The Life Cycle of a species of Crithidia parasitic in the intestinal tract of Gerris fossarum FABR.

By

Captain **W. S. Patton**, M. B. Edin., J. M. S.

(With Plate IX.)

The researches of the late Dr. SCHAUDINN (1) into the relation of the Flagellata to certain intracellular parasites have been the means of drawing attention to those flagellates which lead a parasitic life in the intestinal tracts of Arthropods. Although in 1898 Ross (2) described snch flagellates from the alimentary tracts of the larva, nymph, and adult of varions species of *Culex* mosqnitoes in India, their occurrence in blood snckiug insects has, in more than one instance, led to their being described as further stages in the development of a Haemoflagellate. In addition to the bearing the stndy of these flagellates has on the Trypanosomata I (3) recently pointed ont that certain species of *Herpetomonas* and *Crithidia* have a stage in their life cycles when they are very similar in form to the LEISHMAN-DONOVAN body as seen in the hnman tissnes and that the *Herpetomonas* in particular passes through the same multiplicative processes as the parasite of Kala Azar does in man and in the bed bng *Cimex rotundatus*. The similarity betwen the flagellate stage of *Herpetomonas* and that of the LEISHMANN-DONOVAN body has been pointed out by ROGERS (4), bnt owing to the fact that the non-flagellate stages of *Herpetomonas* and *Crithidia* are very little known their resemblance to the similar stage of the human parasite is less widely recognised. There can therefore be little doubt that the study of these insect flagellates

will prove of the utmost importance in helping one to arrive at the exact biological position of the Leishman-Donovan body.

The present paper is a description of the species of *Crithidia*, which as I have mentioned above, has a non-flagellate stage similar to the Leishman-Donovan body and as there is at present no complete account of a flagellate of this genus the object of the investigation has been to work out in detail its life history especially the method by which infection is acquired. The parasite was first found in January 1907 in the alimentary tract of *Gerris fossarum* [1]) and later in a species of *Microvelia* and in a water bug related to *Perittopus*. These bugs are found either together or separately in all the ponds and tanks in Madras where they live on the juices of insects which fall into the water. As *Microvelia* was readily obtained in large numbers and all the specimens examined were infected I selected it for the study of the parasite. The adult female of this species measures $1^1/_2$ millimetres in length, its head, pronotum, and body beneath are pisceous; the anterior margin of its pronotum, antennae, and legs are ochraceous; the hemelytra are creamy white and reach the apex of the abdomen and the veins dull ochraceous. The female bug lays on an average ten eggs which are placed on a leaf or piece of twig and at the end of three days the nymphe hatch out. They moult five times and after the last ecdysis the male bug fixes himself on tor the dorsal surface of the young female remaining there till she is ready to ovnlate some weeks later.

The alimentary canal of this species of *Microvelia* consists of a long narrow œsophagus which opens into the short sacculated crop. Following the crop is the midintestine which is a short dilated tube and nearly always contains a greenish yellow fluid. The midintestine opens into the small intestine and at the junction of the two four long narrow malpighian tubes arise; the small intestine opens into the dilated colon which is continuous with the short straight rectum.

Specimens of *Microvelia* of all ages were collected and placed in trays the adults being separated from the nymphs. On dissecting out the alimentary tract of a nymph or an adult bug it was mounted in a drop of normal saline solution on a slide nnder a coverslip sealed with vaseline; the parasites were then easily studied with high power objectives in the unruptured or ruptnred crop and midgut. On examining fresh preparations of the intestinal tracts of

[1]) I have to thank Mr. Distant for identifying these water bugs for me.

adult bngs the flagellates were seen not only in the crop and mid-gut but also in the small intestine and hindgut, and on expressing the fluid contents of the latter round nouflagellated forms were seen among the adult flagellates. The discovery of these ronnd forms in the rectum of the adult bng led to a careful search being made for similar parasites in the crops of the nymphs. The intestinal tracts of very young nymphs were dissected ont in normal saline solution. The crop after isolation was ruptured with two ueedles and its contents were made into a thin film with a small piece of glass. The preparations were dried and fixed in Merk's methyl Alcohol and stained with Giemsa's stain it being fonnd that this method of fixing and staining the parasites gave the best results. In the majority of the films the parasites were flagellated and were seen in masses either attached to the cells lining the crop or free in its fluid contents; iu a few instances the ronnd nonflagellated forms exactly similar to those seen in the rectum of the adnlt bug were found and it was possible to study the method of formation and growth of the flagellum. It was however clear from these appearances, that very shortly after the ronnd parasites are ingested by the bngs they flagellate and begin to multiply so that it is only after examining many hundreds of specimens that the earlier stages preceding flagellation can be studied. It was therefore concluded that the flagellates ou encysting in the rectum of the adult bug are passed out into the water where they may be ingested either by the adults or the nymphs.

