent. of divisions, in Monkey 821 on August 22nd, there were 75% of long forms and 37 % of divisions. There is equally a clear correlation between a high percentage of short forms and a low percentage of divisions, and moreover table III of monkey 830 shows strikingly how here as in T. gambiense the depressed period immediately after the fall in numbers is charaterised by the presence of short forms; thus on Sept. 30 th ,the day in question, there were only 4% of divisions and 88 % of all the parasites were below 19 microns,the longest form present was 22 microns and there were only two specimens of this length. That is to say that just as in T. gambiense the survivors who weather the untoward conditions which carry off the majority of the parasites are drawn from the short forms.

It will be well in conclusion to run briefly through the phases of monkey 821 as illustrated in the tables II and IV to show the nature of the cycle.

The 21st and 22nd of Aug. are days of rapid increase in the absolute numbers and are charaterised by a high percentage of long forms. The 23rd shows a sudden drop in the numbers, in spite of an obvious attempt at further multiplication as is evidenced by 22 % of dividing specimens. The 24th is a typical instance of a depressed period, the short forms number 76% . Trypanosomes are very scarce ,and two 3byl films had to be searched for many hours to obtain the numbers requisite., only 3 % of long individuals are to be seen ,a and the divisions are equal to 6% .

From the 25th on there is a slow and steady rise for several days, the 25th itself shows 29% of divisions and a large percentage of intermediate forms, 49% ,though only 20 % of long forms. The long forms increase on the 26th though the divisions are beginning to tail off a little. The rise continues slowly till on the 27th the trypanosomes are very numerous, on the 28th the advance in the numbers is arrested and the types are preponderatingly short and intermediate, the proportion of divisions is low, this is a typical high level period where the cessation of division is characteristically correlated with the marked diminution in the number of long forms. On the 29th the divisions drop still further and there is an absolute diminution in the numbers though it does not amount to a serious clearing out of the trypanosomes from the peripheral blood. By a curious coincidence , although the detailed distribution is different and the absolute numbers are much higher,the relative percentages are identical with those of the 24th, which as we have seen is a typical period of depression. On the 30th the divisions met with are more numerous, but there has only been a slight increase in numbers in the interval

and short forms still preponderate very much. On the 31st a rise from the level of the last two days is definitely underway and just as before the intermediate forms increase as also the long forms. On the 1st of Sept. the rise is maintained but not increased, there is a setting back towards a very high proportion of short forms, and between the 1st and 2nd there has been a slight drop in the numbers, but the increase in the long forms suggests that the check is not to be of long duration. By the 3rd there has been an actual though not very great increase in numbers, this advance is maintained but is not pushed further on the 4th when another typical climax is see; that is to say a period of high absolute nimbers, few divisions ,few long forms and a quite overwhelming majority of short forms. The following day is particulary interesting as it shows a condition diametrically opposed to the one which immediately preceeded it. Between the 4th and 5th there has been a very marked drop in absolute numbers there are 37 % of divisions and 51 % of long forms. This attempt at a rapid rise, which looked as though it would resemble the progress of affairs on the 21st and 22nd of August was in this instance frustrated, and the 5th of Sept. was followed by a depressed period of four days in which there were very few trypanosomes present , and these were of the short type.

It seems quite impossible to escape the conclusion that the long forms are individuals about to divide, that they produce the short forms which are the most stable type and which have almost axclusive possession of the field the moment the divisions are markedly reduced, the intermediate forms being simply the merging of the short into the long types. The irregularity of the cycle should be noted ,the variability depending on the complex interaction of t the resistant forces of the host and the aggressive capacity of the parsites.

Since the completion of the above work in 1912 reflection and the consideration of additional data have led me to question whether the term adult is well chsen to designate the short trypanosome. The evidence as to the significanee of this type in the cycle has been strengthened from quite independent observations. Thus Thomson and Sinton found the blood containing short forms the most favourable material for the cultivation of T. rhodesiense on artificial media, and it should be noted that up to the present all artificial cultivation of trypanosomes derived from the blood of vertebrates corresponds to a reproduction of the intermediate host forms not to a multiplication of the blood types.

In another connection Scott Macfie studying West Coast strains of trypanosomes under natural comditions notes the predominance of infections of low virulence characterised by small absolute numbers of trypanosomes but showing a high percentage of short forms.

Trypanosomes belonging to the polymorphic species upon long [illegible] propagation by syringe passage through unresisting laboratory animals such as rats tend to lose the short forms. The origin of the short forms at division from one of the products of division of a long form or intermediate type can be demonstrated by a patient search for the last stage of division in the blood of the vertebrate. This stage must be passed through rapidly and it is therefore a difficult moment to study as it is relatively a very rare appearance.

Divisions producing two intermediate or long forms are also present and the mechnism for the suppression of of the short forms in these laboratory strains is suggested by the continuous unchecked multiplication that takes place under these circumstances and by the absence of the normal passage through the intermediate host.

(iv) Life-history of Trypanosoma gambiense, with a brief reference to the cycles of Trypanosoma nanum and Trypanosoma pecorum in Glossina palpalis.

Among the Trypanosomes of mammals 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 added a very brief account of T. pecorum and T. nanum, for the sake of comparison. 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 amd 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 nuber 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.

In all the experiments ,the flies used belonged to the Uganda variety of Glossina palpalis, and were hatched out in the Mpumu laboratory from pupae brought from Damba Island in the Lake .

Experiments had been carried out by Dr. Duke and Captain Fraser with several hundreds of these laboratory-bred flies, to ascertain if ~~XXXXXX~~ germinal 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 colleaugues. No further experiments were made, therefore ,in regard to this point, which seems now to be established beyond reasonable doubt.

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 ingected 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 appearto 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,not infrequently undergoes development in the Glossina. T. gallinarum moreover is very readily distinguishable from any phase of T.gambiense.

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 ~~XXX~~ 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 downwards on to Schaudinn's corrosive-alcohol fixing solution. The pre-

paration was subsequently stained by Heidehain's iron haematoxylin method. This treatment of the films gave excellent results, and affords a more accurate account of the parasites than that obtained from the XXXXX method of Giemsa.

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 section, and they will therefore be treated very briefly here.

The course of the endogenous cycle is as follows: the description is drawn from the study of 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 multiplication forms have not been observed at any time in the lung, liver, or spleen.

The forms undergoing schizogony in the lung, described by Via nna, 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 (Sleeping Sickness Bulletin No. 38, vol 4,) there are no details concerning the origin of the strain used.

Intercellular forms have, however, been found in the lung and liver on certain occasions, as will be seen hereafter, but they are involution forms, and possibly latent forms; no sign of multiplication has been observed in any of these individuals.

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 above ,I have been led to the following interpretation of the cycle in the vertebrate.

