Crithidia Leptocoris

by Irene McCulloch

June, 1913

Submitted to the Zoology Department of the University of Kansas in partial fulfillment of the requirements for the Degree of Master of Arts

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LIFE HISTORY AND STRUCTURE OF CRITHIDIA

LEPTOCORIS

Life History and Structure of Crithidia Leptocoris, the Intestinal Flagellate of the Box-elder Bug.

I Introduction.

The organism under investigation is an intestinal flagellate of Leptocoris trivittatus, the box-elder bug very common about the University grounds and buildings. The fact that very few such life histories have been worked out, and the possibility of the connection of these parasitic forms with diseases give a double impetus to the work.

The classification of the form is a point of some hesitation, since authors seem to be at variance in regard to the distinguishing characters of the Herpetomonads and the Crithidia yet I have adopted Calkin's characterization and called the flagellate form Crithidia, because of its possessing a slight undulating membrane during one phase of its life history.

This work has been done under the direction of Miss Nadine Nowlin to whom I here take this opportunity of expressing my indebtedness for many suggestions and much valuable help.

II The Insect Host, Leptocoris trivittatus.

a Distribution.

The Leptocoris trivittati, or box elder bugs are widely distributed over parts of the United States, especially some states of the Mississippi Valley. In Kansas of late years the bugs have been increasing until they are becoming more or less of a pest owing to the fact they they collect in great numbers about the house and other buildings during certain seasons of the year.

b Life History, Habits and Food of the Insect.

The life history of Leptocoris trivittatus or the box elder bug is very simple. The hibernating period, the time spent about buildings, lasts from early October until warm weather in April. This period of their existence is marked by extreme inertness. Artificial heat or the sun's rays may enliven them sufficiently to bring them out of their winter quarters. As for food during this period, the contents of their fat bodies serve that purpose. When warm weather comes, the lethargy disappears and they fly about.

A general migration then takes place in search of food to box-elder trees and grassy areas. At once the reproductive period begins and fertilized eggs are deposited upon dry grass and other debris in groups of four to ten (Plate I A) The eggs are readily found on account of their size and color. They are protected with a thick yellow-brown chitinous covering. Within less than two weeks tiny bright red insects emerge and enter upon a period of very rapid growth. The wings soon appear. By fall the mature insects are again found swarming about the buildings seeking winter quarters. The death rate of these insect during their hibernating period is large. The bodies crumble and soon become part of the rest of the dust stirred up about the house.

c Structure and Infection.

The digestive tract(Plate I B) is made of the oesophagus, the stomach, the intestine and the rectum. There are several glands in connection with the canal, the most important in this study are the malpigian tubules (Plate I B) The walls of the tract are thin and delicate.

Infection by Crithidia leptocoris is confined for the most part to the intestine and the rectum of the digestive tract. The intestine is infected with the pre-flagellate stage and in a few cases with the flagellate forms. The rectum is the main point of infection. Of the hundreds of bugs examined since the investigation of this flagellate was taken up, every specimen showed parasites in this section of the tract and most of them were very badly infected.
A few instances have been found where the flagellate forms have invaded the malpigian tubules.

There is still a question about the eggs being infected by means of flagellate forms which have penetrated the ovaries and encysted within the developing eggs. No definite evidence has been found. As to the infection of the young bugs, the percent infected by flagellate forms is not large. This will be discussed more fully in connection with methods of infection.

III Material and Technique

The studies were made from living forms, permanent smears and paraffin sections.

The general method of getting the material was to clip off the head and tip of the abdomen with sharp dissecting scissors, then with fine pointed tweezers at the posterior end of the digestive tract to pull it gently out upon a slide. It was at once flooded with Ringer's solution and covered with a cover glass. Not only the whole tract came out intact but the digestive glands, malpigian tubules and even the reproductive organs could be removed in good condition. Slight pressure flattened the canal and brought the parasites into view through the thin walls. This was advantageous since it revealed the distribution of the flagellates and its relation to its host in its own successive stages of life history. Again the study of the canal by sections, the oesophagus, the stomach, the intestine and the rectum was found to be profitable. When the parasite was to be studied cytologically a section at a time was clipped off, removed to a separate slide and crushed so as to extract the flagellates. In this way the exact cytological condition of the parasites in various regions could be ascertained. Sometimes the material for these studies was teased out before putting on the cover glass. Living forms were also studied in cultures by means of hollow slides sealed with vaseline. These could be run for days at a time and the changes observed.

The technique for permanent smears was perfected after much experimentation. The methods of Minchin Porter and Robertson were followed with slight modification. For the smears, the material mounted upon slides was teased out with dissecting needles and the large particles picked off. Osmic acid was used for instantaneous fixation. The slide was then allowed to stand exposed to air until almost dry when sublimate acetic, corrosive sublimate or Schaudinn's fluid was put on and allowed to evaporate until only a wet film was left. Subsequently the slide was passed through the alcohols and into the stain. Osmic acid fixation followed by absolute alcohol stained too deep to be satisfactory.

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1 "Protozoology" G. N. Calkins, Ph. D.Columbia U. New York, 1909.

2 "The Structure of T.lewisi in Relation to Micro scopical Technique." E. A. Minchin, Qr. Jr. Micro. Science,1909 July, New Series # 212, Vol 53,

3 " Studies in Ceylon Haematozoa." Muriel Robertson, Qr. Jr. of Micro. Science, #212, Vol. 53, July 1909.

The material for paraffin sections was fixed in sublimate acetic, corrosive sublimate, Flemming's Bucin's and Schaudinn's fluid, and embedded by the regular method. The sections were cut about five microns thick, one canal cutting nearly three hundred sections, and mounted in series. The slides were a valuable study, in that the parasites could be studied in situ: sections of the rectum showed a fringe of attached flagellates on the inner edge of the wall; the stained contents of the rectum showed many developmental stages and the epithelial cells of the wall were stained clear enough to determine whether intracellular forms were present. The one objection to such a study is the amount of time required to look over all the sections of one canal. For this reason longitudinal sections in series would be more desirable. The digective tract could be kept straightened out in the fixation and embedding and thus longitudinal sections of the entire length of the tract could be obtained. No doubt such a section would reveal more of the successive stages of the flagellate's life history.

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4 "Structure and Life History of Crithidia Melophagia." Qr. Jr. Micro. Science, June 1910, Vol. 55, #218. Annie Porter.

In order to study the relative position of the internal organs and to determine whether the organism was confined to the digestive track, attempts were made to section the whole insect. The difficulty here was in getting the fixatives to penetrate the thick chitin and the sections on the whole were very unsatisfactory.

Several stains were employed. Most of the results obtained were taken from smears and sections stained with iron haematoxylin. A series of smears were made using ether and absolute alcohol in equal parts for fixation and staining with Romanosky tricolor stain. The objections to this stain were the same that Minchin found, the trophonucleus and the kinetonucleus were enlarged and very deeply stained and the general cytoplasm showed no structure. Delafield's haematoxylin with a counter stain was used but the results on the whole were less satisfactory than those from the iron haematoxylin process.

For intra vitam stains, Janus green and neutral red were used. No definite nuclear structure was brought out with the Janus green stain; the neutral red proved to be the most reliable for this kind of work. For temporary staining, aceto-carmine gave good results, the only difficulty being that too much of the stain would destroy the material.

The contents of eggs and preparations from young bugs were used for smears upon glass slides in the same manner that the other material was used and stained with iron haematoxylin.

IV Movements.