In a stained preparation of the crop of a nymph in which the uonflagellate forms occnr the parasites are seen as round, oval or pearshaped bodies (Plate IX fig. 1) measuring from 4 μ to 6 μ in length and from 3 μ to 4 μ in breadth. Their protoplasm is granular, stains a light pink towards the centre aud deep blne all along the margin; the nuclei which are usually seen lying towards oue side of the cell are circnlar iu shape, they staiu light pink and contain a uumber of dark chromosomes. The blepharoplasts which are generally situated at some distance from the nuclei towards the periphery of the cells are rod shaped and stain deep magenta and if seen ou eud appear as small black dots. In some of the specimens these round forms had increased in size becoming almost circular, their protoplasm staining a delicate blne and containing a few small circular vacuoles. In some of the films made from the crop contents of the nymphs I was able to study the method of formation of the flagellum. In one of the enlarged vacuolated

parasites a small pink staining area was seen between the blepharoplast and the periphery of the cell, while in others this structure had increased in size appearing as a distinct pink rod attached along the margin of the cell (Plate IX fig. 2). It will be seen then that this pink staining rod, which represents the flagellum, instead of at once projecting freely from the body of the parasite, is attached to its margin by a rudimentary nndulating membrane, which can often be recognised as a faint pink band between the flagellum and the body of the parasite. In all the later stages of the growth of the flagellum it was seen arising from an achromatic area close to the blepharoplast though not attached to it and passing along the periphery of the parasite (Plate IX figs. 3 and 4). Simultaneous with these changes the blepharoplast increases in size and the nucleus becomes full of chromosomes, while in the protoplasm of the cell appear a number of light pink staining granules. As growth proceeds the blepharoplast approaches the nucleus drawing with it the adjacent part of the flagellum; the blepharoplast elongates and divides and at the same time the flagellum thickens and begins to split longitudinally. A further stage is seen where the two halves of the blepharoplast have separated each being accompanied by its own flagellum (Plate IX figs. 5, 6 and 7). In none of the specimens was there any evidence to show that the second flagellum was produced in any other way than by direct division of the original one, and throughout the development of the parasite this method of its formation is maintained. In many of the parasites the two blepharoplasts are connected by a delicate pink filament (Plate IX fig. 7). In some of the cells the division of the blepharoplast was preceded by that of the flagellum (Plate IX fig. 8) bnt in the majority of parasites the blepharoplast and flagellum divide at the same time.

The flagellum when first formed is attached to the margin of the cell and as it grows it passes round the periphery from abont one third to one half the circumference of the parasite after which it begins to protrude freely, at this point the rudimentary undulating membrane apparently ends (Plate IX figs. 5, 6, 7 and 8). Considerable variation however is met with in these appearances for it was found no matter by what method the films were made that the flagella in many instances were torn away from the bodies of the parasites and their free portions appeared much longer than they were in reality (Plate IX figs. 7 and 8). Shortly after the division of the blepharoplast and flagellum the nucleus begins to show indications of division becoming elongated, its two poles containing groups of

chromosomes and the central portion staining lightly (Plate IX figs. 9 and 10). On separation of the two daughter nuclei the parasite splits longitudinally, the nuclei passing to the sides while the blepharoplasts and their accompanying flagella remain on the inner side of each parasite (Plate IX figs 9, 10 and 11). On separation of the two parasites each in turn begins to divide again, the flagellum and blepharoplast, dividing first and lastly the nucleus, so that eventnally rosettes of from eight to forty or more cells are produced (Plate IX fig. 21).

Parasites exhibiting these changes measure from 6 μ to 10 μ in length and from 4 μ to 8 μ in breadth, their protoplasm is granular staining a deep blue and contains a number of vacuoles and gronps of pink staining granules.

It will be seen therefore that division of the parasites does not take place before the formation of the flagellum, but that after flagellation multiplication by consecutive longitudinal division results in the formation of masses of rosettes attached to the intestinal epithelinm. In each rosette the nuclei are situated towards the centre and the flagella although first forming along the inner sides pass out and protrude freely and in fresh preparations are seen in violent agitation. Eventually the parasites break off and swim away from the rosettes. These round flagellated forms have a very characteristic appearance, the nuclens is usually sitnated centrally and the blepharoplast to one side and from a point in close proximity to it the flagellum passes ronnd the circumference of the parasite giving it an undulating contonr (Plate IX figs. 12, 13 and 14). In stained films the posterior or non flagellate ends of many of these parasites were considerably elongated (Plate IX figs. 15, 16 and 17) and in some the anterior ends were seen to be drawn out along the attached flagella (Plate IX fig. 18). This attenuation of the anterior ends of the parasites is most probably due to the further growth of the flagellum and it can therefore be readily understood that the elongation of both poles of the parasites would eventually result in the formation of a typical adult flagellate (Plate IX fig. 19 and 20). In many of the rosettes the parasites were seen in process of elongating so that it is probable the majority of these long forms are produced while still attached to the rosette.

In stained films the rudimentary undulating membrane of these large oval forms can be clearly demonstrated more especially between the undulations of the flagellnm. It is seen as a delicate pink staining band attached on one side to the flagellum and on the

other to the deep blue periphery of the parasite (Plate IX fig. 13 and 14). In all these cells varying stages in the division of the blepharoplast, flagellum and nucleus can be recognised (Plate IX figs. 12, 15, 16, 18 and 19), and if the division has taken place before the cell has fully elongated shorter forms may be produced such as I shall refer to later.

