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by Grace Medes

June 5, 1913

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

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Presented to the Faculty
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ACKNOWLEDGMENT.

In presenting this paper, I would like to acknowledge my gratitude to Miss Nadine Nowlin, under whose direction the work was done.

G. M.

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by

GRACE MEDES,

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INTRODUCTION.

The flies which are the subject of this investigation, have been breeding for several years in the formalin barrels in a store-room connected with this labratory. A number of these flies had escaped into an adjoining labratory, were captured and examined and were found to be heavily infected in the Malpighian tubules with an attached protozoan parasite. Others, taken from the store-room, were likewise examined, but only a small percent proved to be infected and these but slightly with spores and a flagellate form, (figs. 7, 8, and 9), in the oesophagus and salivary glands. A culture from each of these places was started in an endeavor to determine whether these were two distinct types or represented but different stages in the life-history pf the same parasite. Each of these was started with five flies. Flies from these cultures have been opened from time to time during a period of thress months. That from the store-room has shown no infection, while the one from the labratory has gradually developed an increasingly heavy one, both as to percent of flies affected and as to number of parasites. At present about one in ten is entirely

free from the parasites. Just how nearly either of these cultures approximates conditions to be found in natural life it is impossible to say, since all these flies are living under more or less abnormal conditions.

TECHNIQUE.

In studying these flies, a number of methods of observation have been employed. Especial emphasis has always been placed upon the study of the live specimen.

In the larval stage, on account of their exceedingly small size, 1 to 4 mm, and the comparative transparency of their skin, the organs of the living fly could be observed directly under the microscope. Figure 1 is a drawing made in this way. Those in the pupa stage could be studied in the same manner upon removal of the pupa case. Serial sections of flies in both these stages and in the adult condition were made and compared with drawings from the live specimens.

For the study of parasites, smears were found the most satisfactory method of observation. In every case the Herpetomonas were studied as thoroughly as possible before making permanent mounts. This was the more possible on account of the transparent nature of the membranes. Entire alimentary tracts were removed and the condition and location of the parasites observed before teasing the walls and allowing their escape.

Permanent mounts were made in a number of ways. One of the most satisfactory methods of fixation, was to expose the smear to fumes of 4 % Osmic acid until the

slide was almost dry, when it was plunged into 90 % alcohol and hardened for an hour. Smears were also prepared by killing with corrosive sublimate and fixing with Bouin's fluid.

Perhaps the most satisfactory stain for nuclear structure was Haidenhain's haemotoxylin destained with iron alum. Delafield's haemoloxylin and Haem-alum counter-stained with eosin both proved excellent for cytoplasmic structures. Borax carmine was also employed for intra vitam staining with fairly satisfactory results and aceto-carmine for temporary mounts.

NOMENCLATURE.

The Genera Herpetomonas and Liptomonas were established in 1881 by Saville Kent (5) for uni-flagellate parasites found respectively in Musca domestica and a Nematode, Trilobus. His points of distinction are; the greater size of the Herpetomonas, its nonoccurrence in rosettes and its comparatively highly flexible and polymorphic character in contrast with the more persistent shape of the Liptomonas. All these characteristics hold true of Herpetomonas Drosophilae sp . However Kent describes the Herpetomonas as being typically "vermicular", whereas at no time in the life history of Herpetomonas Drosophilae does it approach this shape. The anterior end is always rounded or flattened, and at only one stage, the attached, does the posterior part of the body become elongated and decidedly pointed.

Woodcock, 1906 (11) described a typical Herpetomonas as having the kinetonucleus situated near the anterior, the flagellum not attached to the side of the body at all but becoming free, and correlated with this, no undulating membrane.

Roubaud, 1909 (10) uses the term Herpetomonas for

the biflagellate form described by Prowazek, and Leptomonas for uniflagellate forms with anterior nucleus and trypanosome stage in its life cycle.

Hewitt, (1909) (4) prefers the use of the name Herpetomonas for the flagellate parasite of the domestic fly, at the same time questioning the double nature of the flagellum as described by Prowazek and repeated by Lingard and Jennings 1906 (6), He suggests that the double appearance of the flagellum represents the beginning of longitudinal division.

Chatton and Allilaire, 1908 (1), gave the name Herpetomonas to the parasite of the domestic fly, and called "Léptomonas tous les flagelles acicules du type L. Butchli Kent, L. jaculum Leger, etc.