A quick vigorous vibrating back and forth, and a rotary lashing about of the flagellate end producing a sudden change of direction characterize the movements of Crithidia leptocoris. The movements were studied under a 1/6 objective, an infected piece of the rectum having been teased out in a drop of Ringer's and covered with a cover glass. Three parts of the body aid in the movements, the flagellum, the body itself and the undulating membrane.

Owing to the rapidity with which the flagellate moves, it is difficult to determine just what takes when progression is accomplished. As Crithidia leptocoris moves across the field of the microscope, there is an instant in which the vibrating back and forth of the straightened flagellum is so rapid as to give it the appearance of being double or in the act of dividing longitudinally. Much space comparatively is covered during such an instant. Following this the anterior end executes several quick, spiral rotary movements, the force of which swing the posterior end of the body about and at once the flagellate shoots off in another direction. Looking down upon these while they are moving only the knife blade-like edge with a small circular thickening in the region of the trophonucleus is visible.

At times peristaltic waves pass along the body toward the anterior end. These waves are caused by certain actions of the body itself. For every movement of the anterior end there is a corresponding one of the posterior. It is in connection with the body movemants that the flexibility and rigidity of the organism are shown.

The bodies of the free forms are extremely flexible. A study of these nomad forms, focusing down through the wall, shows parasites pushing and writhing within the cell structure, either the posterior or the anterior end going forward with a serpentine-like movement. In some instances a number of flagellates have been found, the anterior halves of the bodies extending out from a plasmodial mass on all sides, writhing and struggling with great flexibility in their antagonism.

The rigidity of the organism is shown whenever the organism becomes attached in any way or when it comes in contact with others or with foreign material. In forms of the pre-flagellate stage which are attached to the wall of the rectum in great masses (Fig. 153), there is more or less of a rigid appearance of the bodies as they slowly swing back and forth or up and down. Probably the most striking indication of tenseness occurs when a flagellate has hooked its flagellum into some foreign material and the point of attachment serves as the center of rotation for the swinging body. The rigidity of the body may be confined to a certain part of the body, in longitudinal division this is found at the posterior end; when two individuals entangle and come together it occurs in the region of the trophonucleus.

The part played by the undulating membrane in movement is not important because only a small percent of the forms have a membrane. In blood cultures this membrane was very much in evidence. Its presence was indicated by the increased width of the anterior end, the slow flowing movement and the bead-like swellings which passed along in succession from the posterior toward the anterior part of the body.

Movements are not necessarily confined to the individuals. Often great masses of the flagellates and pre-flagellates embedded within a plasmodial-like mass writhe about slowly as a single large organism. Another movement involving a number is that of the aggregation rosette (fig. 100). Here the flagellates being on all sides and pulling about evenly there is little forward movement but a rotary one is brought about.

In addition to the movements already discussed which are more or less confined to the active flagellates, there is an euglenoid movement found among the pre-flagellate and post-flagellate forms. Such forms as (Figs. 40-45-138-140 and 181) show this movement. The most peculiar movement found was that of forms like (Figs. 146-137) where the rounded non-flagellate or posterior end invariably went forward with a quick serpentine-like movement.

V Morphology.

For convenience the life history of Crithidia leptocoris may be divided into three stages. The terms indicating such a division will be the same used by Porter (10), the pre-flagellate, the flagellate, and the post-flagellate. The forms of the one stage gradually emerge into those of another.

a Pre-flagellate Stage.

The life history of Crithidia leptocoris may be taken up beginning with the encysted form, a spore having a diameter from 2.0 to 2.4 microns,(Fig. 1), found in the intestine where it enters upon a period of growth (Figs. 2, 3). Following this there is a question as to just what takes place. Living forms indicate that two forms of unequal size come together and form one larger individual(Figs. 4,5) Stained smears show (Figs. 5-10) a varied nuclear structure, the possibility that only one nucleus breaks up to form the small pre-flagellates. This is similar to a process that Woodcock ('10) found in his study of trypanosomes but such a process is not in accordance with the life histories of the Genus Crithidia that have already been published. Whether conjugation takes place or not, the large forms(Figs. 8-10) form a plasmodial-like mass of pre-flagellates (Fig. 11) which may at once escape and enter upon a period of growth and division (Figs. 12-20) The posterior and the anterior ends elongate about the same time(Figs. 44-46). Evidence has been found indicating the possibility that these small pre-flagellates(Fig. 11) do not at once escape but undergo their period of development here(Figs. 128-129). Not all the pre-flagellates seem to follow the forms of (Figs. 11-20) in their development,(Figs.20-33) are irregular in form and nuclear structure with slight evidence of a transverse division.(Figs. 24-26)

The pre-flagellate forms usually show a kinetonucleus and a trophonucleus. The kinetonucleus is a small,round,deep staining structure which is responsible for the formation of the flagellum. The position and form of this nucleus varies. It is usually found anterior to the trophonucleus. The trophonucleus also changes position and structure(Figs. 43-45) The chromatin is deep staining and abundant.(Fig. 50) shows the chromatin in a small number of granules where the parasi'e is almost a mature flagellate.

b The Flagellate Stage.

The cytology of the flagellate forms is an interesting study in itself, so great is the difference in structure of the several forms. The size of the flagellate forms was found to vary from 8 to 40 microns in length and from 1.9 to 3 microns in width. As to form most of the flagellates can be grouped into three types which are more or less distince. The first type of the mature flagellates have a long, slender body, no indication of an undulating membrane, and the protoplasm is prolonged posteriorly to form a body tapering to a point.(Figs. 67-69) These forms show a vacucle in the region of the kinetonucleus . The second group of the flagellate forms are typified by being more mature, the beginning of an anterior prolongation of the protoplasm to form an undulation membrane, a body medium as to length and width(Figs. 62-64-65-66). The posterior end of the body has begun to draw up increasing the width. The third group shows forms having a distince undulation membrane, a prolongation of the protoplasm about the flagellum, and the posterior part of the body is wider and more blunt.(Figs. 55-59-78-79)

The several parts of the flagellate form studied were, trophonucleus, kinetonucleus, chromidia, undulating membrane, flagellum and the cytoplasm.

The trophonucleus is usually found about the center of the body both longitudinally and transversely. The chromatin material is abundant and deep staining but not so deep as that of the kinetonucleus. The chromatin may be found in the form of a hollow sphere (Fig. 59) or broken up into seven, four, or five granules, (Figs. 65-66-85)respectively. Around this nucleus there is usually a nuclear membrane which stain rather deep. The chromatin may also be found in the center of this with strands radiating out to the circumference.

Above the trophonucleus a short distance the kinetonucleus is most frequently found. The chromatin of this stains very deep and shows little structure. In form, the kinetonucleus may be bar-shaped extending either longitudinally(Fig.61)or transversely, (Figs. 56-68-72) or round (Figs. 59-60-62). In the region of the kinetonucleus there is shown for the most part a vacuole. The relative position of the kinetonucleus in regard to this varies very much. The chromatin of this nucleus may be found at the top,(Figs.57-60), at the side, (Fig. 69) or below the vacuole, (Fig. 82).

Chromidia are found scattered about the cytoplasm especially in the posterior part of the flagellate forms(Figs. 75-122). These according to Porter (10) were formed when the nucleus was broken up during the pre-flagellate stage.

In the more mature flagellates the anterior end of the protoplasm is prolonged in connection with the flagellum and forms an undulating membrane. (Figs. 78-79-110-111) All the stains employed fail-to show any myonemes in Crithidia leptocoris.

The undulating membranes are found in the more mature flagellate stage. When the membrane is present as shown by the forms in the blood cultures, the movement is smooth and slower.