The elongated parasites vary in length from 15 μ to 45 μ and from 2 μ to 4 μ in breadth, their protoplasm stains a delicate blue throughout; their posterior ends which contain groups of small round vacuoles may be either blunt or pointed (Plate IX figs. 23 and 24); their anterior ends are always attenuated being drawn out to fine points to which the flagella are attached by the narrow undulating membranes. The nuclei are oval in shape and are situated in the centre of the parasites, they stain light pink and as a rule contain eight chromosomes arranged along their circumference. The blepharoplasts are always situated from 1 μ to 1,5 μ in front of the nuclei except in the forms about to divide when they are situated close up to the nuclei (Plate IX figs. 23, 24, 25, 26 and 29); they are rod shaped and lie at right angles to the long diameter of the cells. The flagella arise from a point in close proximity to the blepharoplasts but are never directly attached to them and passing along the attennated anterior ends finally project freely. The undulating membranes of the adult flagellates are so narrow that they can only be seen as faint pink bands lying between the dark flagella and the margin of the blue staining anterior ends. Plate X fig. 29 shows a long parasite in which the flagellum has become separated in a great portion of its length from the anterior end of the parasite which is clearly seen terminating in a fine point still attached to the flagellum. These long forms are commonly seen agglomerated together be their anterior ends to particles of food (Plate IX fig. 22) and this appearance must be distinguished from the true rosette which as I have pointed out above is formed by the consecutive division of one cell at a much earlier stage. A reference to Plate IX figs. 20, 23, 24, 26, 27, 28, and 29 will show that there is considerable variation in the size and shape of the elongated flagellates, and it is evident that no definite form can be taken as a type of this stage so that no importance can be attached to the length of the parasite. In the shorter as well as in the thinner forms all stages of longitudinal division were observed. The blepharoplast enlarges, approaches the nucleus and may be seen as a thick rod or a round dot measuring as much as 1 μ in diameter

(Plate IX figs. 24, 25, and 27); at the same time the flagellum thickens and begins to split (Plate IX fig. 26) and later the blepharoplast separates into two. The nucleus elongates and as described above finally divides into two. The protoplasm of the parasite begins to divide towards the anterior end (Plate IX fig. 31) and as this process proceeds the rapid vibration of the flagella undoubtedly facilitate further separation (Plate IX fig. 32); the anterior ends of the parasites become completely separated and as shown in Plate IX fig. 33 they may be seen attached by their posterior poles. In numerous instances this process of division was followed from its commencement till the newly formed parasites broke away and they in turn again began to divide.

When an oval flagellate divides prior to elongation two shorter forms are produced (Plate IX figs. 34, 35, and 36). These shorter forms are themselves capable of longitudinal division without first elongating (Plate IX figs. 36, 37, 39, 40 and 41) giving rise to very short parasites measuring from 2 μ to 4 μ in length and 1 μ to 1. 5 μ in breadth. In these minute parasites the nucleus is situated in the centre and the blepharoblast about 1 μ anterior to it; the flagellum measuring from 5 μ to 10 μ in length is attached along the attenuated anterior end (Plate IX fig. 42) though owing to the small size of these flagellates it was often difficult to demonstrate the extent to which the anterior end was actually drawn out.

As a result of the further longitudinal division of the long thin forms, parasites more and more attenuated an produced which are themselves capable of division (Plate IX figs. 43, 44, 45 and 46) till very thin spirochæte-like flagellates are formed (Plate IX fig. 47). These parasites often measure less than 1 μ in diameter and from 8 μ to 10 μ in length. The nucleus consists of a number of granules while the blepharoplast is seen as a small rod from 1 μ to 1.5 μ anterior to it.

In the extremely thin forms it was impossible to recognise the attenuated anterior ends and they appeared to consist only of long stout flagella. The marked polymorphism exhibited in the flagellated stage of this parasite is undoubtedly due to the fact that there is no regularity observed in its development and that the repeated longitudinal division results in minute as well as long forms.

I have devoted special attention to the movements of the free flagellated forms of this parasite. The oval flagellates shown in Plate IX figs. 12 to 17 progress very slowly by a rolling movement, the flagellum can be observed vibrating all along the margin

of the parasite and its free end lashes from side to side. The long stout flagellates move much more rapidly, the body revolving round and round, the flagellum drawing it along by its rapid vibrations. In the very long forms the anterior end exhibits a lashing movement while the posterior end is seen to bend from side to side. The small and attenuated forms move very rapidly darting across the field of vision. The study of the long forms during division was of great interest (Plate IX figs. 30 to 33); it is most probable the parasite attaches itself by its posterior end till division is completed. At first the single flagellum is seen moving from side to side coiling and uncoiling itself and on the formation of the second flagellum division commences at the anterior end; as division proceeds the two parasites are seen moving freely and on the posterior ends separating swim way. This process is a very rapid one lasting from three to four minutes. I have never been able either in fresh or stained preparations to observe any process of unequal longitudinal division, and any disparity between the size of two opposed parasites is explained by a more rapid growth of the larger.

From the above it will be seen that the parasites shown in Figures 7 to 18 do not represent the so-called Gregarine or resting stage but are young growing forms. I have been unable to observe any sexual dimorphism in the elongated flagellates as the extreme differences in size only represent early and late stages of longitudinal division and this view is supported by the failure to find any sexual process. This then concludes the description of the active multiplicative stages in the development of the parasite and it now remains to follow the method of encystment.