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 vertebrate 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 group chiefly to be recognised by the fact that they have increased in length and have a longer free flagellum, but are much the same average breadth as the short forms, namely 2 to 2.5 microns. The long slender forms are the individuals about to divide. 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 already,it suffices XXX 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, and obvious relation to the rapidity of the rise in numbers. An important feature of the depressed period is that ,XX

frome 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 distruction is difficult to follow , phagocytosis probably plays and important .but there seems also to be a certain amount of trypanolysis ocurring in the blod-stream. The liver and lungs have shown in the case of a very teeming infection investigated at this period, both degenerative appearances and what one is inclined to call involution-phases (fig 378). The liver of this monkey (633) showed many rounded -off individuals,posessing a trpphonucleus and a kinetonucleus,but no flagellar apparatus. These forms seemed for the most part to be between the cells, but this is a point very difficult to determine 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 chronis 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 ten days orio to the taking of the films . The infection

had shown the usual short depressions, but these had been lessfrequent 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 the persistence in the latter seems less probable. Neverteless ,the three cases just cited above do not bear this out,and the evidence ,so far as I have been ab,e to see,is all in favour of the rounded forms being destined to destruction. The pojnt must, hoever ,be borne in mind as the possible survival of latent forms,hoever scarce in conatct with or enclosed within the cells of any pert of the body is exceedingly important from the therapeutic point of view.

The question of a sexual interpretaion of the polymorphism of T. gambiense has very frquently 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 forms are males ,the short forms demales ,and the intermediate individuals are indifferent ,or simply non-sexual forms. This is based primarily on a vague analogy with the life-cycle of other protozoa of widely different groups , and on the supposition tha the long XXXXXXXX and short forms play the part of gametes or gametocytes in the transmittimg 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.

Dismissimg ,then, the division of the long and short forms in the blood of the vertebrate into sex-categories,there remains to to be considered if there is a sexual differentiation among the short forms. I have not been able to

detect any morphological distinction that could be reasonably 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 id 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 haematoxylin 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 preparations stained by Giemsa's method 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 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 wet method , and never dried in air at any period during the preparation of the slide, shows conditons 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 sXXXXXX delicate and highly flexible organism occupying three dimensions is swiftly flattened out into the nearest approach possible to two dimensions, quite regardless of its attitude at the time, disasters are liable tooccur. In dry preparations the nucleus has simply been burst. 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 medium 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. 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 part 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 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 basal granule of the flagellum (blepharoplast) is not clear, but Fig.6 suggests that it has the centrosomic function to be noted in the division of the trypanosome in the gut of the fly. The flagellum appears to split but this may really be an out growth of the new flagellum within the sheath of the original one ,the split is is never carried through the whole legth. The flagellum of the new organism becomes free about two-thirds of the way down, and the flagellum of the daughter- individual is usually shorter than that of the parent. The trophonucleus shows one very interesting feature before division. At a time when only the kinetonucleus and the flagellum have as yet begun to show 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 karyosomeThe other strands passing from from the karyosome to the membrane persist for a while ,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 unable to say if they arise from the karyosome. The division-figures are shown in Figs,5 to 8 .

There is no equatorial plate.

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 , theat I shall not tpuch upon them. Following the practice of the Uganda Sleeping Sickness Commission of 1908, I have in considering the infected flis, div ided 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 intestine,the middle and the hinder intestine.

From the dissection of a considerable number of wild teetse 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 od at least two pr three days possibly of a very much longer duration; in captivity flies will live from nine to twelve days without food if the coditions of moisture are suitable. A large number of experiments of long duration were condutcted 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 flies do not feed in nature at such very close intervals of time as seems to be implied from the usual laboratory treatment of 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 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 first passed 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 any 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 monkeys 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 first feed from both crop and gut alike. [illegible] . In a large number of cases, however, some of the first feed is [illegible] 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 repaced 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 th 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 Capt. Fraser.

The role 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, onr 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 buuble of gas.

Now, if a newly hatched fly has received tryapnosomes 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.

(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 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.

(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.

(5) The trypanosomes may persist in greater or less numbers in the gut and 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 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 well 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 gut crop into the gut.

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

(6) seems to be pretty definitely a condition that will lead to a permanent infection of the fly. Once the 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 imfection 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 produc-

tion of negative flies is shown by the very much higher percentageof 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 to 12 th day. Out of 103 starved flies, 22 showed trypanosomes between the 6th and 12th day. Neglecting these 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. 15.5 % . 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 % of positive flies.

To quote a typical experiment. Jars Nos. 16 ,17 , amd 18, each contained 15 flies, they 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 second day after the infecting feed. Jar No. 18 was starved outright after the infecting feed; between the 9th and the 12th days three flies showed trypanosomes in the gut, and three showed trypanosomes in the crop-- altogether 6 out of the total 15 flies 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.

(A) Mere persistance 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 to 84 haurs, and is chiefly to be observed when the trypanosomes were numeraous in the infecting blood.

(B) Cases where the trypanosomes persist in small numbers in some part usually of the mid-or hinder intestine and multiply, but are neverteless, unable to establish themselves permanently. These are mostly cases where the apparently advrse 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 eiter 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 tenth or twelfth 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 sveral 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 early developmental processes.

(C) 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 higher 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 parsites,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 already been mentioned , a 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 p percentages of infected flies produced, this being dur to the greater or less vigour of the trypanosomes interacting with the inhibiting forces of the fly. The negative periods are just as marked in very vigourous 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, profe ssor 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 s 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 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 once 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 trypanosomes are ready to infect another vertebrate, nevertheless the duration of this cycle of cganges 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 14th 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 developm nt 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 developming and not merely persisting, is that of a fly of the second day. . That is to say, the trypanosomes had been in thegut 36 to 48 hours. A provedly developing infection of so early a date is merely obtainedby 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 5th ,597 showed no trypanosomes in the course of the examination of the live film. Prolonged examination of a stained film, 3 by 1 in., showed very rare parsites. The fly in question (No,8) died during the night of March 6 to 7 and was dissected at 9 am. 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 of the contents of the fly amd multiplying individuals were observed. Figs. 9 – 12 are from this fly. Although special attention was paid to this fly from Expt. 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 thr trypanosomes are taken into the gut of the fly. Degenerating forms are presentin 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

There is no marked division into slender and brcad 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 however, emerges which is I think significant namely, that the dividing individuals at this early stage are all relatively broad forms. Figs. 11 & 16 are typical of the process. A broadening of the individuals abou t to divide is not characteristic of the development in the gut of the fly as a whole, as a glance at Figs. 20 & 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 temnds 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 however is worthy of notice, and that is the relations of the kinetonucleus of the daughter individuals in figs 11 & 16 . In both cases the young individual is passing through a condition in which the relations of the kinetonucleus and trophonucleus are those of a crithidia; this is a very transitory state,as it develops almost invariably into a trypanosome before it is set free. (see figs 12 from 2nd day.) This is only characteristic of the earlier divisions,and(with the exception of a rare and very slender form to be discussed later on) is the only sign of a crithidial phase to be observed in t the ~~gx~~ development in the gut. There is ,as will be seen hereafter , a most marked crithidial stage in this,as in most trypanosome life-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 to surpass as a rule the maximum length of the blood types. i.e. 34 to 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 to 13th day flu (mid-gut) , corresponds apparently to the "male" forms of Kleine and Taute. There is no evidence of this being a sexual form at all; it has never been found in conjugation in spite of much searching, either by Kleine & Taute or by myself. In dealing with flagellates, there is no support to be found in any of the well worked out cycles in which conjugation has been established, for the theory that markedly long slender forms are male gametes. In flagellates, all cases of conjugation involving holo-gametes (as must obviously be the case here) are practically isogamous. There is no sound argument by analogy in favour of these long forms being males to be drawn from the study of flagellates as a whole and there is also no direct evidence in the particular cycle under consideration.