In this stage of the life history of Crithidia leptocoris the flagellum is found extending in most cases almost the entire length of the body. At the posterior end of it there is usually found a basal granule and at the extreme anterior end of the body another granule is found occupying a postion similar to that of the diplosome of other parasitic flagellates. (Fig.88) The flagellum may either pass through the center of kinetonucleus (Figs. 56-58-59) or touch the end(Fig. 66).

The protoplasm of Crithidia leptocoris is alveolar(Fig. 83) especially in the posterior end of the body. Such forms as (Fig.76) show a hyaline structure in their protoplasm. Many small granules and chromidia are scattered about in the protoplasm. The posterior part of the body is granular but the anterior part frequently stains more densely. There is usually a large vacuole present in the region of the kinetonucleous(Fig.60) and numerous small ones found in the posterior part of the body.

Not all of the post-flagellate forms have been definitely worked out yet and more work will be done in the future along this line. The life history of Crithidia leptocoris seems to more complex than the other life histories. One life cycle, evidence goes to prove this, of what I shall call the free flagellate form of this stage is completed in the insect while the life cycle of the attached forms of this stage forms a cyst and completes the life cycle in another insect.

The large nomad flagellate forms (Figs.56-75-76) are the parasites which complete their life cycle within the body of the same insect. The first indications of the post-flagellate stage are found when the body grows shorter and the flagellum together with the membrane begins to disappear(Figs.130-132). The body is no longer a thin blade-like form but it begins to grow thicker, and the nuclear structure undergoes a change. Smaller nuclei are formed and the flagellum is slowly being absorbed.(Fig.137).

Fig.137 indicates another possibility of a sexual form of reproduction which is in accordance with Woodcock ('10). These large free forms finally become circular(Fig. 135)(136) and the nuclei break up to form small pre-flagellate forms, and the life cycle is completed within the rectum of the same insect.

The flagellate forms which attach themselves to the wall of the rectum before attaining any great period of growth(Fig. 154) undergo a rapid division period wherein the size of the individuals is gradually reduced. The attached forms also indicate other changes that are taking place(Figs. 152-163), the posterior part of the body is drawing up, the width is increasing, the flagellum is gradually being absorbed, and changes are taking place within the nuclear structure. (Figs.164-169) were drawn from a cross section of the rectum. Fig. 172) shows a form that has been detached from the rectum wall and shows the form of the parasites as they are about ready to begin to encyst. (Figs. 178-186) show the final steps in the disappearance of the flagellum. (Figs.187-199) show the later stages of the development of the encysted form or spore within the contents of the rectum. (Fig. 200) is the spore as found in the feces.

VI Longitudinal Division.

Longitudinal division is found early in the life history of Crithidia leptocoris among the minute pre-flagellate forms(Figs.15-17-19-30). There is little movement in connection with the process at this stage of the life cycle. The constriction of the protoplasm into two parts begins at the smaller or anterior end, the nuclei having already divided into two and migrated toward the periphery of the organism. In such forms as shown in (Fig. 17) the division has taken place rapidly enough to form small division rosettes, the posterior ends being slightly connected for a time. The pre-flagellate stage is a period of rapid longitudinal division and growth until the flagellate forms (Fig.52) are formed. The division continues throughout the life cycle but the period of constant increase in size with each division if confined to the pre-flagellate stage.

The series of movements on the part of the daughter flagéllates during during division is the most interesting thing in connection with the longitudinal division of the flagellate forms. Stained forms(Figs. 86-95-96) show several flagellates about ready to divide, the body is unusually wide, and the nuclear structure is in the act of constricting. The kinetonucleus divides first and then the flagellum at this point begins to split and continues to split in both directions until there are two flagella the entire length of the body. The basal and the anterior granules also divide into two in connection with the flagellum if they are present. The trophonucleus is the last to constrict into two parts and the parts like those of the kinetonucleus immediately migrate toward the periphery. From this point the process may be best studied from living forms. The two flagella indicate that the the process has begun and the movement of the two flagella as they struggle and writhe about in their antagonism toward each other hasten the separation of the protoplasm into two.

The movement of a dividing flagellate as one individual is almost as rapid as that of the normal one as it shoots off across the field. Fig. 92 shows the two flagellates about ready to assume the horizontal position and Fig. 93. shows the horizontal position taken and the at point the two struggle and pull in opposite directions in their effort to bring about the separation. As the two finally pull apart the protoplasm is torn and ragged particles are found adhering to the posterior ends of forms that have just divided.

Forms frequently come together and in some way entangle their flagella, and if this condition continues long enough longitudinal division of the forms takes place and an aggregation rosette is formed. (Figs. 95-97) show the several steps in the formation of such a cluster of parasites and some flagellates of the smaller rosettes show that division is about to take place. The aggregation rosette is not like the division rosette, in that the rapid division is the initial beginning of the division rosette while an entanglement is the beginning of the aggregation rosette. The true rosette formation or the division rosette is not found among the flagellate forms very often because the rate of division is slow among the flagellate forms. In a few instances the flagellate forms have been found where the constriction of the mother form into two daughter flagellates began at the posterior end instead of the anterior.

Among the post-flagellates, the attached forms, the process of longitudinal division is similar to that of the flagellate forms. The nuclear structure is constricted into two and the constriction here usually begins at the posterior end and rarely at the anterior end. When a flagellate form becomes attached to the rectum wall a period of rapid longitudinal division takes place and a great mass is formed. (Fig.153)

Longitudinal division for the most part results in the formation of two daughter forms of equal size. Porter ('10) observed the process of longitudinal division where two forms of unequal size were the results. In Crithidia letocoris this form of longitudinal division has been found (Figs.99-101) . The constriction in such a process begins at the posterior end and one longer slender form and a shorter circular form result.

In the pre-flagellate stage evidence has been found that the small forms may divide transversely. (Figs. 24-26) are the nearest evidence of such a process found among the stained forms.

VII Infection.

Infection of insects by the genus Crithidia takes place by one of two methods or by both, the hereditary and the casual method. The infection of Leptocoris trivittatus, or the box elder bug by Crithidia leptocoris has been investigated to some extent but more work will be done along this line.

a Hereditary Infection

Hereditary infection is usually brought about by flagellate forms which leave the digestive tract and penetrate through the tissues into the ovaries where they encyst within a developing egg.

The flagellate forms were studied carefully to determine whether they left the tract and penetrated into the reproductive organs and no evidence of such was found. In addition to this the contents of eggs taken direct from the insect were taken and the contents examined in Ringer's in the usual manner for the study of the parasites for living forms, spores or flagellates. Not finding anything that could be called parasites the smears were stained and examined again. In addition to this material eggs taken from dry grass and other debris were examined and the contents of the tract of young insects were like-wise intestigated. Nothing definite was found to show hereditary infection.

b Casual Infection.

The tip of the abdomen of a mature insect was clipped off without any of the rectum and put upon a slide in a drop of Ringer's After teasing and scraping the bit of chitin the smear was stained and fixed. A few encysted forms were found. The feces were also examined for parasites, both living flagellates and post-flagellates were found. Young insects which had been feeding for a few days were examined and the percent of infection showing flagellate forms was very low. Some young insects were put into a glass bottle containing some living mature box elder bugs and the young were from time to time examined for parasites. The results showed that the older the insect the more the infection and the percent of infected bugs in this culture was higher than that of the insects without. The young insects feed upon feces as well as the green vegetation, and the evidence so far would go to show that the infection in this case if casual for the most part.

