OBSERVATIONS ON *EUGLENA LEUCOPS*, SP. NOV., A PARASITE OF *STENOSTOMUM*, WITH SPECIAL REFERENCE TO NUCLEAR DIVISION [1]

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Introduction

A *Stenostomum* heavily infected with a colorless euglenoid parasite was found in a collection made near the University of Virginia. The morphology of this curious flagellate has led me to place it in the genus *Euglena* Ehrbg.

My purpose is to describe this new form, its nuclear division and its relation to its host; discuss its systematic position; and record some further observations upon its life history.

Historical

Euglena-like parasites of rhabdocoels have been reported on previous occasions. Haswell (1892) reported from an undetermined species in Australia an intracellular, colorless flagellate that had neither stigma nor flagellum. In 1907 the same author reported from the same continent another colorless form, from a mesostomid. It possessed a stigma and when liberated from the host, it no longer progressed by metabolic or euglenoid movement but became somewhat bottle-shaped and swam spirally by a flagellum. This parasite was found not only inside the cells but also in the space between the gut and body wall. He did not attempt to identify either of these forms.

In France, Beauchamp (1911) described *Astasia captiva*, a parasite within the "pseudocoele" of *Catenula lemna*. His description is fairly complete. This form retained its flagellum in the host and bore a colorless "rudimentary" stigma. It measured 30 to 40 microns in length and even when liberated from the host moved only by a rapid metabolic (euglenoid) movement. He speaks of, and his figures show, the oblique surface striations and spoon-like depression near the posterior end of the body. He mentions the "conduit buccal" and nucleus as being typical of the "Eugleniens."

[1] The writer is greatly indebted to Dr. B. D. Reynolds, under whose direction this investigation was carried out at the Miller School of Biology and the Mountain Lake Biological Station of the University of Virginia.

Materials and Methods

The infected *Stenostomum* (a new species soon to be described) was found in one of my aquaria in October 1929.[2] Although there were several other *Stenostoma* of the same, as well as of other species, in the aquarium, none were found to be infected. No free-living flagellates were found in the water that resembled the parasite. Other old aquaria in the laboratory as well as fresh collections were then examined. Of the hundreds of rhabdocoels examined, only two species were found infected and these were both species of *Stenostomum*. The other *Stenostomum* found infected is *S. predatorium* (Kepner and Carter, 1930). The infection is very rare in nature.

Fortunately, one of these *Stenostoma* was cultivated very easily in rather large glass dishes in which there were wheat cultures of small flagellates and ciliates. A half dozen of these flatworms added to a thriving protozoan culture will produce a hundred or more in two or three weeks. The worms were examined frequently to see if any of the free-living Protozoa had become established as parasites. Negative results are reported. Upon the addition of two or three infected *Stenostoma*, practically all the flatworms were infected in a week. In this way it was possible to have on hand as many specimens of *Euglena leucops* as were desired.

Various killing and fixing agents were employed. The best fixation of the chromatin was obtained with Carnoy's aceto-alcohol mixture. Absolute alcohol saturated with corrosive sublimate gave good results, as did Bouin's picro-formol solution, Allen's modification of the former, Schaudinn's and Zenker's fluids. Nuclear division was best studied in sectioned hosts, although smear preparations were also used. Heidenhain's iron alum haematoxylin was found to be the best stain for the nucleus, flagellum and blepharoplasts. The alcohol-xylol-paraffin method was employed.