In order to study this stage it is necessary to keep a large number of adult bugs for a considerable time and to dissect them at varying intervals. As was mentioned above the mature flagellates of all sizes are found not only in the crop but also in the midgut, small intestine and rectum. On smearing out a number of rectums and their contents as described above, and staining the films with Giemsa's stain, in addition to the ordinary flagellates there were seen a number of forms exhibiting the earliest changes towards encystment. The central portion of the bodies of some of the flagellates were globular (Plate IX fig. 48) and in addition to this the posterior ends of some were shortened appearing as short processes projecting from the globular body of the parasite (Plate IX figs. 51 to 56); at the same time the anterior end which stains less deeply shortens and parasites exhibiting all the stages in this

process up to their final rounding up are shown in Plate IX figs. 53 to 56. While these changes are taking place the nucleus which at first was situated centrally now occupies one end of the parasite, it stains uniformally deep red and is often surrounded by a clear zone (Plate IX figs. 53, 54 and 55). The blepharoplast which was at first towards the periphery of the cell passes closer up to the nucleus. As the anterior end of the parasite shortens the flagellum gradually becomes more and more free so that eventually it is only attached by a short intracellular portion (Plate IX figs. 50 and 51). The parasites are now oval or circular in shape and measure from 4 μ to 6 μ in length and from 3 μ to 4 μ in breadth, their protoplasm stains dark blue, is granular and slightly vacuolated. The attached portion of the flagellum next becomes absorbed as it can no longer be seen passing into the body of the cell but ends abruptly at the margin of the parasite (Plate IX figs. 57 to 63) and in one instance it was separated and seen lying close to the cell (Plate IX fig. 62). The encysted forms vary considerably in size so that it is very probable they originate from more than one type of elongated flagellate (Plate IX figs. 64, 65 and 66). The periphery of these cells stains pink with Giemsa's stain and is of the nature of a periplast. The fluid contents of the hindguts of adult bugs can readily be obtained without dissection by exerting pressure on the abdomen with a needle and in the fluid thus obtained the various long flagellates together with all the stages of encystment described above can be recognised. Many of the flagellates in faeces of the bugs judging from their indistinct outlines and faintly staining bodies were undergoing degeneration but such parasites can be easily distinguished from the true encysting forms. It would therefore appear that a large number of flagellates together with the encysted forms are discharged in the faeces.

Although I examined many specimens of *Gerris fossarum*, *Microvelia* sp.; and the water bug allied to *Perittopus* I was unable to find an uninfected one and judging from the fact that flagellates in all stages of development were found in their intestinal tracts it is probable many had become reinfected. A careful search was made for the parasites in the ovaries and eggs with negative results and they have never been found in any other situation but the intestinal tract so that it is extremely doubtful whether these bugs inherit the infection. As the nymphs become infected very early in their development the male bugs are also infected. Another possible method of infection must be mentioned. These bugs are in the

habit of attaching and killing each other and then feeding on the dead bodies and as I have frequently oberved that the flagellates remain alive for at least twelve hours in the intestinal tracts of the dead bugs they may in this way be sucked up.

Summary and Concluding remarks.

Like the *Herpetomonas* of *Culex pipiens* this flagellate of *Gerris fossarum* begins its life cycle in a form similar to that of the Leishman-Donovan body; it then increases in size and before dividing passes on to flagellation so that in the majority of the water bugs examined it is seen in this condition. The flagellum develops at the margin of the cell in close proximity to the blepharoplast and as it grows passes along the periphery of the parasite being attached to it by a narrow undulating membrane. While adhering to the intestinal epithelium the flagellates divide again and again resulting in the formation of masses of rosettes until almost the whole epithelium of the crop and midgut is covered by them. In each rosette the nuclei lie towards the centre, the blepharoplasts to one side while the flagella first developing along the inner sides pass out externally. The parasites elongate, the anterior end being drawn out as the attached flagellum increases in length so that groups of long forms attached by their posterior ends are produced; these elongated forms separate later and swim away but may often be seen agglomerated together by their flagellar ends. Longitudinal division proceeds rapidly, the blepharoplast and flagellum dividing first and then the nucleus resulting finally in small short forms and long thin ones. The parasites next pass down the intestinal tract where they may be seen in masses; some shorten, the posterior end being drawn in and later the anterior, until at length they are seen as round or oval bodies with long free flagella. The attached portion of the flagellum next becomes absorbed and is finally detached and the cyst appears as a round body with a circular nucleus and a rod shaped blepharoplast. This flagellate then passes its complete life cycle in the intestinal tracts of *Gerris fossarum*, *Microvelia* sp. and the species allied to *Perittopus*.

It is obvious from the very rich infections of these bugs that their intestinal tracts are well adapted to the life processes of the parasite. Although three species of mosquitoes, *Culex pipiens*, *Culex sp.* and *Anopheles barbirostris* were breeding in the same pond with the water bugs they never became infected.