VIII Experiments.

1 A bright, intense light through the microscope increased the activity of the flagellate forms very much.

2 Neutral Red reacted upon the post-flagellate attached forms and caused them to become circular in shape and begin the process of encystment. They also lost their attachment to the rectum wall.

3 Blood cultures, prepared by putting a small drop

of blood into a hollow slide filled with Ringer's solution and numerous flagellates, Crithidia leptocoris. Observations were taken from time to time. The activity of the fla- was noticeably increased at first, and the tendency to form an agglomerated mass was noticed later. The cover slip was sealed on with vaseline and culture put away. The second showed many forms with a well developed undulating membrane, the movements were slower and more even on the whole. At times the body became curved and rigid and then sprang across the field.

4 Dead bugs were examined for living forms and spore forms in the dried intestine. In the winter liv- forms were found after three weeks. Dead bugs were examined at stated intervals to study the process of encystment of the flagellate forms. Such a process was not found.

5 A frog was taken, a microscopic examination was then made of its blood for blood parasites and fed upon the box elder bugs for a week. The This experiment wasnot completed because the

frog escaped just before the final examination of its blood was made. Lack of time prevented a repetition of the experiment.

IX Summary.

1 Crithidia leptocoris is an intestinal flagellate found in the alimentary tract and rarely in the malpigian tubules of Leptocoris trivittatus or the box elder bug.

2 The life history of the parasite may be divided into the pre-flagellate stage (Figs. 1-52), the Flagellate stage (Figs. 53-85) and the post-flagellate stage (Figs. 86-200). The first two stages may be found in the intestine but most of them are found in the rectum together with the post-flagellate stage.

3 The development of the pre-flagellates from either a spore or a mature flagellate form takes place fapidly (Figs. 1-52) Longitudinal division is found in this stage(Figs.17-20), indications of transverse division(Figs. 24-26) and also of sexual process(Fig. 1) are found.

4 Flagellate forms vary from 8 to 40 microns in length and from 1.9 to 3 in width. The general protoplasm is alveolar, both trophonucleus and kinetonucleus are found, chromidia, flagellum and an undulating membrane on some forms. Longitudinal division takes place and aggregation rosettes are formed. A basal granule is frequently found at the base of the flagellum which extends usually throughout the length of the body.

5 The post-flagellate stage shows individuals undergoing two methods of completing the life cycle. The large mature flagellate forms having an undulating membrane(possibly fertilized by by a small form fig. 137) at once become circular, lose flagellum and membrane and the nuclei break up into smaller ones which develop into the pre-flagellate forms (Figs. 163-127)

The attached flagellate forms undergo a period of division and reduction in size while attached to the rectum (Figs. 154-168), the body changes shape and the flagellum after the parasite ceases to adhere to the rectum wall, begins to disappear (Figs. 169-194). The final process is shown (Figs. 195-200) where the spore which passes out through the anus is completed.

6 The multiplication of Crithidia leptocoris is chiefly by means of longitudinal division.

7 Evidence so far in the investigations goes to show that Leptocoris trivittatus or the box elder bug is infected with Crithidia leptocoris by casual rather than the hereditary method.

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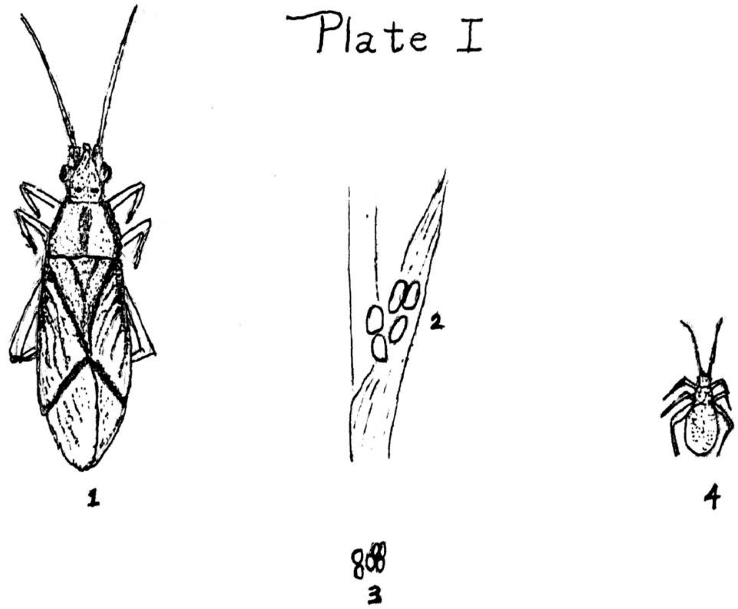
Plate I



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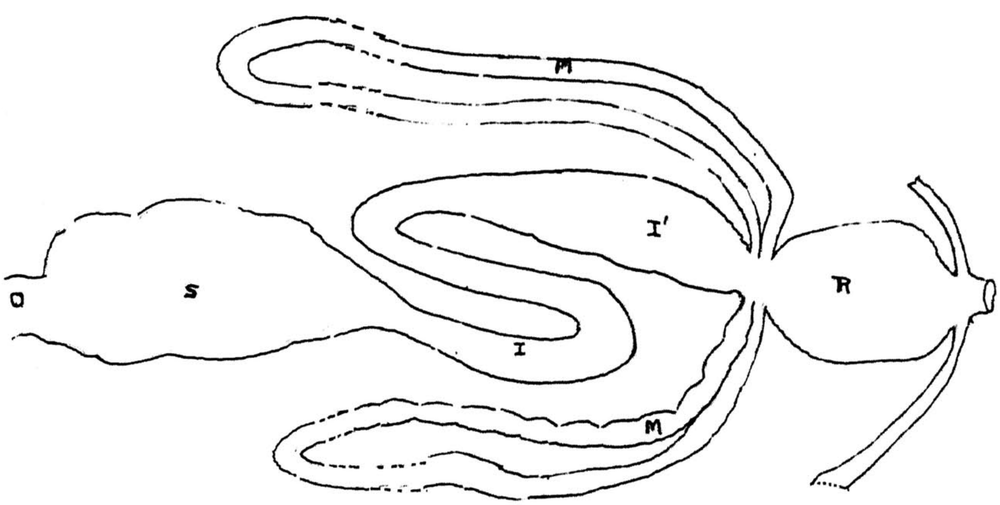


B

Explanation of Plate I.

A Drawings showing the life history of Leptocoris trivittatus, or Box-elder bug.

1 Sketch of mature insect, wings dark brown with red markings, body red. X 3

2 Group of eggs deposited upon dry grass x3

3 Eggs, natural size.

4 Young insect a few days old, wings have not begun to grow out.

B Sketch of the Digestive tract of Leptocoris trivittatus, or Box-elder bug.