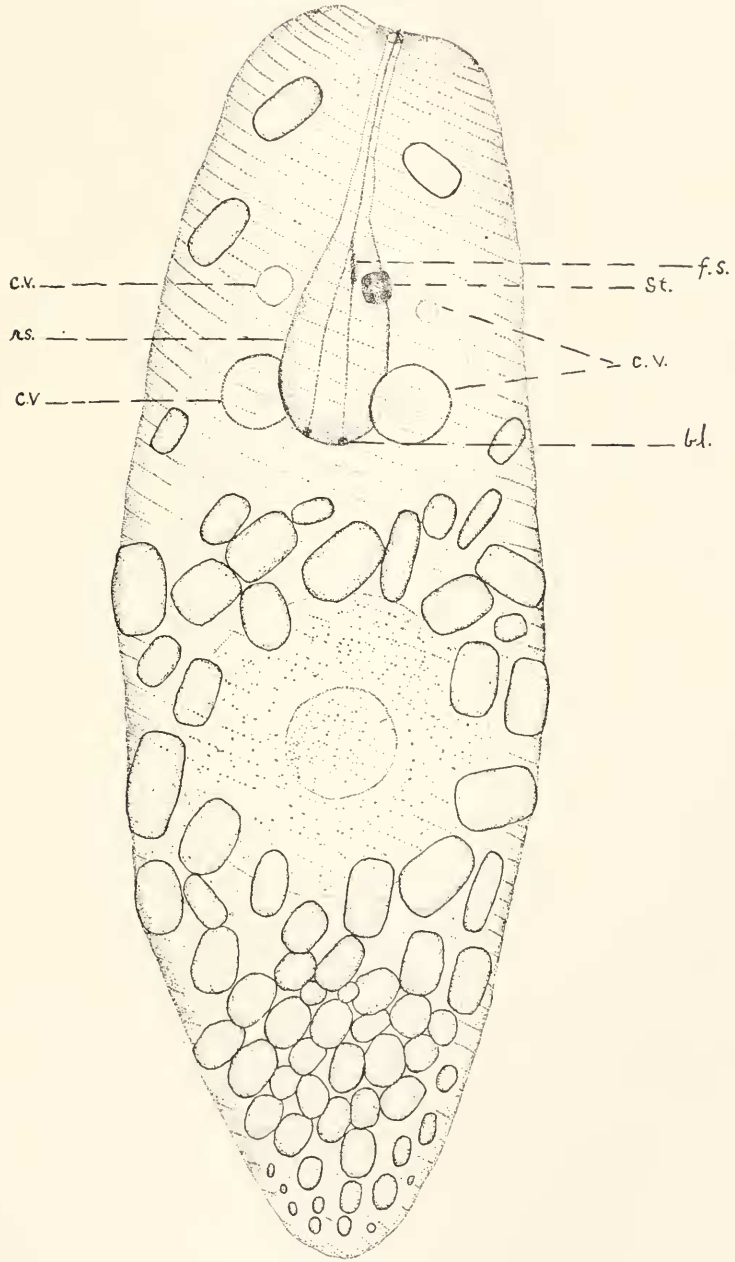
[2] I am greatly indebted to Dr. W. A. Kepner for help in the identification of the rhabdocoels employed in this investigation.

Plate I (× 8,000)

Fig. 1. Drawing of a non-flagellated living specimen of *Euglena leucops* sp. nov. as it appears immediately after liberation from the host.

The two rami of the flagellum can be seen to end at the blepharoplasts (*bl.*) at the base of the reservoir (*rs.*). The flagellar swelling (*f.s.*), a little anterior to the stigma (*st.*), is barely visible. The oblique surface striae are shown as well as both face and side views of the paramylum grains. Four contractile vacuoles (*c.v.*) are figured, two extended and two contracted. Details of the living nucleus other than the endosome can not be clearly made out. The stigma illustrated is composed of four distinct particles.

PLATE I



General Morphology

The organisms vary as to size. In a contracted condition the range in length is from 22 to 29.6 microns. Elongated, the range is from 33 to 44.5 microns. In width, the range is from 9 to 13 microns for resting individuals. In a resting condition the parasites are approximately two and one-half times as long as wide and rather blunt on both ends. The anterior end bears a slight depression in its contour where the gullet has its outlet. Most of a population are of average size. The forms just before cell division present the maximum size, but the daughter cells produced are often larger than others seen. The wave formed during metabolic movement may be as great in diameter as 18.5 microns. During metabolic movement the anterior end decreases only slightly in diameter while the posterior end becomes quite pointed.