Patton, also 1909 (8), takes the same interpretation of the double flagellum, and classes all uniflagellate parasites of insects, without undulating membrane and with kinetonucleus anterior to the trophonucleus as Herpetomonas. He calls those Crithidia which possess undulating membrane and the kinetonucleus posterior to the trophonucleus.

Dunkerly, 1911 (3) decides that "Léptomonas would appear to be the correct name for uniflagellate parasites found in the gut of non-sanguivorous insects, including house flies, Pyconogonum, Bombyx, and some plants, while Herpetomonas may be retained as a provisional name for a large form with a peculiar flagellar apparatus and a

complicated life history described by Prowazek.

The relative position of the trophonucleus and kinetonucleus can scarcely be accepted as a positive method of identification since in many of these forms their relative positions may become reversed at certain stages in the life history of the flagellate. Dunkerly 1909, Léptomonas Muscae domesticae, Chatton and Leger 1911, Léptomonas Drosophilae confusae, Annie Porter 1910 Crithidia Megophaga, etc. The absence or otherwise of the undulating membrane also seems to vary. Leger, Tabanus glaucopis and Haematopota italica describes a Herpetomonas with undulating membrane.

In view of this confusion of names, I have returned to the term Herpetomonas for the form I have described, since it corresponds most closely to the main characteristics which seem to be generally accepted: Kinetonucleus anterior to the trophpnucleus, a peculiar flagellar apparatus from which the flagellum arises, and no undulating membrane.

DISTRIBUTION.

The Herpetomonas of Drosophila seem to live entirely in the alimentary tract. Regardless of how great the infection, they never appear to be able to break through the rather tough walls and to reach the surrounding tissues. Consequently I have never observed any parasites in the ovaries or testes and genital infection never seems to occur. As a result, the parasites must be taken in with the food. This often happens in the very earliest stages, as I have found the spores in larva of 1½ mm. In a few instances these were still remaining in the bundles, fig. 4. Usually, however, the sporozoites separate as soon as they enter the freshly infected insect; for often the entire alimentary tract, including the salivary glands, hasbeen lined with the separate sporozoites, while not one of the bundles was to be found.

The spores, after entering the mouth, pass from the pharynx either into the oesophagus or through the salivary ducts into the salivary glands. It is in these places that transformation from sporozoites into flagellate stage generally takes place. In larvae very heavily infected, however, the spores may be found forming an almost complete lining, fig. 8 , of the alimentary tract as far back as the hind gut. The period during which these spores

remain in this condition must be of comparatively great duration, as I have a number of times observed the walls of the alimentary canal fairly lined with sporozoites, while not one has attained the flagellate condition.

Wherever the spores may be located, the young flagellates immediately begin to push their way back along the alimentary canal until they reach the openings of the Malpighian tubules, where they become attached and enter upon the third stage of their life-cycle. They continue to stream into these tubules until the entire lumen seems to be so nearly filled with their swaying, lashing bodies now attached to the walls of the tubules, that the freshly arriving flagellates can scarcely push their way among them. When the passage becomes so nearly filled that there seems to be no more room for attachment, the flagellates begin to find permanent location along the sides of the gut, passing both backward and forward from the openings of the Malpighian tubules. In one instance I found the entire alimentary canal from oesaphagus to the rectum lined with these forms. So great a degree of infection however, I believe to be rare, as I have never, in flies captured outside of my infected culture, found the attached forms other than in the Malpighian tubules.

Although the transition from the flagellate to the following stage is very rapid, the period of attachment

seems to be the longest in the life-cycle of the parasite. Spore formation takes place wherever the attached parasites are located, and since the earliest infection seems to be in the Malpighian tubes, the spores first appear there, and later in regions of the alimentary canal farther removed from the openings of these tubules.

This early appearing of the spores in locations first infected argues a definite period for this phase in the life-cycle, rather than as at first was thought possible, that the life-cycle of the parasite corresponds to some definite phase in the life-history of the host. I have observed these mature spores with scarcely an active parasite present, in larvae about to enter the pupa stage. They then pass through the intestine and rectum, and find exit with the faeces.

I could not at any time determine that the parasites exerted any especially harmful effect upon the host, and a careful study of the epithelial lining of the Malpighian tubules after the Herpetomonas had formed spores and become detachedfailed to reveal any pathogenic cnndition. In this event it could be quite possible for a fresh invasion of the parasites again to enter the host.

