DESCRIPTION OF A *HERPETOMONAS* PARASITIC IN
THE ALIMENTARY TRACT OF THE COMMON GREEN-
BOTTLE FLY, *LUCILIA* SP.

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With Plates VIII and IX and 2 Text-figures.

A *Herpetomonas* has been previously recorded in species of *Lucilia* by Patton in India (1908) and by Roubaud in the Congo (1908), but neither observer has described the parasite.

The following paper is concerned with the description of a *Herpetomonas* found in two species of *Lucilia* which infest butcher's shops in Cambridge. It is set out under the following heads:

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Note on the digestive tract of Lucilia.

The alimentary tract of *Lucilia* is similar to that of *Musca domestica* and allied Diptera. The oesophagus (Fig. 1) leads to the midgut, which commences at a valve-like organ, the proventriculus. Before reaching the proventriculus, however, it branches to form the oesophageal

diverticulum, which is at first tubular, but later ends in a large bilobed sac, sometimes, though incorrectly, referred to as the 'crop.' The midgut is dilated slightly, and is succeeded by the small intestine, which terminates at the point of entry of the Malpighian tubules. The hindgut commences at this point, and ends in a dilated portion termed the rectum, which opens to the exterior at the anus.

In this paper the term 'crop' will be retained only for the sake of brevity.

Fig. 1. Showing the anatomy of the alimentary tract of *Lucilia*.

The methods of examination of the gut contents.

The parasites were studied both in the living state and in stained preparations. Living specimens were obtained by dissecting out the flies' intestinal tract in saline solution, removing portions of it to a slide, and protecting with a cover-glass. Stained preparations were made from isolated portions of the gut, opened up in a small drop of salt solution and smeared out in the usual way. They were fixed in absolute alcohol, and finally stained with Giemsa diluted 1:20. It was found that a weak solution of the stain gave less diffuse staining than a stronger one.

Morphology.

The general character of the parasite varies in the different regions of the intestine in which it is encountered. The description of the various forms may therefore be conveniently arranged according as they are found (i) in the oesophageal diverticulum or 'crop,' (ii) the midgut,

(iii) the hindgut and rectum, although intermediate forms between the predominant types occurring in each region are occasionally found. The scarcity of such intermediate forms probably depends upon rapid and marked changes of form occurring in the parasites as they pass from one region of the intestine to another.

I. THE PARASITE IN THE OESOPHAGEAL DIVERTICULUM.

In this region, we find (1) *small truncate pyriform bodies* (the so-called cysts), (2) *oval forms without a flagellum*, and (3) *oval forms with a short flagellum*. These forms are connected by intermediate stages, and none of them show any signs of division. They have the following characters:

(1) The cysts, as seen alive, are small truncate pyriform bodies about $3.5 \times 1.5 \mu$ in size. Their truncated appearance is due to a small cup-shaped depression in the protoplasm, at what we consider to be the anterior extremity of the cell. The protoplasm can readily be seen to consist of an inner core of endoplasm, invested by a layer of ectoplasm, which however does not cover in the endoplasm at the site of the above-mentioned depression at the anterior end of the cell.

In stained preparations, the ectoplasm, or 'periplast,' appears as a dense blue-staining coarsely sponge-like structure, while the endoplasm is more homogeneous, and appears pale-blue through the periplast, but in cells which have ruptured it can be seen to be stained pale-pink. As was seen in the living cell, the periplast does not completely cover in the endoplasm, but leaves a tiny area where the latter comes to the surface at the truncated end of the cell. The pale-staining endoplasm may be called the *cytopharynx*, and its cup-shaped entrance the *cytostome*. The *lip* of the cytostome consists of periplast. In those cysts which have the most differentiated protoplasm, the lip of the cytostome is inverted, probably by the action of the retracting endoplasm, so that only a minute aperture, or *micropyle*, is left opening into the cytostome. The periplast is thus made to fold over the exposed endoplasm. Embedded in the ectoplasm are the nucleus, blepharoplast, and 'chromidia'; and in the endoplasm traces of a flagellar apparatus can sometimes be seen as a faintly-staining red line. (See Pl. VIII, figs. 1-2, 23-25.)