Very fine surface striae run obliquely from left to right (Plate I, Fig. 1).

There are no chromatophores in the cell.

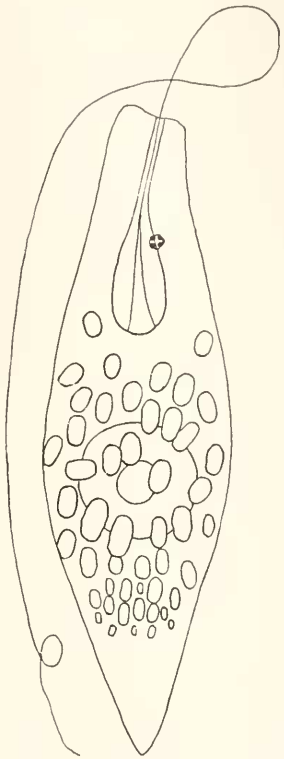
A gullet and reservoir can be seen in the living animal. The two together present a flask-shaped outline with a narrow passage, the gullet leading anteriorly. Associated with the reservoir are a series of contractile vacuoles that open into it. As many as four vacuoles have been counted in living specimens (Plate I, Fig. 1).

Flagellum

When the parasite is in the host the flagellum does not extend beyond the opening of the gullet (cytostome). Even in living individuals the flagellum can be seen to bifurcate as soon as it has passed the narrow passage of the gullet, the two rami proceeding to the blepharoplasts at the bottom of the reservoir. There is a "flagellar" swelling at the point of bifurcation, which is approximately at the level of the stigma (Plate I, Fig. 1).

When the parasite is liberated from the host and put into tap or spring water, a flagellum can be seen beginning to grow out of the "stump" in less than ten minutes. In twenty-five or thirty minutes in this medium it has reached its maximum length, a little longer than the body. Even before it has attained a length one-half as great as the body, the parasite ceases its metabolic movement and progresses feebly by the flagellum. In this condition the organism is dumb-bell-shaped, moving forward irregularly in very small spirals. The cell is still blunt on the posterior end at this stage. By the time the flagellum has reached its full length, the shape of the cell has changed greatly. It is now bottle-shaped, moving vigorously and swiftly by means of the newly acquired organelle of propulsion (Text figure 1).

The animal displays a marked faculty to replace a lost flagellum, since, as pointed out below, this structure when lost will be regenerated if the conditions of osmotic pressure are made favorable.



TEXT FIG. 1. Outline drawing of a flagellated specimen of *Euglena leucops* sp. nov.

Paramylum Bodies

These bodies are distributed irregularly over the cell, being most abundant in the posterior third. Only one to three small ones may be seen anterior to the stigma (Plate I, Fig. 1). These granules sometimes measure 4 microns at the greatest diameter. They are found to be disc-shaped and to decrease regularly when the parasite is subjected to inanition.

Stigma

The stigma, dark reddish-brown in color, stands out distinctly in the anterior region, just lateral to the gullet. It is not large, measuring less than two microns at the greatest diameter.

Much variation in size and form of the stigma has been observed (Text figure 2). The stigma is seen to be composed mainly of four to seven distinct bodies which appear to be connected by fine strands and the whole embedded in a colorless matrix. This morphologically degenerate stigma may represent a stage in the complete loss of this organelle.

A large number of observations were made upon the stigma of dividing individuals. While the nucleus is in the early prophase, the stigma enlarges slightly but usually the number of colored bodies of which it is mainly composed does not increase. These granules push apart somewhat and, keeping their identity, usually about one-half of them migrate, probably attached to each other, to the opposite side of the cell, never wandering far from the reservoir. They finally come to lie in a position lateral to the new gullet which has formed in the meantime.

This account of the origin of the daughter stigmata agrees somewhat with the observations of R. P. Hall and Jahn (1929*b*) on *Euglena gracilis*, except that they found that the stigma breaks up into a large number of smaller granules which become more widely separated.