In 1902 Léger (5) created the genus *Crithidia* for a flagellate organism which he found in the intestinal tract of *Anopheles maculipennis* naming it *Crithidia fasiculata* and in his description of the parasite he recognised two species oval and attenuated. The oval form was a short truncated parasite with a centrally placed nucleus and a blepharoplast situated close to it; a flagellum usually the length of the parasite protruded from the anterior end and was prolonged into its body up to the blepharoplast. The attenuated form was considerably elongated, the anterior end to which the flagellum was attached exhibiting an undulating contour which Léger believed was due to the presence of an undulating membrane; the blepharoplast was always situated anterior to the nucleus. In their recent paper Novy, Mac Neal and Jovrey (6) have described a flagellate from the intestinal tracts of a number of *Culex* mosquitoes in America and they have been able to cultivate it on blood agar for a considerable time. In the mosquitoes as well as in the culture medium two forms were recognised, a short oval body and a longer cylindrical one. In the shorter parasites the anterior ends from which protruded short straight flagella were truncated; the nuclei were at the posterior ends while the blepharoplasts lay close beside them.

In the longer forms the anterior ends were rounded, the nuclei were situated centrally and the blepharoplasts some distance anterior to them; the long flagella protruded freely so that no undulating membrane could be recognised. But this parasite in its adult flagellate stage differs from that of *Crithidia fasiculata* in that its anterior end is not drawn out the undulating membrane being absent. It is clear then tat the flagellate described from *Anopheles maculipennis* by Léger and that from *Culex* mosquitoes by Novy and his collaborators must represent two distinct parasites. In his description of *Crithidia fasiculata* Léger based the genus on the fact that the parasite was usually seen as a short truncated organism attached in bundles to the intestinal wall of *Anopheles maculipennis*, but as Novy Mac Neal and Jovrey have shown that at least two species of flagellates frequently occur in the same mosquito, the short forms of any one of them can hardly be taken as the type on which to base a new genus. I have shown in the present paper that such short forms only represent young growing parasites and unless all the intermediate stages as well as the adult flagellates are studied it is impossible to differentiate between two species when occurring in the same insect. It is most probable as the American observers point out that Léger was dealing with two distinct flagellates in

Anopheles maculipennis. This view is supported by thy fact that the adult flagellate of *Crithidia fasiculata* is very similar to the adult form I have described above, while the young forms of Léger's parasite suggest a young *Herpetomonas* similar to that of *Culex pipiens* and have none of the appearances of a young *Crithidia.*

The genus *Crithidia* of Léger is therefore for the present best represented by all such flagellates in which the adult form is characterised by an attenuated anterior end to which the flagellum is attached by a rudimentary undulating membrane; the blepharoplast in all such flagellates is situated close up to the nucleus, never posterior to it. Those flagellates in which the undulating membrane is completely absent and in which the anterior end is round or truncated, the blepharoplasts nsually sitnated some distance anterior to the nucleus, should be regarded as belonging to the genus *Herpetomonas.* [1])

The flagellate which Novy, Mac Neal and Jovrey have recently described as *Trypanosoma culicis* readily falls into the genus *Crithidia* as in its adult form the anterior end is drawn out and the flagellum is attached to it by a rudimentary nndulating membrane. The parasites which they describe as spherical or club shaped (Ref. Plate II fig. 1 and Plate XII figs. 3 and 4) at once suggest the large round or oval flagellates (Plate IX figs. 12 to 17) with the only difference that *T. culicis* has a diplosome. The forms of this parasite from the blood agar corresponded in every respect to those observed in the mosquitoes and from this the American observers conclnde that the flagellates in these insects represent cultural forms and that if able to grow in the blood current they would probably give rise to typical trypanosomes. It will however be seen that they have not studied the non flagellate stage of *T. culicis.* The fact that these flagellates are true insect parasites and have no connection with any blood flagellate makes it certain that in each case their complete life histories can only be studied in their insect hosts and I have already pointed out it is in the alimentary tract of the larva, nymph or adult as the case may be that the non-flagellate stages are to be looked for.

---

[1]) I have recently had the opportunity of stndying *Herpetomonas muscae domesticae* and *Herpetomonas sarcophae* and in each case I have been nnable to demonstrate a donble flagellnm in the adult flagellates as described by Prowazek (7). A careful stndy of stained and fresh specimens of these parasites has shown that the appearance of a donble flagellnm only represents an early stage of longitudinal division as shown in Plate IX figs. 34, 35, 43 and 44 of the present paper.

As the flagellate of *Gerris fossarum* goes throngh a cycle differing in many respects from that of any known vertebrate trypanosome and in its adult stages the blepharoplast never passes back posterior to the nucleus owing to its rudimentary undulating membrane, it will be best for the present to place it in the genus *Crithidia* and I propose naming it *Crithidia gerridis.*