The *nucleus* is round and placed eccentrically against the side of the cell. It consists of a delicately reticular matrix, which stains

pale-pink, and it is devoid of chromatin. It causes a slight projection of the periplast into the cytopharynx.

The *blepharoplast* is a small oval body constantly situated in the periplast at the posterior end of the cytopharynx, and when any traces of a flagellar apparatus are present in the cytopharynx, they are seen to run up towards it. In structure, the blepharoplast consists of a homogeneous-looking matrix, which stains rosy-red, and is devoid of chromatin. It can be distinguished from the chromidia, by which it is often surrounded, only by its staining reaction, the chromidia appearing deep carmine.

The *chromidia* consist of a group of deeply-staining carmine-coloured granules, each of which is often as large as the blepharoplast. The group has a characteristic position at the extreme posterior end of the cell, but very often an isolated granule occurs close to the blepharoplast, the significance of which is not obvious. The chromidia are often obscured from view when the periplast is densely stained. (Pl. VIII, figs. 23-25.)

(2) The *oval forms* are connected with the cysts by intermediate forms which are about the same length as the cysts, but broader. The typical oval forms are longer and possess a pointed anterior extremity, corresponding in position to the cytostome of the cyst. In stained specimens the protoplasm is seen now to be undifferentiated into the periplast and cytopharynx. The chromidia are scattered throughout the cell, and the nucleus and blepharoplast are partly chromatinised. It is consequently very difficult to distinguish the blepharoplast from one of the chromidia, but it lies to the side, or in front, of the centrally placed nucleus. (Pl. VIII, figs. 1-2.)

(3) The *oval forms with a flagellum* differ from the preceding forms only in possessing a free flagellum, the length of which equals that of the body of the cell, and in the nucleus and blepharoplast being more chromatinised.

The flagellar apparatus consists of (i) a rigid rod-shaped and heavily chromatinised body, the rhizoplast, lying between the blepharoplast and the anterior end of the cell, but having no chromatin connection with the blepharoplast; and (ii) the young flagellum, which consists of a delicate prolongation of the cell-protoplasm, staining pale-pink, investing a filament of chromatin, staining deep carmine, which runs into the anterior end of the rhizoplast (Pl. IX, fig. 6). In those cells possessing a divided rhizoplast, two chromatin filaments may be present in the protoplasmic prolongation of the cell, each arising from one of the

halves of the rhizoplast (Pl. VIII, fig. 5). Usually the blepharoplast shows no signs of division, but forms may occur in which it has divided, although there are no signs of division elsewhere.

Other forms may be seen with two chromatin filaments in the flagellum, as described above, but with no signs of division in the rhizoplast. (Pl. VIII, figs. 3-6.)

II. THE PARASITE IN THE MIDGUT AND SMALL INTESTINE.

The parasites found in this region of the gut are quite distinct from those seen in the 'crop,' and intermediate forms are difficult to find.

Both in living and stained specimens, they are seen to be in a state of very active division, so that a portion of the gut sometimes contains literally myriads of them.

In the living state, the form of the parasite is seen to be very variable, but often it is like a greatly elongate cone with the flagellum at the base of the cone. The flagellum is long and wavy. The body of the cell measures 15 to 20 $\mu \times 3 \mu$; the flagellum is 25 to 40 μ long. Dividing cells are broader.

The following structural details are observable in stained specimens :

(a) The cell protoplasm is finely alveolar and stains bright-blue, there being no differentiation into periplast and endoplasm. It contains the nucleus, blepharoplast, rhizoplast, and chromidia ; and forms a fine membrane about the chromatin of the flagellum. In dividing cells the protoplasm has a coarser structure.