In the case of T gambiense, one must first establish clearly that these forms (fig 43) do enter into conjugation before they can be held to be male gametes. They have never been seen in conjugation with broad or other forms. They are of a rare occurrence, and appear in the middle period of very active multiplication, . Moreover, their nucleus and protoplasm has almost always a pathologi cal appearance,and one is inclined to consider tham as degenerating slender forms. ((compare Figs. 40 - 41)

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 trophonucleus sometimes seen in these forms seems to be due to the tendency ,so often to be observed in degenerating trypanosomes for all the protoplasm and its contents to aggregate at the posterior end (fig 41) .

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 multiplicatives (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 basal granule (or blepharoplast) 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 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 ironohaematoxilyn 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 the division of the karyosome are to be observed. The beha-

viour of the nuclear membrane is not very clear it becomes very faint though ~~but it~~ is difficult to be certain ~~XXXXXX~~ ,it does not ~~XXXXXXX~~ seem to disappear entirely at any stage. The chromatin material of the nucleus draws out along the lengthening centrodesmose into a somewhat irregular spindle , there is no formation of chromosomes , 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 the fig 34) are absorbed in the protoplasm. The fate of the cetrosomes 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 divisiom of the protoplasmic body is very characteristic,. There is no longitudinal splitting of the parent-organism , but he 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 occurs in many trypanosome-divisions . This behaviour is very constant ; the young individual is usually somewhat smaller than the parent.

When 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 - 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 nuclear 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 chro-

matic 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 individuals (fig 38) but seems to be inhibited in types such as those shown in figs 36 & 37

The infection as a whole generally develpes in the hinder intestine or the posterior part of the middle intestine. I have never found trypanosomes really 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 anterior intestine. The anterior portion of the anterior intestine and the proventriculus show the typical long slender forms (figs 36-37) described in the preceeding 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 reaapture their position again. If, however , the new blood is taken in while they trypanosomes are still in the proventriculus, they retain their position and are apparently little affected by the influx of fresh blood.

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

In very numerous infections the trypanoeomes sometimes overflow from the proventriculus into the crop, and may be found there in considerable numbers; they are typical slender proventriculus types. 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 these produced during the first 12 days. Ther does not appear to be much, if any, multiplication among the very slender individuals, and the hinder and middle intestine seem to serve as a reservoir from which the more slender types are being constantly drawn.

In many trypanosomes, notably those of fish and reptiles, this slender form id 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 on in the gut still remains to be gone through in the saliwary 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 amoeboid, and the flagella are more or less adherent to the prtoplasm; others have no flagella at all and are quiescent; and still others are obviously wriggling masses of half fused trypanosomes. Figs, 2/1 14 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~~

seen in live films to be caused by the fusion of soft rather unhealthy-looking trypanosomes under the unfavourable conditions 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 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 comjugation,. 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 0.5 % salt solution, trypanosomes were observed to come together from opposite sides in pairs so asto overlap about one-third of the body length as in fig. 39A, whichwhich is 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 same morphological type as that found in the blood of the vertebrate.

This is the sum of the direct evidence from live observations 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 coverslip to give satisfactory opportunities for this kind of observation. The stained preparations do not reveal any process of conjugation.

It must also be considerd whether any whether conjugation might not take place in the salivary gland. There is no direct evidence of this ,and live observations are even more hopeless here than in the case of the gut, as the trypanosomes from the glands are extraordinarily sensitive to the unfavourable conditions of the slide. As will shortly be seen ,there are important data for considering the salivary gland development as the really essential part of the whole cycle , and it is for that reason that one might 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 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 chapter, --- 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-cycle as a whole the part passed through in the fly plays a definite and essential role on 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 doubt as to the sequence of development. The easiest way to get at the thin duct of

of the glands, which is the place where the first stages are to be found, is to starve the fly for two full days , then to cut off the posterior tip of the abdomen, snipping of a slightly larger portion than 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 right out up to the narrow duct,and the same is done for the other side. The glands are transferred to a clean slide wit a drop of normal salt solution or monkey serum; they are transferred again to a clean slide and examined. [illegible]

The pulling out of the gland without breaking it 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 an 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 tthere as slender free-swimming creatures.

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 [illegible] 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 at this stage quite free from trypanosomes. At first they are slender forms, which may sway forwards and backwards attached by their flagellum to the wall of the gland (figs 44 ~ 45) . Later on they become broad round-ended , crithidial flagellates. They multiply, and gradually, what with divisions and ffresh arrivals , finally fill, up large portions of the gland with flagellates in all stages, from f[illegible] 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 average 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 ~ 57)The cytology of the gland forms calls for no special notice, except that there seems to be an increase once more of the amount of chromatin within the nucleus ,and there is a tendency for the karyosome to increase in size. This feature of the nucleus it will be remembered was noted to be reduced in size in the long slender forms which invade the salivary glands. 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 also be found in the free state, but it seems probable 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 parsites. 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 the behaviour of an individual fly,this time is naturally only

an estimate made from the dissection and consideration of a number of only more or less similar cases. It appears clear beyond question that only when the salivary gland shows trypanosomes is the fly infective. Early positive transmissions are always accompanied by a rapid virulent infection 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 15th day infected galnds are found in rare cases, and after the 20th day they become the trypanosomes are increasingly frequent. Once established in the salivary gland the fly remains infective until its death .

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

It is interesting to compare the general course of development in T. nanum and t. pecorum with that of T. gambiense. The count given here is extremely brief and only the most salient features a re touched upon.

The development of T. nanum in Glossina palpalis has many points of resemblance with with the condition of affairs in the case of T. gambiense. The infection starts in the hinder 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 forms, and have nuclear relations practically indentical with those of T. gambiense (FIGS 61-64). The method of division corresponds in such minute detail with that described for T. Gambiense that it is needless to recapitulate the description. Slender forms begin to be produced from 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 uniformity of type ,nor are they so thread-like ,as in the case of T. gambiense. Moreover the characteristic changes in the nucleus of T. gambiense shown in figs. 36-37 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 made from dried preparations stained with Giemsa's method) ,as it was found that the trypanosomes could not be made to the coverslip in the wet preparations except in veru 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 organisims wre observed , sometimes in the hypopharynx and sometimes in the labrum.

As in T. gambiense, the gut forms do nto attach themselves to the wall of the alimentary canal , nor are crithidial forms seen in the gut cycle. The salivary glands are never invaded in the case of T. nanum, the proboscis infection apparently playing the role of the gland phases in the cycle of T. gambiense.

Trypanosoma pecorum develops in G. palpalis , but thecycle is so extraordinarily slow , and the transmission si difficult to effect, that it appears from Dr. Duke's experiments that this fly is at most only a facultative host at least under the conditions of temperature etc. obtaining during the carrying out of this work. For this very reason, the conditions of the cycle have some points of special interest ,as will be seen later on. The course of t 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 changesremarked upon in the corresponding stages in T. gambiense occur here also.(fig 72).