O Oesophagus

S Stomach

I Fore part of the Intestine

I' Hind part of the Intestine

R Rectum

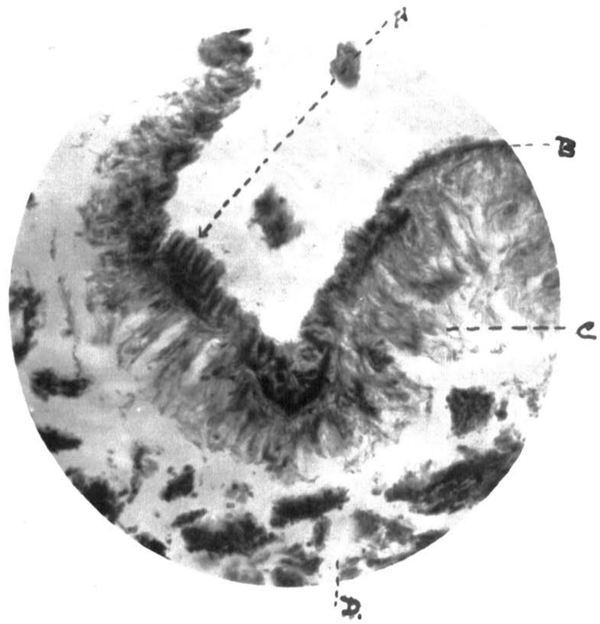
M Malpigian Tubes

Plate II

Explanation of Plate II

Plate II is a photomicrograph of a portion of a cross-section through the rectum, where the Crithidia leptocoris attach themselves to the wall of the tract. The numerous bodies of the parasites projecting out into the interior of the rectum make an irregular line at A. The rest of the wall below B has been more or less torn away. x 250.

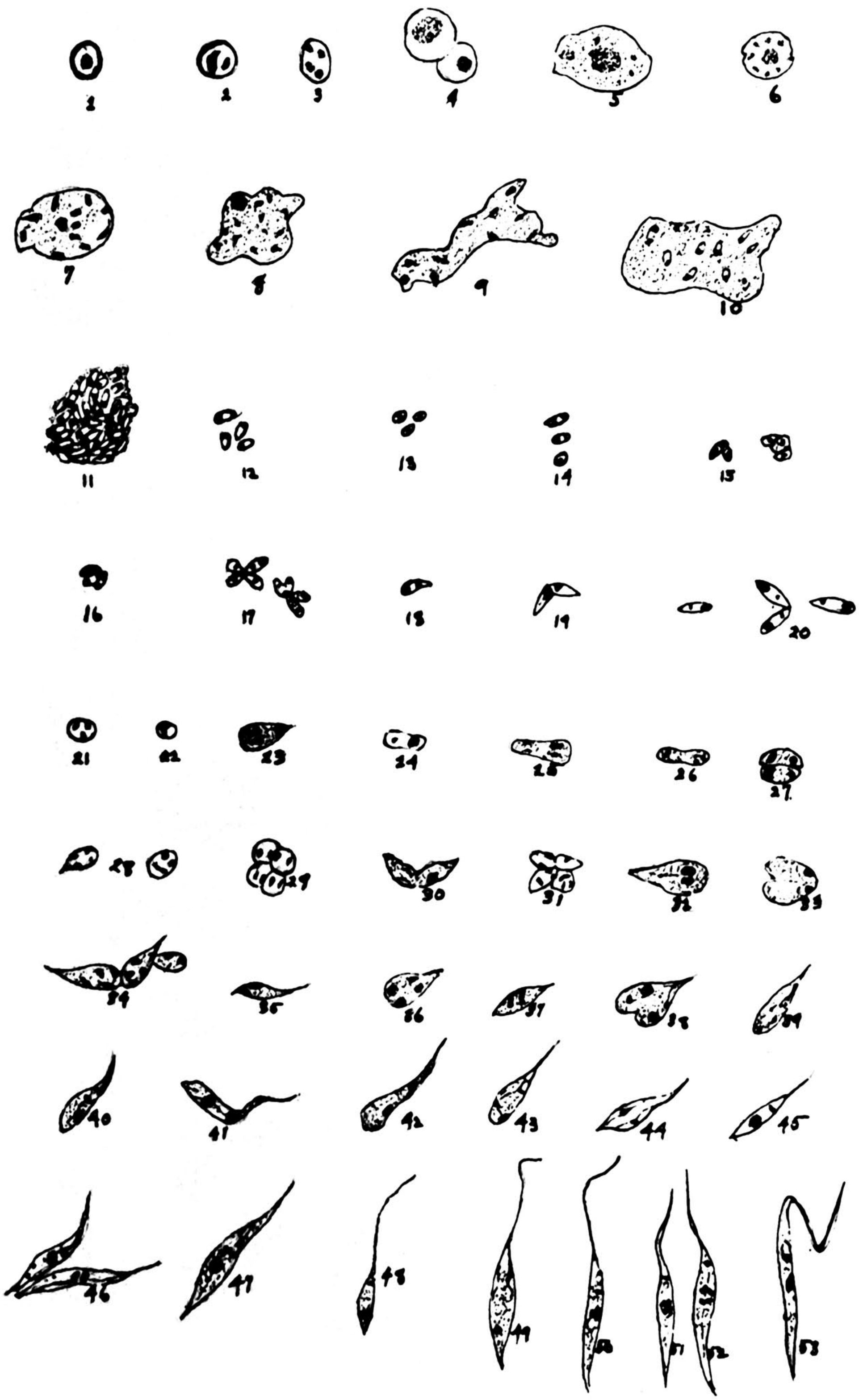
Plate III



Explanation of Plate III.

Another photomicrograph showing the rectum wall more or less intact and a mass of attached parasites at A. In mounting the section the wall at this point was turned over and the posterior end of the bodies turn toward the outside rather than toward the inside as they should. x 250

Plate IV



Explanation for Plates 4 --10.

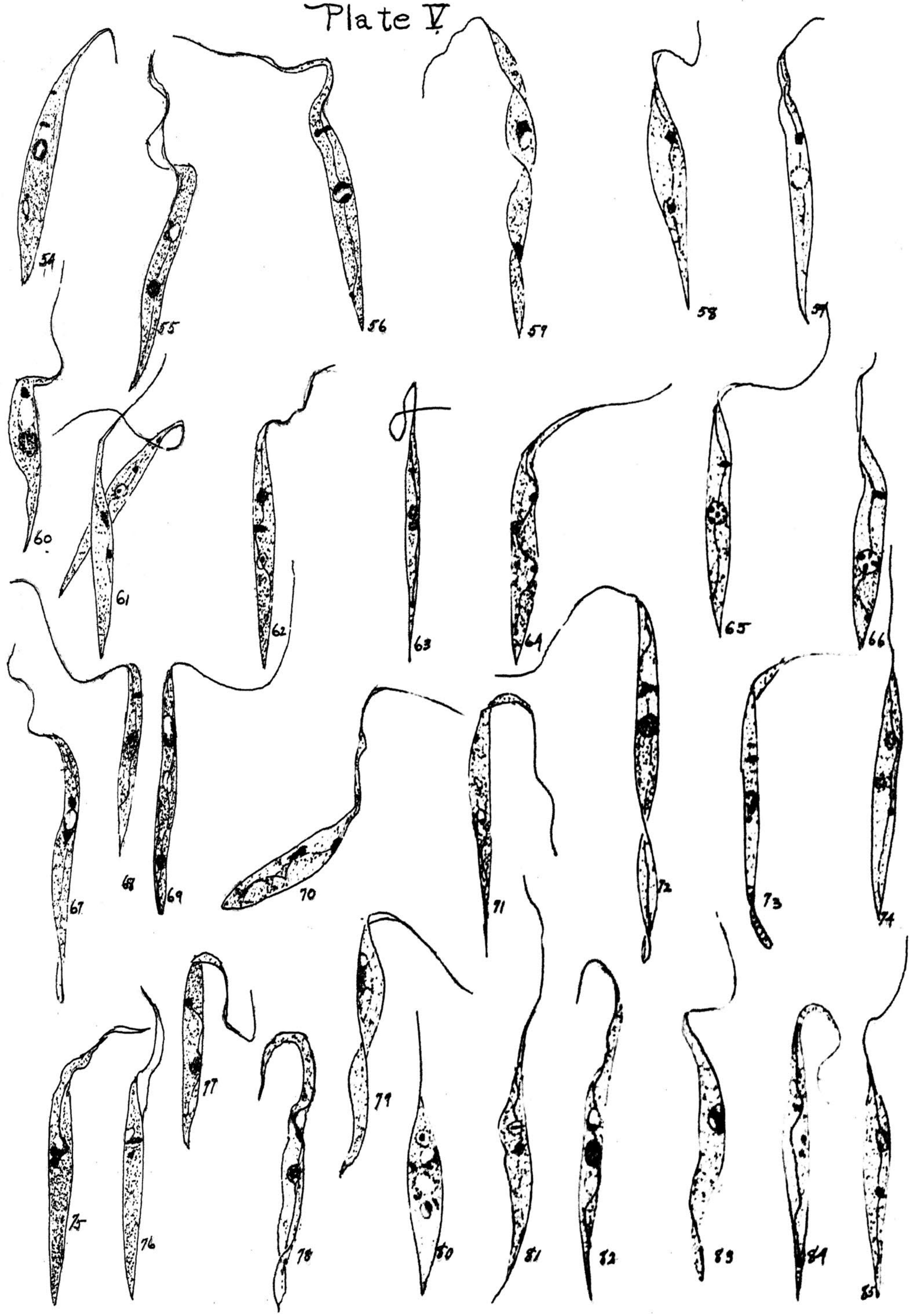
Note: All figures were outlined with an Abbe-Zeiss camera-lucida, using a 1/16 oil immersion (Leitz) objective, and an 8 compensating ocular. The magnification in all cases is approximately x 2000 Unless otherwise mentioned the stain used in all these figures was iron haematoxylin.