(b) The nucleus is spherical and situated in the centre of the cell. It consists of a reticulate achromatinic matrix, staining pale-pink, in which lies a network of chromatin, thickened at the nodes of the reticulum. The following stages in the structure of the dividing nucleus may be observed :

(i) The nucleus consists of the achromatinic matrix without the finer parts of the chromatin network, so that the latter appears coarser and the nodal points thicker. (Fig. 2 b.)

(ii) The nucleus has an oval form, and lies across the cell ; the chromatin granules are aggregated around the periphery of the nucleus, and also in a transverse line across its longest diameter. (Fig. 2 d.)

(iii) The nucleus shows chromatin granules which have become flattened out, forming a peripheral ring of chromatin, and a transverse band lying nearly across the nucleus, but not touching the peripheral ring. (Fig. 2 f.)

(iv) In this stage the transverse band of chromatin is divided into two equal parts. (Fig. 2 *g*.)

(v) The nucleus is seen to have divided into two spherical daughter nuclei, each with a peripheral ring of chromatin and a short transverse band of chromatin. (Fig. 2 *h*.)

(vi) The further stages are represented by nuclei in which the chromatin network is reformed by fragmentation of the chromatin bands and the dissemination of the fragments to form a fine network in the nucleus. (Fig. 2 *i, k, l*.)

Fig. 2. Showing the structure of the dividing nucleus of *Herpetomonas luciliae*.

(c) The blepharoplast lies midway between the nucleus and the anterior end of the cell, and is of very variable form (Pl. IX, figs. 1-14). In the 'resting' cell it is apparently a homogeneous mass of chromatin; but in dividing cells, the chromatin splits into two halves, which separate and reveal a lighter-staining intervening matrix.

(d) The rhizoplast is in the same position as in the 'crop'-forms, but it is thicker and contains more chromatin, and therefore stains very densely. In some forms the rhizoplast is split longitudinally, and in such cases it may be connected with either one or two chromatin filaments in the flagellum.

(e) The flagellum, in 'resting' cells, consists of a chromatin filament surrounded by an extension of the cytoplasm, as in the 'crop'-forms. In some cases, whether the rhizoplast be divided or not, the flagellum contains a second filament of chromatin which has no connection with the first filament. The two filaments, which may be very different in length, are connected together by a pink staining cytoplasmic membrane. In some of the forms containing two chromatin filaments the membrane stretched between the filaments is split either proximally, or distally, or both; and in other cells the division is complete, the cell then possessing two independent flagella. In such

cells none of the organella may be divided, a unicellular biflagellate creature being the result, but these forms are very rare. In yet other cells we find a cell, incompletely divided, with *four* chromatin filaments in the flagellum, which must be interpreted as cells in which redivision is taking place prior to the separation of the daughter cells.

(*f*) The chromidia are scattered throughout the cell. Relatively to the other structures they are very much smaller than in the 'crop'-forms. The more actively dividing parasites seem to contain the most granules.

The structure of the parasites in this region is therefore very variable, in that they do not form a gradual series of forms. This indicates that the sequence of division of the various organella is not uniform; for instance in some cells only the flagellum has divided, in others only the rhizoplast. (Pl. IX, figs. 1-14.)

III. THE PARASITE IN THE HINDGUT AND RECTUM.

In this region the forms of the parasite fall into three main groups, which are connected by intermediate forms: (i) large oval flagellates, (ii) round flagellates, (iii) small oval forms, or 'cysts.' None of these show any signs of multiplication, either in living or stained preparations.

(i) The *large oval* forms are found in the hindgut immediately below the opening of the Malpighian tubes. They are less numerous than the other types, probably because this stage is very transitory. They measure 8 to $10\mu \times 2$ to 3μ . The cytoplasm, as in the midgut forms, is not differentiated into periplast and cytopharynx, and stains pale-blue. The nucleus, blepharoplast, and flagellar apparatus contain less chromatin than the corresponding structures in the midgut forms, and the matrix of the nucleus, which stains pale-pink, is more evident. The blepharoplast still occupies the same relative position to the nucleus as in the midgut forms, that is, well in the front end of the cell. The flagellum is much shorter than in the midgut forms, and stains less deeply owing to its being partly dechromatinised. The chromidia have a tendency to become grouped in the posterior end of the cell. (Pl. IX, fig. 7.)