The first invasion of the proventriculus was observed upon the 45th day of the cycle, and no proboscis-infection was foud before the 76th day. The proboscis infections were generallt slight, and the usual difficulties were experienced in getting preparations from this situation. Figs. 73 and 74 show two individuals fixed in Shaudinn's fluid, and the nuclear relations recall those of the gland-stages of T. gambiense. The salivary glands never became infected.

The most remarkable feature of the cycle of T. gambiense is the curious double develpment, first in the gut, culminating in the long slender forms, and then in the salivary glands, where the crithidial stage so typical of all trypanosome cycles is produced. 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 parsitism in the case of T. gambiense is obviously more complete, 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 chapter. So long as the

parasites are established in the gut only , their presence is infifferent 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.

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

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 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 2-7.

The figures are all drawn at an approximate magnification of 3,000 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.

Figs. 40-45.—Early slender forms degenerating in the middle intestine; 43 seems to correspond to the "male" type of Kleine.
,, 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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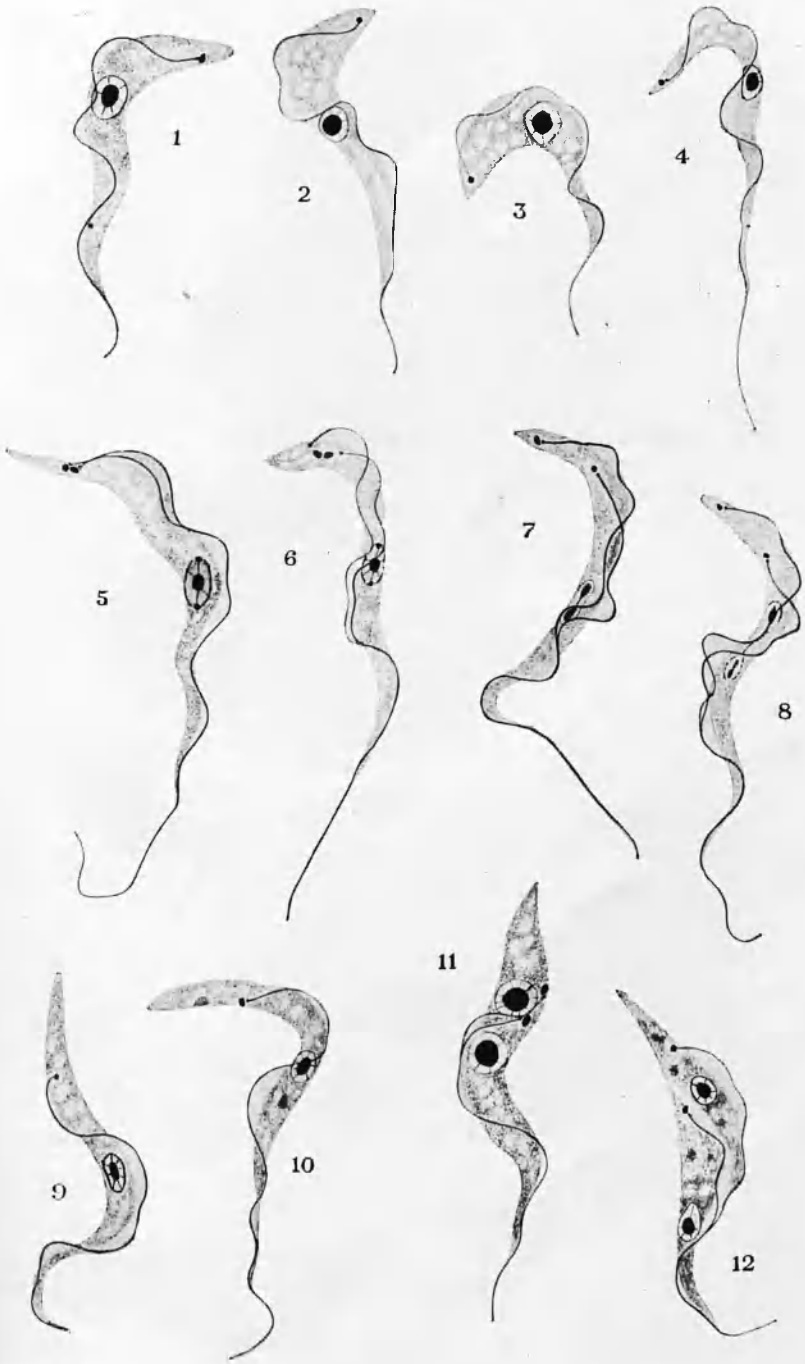
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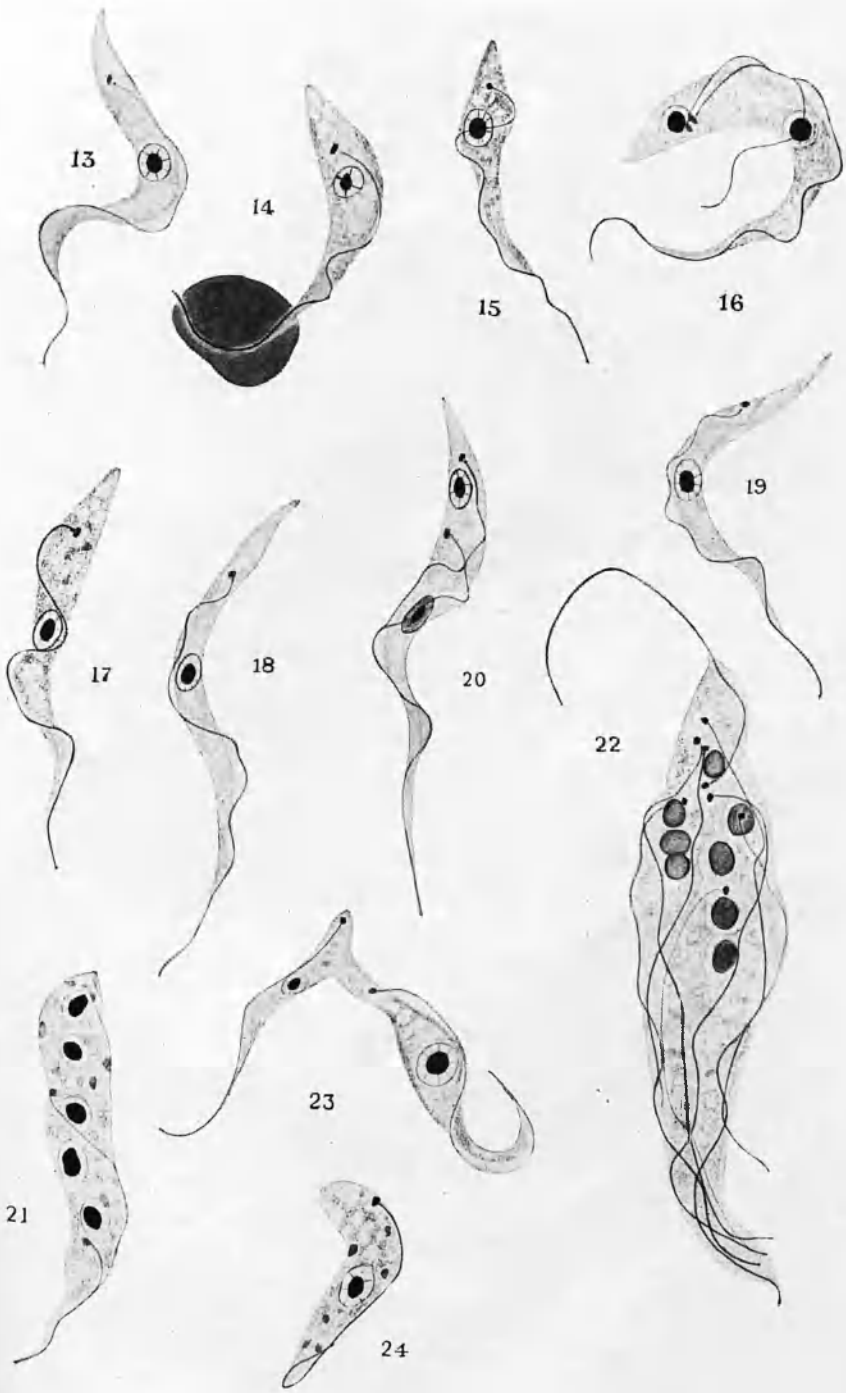
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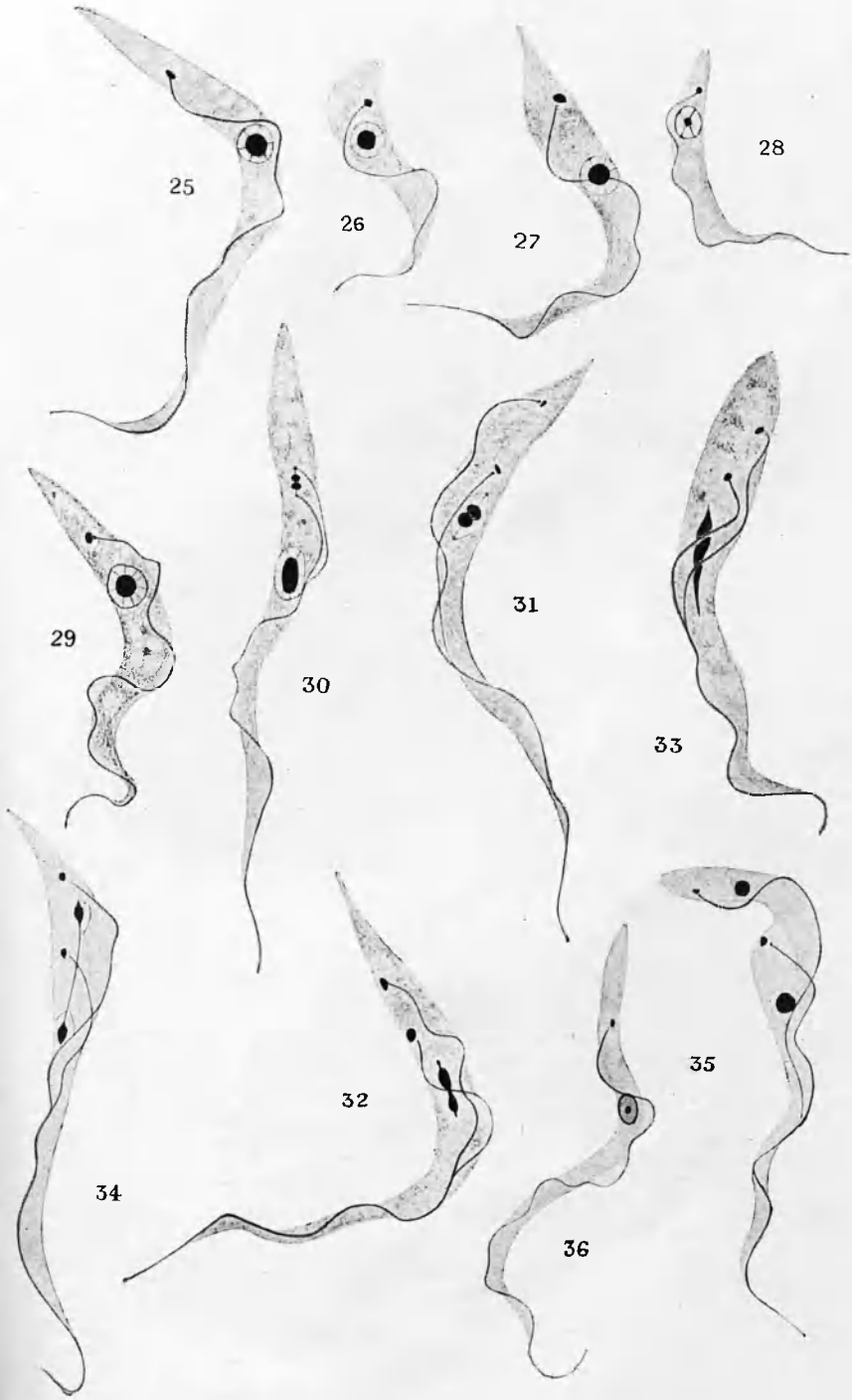