In a recent paper Miss Robertson (8) has described a flagellate from the intestinal tract of *Pontobdella muricata* this parasite is of great interest as in the early stages of its development it is similar to *Herpetomonas* and *Crithidia* while in its adult stage it is allied to a true Trypanosome. In the crop and intestine of o newly fed leech nonflagellate forms are seen which are similar to the non-flagellate stages of *Herpetomonas* and *Chrithidia.* In the intestine of the leech these forms develop flagella and then appear as ronnd or oval bodies with free fiagella of varying lengths and suggest a young *Herpetomonas,* there being no evidence of an nndulating membrane. In the next stage which is also found in the intestine of the leech the blepharoplast is seen passing back behind the nucleus drawing the flagellum with it. In some of these stages the parasite has the appearance of a yonng *Crithidia* (Ref. Plate VI figs. 40 and 41) the blepharoplast being alongside the nuclens and the flagellum is attached along the margin of the parasite. On the further lengthening of the parasite and the migration of the blepharoplast to some distance posterior to the nncleus the trypanosome appearance is prodnced. Miss Robertson makes no mention of the undulating membrane in these forms but from her fignres it appears to be formed as the blepharoplast travels backwards. In her fignres of the adult try-panosome the undulating contonr so charateristic of the majority of vertebrate trypanosomes is absent the parasite appearing much stiffer; this suggests that the nndulating membrane is extremely narrow and the flagellum therefore does not exhibit an nndulating contour. The flagellate of *Pontobdella muricata* also shows the polymorphism in its adnlt stage spirochaete- like forms being produced as in the case of *Crithidia gerridis.* The life cycle of this leech flagellate suggests that it is in no way connected with *Trypanosome raiae* but is a true parasite of the leech. Further study of such forms will nndoubtedly help in the classification of the Trypanosomata which at present it is difficult to separate on morphological differences alone.

Madras, November 1907.

References to Literature.

1) Schaudinn: Generations- und Wirtswechsel bei Trypanosoma und Spirochäte. (Vorläufige Mitteilung.) Arb. a. d. kais. Gesnndheitsamte 1904 20 pp. 387—439 20 figs.

2) Ross: Notes on the parasites of mosqnitoes fonnd in India between 1895 and 1899. Jonrn. of Hyg. 1906 6 p. 101—108.

3) Patton: Preliminary note on the life cycle of a species of Herpetomonas found in Culex pipiens. British Medical Jonrn. July 13th 1907.

4) Rogers: Further work on the development of the Herpetomonas of Kala Azar and cachexial fever from Leishman-Donovan bodies. Proceed. Roy. Soc. Biol. vol. 77 1906.

5) Léger: Snr nn Flagelle parasite de l'Anopheles macnlipennis. Compt. Rend. de la soc. de Biol. p. 354 1902.

6) Novy, MacNeal and Jovney: The Trypanosomes of mosqnitoes and other insects. Journ. Infect. Diseases vol. IV No. 2 April 1907 p. 223 to 276.

7) Prowazek: Die Entwicklnng von Herpetomonas, einem mit den Trypanosomen verwandten Flagellaten. (Vorläufige Mitteilnng.) Arb. a. d. kais. Gesnndheitsamte 1904 20 p. 440—452.

8) Robertson: Stndies on a trypanosome fonnd in the alimentary canal of Pontobdella mnricata. Proceedings of the Royal Physical Society vol. XVII No. 3 August 1907.

Explanation of Plates.

Plate IX.

Figure 1. A gronp of yonng parasites from the posterior portion of the mid gut of a nymph. — a. Pear-shaped form with the blepharoplast at the periphery. b & c. Round forms with more central blepharoplasts. d & e. Two pear-shaped forms. f. A small round form with dark blne protoplasm.

Figure 2. A round form shewing the early formation of the flagellnm at the margin of the cell, gronps of grannles are begining to appear in the protoplasm.

Figure 3. A ronnd parasite with the flagellnm seen as a distinct rod arising from a point close to the blepharoplast, the nnclens shews an increase in size.

Figure 4. A ronnd parasite shewing further development of the flagellum.

Figure 5. An enlarged ronnd form shewing the thickened blepharoplast passing towards the nncleus and drawing with it the adjacent portion of the flagellnm; the free end of which is lying on the parasite.

Figure 6. A ronnd form shewing the blepharoplast abont to divide, the flagellnm has grown round the margin and is begining to protrnde freely.

Figure 7. Another ronnd parasite shewing division of the blepharoplast and flagellnm and the early changes in the nnclens preceding division.

Figure 8. A ronnd parasite shewing the flagellnm dividing hefore the blepharoplast.

Figure 9. A mnch enlarged ronnd form in which the blepharoplast and flagellnm have divided and the nncleus is abont to do so.

Figure 10. A large oval form; a flagellum is seen passing along each side of the cell.

Figure 11. Complete longitudinal division of an oval parasite into two, one of which has grown more rapidly and is about to divide again.

Figure 12. A large oval form showing one end more pointed; note that the second flagellum is shorter than the original one, as division does not take place throughout the whole length.

Figure 13. A large oval form shewing the narrow undulating membrane lying between the undulations of the flagellum.

Figure 14. A similar parasite.

Figure 15. A round parasite shewing one end becoming pointed.

Figure 16. Further stage of the same.

Figure 17. A similar parasite in which the second flagellum is shorter than the original one, as seen in figure 12.

Figure 18. A large parasite shewing growth of the posterior end and further elongation of the anterior.

Figure 19. Further stage of the same which also shews commencing division into two shorter forms.

Figure 20. An elongated form with a long flagellum attached to the attenuated anterior end.

Figure 21. A true rosette consisting of eight parasites, in one of which division is already far advanced; note that the nuclei are towards the centre, the blepharoplasts are at the side of some and more towards the anterior ends in others, and the flagella which at first developed along the inner sides of the opposed parasites have passed out externally.