Bananas soaked in yeast water is the food upon which the flies lives and in which they bred. Particles of this taken from the infected culture were examined, and in it were found spores and sporozoites of Herpetomonas Drosophilae.

MORPHOLOGY.

The life-cycle of Herpetomonas Drosophilae may be conveniently divided into four phases, preflagellate, flagellate, post-flagellate, and spore. Each of these stages seems to be of long duration compared with the rather quick transition. Indeed, so sudden is this transformation and so great seems to be the difference in structure between the free swimming, i. e. the flagellate, and the attached stage, that it long remained a serious question as to whether there existed one form or two. However through the study of the few intermediate forms obtained, and a consideration of the locations in which the stages occur, I now feel convinced that all the forms to be described belong to the life-cycle of one individual.

PREFLAGELLATE STAGE. The sporozoites of Herpetomonas Drosophilae sp. , upon first emerging from the spore bundles are somewhat retangular with rounded ends and a generally uniform size of about 5 x 1½ μ. As seen in fresh smears, they present a clear glassy appearance of a slightly greenish color. Their covering seems to be tough and rigid, and no structure, nucleus, vacuoles, nor granules of any kind can be discerned. This covering is also highly resistent to stains, since Delafield's Haematoxylin and aceto-carmine do not penetrate. In specimens stained with Heidenham's Haematoxylin, however,

a large oval, granular nucleus, slightly to one end of the center may be observed, and other darkly staining bodies scattered through the cytoplasm. Some of these always seem to lie at one end, and one of them may possibly represent the kinetonucleus.

During this period a considerable growth occurs, and by the times the first signs of life are evident, they may have obtained the size of 7μ x 2μ. At first the sporozoites are straight and rigid, fig. 6, , Often one end grows broader and flatter, while the other, the posterior, becomes somewhat more pointed, fig. 8.

FLAGELLATE STAGE. Gradually at one end, the larger when of different sizes, a small knob like prominence becomes visible, fig. 8 , and from it the flagellum begins to emerge. This flagellum increases rapidly in size and soon obtains a length of 12μ, or about one and one half times the length of the body.

The flagellum is very wide, and seems to spread nearly across the anterior end of the parasite. It often looks heavier and thicker on the sides, and thus presents somewhat the appearance of the double flagellum described by Prowazek (8).

Sometimes this broad base, instead of narrowing gradually into the flagellum, presents the appearance of a knoblike ball of cytoplasm from which the flagellum

protrudes as a separate structure. Sometimes again, it presents somewhat the appearance of a loose membrane, or even a circle of very fine cilia. Its exact structure I have not been able to ascertain, but expect to continue some experiments with a view to obtaining a stain which will make this possible.

As the parasite now begins its career as an active flagellate in the lumen of the canal, a slight change in its shape takes place. It increases in size, reaching a length of about 12μ, Its anterior end becomes still broader and more flattened, and its posterior more pointed. This is the characteristic form in which it moves about in the digestive tract of the host and finally attaches itself to assume a fixed position.

During this period, the trophonucleus maintains a position slightly posterior to the center, and two small darkly staining granules lying at the base of the flagellum represent an already divided kinetonucleus. This double kinetonucleus might also lead one to believe in the double nature of the flagellum, since it appears as two structures at a very early stage.

In this as well as in the following period of its life history, great difficulty has been experienced in studying the nuclei, owing to the large number of granules present in the cytoplasm. These are fairly regular as to size, and are about 1μ in diameter. The cytoplasm in the anterior part of the body appears denser than that

back of the nucleus, although these large granules may be scattered through both regions. There are numerous vacuoles present. They also are of fairly regular size, and often are arranged in definite longitudinal rows, fig. 10, a.

POST FLAGELLATE STAGE. When first attached the Herpetomonas hang by the ends of their long flagella far into the lumen of the canal in which they have settled for their fixed position. The flagella, however, seem to be rapidly resorbed, for soon the parasites come to lie with their anterior ends of their bodies attached to the wall of the gut or Malpighian tubule. When these tubules are freshly removed from a fly and viewed through the wall, the entire inner surface may be seen covered with small circles packed closely together, representing the attached end of the parasites. But although the Herpetomonas loose their flagella, they retain the peculiar membrane-like structure described above. In fresh smears made by teasing out one of the Malpighian tubules, the parasites may be seen floating about in this condition, fig. 11.