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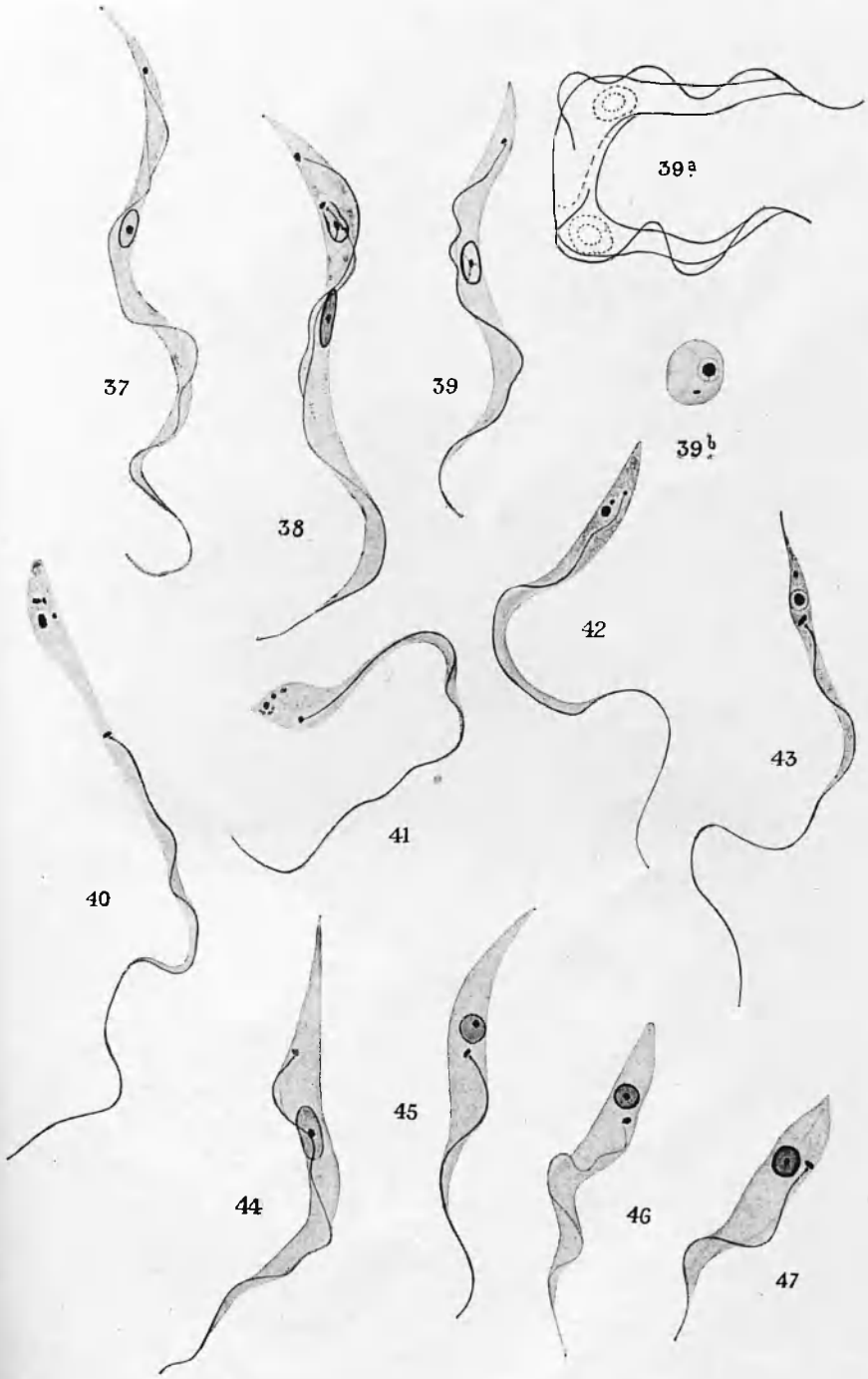
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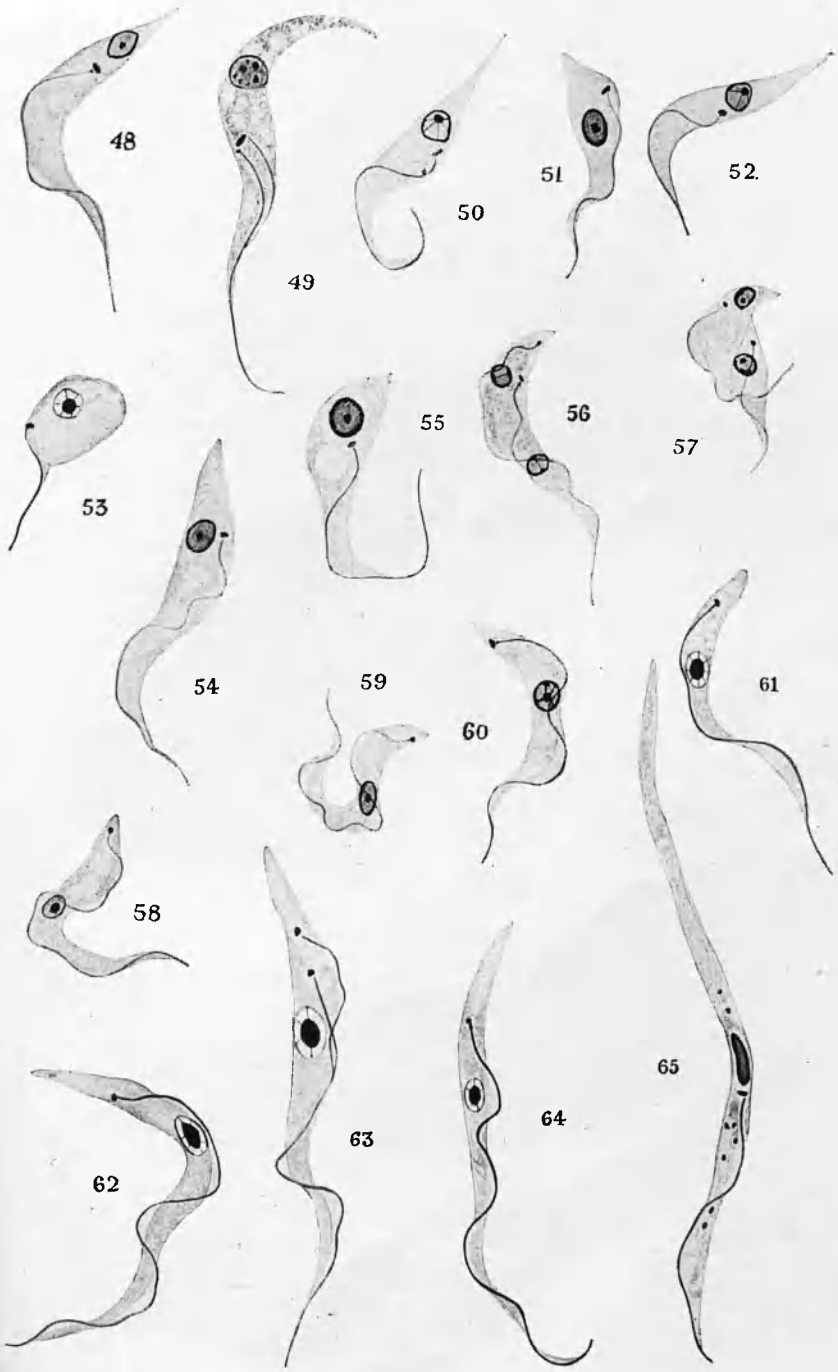