The stigma does not always divide equally. A number of times the daughter cell has been seen to receive only two-fifths or even

TEXT FIG. 2. Variations in the stigma of *Euglena leucops* sp. nov. Drawings from several specimens. × about 16,000.

one-fourth of the dark granules. This unequal division aided by natural selection perhaps accounts for the decreased size and degeneration of the stigma.

Nucleus

The nucleus can be seen in the living organism as a relatively large spherical body (8 to 9 microns in diameter) lying usually near the center of the body. Upon fixing and staining, its chief characteristic is the presence of usually one comparatively large, deeply-staining, central body, the endosome (karyosome of some authors). Immediately surrounding the endosome is a hyaline area, apparently lacking in chromatin. The twenty-two to twenty-five chromatin bodies which

have been called "chromosomes" by Keuten (1895) and which give rise to the same number of chromosomes, remain distinct even in the nucleus of the interphase or the resting nucleus. Their distal ends lie close to and probably join the well-defined nuclear membrane. Fine strands are distinctly seen connecting the proximal ends of these bodies with the endosome (Plate II, Fig. 1).

These observations on the chromatin in the resting nucleus are not in agreement with those of most other workers on the euglenoids. Numerous small granules arranged at the nodes of a linin network have been described by Hartmann and Chagas (1910) for *Peranema*, by Tschenzoff (1916) for *Euglena viridis*, by Bělař (1916) for *Astasia*, by R. P. Hall (1923) for *Menoidum*, by R. P. Hall and Powell (1928) for *Peranema*, by Baker (1926) for *Euglena agilis* and by Ratcliffe (1927) for *Euglena spirogyra*. It is not difficult to see how the "numerous small granules" idea became prevalent, since this impression is frequently given by the nucleus after a casual observation, particularly following fixation in Schaudinn's fluid, the usual method employed. What I take to be the correct condition of the chromatin is better followed after the use of Carnoy's fixative. I have been able to observe the distinct "chromosomes" in the resting nucleus of *Euglena leucops* following fixation with all the agents mentioned in the section on materials and methods.

My observations are supported by Keuten (1895), who has figured chromosomes in the resting nucleus and in the late telophase for *Euglena viridis*. R. P. Hall (1925) has described the nucleus of the dinoflagellate *Oxyrrhis marina* thus, "Around the endosome the chromatin appears in the form of chromomeres, arranged in string-of-beads fashion in rows, or chromosomes; such an organization seems to be evident even in the resting nucleus, as characteristic of the dinoflagellates 'where there appears in the nuclei . . . to be a persistent organization of beaded chromosomes with subparallel or even spiral arrangements within the nucleus.'" [3]

In preparations fixed with Schaudinn's fluid and stained with iron haematoxylin, the endosome resists destaining very much longer than the chromosomes. After Carnoy's fixative and iron haematoxylin, the endosome decolorizes more readily than the chromosomes. The ability to destain the endosome almost completely has made possible a careful study of the resting nucleus and division stages.

A nuclear membrane is distinguishable in the resting condition and can be followed through mitosis.

Frequently, the endosome is seen to be in two, three or more

[3] Hall is quoting from Kofoid (1923).

fragments. R. P. Hall (Hall and Powell, 1928) noticed a multiplication of the endosome in *Peranema trichophorum*. Wenrich (1924), in his description of *Euglenamorpha Hegneri*, says: "In the pellucid variety there is a marked tendency for the nucleus to hypertrophy. . . . This hypertrophy is accompanied by a multiplication of the caryosome, as many as four have been found in one nucleus. Hypertrophy apparently leads to amitotic division of the nucleus which is probably followed by division of the body. Such amitotic stages have not been found in the green variety." No explanation is offered here for fragmentation of the endosome occurring in *Euglena leucops*. However, no evidence whatever for amitosis was found.

Behavior

Euglena leucops is stimulated to extremely vigorous movement by light, heat, and a change either way in osmotic pressure.