Figure 22. A pseudo rosette of elongated parasites agglomerated by their anterior ends to a particle of food.

Figure 23. An elongated form with a blunt posterior end.

Figure 24. Another elongated form with a pointed posterior end.

Figure 25. A large form shewing the blepharoplast in close proximity to the nucleus.

Figure 26. A similar form shewing the blepharoplast and flagellum about to divide.

Figure 27. A very long form with a circular blepharoplast, the flagellum is torn away from the greater part of the anterior end.

Figure 28. Another long form shewing division of the blepharoplast and flagellum.

Figure 29. A very long form, with blepharoplast and flagellum about to divide.

Figure 30. Two elongated forms showing divisional changes.

Figure 31. An elongated parasite commencing to separate at the anterior end.

Figure 32. Shewing further separation.

Figure 33. Two elongated forms attached by their posterior ends alone.

Figure 34. A short form shewing division of the blepharoplast and flagellum.

Figure 35. A club-shaped form shewing similar appearances.

Figure 36. A short parasite with a pointed posterior end about to divide.

Figure 37. Division of a similar parasite.

Figures 38 to 42. Stages in the division of the short forms, which eventually result in minute parasites.

Figures 43 to 47. Stages in the division of the long thin forms which eventnally result in thin spirillar-like parasites.

Figure 48. Earliest changes towards encystment, the body of the parasite is becoming globular.

Figure 49. Retraction of the posterior end.

Figure 50. Retraction of the anterior end.

Figure 51. An oval form, both ends having retracted.

Figures 52 to 56. Various stages in the retraction, of the anterior end the posterior end having already become ronnd.

Figure 57. A ronnd cyst shewing the flagellnm still attached by a short intracellular portion.

Figure 58. Absorption of the intracellular portion.

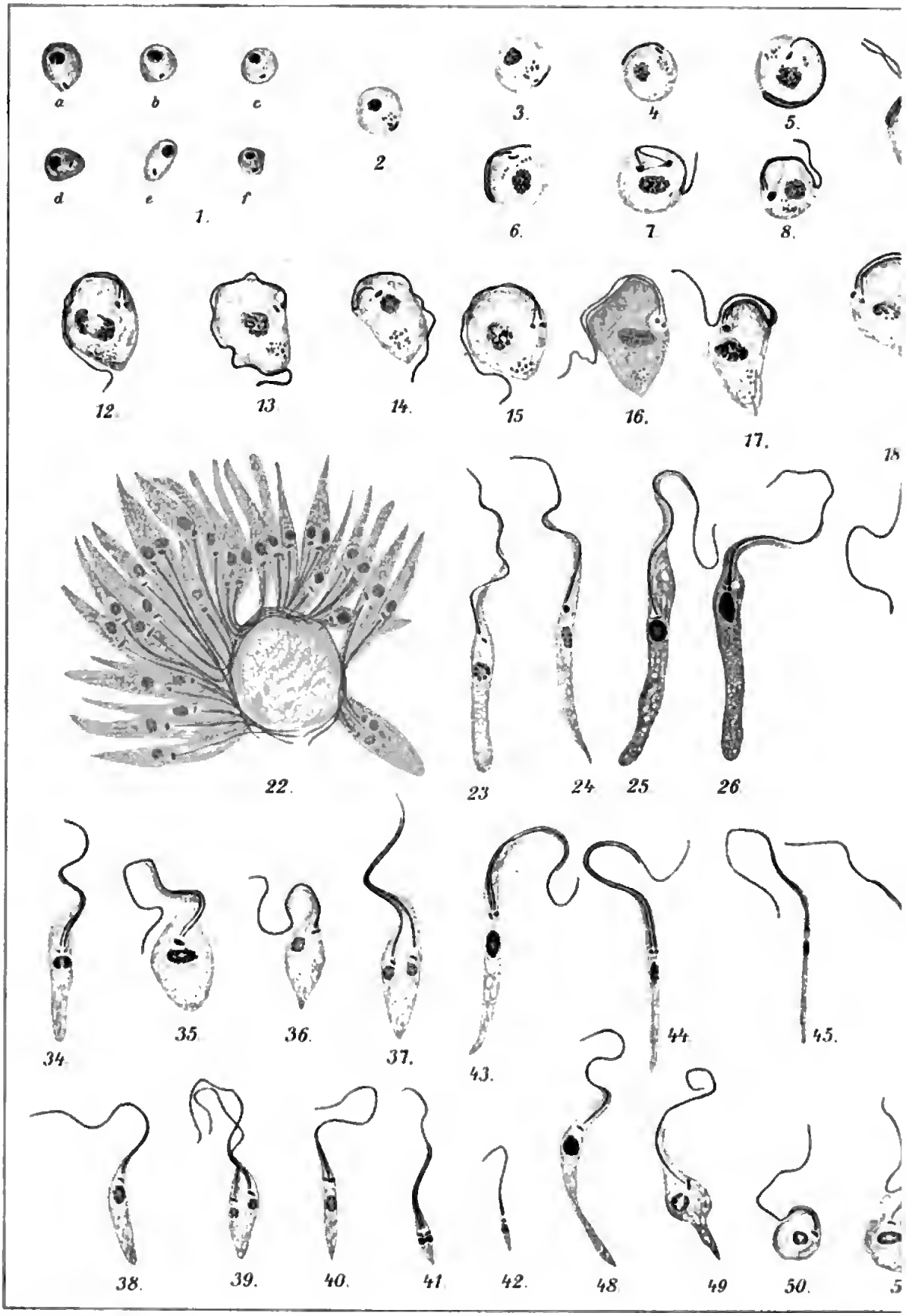
Figures 59 to 61. Similar forms.