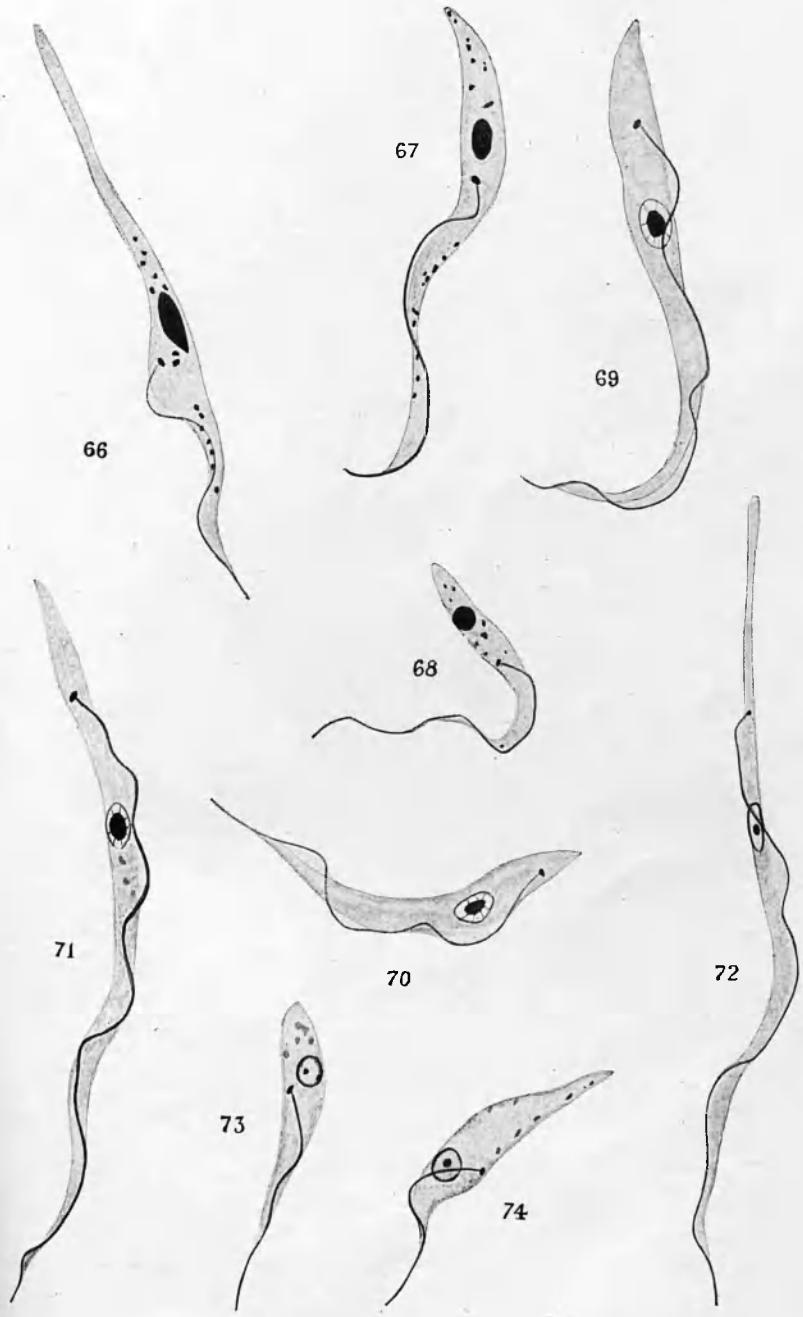
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(v) General Considerations and Conclusion.