(ii) The *round* forms are chiefly found in the posterior part of the hindgut, and in the rectum. In this category we include forms which are not round but oval, for the only difference between them is their shape. They are of the same length, about 5μ , but whereas the round cells are about 5μ in breadth, the oval cells have contracted to about

2 μ . The latter are very abundant indeed in the rectum. It appears that the large oval forms become rounded up, and that they then contract from side to side.

The cytoplasm is differentiated into ectoplasm and endoplasm: in forms probably representing a later stage of development, the differentiation is extreme, a very dense-staining periplast being formed. The appearance thus presented is the same as that of the forms occurring in the 'crop,' which have been described above as cysts. The nucleus, blepharoplast, and flagellar apparatus have even less chromatin than in the large oval forms. The nucleus, blepharoplast and intracellular part of the flagellar apparatus may even lose all their chromatin; the nucleus then staining pale-pink, the blepharoplast rosy-red, and the intracellular flagellum being invisible, probably because the dense periplast obscures such a delicate structure.

The blepharoplast is now situated to one side of, or even just behind, the nucleus which is slightly eccentric. The rhizoplast as long as it contains chromatin is seen to follow the blepharoplast as this passes backward, although there is no visible connection between them.

The extracellular part of the flagellum is faintly stained but is seen often when the intracellular part is invisible. The chromidia are very large in those cells in which the organella have lost most chromatin: they are often with difficulty distinguished when the periplast is densely stained. A single chromidium away from the others is sometimes seen close to the blepharoplast. The others are grouped at the posterior end of the cell in the periplast. (Pl. VIII, figs. 10-22.)

(iii) The *cysts* are found in the lower end of the rectum in very great numbers, but are always discrete. They have been described above under the 'crop'-forms. (Pl. VIII, figs. 23-25.)

Taxonomy.

The parasite above described must be classed with those described by Prowazek, Patton, Mackinnon, and others, and named *Herpetomonas*.

It has been recently suggested by Miss Mackinnon (1910) that there has been needless subdivision of this genus into species without consideration of the adaptability of perhaps one species to various hosts. I had this in mind at the time (1909) of conducting the observations on this parasite, and accordingly dissected some *Musca domestica*, and *Calliphora coerulea*, from the same source, two butcher's shops, as the *Lucilia*, but was unable to find herpetomonads in them.

This species is therefore probably distinct from *H. muscae domesticae* at least, and I therefore propose for it the name *Herpetomonas luciliae*.

It has been said that such parasites as these are 'biflagellate,' because certain forms appear to have two flagella. The appearance, however, is deceptive, and these parasites in reality only have one flagellum in which the two chromatin filaments stain very prominently. The term 'biflagellate' is only applicable to those forms, rare in *H. luciliae*, in which there are two *free* flagella.

Prowazek, Chatton, and others would use this inconstant form of the parasite—the so-called biflagellate form—with which to define the genus *Herpetomonas*, and would separate it from *Leptomonas*, which has a slightly different type of flagellar apparatus.

Miss Mackinnon, however, has shown that a '*Leptomonas*' in the larval host may become a '*Herpetomonas*' in the imaginal host, and that the difference in the flagellar apparatus is only a matter of the time at which the flagellum divides. It seems therefore wrong to class these parasites into two genera.

They would thus all be named *Herpetomonas*, sensu Pattoni, who defines them as having no undulating membrane and the blepharoplast near the anterior end of the cell.

The method of infection of the fly.

The experiments performed in order to discover how the parasite is transmitted from fly to fly were conducted in the following manner.

An attempt was made to raise 'clean' flies in the laboratory, but owing to the cold summer (1909), and also because the experiment was begun rather late in the year, the pupae obtained did not hatch out. Recourse was therefore had to flies caught wild, and in estimating the results obtained with these it was borne in mind that 76% of them had been previously found infected.