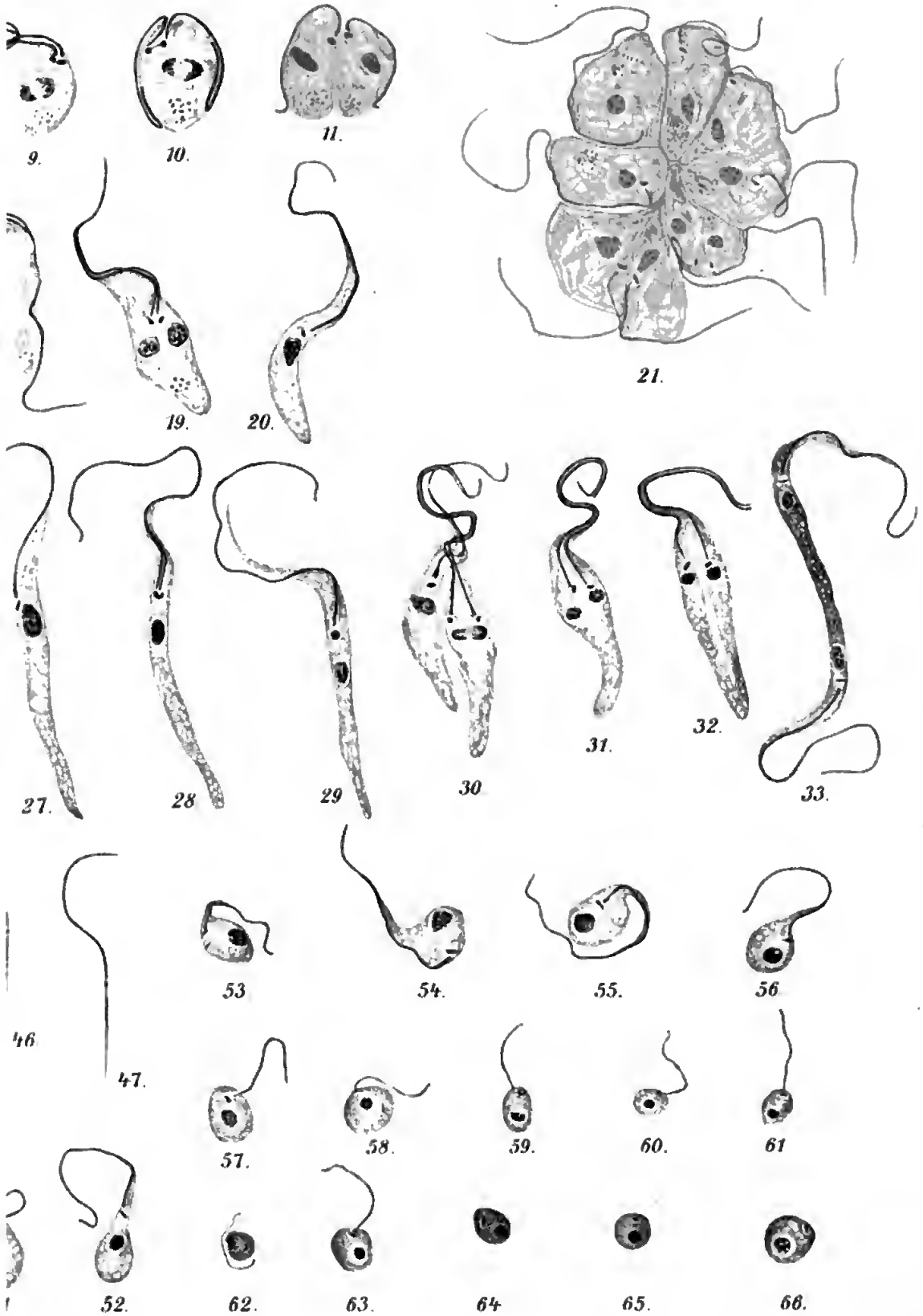
Figure 62. Flagellum become detached.

Figure 63. A ronnd form with the flagellum abont to he shed.

Figures 64 to 66. Cysts of various stages showing the circular nuclei sitnated at one end and the rod shaped blepharoplasts towards the other; no distinct capsule can he recognised.

All the parasites were drawn with a camera lncida and, excepting figure 22 which is magnified 550 diameters, are magnified 950 times.


a
b
c
d
e
f
1.
2
3.
4
5.
6.
7
8.
12.
13
14.
15
16.
17.
18
22.
23
24
25
26
34
35
36.
37.
43.
44
45.
38.
39.
40.
41
42.
48
49
50.
5



Fischer in Jena.

Lith. Anst. v. Johannes Arndt, Jena.

intestinal traet of Gerris fossarum Fabr. 131-146