The foregoing chapters have yielded a collection of data which may now be considered from a more general standpoint.

The genus trypanosoma shows a very high degree of uniformity in its morphological characters ,only a few species such as T. rotatorum departing even superficially from the general type. The difference between the divergent species being confined chiefly to the size and bulk of protoplasm and to slight differences in the position of the trophonucleus and the kinetonucleus and the relations of the undulating membrane. The genus comprises only parasitic members, the life being passed in the great majority of cases in the blood and lymph spaces of a vertebrate host and the alimentary tract of an invertebrate which acts as the transmitting agent.

It is a futile speculation as to whether the vertebrate or the invertebrate is the original host of the ancestral monogenetic types which must have preceeded the digenetic types of today. Very good cases have been put up for both hypotheses. On the whole the weight of evidence seems to incline towards the side of the invertebrate.

All the life-cycles described in this paper and indeed all known cycle of t trypanosomes agree in one point ,namely in the temporary appearance of a herpetomonad or crithidial stage. This appears in the part of the life-history passed through in the alimentary tract of the invertebrate and is very clearly a quite fundamental part of the development.

It is difficult to frame an explanation of the herpetomonad and crithidial stage or to appreciate the significance of this phase in the life-cycle. It is not a question of mere multiplication as the polymorphic trypanosomes such as T. gambiense

show an enormous amount of division in the gut of the fly without the production of these forms. One is tempted to consider that it is a reversion to an ancestral type, but this conception does not afford any particular illumination of the question. These stages do appear to me to arise in the cycles treated in the foregoing chapters, in response to a chemical of physical stimulus. In the species transmitted by leeches this stimulus operates in the crop or intestine and I consider that in these cases one of the factors at work is certainly the lowering of the osmotic pressure of the fluid surrounding the trypanosomes.

In all the trypanosomes normally transmitted by Glossina palpalis the herpetomonad and crithidial phases are passed through in the salivary fluid. T. gambiense goes through a prolonged and very prolific period of multiplication in the gut and then on invading the salivary glands goes through the herpetomonad development. T. pecorum and T. nanum likewise multiply actively in the gut and then on settle in the proboscis where bathed in the salivary fluid, they undergo this part of the development. T. uniforme and T. vivax which are also transmitted by Glossina species cut out the indifferent multiplication in the gut altogether and proceed directly with the herpetomonad phases ,attaching themselves in the proboscis and never invading either the gut or the salivary glands.

In this connection it is interesting to note that T. gambiense will multiply and show the slight morphological alterations characteristic of the early gut stages in the crop where the conditions are such that no visible change takes place in the appearance of the blood -corpuscles ingested. The blood can be retained in the crop for many days without suffering any change in shape or appearance of the corpuscles which implies that the osmotic pressure must correspond to that of the blood stream. Whatever the conditions of the gut may be in regard to the osmotic pressure the crop furnishes evidence that the

mere multiplication of these trypanosome phases is not dependent on a change in these conditions. The salivary fluid of the fly evokes the herpetomonad and crithidial phase and one is naturally tempted to consider that the surface tension of this fluid is different from that contained in the gut and that the striking change in the behaviour is at least in part connected with this. I have however no knowledge of the actual physical properties of the salivary fluid in Glossina and the whole question remains for thepresent a matter of speculation.

This herpetomonad series of changes is the great common feature in all the cycles; it is an essential development and until it has been passed through , the trypanosomes are not ripe for passage back into the blood stream of the vertebrate . A modification of this statement is however necessary in the case of T. lewisi where the crithidial phase is itself infective. It may be noted in passing that the direct subcutaneous and intramuscular injection of herpetomonad and crithidial phases from cultures of T. lewisi into rats will produce excellent infections. I have also found that as a rule from about the 5th or 6th day on the cultures cease to produce infections when injected until the herpetomonad and crithidial phases appear. This happens ,under the conditions of culture which I used ,about the 16th to the 20th day. Successful cultures are in my experience swarming with herpetomonad and crithidial types about the 21st day,and are highly infective. In practice T. lewisi strains are best maintained in laboratory infections (in the absence of transmission by flaas) by alternating from the culture tube to the blood of the rat. Direct propagation by syringe from rat ti rat is often very uncertain .

It is to this herpetomonad and crithidial development that I am inclined to attribute the stabilizing effect of the passage through the natural invertebrate host.

In spite of the very interesting differences in the order and sequence of events in the different groups of trypanosomes some of which are clear adaptations to the conditions of life of the particular two hosts, the main broad features of the cycles of the whole genus shows a fundamental unity of proceddure. The differences of behaviour in the various groups are never theless stable arresting features which can be used as a very good means of dividing the genus into groups. The question of species is however a matter of the greatest difficulty. The current methods of dealing with the nomenclature ~~errs~~ in the creation of far too many species. There is for instance no zoological reason for breaking up the trypanosomes of fresh-water fishes into many species. The polymorphic trypanosomes of man and domestic animals are habitually divided into such species as T. gambiense, T. rhodesiense , T. brucei etc, these show difference of virulence and of behaviour but there are no sound zoological grounds in the morphology of these types to divide them into different species. The life-cycles show slight distinctions in rapidity and vigour of development but no single point of significant disagreement occurs.