The flies were kept in a large fly net, but they were with difficulty kept alive. They were fed on fresh meat contaminated with the rectal parasites of other flies, and afterwards were killed at definite intervals and examined for parasites.

The result was meagre, but three flies which had been fed as described above showed actively growing cysts in the 'crop' two days later; whereas of flies examined straight from nature, I never found one containing these forms in the 'crop.'

The fact that rectal cysts are found in the 'crop' of the fly indicates that the parasite is transmitted from fly to fly by contamination of its pabulum.

Hereditary transmission does not take place. Several larvae from eggs laid by infected flies were dissected, but parasites were never found. Neither do these larvae become infected if they feed on contaminated meat, this being in contrast to Miss Mackinnon's observations (1910) on the life history of *Herpetomonas* from dung-flies.

In the case of *H. luciliae*, therefore, I conclude that infection is usually produced in the imago by its feeding on pabulum that has been contaminated with cysts, derived from other infected flies.

Biology.

Movement. The parasites that occur in the 'crop' and hindgut, each possessing only a short flagellum, have little power of progression. The flagellum waves feebly to-and-fro, but without causing much movement. Those with a long flagellum, as in the midgut, can only with difficulty be kept within the field of vision, the flagellum being generally directed forward under these circumstances. The 'cysts' are quite inert, floating about in the fluid in which they are examined.

Growth and division. At various stages in the life-history of the parasite, the relative rapidity of its growth and its change of structure can be estimated by noting the comparative numbers of the parasite at that stage. In the 'crop' the cysts and the oval forms without a flagellum are the rarest, so it must be assumed that these forms are the most rapid in their growth. In the midgut the parasite does not remain long without division; while in the hindgut and rectum the cysts and the small oval forms with a flagellum are found in enormous numbers, and consequently represent the slowest stage of growth.

It seems therefore that rapidity of growth and change of structure is most rapid in the upper part of each subdivision of the gut, while at the lower end it is slowest and there is not much change of structure in the parasite. Division only occurs while the parasite is in the midgut.

Individual variability. The study of stained specimens of the parasite shows that it is very variable in structure, particularly in the forms in the midgut, which we may regard as at the most plastic stage.

Physiology.

Cytoplasm. The cytoplasm of the midgut forms shows no differentiation in structure, and therefore its function is probably generalised; but in the forms occurring in the 'crop' and hindgut, it is more or less differentiated into ecto- and endoplasm. The ectoplasm forms the periplast, the function of which is doubtless to resist desiccation of the cell. It is accordingly most marked in the cysts which, when voided in the excreta, have to withstand the effects of atmospheric drying, perhaps for long periods. From its structure it seems to be formed out of the more solid spongy elements of the cytoplasm. When water and food are plentiful, as in the 'crop,' the periplast soon disappears, which proves that this structure is ill-adapted for the absorption of water and nutritive substances. On the contrary, in the hindgut, where the excreta of the fly are more and more concentrated, the periplast is rapidly formed.

The reduced metabolism of the cell is then carried on by the endoplasm, acting through the small area which comes to the surface of the cell at the 'cytostome.' The endoplasm is thus well-named the cytopharynx. It seems, from its structure, that this endoplasm is derived from the more fluid 'hyaloplastic' part of the cytoplasm.

Later on, as shown by the movements of the chromidia, the ectoplasm and endoplasm diffuse into one another, and then the whole surface of the cell is available for absorption. The cytostome would be a weak spot in the armour of the cell under conditions of excessive dryness, were it not for the following changes. As more dryness is experienced the cell loses water, thereby causing the cytopharynx to retract, and consequently deepening the cytostomic pit. The effect of this is to invert the periplastic lips of the cytostome over the vulnerable area, with the exception of the tiny micropyle described above. The cell is thus enabled to withstand prolonged desiccation.

Rhizoplast. The function of the rhizoplast seems to be the secretion of the chromatin filament in the flagellum. This is probably effected by the protoplasmic achromatic basis of the rhizoplast. When the rhizoplast loses its chromatin this protoplasmic basis is possibly retracted into the blepharoplast, in view of the close connection which exists between the two structures.