At this stage, Herpetomonas Drosophilae may present considerable variation in form. At first they generally become long and slender, about 25 , and may even attain a length of 30 , fig. 11. In this

form they spend the greater part of their attached existance. The kinetonucleus continues to lie against the anterior wall of their bodies, and the trophonucleus also remains about the distance from the anterior end, and accordingly is now considerably in front of the centre.

As the parasite approaches the spore forming stage a marked change in form occurs. The anterior end becomes expanded into a large rounded gregarine-like form, and the posterior becomes still more slender, resembling a tail like appendage, fig. 13. Gradually this tail shortens and becomes resorbed, until the parasite now presents a large rounded shape with slightly pointed posterior end, fig. 14.

There is naturally, considerable variation in the exact period in which these changes occur. Sometimes the parasites become attached and lose their flagella while they are in the early form presented when first they emerge from the sporozoite condition. At other times they seem to remain longer in the free swimming condition. In this case they may be seen moving about, flagellum present and oval bodies bearing the tail-like appendage, fig. 11, a. . These latter types however seem to be rather aberrant, and that described above is the typical course of development.

In many of the specimens teased out into fresh smears, the parasites float out in rosette like groups. I have no evidence for believing that these correspond

to true rosettes, since I have never observed them in this condition in the lumen of the unbroken tubules. I am rather inclined to think that they represent groups of parasites torn loose from the walls of the tube and still clinging to some of the tissue.

SPORE FORMATION. I have not been able to follow as completely as I would like all the stages in spore formation. This spore-formation period must pass exceedingly rapidly for although in nearly all the infected flies that I have examined, the Malpighian tubules are either lined with the attached parasite, or covered over the surface with spores, I have not been fortunate enough to open many in which transition stages were present. But it seems to me that the following reasons are weighty evidence in favor of the belief that the spores are formed directly from these attached parasites. First, that the newly formed spores are always found in the Malpighian tubules where the parasites are attached. And second, that each spore corresponds in size to that of the Gregarine-like Herpetomonas in the latest stages.

Moreover a few intermediate types have been observed. Figure 16 shows a group of four individuals all in the act of dividing. Each of these were so much smaller than any of the surrounding parasites that it seems possible that they may originally have arisen by division from one individual. In the spore forming divisions the individuals

do not separate as in earlier divisions, but lie with their adjoining surfaces closely approximated. Figure 15 shows the first division in this process. Sometimes three all apparently of equal size, fig. , are found lying in this position, but I am unable to say as to the type of division by which they are produced. This may explain the frequency with which the spores are found composed of 6 and 12 sporoziotes. I have observed eight of these individuals, fig.18 , still smaller, approximating in size the sporozoites. These eight individuals lay close together, arranged like the sporozoites in a spore, fig. 19 . They were shaped somewhat like sporozoites, but their contours not quite so regular, and the surrounding membrane thinner and apparently not so hard.

These sporozoites lie together in little bundles without any surrounding membrane to bind them together. Yet they seem to cling together with considerable tenacity, since they are often found unseparated in newly infected flies. There is no definite number of sporozoites in these bundles, nor do they have any definite arrangement. Eight seems to be the predominating number, although I have at times counted twelve and sixteen.

Often in teased out fly intestine, I have found groups of these spores, as shown in figure 20 apparently surrounded by a membrane. The significence of this I am

unable to say, since it seems to be rather unusual. It may possibly be associated with some pathogenic condition in the host. Again the spores contained in it may represent the parasites arising by division of one individual in the earlier stage of attachment, and still clinging together.

DIVISION.

In both of these stages, the flagellate and the attached, division takes place. In the latter it seems to be by far the more common. There are two methods of division, longitudinal and transverse. Transverse division is the less usual, especially during the flagellate condition, where I have found it occuring only a few times. I have never succeeded in staining one that shows the nucleus in the process of dividing, as the few examples I have seen of this type show the completed cells almost ready to separate, fig. 27. No sign of flagellum could be distinguished in the posterior, and a new one probably develops as in the young active forms. When this mode of division occurs in the attached form, I have observed the nucleus dividing as in longitudinal division.

The longitudinal method of division occurs always beginning at the anterior end, and proceeding backward. At first a slight constriction appears which gradually increases into a split extending the length of the parasite. This type of division occurs at any time from the the early flagellate stage, until spore formation. When it takes place in the flagellate stage, the anterior part of the body broadens, and becomes constricted downward, while at the same time a split appears in the base of the flagellum, and posteriorally through the body. The process is

practically the same through the attached stage whatever the shape of the parasite. Figures 21 to 26 show different types of parasites in various stages of division.