Plate IV

Figs. 1 -- 53.-- Pre-flagellate Stage.

Fig. 1 --Encysted form or spore, beginning of pre-flagellate stage, one nuclear structure.
Fig. 2 --Pre-flagellate. Trophonucleus. Kinetonucleus.
Fig. 3 --Preflagellate with nuclear material broken up.
Fig. 4 --Slight evidence of conjugation. Forms unequal.
Fig. 5 --Shows possibility of 4 becoming 5
Fig. 6 --Beginning of the breaking up of Nucleus to form small pre-flagellates.
Figs.7-10 Same as 6 showing different stages of their formation
Fig. 11--Plasmodial-like mass of pre-flagellates. Both Trophonucleus and Kinetonucleus present. Individuals free themselves at different inter vals.
Figs 12-16 Several groups of pre-flagellates.
Fig. 15 - Also shows longitudinal division before flagellate stage is reached.
Fig. 17 Division rapid enough to form division rosette
Fig. 18 Single Individual, ends elongating.
Figs.19-20 Longitudinal division, nuclear structure different.
Figs.21-22 Another form of pre-flagellate.
Fig. 23--Same form as 20 after a period of growth Division about to take place.
Figs.24-34 Forms irregular,steps in their formation not yet determined definitely. Fig. 26 may indicate transverse division, which seems to occur.

Plate V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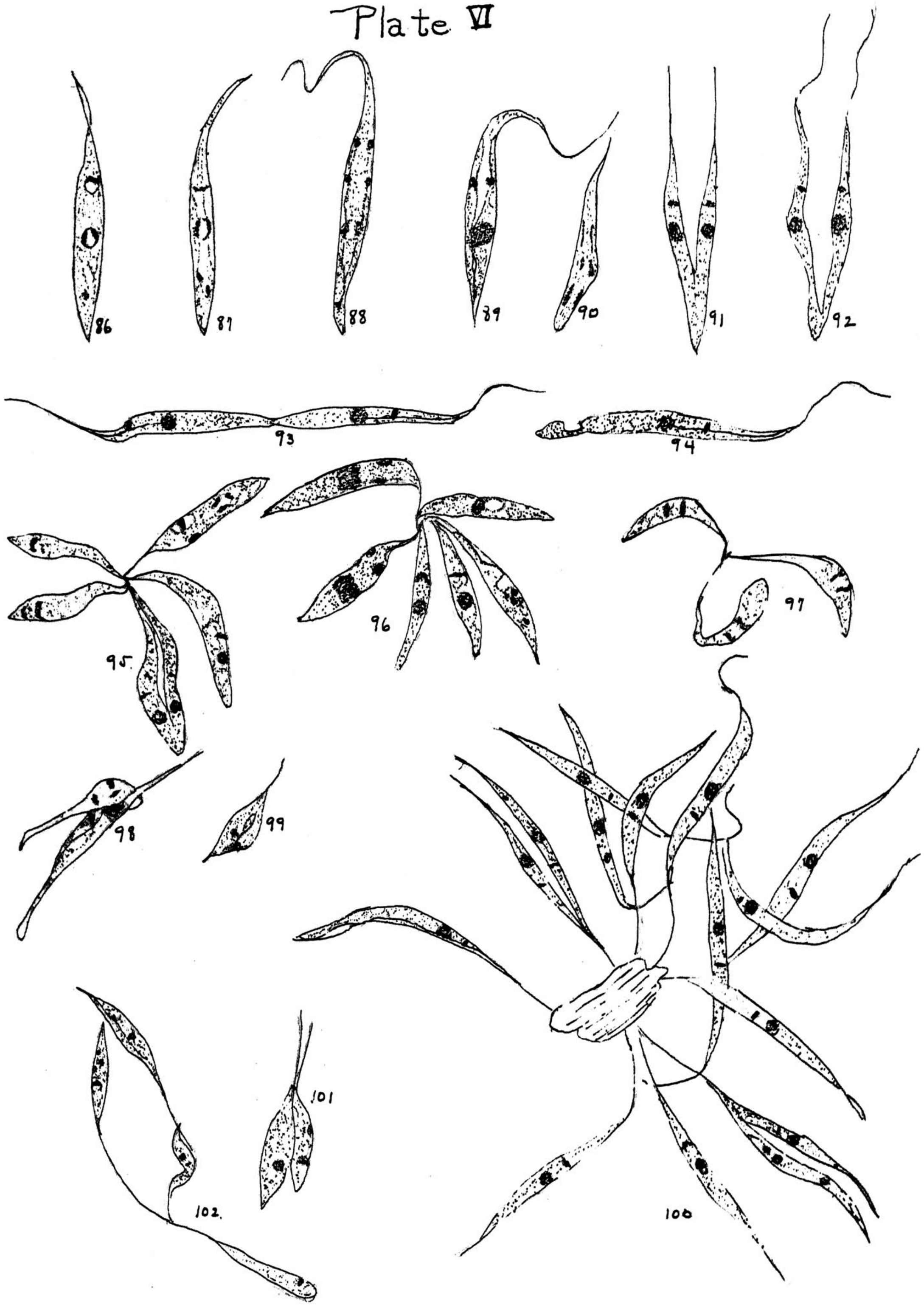
Explanation of Plate V. Flagellate Stage.