Flagellum. The flagellum, which, it must be reiterated, consists of two distinct parts (i) the protoplasmic basis, and (ii) the chromatin

filament, is lost in the following manner. The chromatin filament first gradually disappears, but before this change is complete the rhizoplast has passed backward in the cytopharynx, drawing the flagellum after it. By this means the free part of the flagellum is shortened. It constitutes an *active* shortening of the flagellum. Prowazek has previously described this process in the flagellum of *H. muscae domesticae*. The chromatin filament in the flagellum is then absorbed centripetally, so that in some cells only the chromatin of the intracellular part remains, and, as this process takes place, the protoplasmic basis of the flagellum is retracted *pari passu*. The protoplasmic basis does not remain when the chromatin has been absorbed. This constitutes a *passive* shortening of the flagellum.

These observations on the structure and changes in the flagellum seem to show that the chromatin is devoted to sustaining the flagellum in a filamentous form, which the protoplasm surrounding it has no power *per se* of doing.

The function of movement is, we think, subserved by the protoplasmic basis of the flagellum. It is obvious that the protoplasmic basis is homologous to the undulating membrane of *Crithidia* and *Trypanosoma*, although it has been seen that when the flagellum is drawn up into the cell by the posterior movement of the blepharoplast it does not form an undulating membrane as such but remains within the endoplasm. The undulating membranes of the Trypanosomatidae contain myonemes, so that it is most probable that the function of movement is subserved by the protoplasmic basis of the flagellum. The staining reaction of the protoplasmic basis, or of the *kinetoplasm*, as I propose it be called, is the same as the endoplasm of the cell, and is obviously derived from it, so that it is interesting to see that the more hyaloplastic constituents of the cell are employed for the function of movement.

Chromidia. It has been seen how the size and number of the chromidia vary inversely with the amount of chromatin present in the nucleus and other organella. There can therefore be little doubt that they represent the supply of chromatin for the use of the cell. Swellengrebel has shown that they do not consist of chromatin itself, but of a slightly altered substance named 'volutin.' They probably are concerned in the following manner. When the parasites are undergoing involution the chromatin in the organella is dissolved by the protoplasm and circulates in the cell, and is then taken up by certain plastids, and resecreted as 'volutin,' in the form of the chromidia.

The reverse process takes place when the cell is on the 'up-grade,' the volutin being dissolved and resecreted by the protoplasmic basis of the nucleus, blepharoplast, and flagellar apparatus, respectively.

The process is familiar in certain plant-cells, such as *Spirogyra*, in which the carbohydrate metabolism is similar.

The presence of volutin has been shown by Nuttall (1910), and by Hindle (1910), to be associated also with degeneration of the cell, but in this *Herpetomonas* the most vigorous parasites are seen to contain the most volutin granules, or chromidia.

SUMMARY AND CONCLUSIONS.

1. The parasite described inhabits the alimentary tract of two species of *Lucilia*. In addition it has a resting stage—the 'cyst'—which is passed in the voided excrement of the insect.

2. When the cyst is ingested by a fly it grows in length and becomes flagellated in the 'crop'; in the midgut it greatly elongates and multiplies rapidly by division; in the hindgut it first shortens, then becomes spherical, and finally oval and cyst-like, meanwhile losing its flagellum.

3. The cysts measure 3μ , the fully flagellate forms 20μ , in length.

4. The cysts possess a *cytostome* and *cytopharynx*, the functions of which are probably nutritive.

5. The flagellum consists of two parts (i) a cytoplasmic part, which probably subserves the power of movement and which I therefore propose to call the *kinetoplasm*, and (ii) a thread of chromatin secreted by the rhizoplast.

6. The function of the chromatin in the flagellum is probably to sustain a filamentous form.

7. The nucleus divides by a definite process something akin to mitosis.

8. The chromidia serve as reserve material for the supply of chromatin in the cell. They are secreted probably by cell-plastids.