Division always seems to be proceeded by widening of the body. The nucleus divides early, and when the first indication of a constriction appears at the anterior end of the parasite, two nuclei already are present in the cytoplasm.

MOVEMENTS.

As I have mentioned above, the period during which these parasites remain in the spore stage, seems to be of comparatively great duration, as indicated by the vast quantities in which they may be found lying in apparently a lifeless condition in the digestive tract of the host. Among them however, a few sporozoites may begin to reveal a slight quivering motion. This may commence either while the sporozoites remain in their characteristic bundles, or later when they have separated. This quivering may increase so greatly, that the sporozoite may become slowly disentangled from the mass of others surrounding it, and drift from place to place. Meanwhile, the hard covering of the sporozoite remains apparently stiff and there is no flagellum or other motile apparatus to direct the movements.

Soon, however, the flagellum begins to grow out, and as it keeps up a continuous lashing, the motion becomes more and more jerky. Gradually the body becomes less rigid until it bends and sways with the constant beating of the flagellum. THe movements of the Herpetomonas in this stage, may be rapid, but is never smooth or gliding.

As the parasite dashes about in the alimentary tract

of the host, it may become temporarily attached, sometimes by the tip of the flagellum, sometimes by the posterior end, while the protruding flagellum keeps up a continuous lashing. When finally the flagellates settle down to a permanently attached condition, the tubule becomes completely carpeted with their swaying, beating bodies. Gradually, however, this motion grows less violent, and their bodies inflexible until they seem to hang attached inertly to the walls of the canal. From this time on, they never exhibit any powers of motion, other than the characteristic change of shape they undergo in the course of development, and, if disrupted from their position are unable to regain attachment.

SUMMARY.

1. The parasitic flagellate, which is the subject of this paper is found in Drosophila sp , a species of fruit fly, found breeding in the formalin barrels of this labratory.

2. I have named this flagellate Herpetomonas on account of its polymorphism, its peculiar flagellar apparatus, and entire absence of undulating membrane.

3. Its life-history falls conveniently into four periods, the preflagellate, the flagellate, the post-flagellate, and the spore stage.

4. At the beginning of the pre-flagellate stage, the spores are of a rectangular shape with rounded ends and a clear glassy appearance, and they lie at first motionless in the salivary glands or oesophagus of the newly infected host. As transformation from spore to active flagellate takes place, a slight quivering motion may be observed, which gradually becomes more violent and jerky as the flagellum appears. Growth occurs during this period.

5. During the active flagellate period, the parasites find their way along the fore-gut and mid-gut of the fly until they pass into the Malpighian tubes, where they attach themselves by their flagellar end and settle down to a permanently fixed condition. During this flagellate

stage their bodies become more elongated, with anterior end broader and flatter, and posterior extremity more pointed.

6. Following attachment their bodies become still more elongate, and remain in this characteristic shape until spore formation approaches. Then the anterior part of their bodies becomes larger and round, while the posterior tail-like portion becomes at first more slender, then shorter until it is entirely resorbed.

7. During these two phases division may occur, being more frequent in the latter stage. It is generally longitudinal, a slight depression at the anterior end gradually growing downwards into a split which divides the parasite throughout its entire length. Transverse division may also occur.

8. As spore formation approaches, the parasites begin to divide. They are now so short and Gregarine-like in form they look heart shape as the constriction first appears at the anterior end. Apparently each individual goes through sufficient divisions to form eight, twelve or sixteen individuals, each of which represents one sporozoite. These latter usually remain in bundles and so pass out with the faeces. On account of the absence of spore membranes however, they may become separated, and find exit in this condition.

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EXPLANATION OF PLATES.

All drawings were made from Camera-lucida outlines. Figure 1 is magnified 44 times. All others were made with Spencer ocular 8. Drawings 2 and 3 were made with 1/6 objective, and magnified 1250 times. All following were done with 1/12 oil-immersion lens, and magnified 2500 times.

EXPLANATION OF PLATE I.

Fig. 1. Larva of fly. c. coeum or intestinal gland. c. m. Common Malpighian duct. m. t. Malpighian tubule. ph. pharynx. p. v. Proventriculus. oe. Oespphagus. r. Rectum. s. d. Common salivary duct. s. g. Salivary gland. v. Ventriculus.

Fig. 2. End of Malpighian tubule of fly showing parasites attached.

Fig. 3. Malpighian tubule of fly, showing spores in lumen.