Fig. 54--Large form no definite flagellum.
Kinetonucleus divided. Anterior granule.
Fig. 55--Longsslender flagellate. Membtane. rectum.
Fig. 56--Typical flagellate. Basal granule. Transverse kinetonucleus. rectum
Fig. 57--Large flagellate showing knife-blade-like form
Fig. 58--Broad flagellate. Chromatin at center of trophonucleus. Vacuole-like area present.
Fig. 59--Little chromatin in Trophonucleus
Fig. 60--Large trophonucleus. Round Kinetonucleus vacuole.
Fig. 61--Two forms found together. Note difference in trophonucleus and the bar-shaped kinetonucleus lying longitudinally.
Fig. 62--One large chromidia in vacuole-like area. Bar-shaped trophonucleus.
Fig. 63--Trophonucleus almost intwo. Basal granule. Small kinetonucleus.
Fig. 64--Large flagellate. Basal granule. Chromidia.
Fig. 65--Trophonucleus with seven chromidia.
Fig. 66--Basal granule. Four chromidia in trophonucleus.
Fig. 67--Characteristic position for two nuclei with respect to vacuole.
Fig. 68--No Membrane. Trophonucleus large.
Fig. 69--Large basal granule. Vacuole. Peculiar trophonucleus.
Fig. 70--Both nuclei bar-shaped. Anterior and basal granules
Fig. 71--Long body. Little nuclear structure.
Fig. 72--Long body showing knife edge
Fig. 73--Slender body. Peculiar nuclear structure.
Fig. 74--Slight membrane.
Fig. 75--Dense cytoplasm. Nuclei together. Chromidia.
Fig. 76--Membrane. Nuclei near together.
Fig. 77--Trophonucleus nearer the posterior end
Figs. 78-79 Peculiar forms as to position and structure of single nucleus. Membtanes present.
Fig. 80--Vacuoles. Chromidia. Shape of both nuclei.
Figs. 81-83-84-85 Types of trophonuclear structure.

Plate VI



Explanation of Plate VI. Flagellate Stage.

Fig. 86--Width of body and nuclear structure indicate division.
Fig. 87--Kinetonucleus, trophonucleus and basal granule dividing.
Fig. 88--Form showing the anterior granule also dividing.
Fig. 89--The trophonucleus is the last to divide
Fig. 90--Form with peculiar nuclear structure in the dividing stage.
Fig. 91--Two daughter flagella separating.
Fig. 92-- Separation a little more advanced.
Fig. 93--The two flagellates pulling oppositely
Fig. 94--Cytoplasm at posterior end indicates that division has just taken place.
Fig. 95--Small aggregation rosette.

Fig. 96--Aggregation rosette showing forms about to divide.
Fig. 97--Rosette with forms having bar-shaped nuclear stbructure.
Fig. 98--Forms that adhered to eatch other.

Fig. 99--Unequal division

Fig. 1oo-Large aggregation rosette.

Fig. 101-Unequal division

Fig. 102- Aggregation rosette just forming.

Plate VII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Explanation of Plate VII Flagellate Stage.

Fig. 103- Flagellum disappearing. Body shorter and wider.

Fig. 104- Flagellate, body increasing in width.

Fig. 105- Flagellate showing vacuole area.

Figs. 106-108 Flagellum disappearing. Membrane.

Fig. 107 Large flagellate form

Fig. 109 Flagellum present. Nuclei like 106.

Figs. 110-112 and 116 Membranes present.

Fig. 113 Membrane also has disappeared and nucleus is breaking up.

Figs. 114,115 Stages in the degeneration of the Flagellum.

Figs. 119-120-121-122-136 Indicate degeneration of of the flagellum.

Fig. 117 Post-flagellate. Nuclei have broken up into smaller ones.

Fig. 118 Rectum. Post-flagellate.

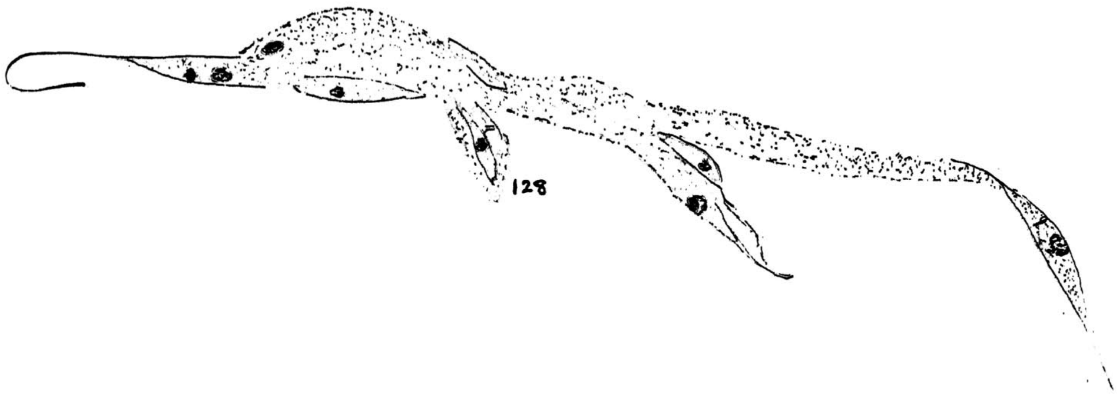
Fig. 123 Rectum. One nucleus present. Post-flagellate

Fig. 124 Post-flagellate.

Fig. 125 Post-flagellate. Vacuole.

Fig. 127 Post-flagellate, Pre-flagellate forms within the plasmodial-like mass.

Plate VIII



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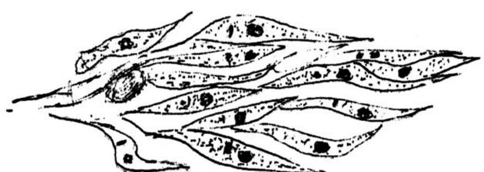

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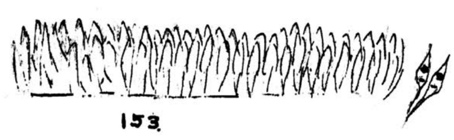

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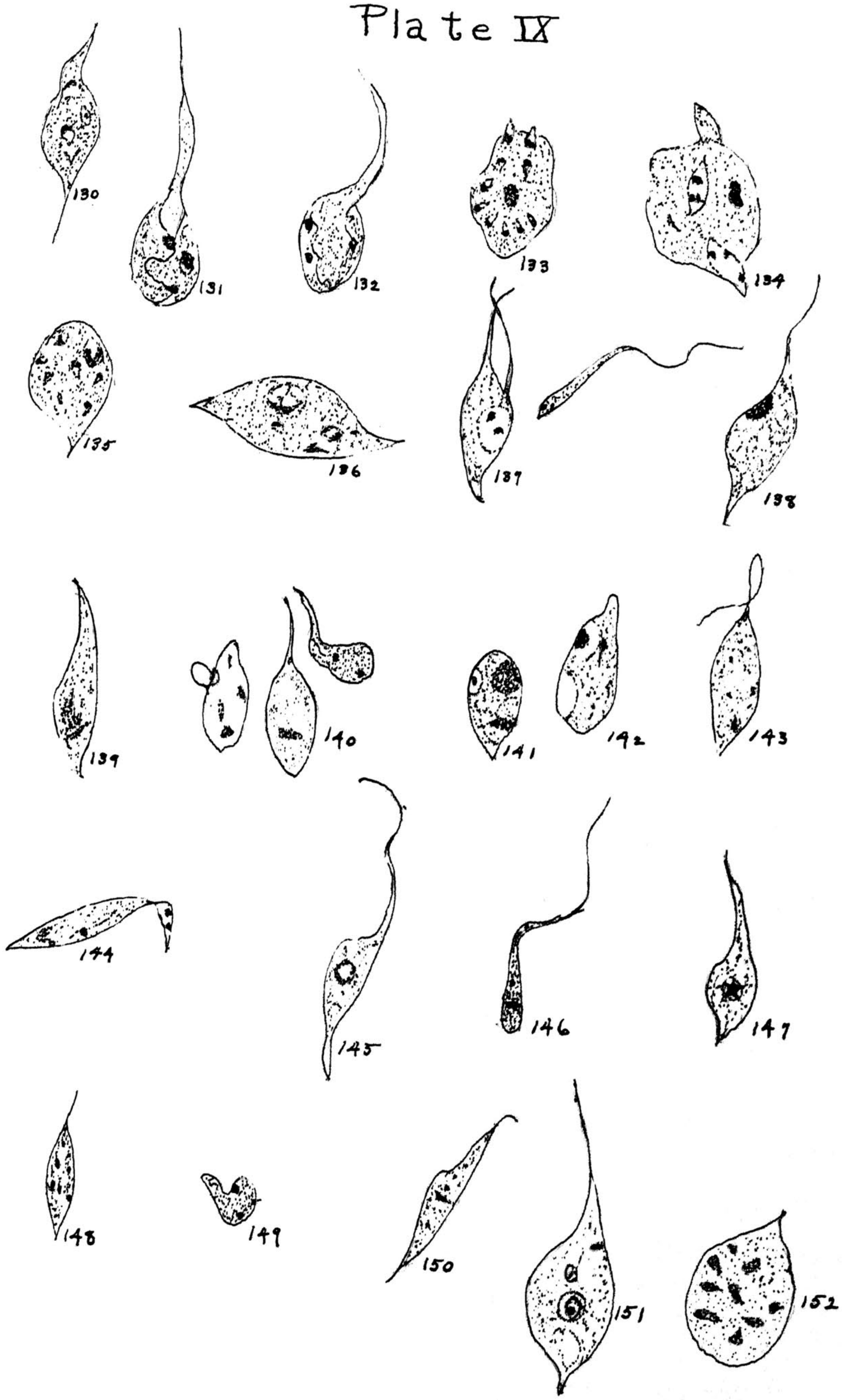
Explanation Plate VIII.