9. I propose the name of this parasite be *Herpetomonas luciliae*, and that the genus *Herpetomonas* should be defined so as to include the forms described as *Leptomonas*.

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EXPLANATION OF PLATES VIII-IX.

PLATE VIII.

Figs. 1-6. Forms found in the 'crop.'

Fig. 1. Oval form without flagellum. The chromidia are scattered throughout the cell. The blepharoplast is at the extreme posterior end. It can hardly be seen in the figure.

Fig. 2. Oval form without flagellum. Nucleus slightly chromatinised.

Figs. 3-6. Oval forms with flagellum.

Fig. 3. Shows the blepharoplast dividing, and a single flagellum.

Fig. 4. Shows the cell with the rhizoplast undivided and the flagellum with two chromatin filaments, between which is stretched the kinetoplasmic membrane.

Fig. 5. Shows the rhizoplast divided, as well as the 'double' flagellum.

Fig. 6. A densely staining form in which the nucleus cannot be seen.

Figs. 7-22. Forms found in the hindgut and rectum. In all the cytopharynx is well marked, but the nuclei are badly marked in the figures.

Fig. 7. Large oval form with small chromidia.

Fig. 8. Shows the chromidia in a group at the posterior end of the cell.

Fig. 9. Shows the nucleus, blepharoplast and flagellum all more or less dechromatinised.

Figs. 10-14. Round forms.

Fig. 10. Shows commencement of the formation of the cytopharynx, the blepharoplast lateral to the nucleus.

Fig. 11. Shows well the blepharoplast at the posterior end of the cytopharynx. The nucleus shows one of the stages of mitosis.

Fig. 12. Partial retraction of the cytopharynx has occurred.

Fig. 13. The chromidia are grouped at the posterior end of the cell. The rhizoplast is still chromatinised.

Fig. 14. Shows the differentiation of the protoplasm into perioplast and cytopharynx.

Figs. 15-22. Oval forms with a flagellum. They all show the further differentiation of the protoplasm, and the consequent formation of the truncate end of the cell. The flagellum is short in all cases and is nearly dechromatinised; it cannot be seen when

it becomes intracellular. The blepharoplast is always at the bottom of the cytopharynx and stains less deeply than the chromidia.

Figs. 23-25. Cysts found in the rectum or 'crop.' The cytostome and micropyle are well seen; also the characteristic position of the nucleus, blepharoplast, and chromidia.

PLATE IX.

Figs. 1-14. Various forms of the flagellate found in the midgut.

Fig. 1. Small cell in a resting condition.

Fig. 2. Cell showing two free flagella, the rhizoplast slightly divided.

Fig. 3. Cell in which the blepharoplast is dividing, showing the achromatic matrix.

Fig. 4. Cell showing formation of a second chromatin filament, with a protoplasmic membrane (kinetoplasm) between the two filaments. Nucleus showing mitotic figure.

Fig. 5. Cell showing division of the blepharoplast and rhizoplast; also the formation of a second chromatin filament.

Fig. 6. Shows division in nucleus, blepharoplast and rhizoplast. Flagellum with one chromatin filament in a thick sheath of kinetoplasm.

Fig. 7. Cell in which the nucleus shows mitosis; the rhizoplast is divided and the flagellum contains two chromatin filaments.

Fig. 8. The blepharoplast is dividing and the flagellum contains two chromatin filaments.

Fig. 9. Cell showing complete division except between the two chromatin filaments.

Fig. 10. Shows clearly the kinetoplasmic membrane.

Fig. 11. Flagellum twisted on itself. Rhizoplast and blepharoplast dividing.

Fig. 12. Cell showing complete division of all the organelle, but not of the cell protoplasm.

Fig. 13. Complete division of all parts except the flagellum which is only split partially, both proximally and distally, new chromatin filaments being laid down by the side of the old.

Fig. 14. Cell showing a further stage of division. The two cells have completely divided and the formation of the new chromatin filaments is continued. The respective rhizoplasts of the two daughter cells have divided.

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