EXPLANATION OF PLATE II.

Fig. 4. Spore unstained, containing usual number of sporozoites. a. Sporozoite, stained.

Fig. 5. Spore viewed from end of bundle, showing characteristic number of sporozoites.

Fig. 6. Spore containing 12 sporozoites.

Fig. 7. Sporozoites beginning to emerge from bundle.

Fig. 8. Sporozoites increasing in size and becoming U-shaped.

Fig. 9. Transformation into flagellates, showing individuals with flagella of various lengths.

Fig. 10. Group of attached flagellates. Some are permanently attached by their flagellar end, others temporarily by their posterior ends. a. Individual showing peculiar flagellar membrane and flagellum.

Fig. 11. Group of attached Herpetomonas with flagellum resorbed. a. Abundant type, in which the body is drawn up into a Gregarine-like anterior portion and tail-like posterior portion, while it still retains the menbrane and flagellum.

EXPLANATION OF PLATE III.

Fig. 12. Group in which the individuals have begun to prepare for spore formation. The anterior parts of their bodies are rounded.

Fig. 13. Group in still later state of development. The tail-like posterior parts of their bodies show clear and non-granular.

Fig. 14. Gregarine-like group just before beginning of spore formation. a. b. c. d. showing steps in division of trophonucleus.

Fig. 15. Group showing individuals about to divide. a. nucleus almost divided. B. nuclei divided.

Fig. 16. Next division.

Fig. 17. Still later division.

Fig. 18. Group of individuals in late stage of spore formation.

Fig. 19. Group of individuals each one becoming sporozoite.

EXPLANATION OF PLATE IV.

Fig. 20. Mass of spores surrounded by a membrane.

Fig. 21. Flagellate in early stage beginning to divide.

Fig. 22. Flagellate of later stage in process of division.

Fig. 23. Attached form, dividing.

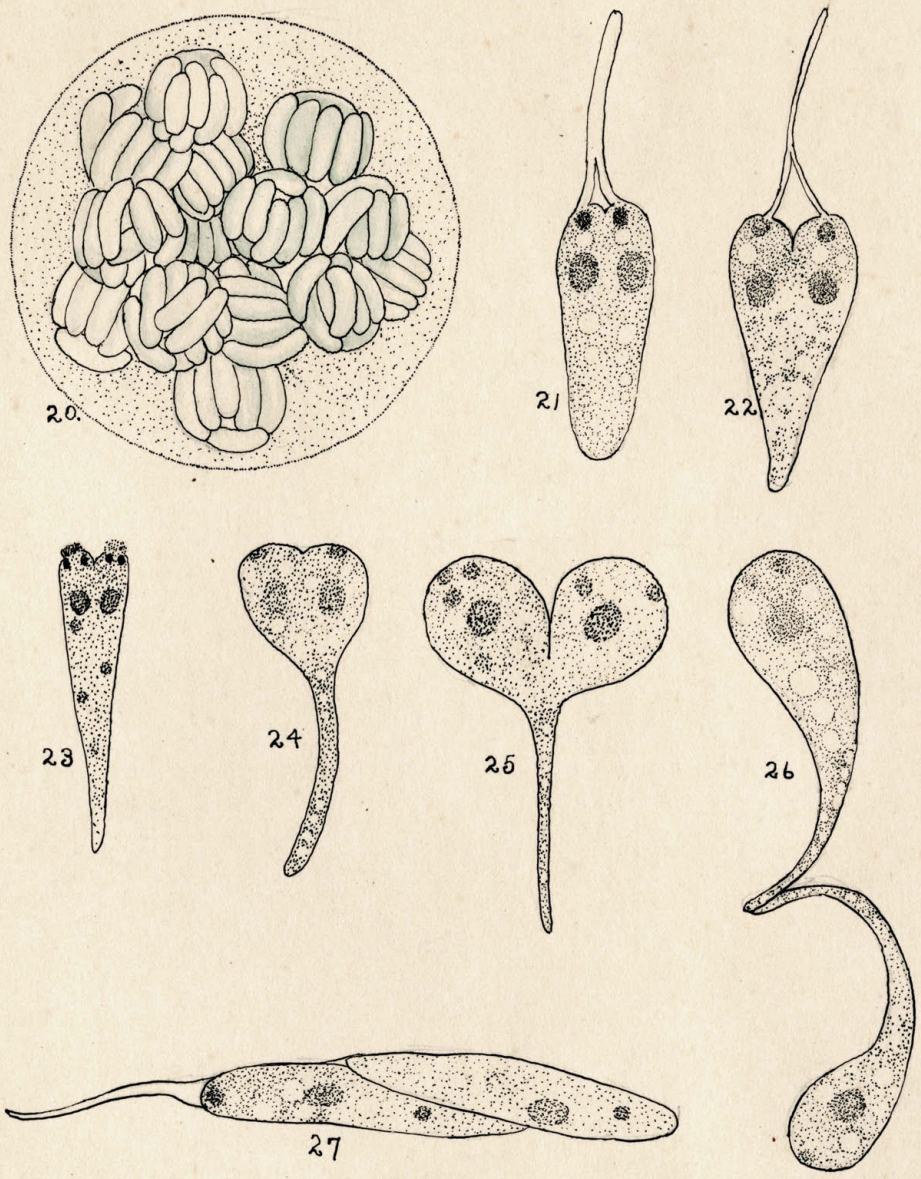
Fig. 24. Attached form, later stage, beginning division.