Fig. 128. Plasmodial-like mass containing mature flagellates. Mass has been pulled out of shape in fixing.

Fig. 129 Group of individuals, mature flagellates which seem to have come from a mass similar to the one above, fig 128.

Fig. 153 Sketch showing the attached parasites, Crithidia leptocoris on the inner wall of the rectum in the anterior part.

Plate IX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Explanation, Plate IX. Post-flagellate.

Figs. 130, 131, 132, 136, 138 show various stages in the degeneration of the flagellum.

Fig. 135 Large Nuclei breaking up.

Fig. 137 These forms might indicate sex and fertilization process before the breaking up in fig. 135.

Fig. 139 Large form with one nucleus.

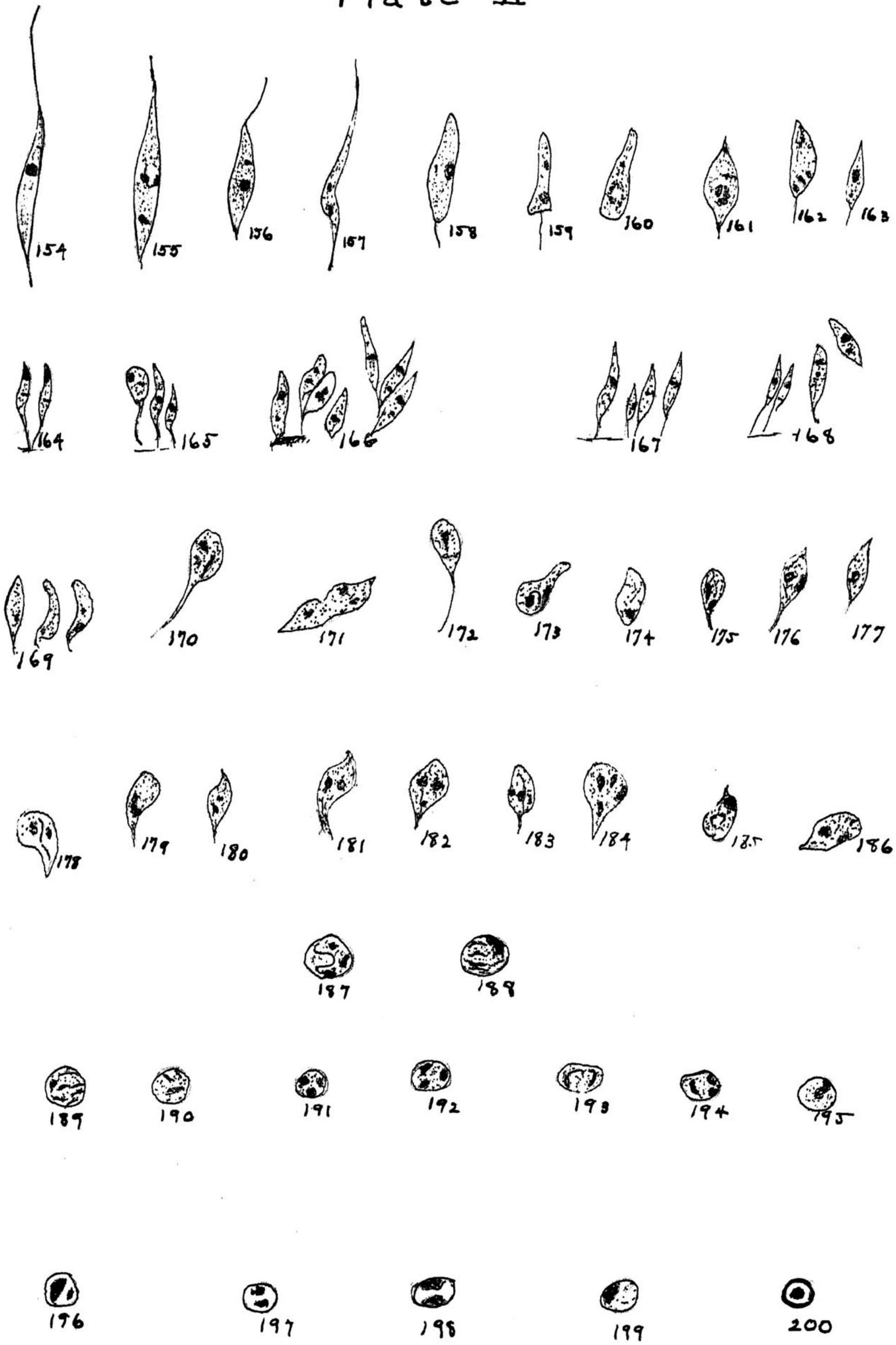
Fig. 140-145 Post-flagellates.

Fig. 146 Peculiar form which moves with blunt end forward, serpentine movement.

Figs. 147-151. Various stages of post-flagellate development.

Figs. 152, 133, and 134 Post-flagellate forms with pre-flagellates in the process of development.

Plate X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Explanation of Plate X. Post-flagellates.

Attached forms.

Fig. 154-- A small flagellate form that has just attached itself by anterior end to rectum wall.

Fig. 155--A form like fig. 154 about to divide.

Fig. 156--Body getting shorter.

Figs. 157--163. Stages of reduction in size of flagellate forms.

Figs. 164-169. Post-flagellates attached. Groups drawn from rectum section.

Figs. 169-177 Contents of rectum. Series of stages preparatory to encystment.

Figs. 178-186-Post-flagellate. More advanced stages preparatory to encystment. Disappearance of flagellum.

Figs. 187-194- Disappearance of flagellum.

Figs. 196-199 Final stages in the formation of the cyst.

Fig. 200 Cyst.