This labile character and the tendency to form strains are pretty clearly due to the high degree of adaptibility and the great capacity for multiplication, i.e. the large number of generations run through in a very short time. The study of the recidives sheds additional light on this. The resistance put up by the host is a very important feature . There is a constant and repeated elimination and destruction going on and the successive recidives are produced by the rapid multiplication of the individuals selected by the capacity for surviving the adverse conditions set up by the various protective reactions of the host, such as the production of immune body , rise of temperature and so on. In the end stages of trypanosomiasis in a host with some capacity of resistance , as for instance, T. gambiense in monkeys or T.

pecorum in cattle , a very interesting state of affairs is produced . The trypanosomes are few in number and held in check by the reaction of the host --- the host however is profoundly intoxicated ,is quite unable to eliminate the infection and ultimatelt dies. The behaviour of a virulent strain of ,for instance, T. brucei on artificial passage through unresistant laboratory animals like rats, shows an astounding and continuous capacity for multiplication, we are thus justified in assuming ,certainly in these polymorphic trypanosomes , that where the multiplication is checked and numbers are persistenetly low the inhibiting factor is the active resistance of the host.

It seems at first sight difficult to determine whether the low numbers found in many apparently non-pathogenic trypanosome infections ,is due to the resistence of the host or to a low capacity for multiplication od the part of the parasite. The evidence at present is in favour of the low numbers being due at least to a very considerable extent to the reaction of the host.

A very interesting light was thrown upon this question by some slides kindly sent to the Mpumu laboaratory from the veterinary department in Nairobi. There is a very large non-pathogenic trypanosome present in the blood of cattle in East Africa --- probably identical with T. theileri found in South Africa-- it produces no symptoms of disease and is very scarce in the blood, s so scarce that ordinary inspection of the blood with the microscope is no criterion of the presence or absence of the infection.

Duribg the production of immune serum by the inoculation of the virus-bearing blood of animals infected with Rinder-pest into normal susceptible cattle , there was produced a swarming infection with T. theileri in the blood of one of the bullocks used. There were as many as three or four Trypanosomes to a field on the film taken. This condition of affairs had arisen by the artificial passage of thetrypa nosomes ubder conditions which would cause a disturbance of the natural resistence of the cattle.

The presence of the Rinderpest virus had altered the capacity for resistance on the part of the host and the parasite usually so scarce had been able to multiply in the manner noted.

On another occasion somewhat similar evidence presented itself in connection with this same species. I had examined the blood of three Uganda bullocks which happened to be grazed near my laboratory on Kampala, every few days for many weeks. I had on one or two occasions found the large trypanosome in the blood of one of the animals. The flagellate was very scarce and it was a pure matter of chance that I had ever seen it at all, even taking into account the large number of examinations made. The bullocks developed foot and mouth disease and the trypanosome was readily found on every examination implying a very great increase in the number of parasites present. Here the appearance of another disease altered the conditions in the bullock in favour of the trypanosome.

From these two cases one is inclined to conclude that even in the non-pathogenic trypanosomiasis where the numbers of parasites are low this condition is brought about by the active reaction of the host.

In conclusion it may be remarked that trypanosomes afford some of the best examples of the perfect adaptaion to the parasitic habit of life. The vast majority of trypanosome species carry on a most successful existence the two hosts at whose expense they live suffering no serious damage. So perfect is this adjustment in many cases that the presence of the flagellate has been difficult to demonstrate and yet the species persists in a flourishing and prosperous condition. One of the best instances is the trypanosome of the sheep,--- this is an almost universal parasite in England. The flagellate was first found in the alimentary canal of the sheep-ked (Melaphaga) which is the transmitting host. It was considered to be purely a parasite of

the insect and used to be cited as an example of a monogenetic insectan infection. Woodcock first saw the trypanosome in the blood of the sheep and now Ritchie has demonstrated that it is an almost universal parasite in healthy sheep in this country.

Even the highly pathogenic trypanosomes owe this quality to some disturbance of the natural economy of the species. T. gambiense which in East Africa is highly pathogenic to man, causes no disturbance to the health of the antelope which is its natural host. T. pecorum which causes such heavy losses in cattle is likewise perfectly harmless to antelope. Trypanosomes are a late and very perfectly adjusted example of the of the opportunist method of the successful parasite. The already existing balance of conditions obtaining between the blood-sucking insect and its vertebrate host has been made use of by the protozoan genus without the sacrifice of either of the metazoan protagonists.

List of papers referred to.

1. Brumpt. De l'heredite des infections a trypanosomes et a trypanoplasmes chez les hotes intermediaires. C.R.Soc Biol. vol 63 ,p. 176.
2. Doflein. "Studien zur Naturgeschichte der Protozoen," Arch f. Prot. , 3 vol. 19, 1910.
3. Doflein. "lehrbuch der Protistenkunde." 1909 and subsequent editions.
4. Dutton. Todd and Toby Ann .of Trop. Med & Parsit. No. 3 1907.
5. Friedrich. "Trypanplasma helicis." Arch. f. Prot. vol. 19 1910.
6. Franca. Bull. Soc. Portug. Sc. Nat. 1907.
7. Hamburger. Osmotischer Druck und Ionenlehre, "vol 1 p. 182, 1902.
8. Harding, "A revision of British leeches," Parasitology vol 3 No. 2 ,1910.
Hoeber, "Weitere Mittheilung uber Ionenpermeability bei Blutkorperchen," Pfluger's Arch. vol 7 102 ,1904.
10. Keysselitz. Generations und Wirtswechsel von TRYpanoplasma borreli." Arch. f. Prot. vol 7. 1906.
11. Klebs, Crooniam lecture,1910 , Proc.Roy. Soc. B. vol. 82.
12 Kleine &Taute, Arbeiten aus dem kaiserlichen Gesundsheitamte,vol 31 partLL 1912.
13. Laveran &Mesnil, Trypanosomes et Trypanosomiasis. Paris 1912.
14. Leger. Sur la structure et la mode de multiplication des flgelles du genre Herpetomonas, Kent. " Compt. Rend. Acad Sci. Paris,vol,134 No. 14 p. 781.
15 Loeb. Untersuchungen uber kunstliche Parthenogenese.1906.
16. Luhe. " mense's Handbuch der Tropenkrankheiten," vol 3. 1906.
17 Minchin, Q.J.M.S. May 1908 vol 52.
18. Minchin, "Observations on the flagellates parasitic in the blood of freshwater fishes. Proc.Zool. Soc. 1909.
19. Minchin and Woodcock, " Observations on certain Blood-parsites od fishes occurring at Rovigno", Q.J.M.S. vol 55 p. 113 , 1910.
20 McConnell G. fuscipes, Sleeping Sickness Bull. No.38 vol 4.
21. Peters " Metabolism and division in Protozoa. Proc. Amer. Acad. vol 39 , 1904.
22½ Petrie Observations relating to the structure and geographical distribution of certain Trypanosomes. Journ. of Hygiene. vol.5. 1905.
24. Plehn. Trypanopasma cyprini.Arch. f. Prot. vol 3.
25. Porter. Parsitology. vol 2 no.4. 1910
26. Sleeping Sickness Reports of the Royal Society.
27. Shhuberg & Kunze. Verh. Deutsch. Zool . Gesell. 1906.
28. Thomson. Cultivation of the Trypanosome found in the blood of the gold-fish. Journ. of Hygiene. vol 8, p. 75 ,1908.
29. Pienantoni. Arch. f. Prot. vol 16 p. 82.