Fig. 25. Attached form, same stage as above, more nearly divided.

Fig. 26. Attached form almost divided.

Fig. 27. Transverse division of flagellate form.

Fig. 28....32. Stages in spore formation.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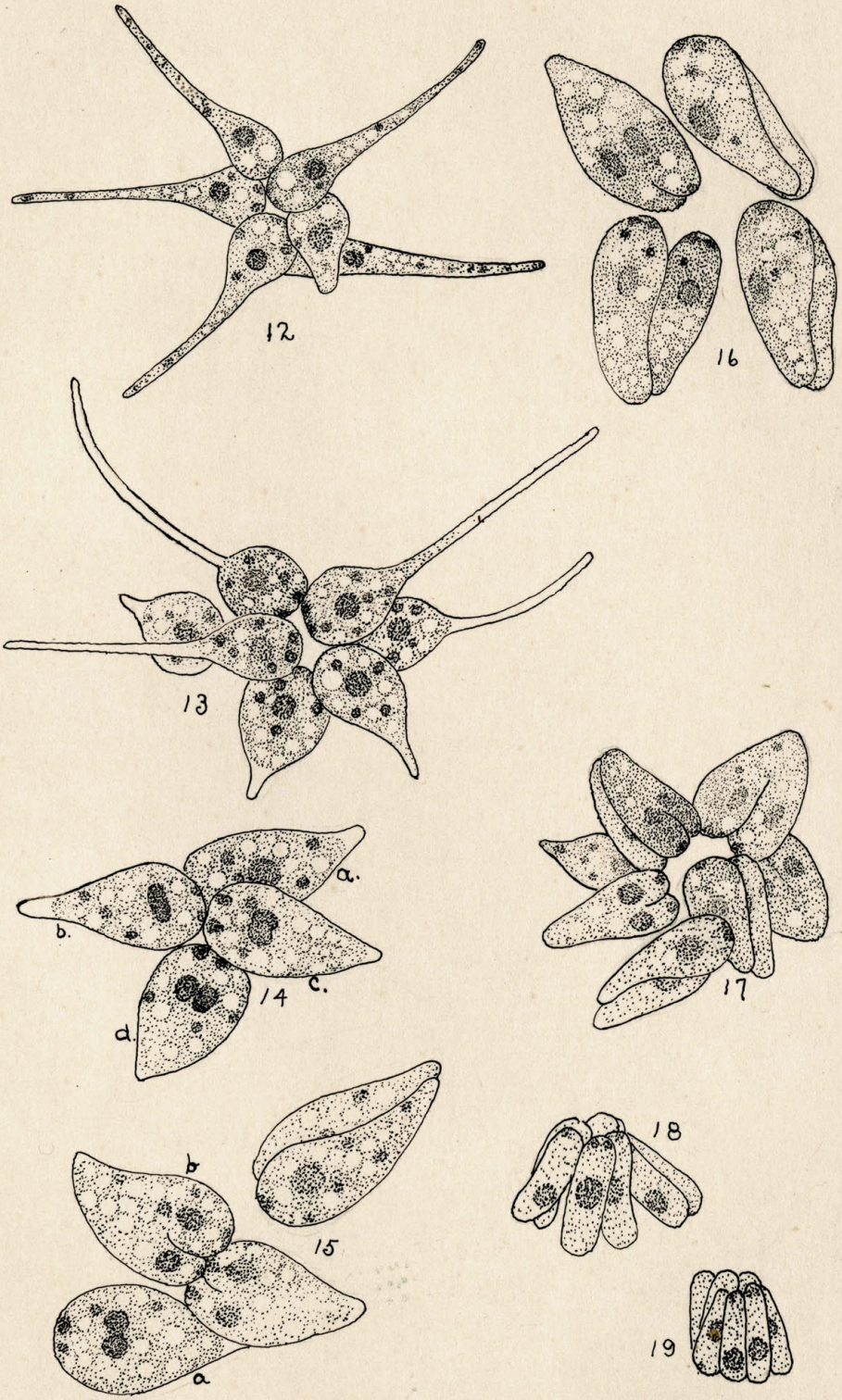
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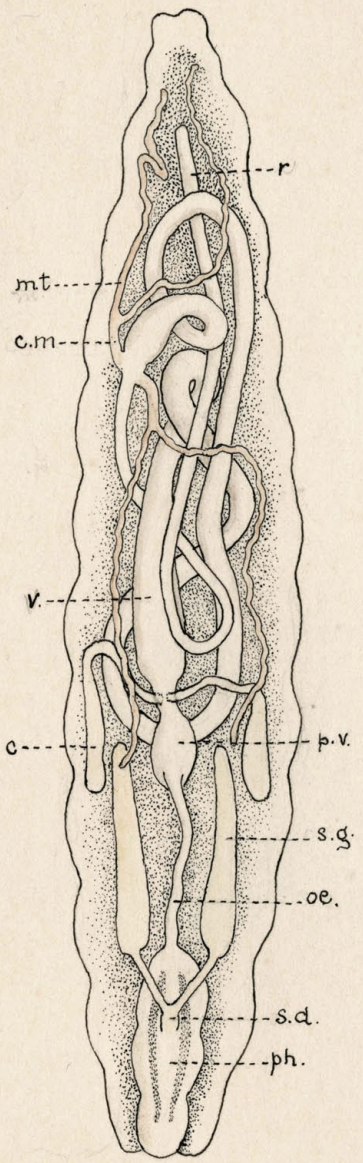

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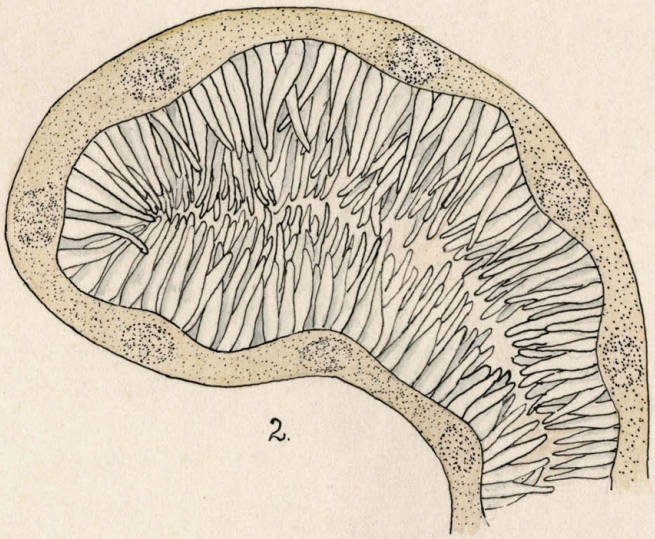

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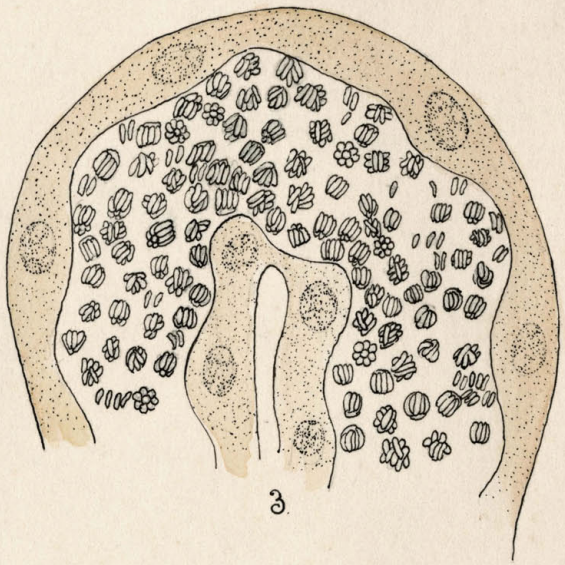

3.