Thigmotropism is exhibited in non-flagellated individuals. Flagellated organisms were found to be negatively phototropic.

Plate II (× 10,000)

All the figures were drawn from specimens fixed in the host with Carnoy's aceto-alcohol mixture and stained with Heidenhain's iron alum haematoxylin.

Fig. 1. A typical nucleus of the interphase. Twenty-two chromatin bodies or "chromosomes" are figured. Note the attachment of these to the endosome.

Fig. 2. A very early prophase. The "chromosomes" and endosome are beginning to enlarge.

Fig. 3. The nucleus is becoming noticeably larger and the "chromosomes" and endosome are beginning to elongate.

Fig. 4. Note the blunt bifurcation of one end of the endosome.

Fig. 5. The nucleus, chromosomes and endosome are all distinctly elongated. Most of the chromosomes have apparently lost their attachment to the endosome.

Fig. 6. This is the first stage in the metaphase. The chromosomes are quite narrow and extend the length of the elongate nucleus. The blunt bifurcation of one end of the endosome is distinctly pronounced by now and both ends have become enlarged.

Fig. 7. Metaphase and the beginning of the anaphase. The chromosomes, having spun out practically the length of the elongated nucleus during the early metaphase, now divide transversely as the extremities of the nuclear membrane and endosome further pull apart. Note the constriction in the membrane and endosome. Fine strands are seen in the mitotic figure at this stage, most of which appear to be a connection between the two broken ends of the chromosomes.

Fig. 8. The chromosomes are beginning to round off somewhat and are apparently attracted toward the two ends of the membrane. The nucleus and endosome are now decidedly elongated and constricted in the center.

Fig. 9. The chromosomes are beginning to assume their interphase shape and their attachment to the ends of the dividing endosome again becomes evident. Notice the upward turn of the nuclear membrane and endosome.

Fig. 10. The end of the telophase. Most of the chromosomes have regained their attachment to the endosome. The daughter nucleus and endosome now round off as the interphase approaches.

I am indebted to Miss M. E. Hill for help in the preparation of the drawings.

PLATE II

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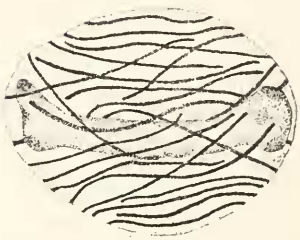

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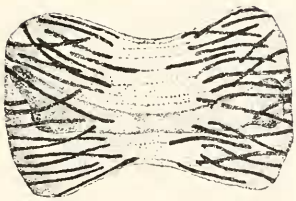

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The fact that *Euglena leucops* elaborates a flagellum soon after being liberated from the host and is negatively phototropic,—the host reacts similarly—makes it particularly well adapted for swimming away and finding a new host.

Division of the Body

When an infected host is macerated in spring water, at any hour of the day, numerous dividing parasites are encountered after a few minutes. Frequently as many as 10 per cent of the flagellates, after ten to fifteen minutes exposure to this solution, will start dividing and complete the process within seventeen to twenty minutes. Now if an infected host is macerated in a solution approximately isotonic with the host's plasma, no more dividing forms are seen in this medium than normally appear in the host and twenty-five to thirty minutes are required for the parasite to complete the division. Since the spring water is of a lower molecular and ionic concentration than the host's plasma, it would appear that the change in osmotic pressure both initiates and accelerates cell division.

Division in *Euglena leucops* seems to be periodic, as is the case in *Hydramoeba hydroxena*. Reynolds and Threlkeld (1929) observed that division in this amoeba occurred most often about 3 A.M. Observations upon more than three hundred dividing forms of *Euglena leucops* have shown that division, while sometimes occurring at other times, most often takes place between 10 P.M. and 2 A.M., with the peak about midnight.

A short time before the longitudinal split occurs, there is a duplication in the number of gullets, reservoirs, flagellar "stumps," stigmata, and nuclei. The split begins at the anterior extremity and continues posteriorly along the median plane of the body until the two daughters are completely separated. There is a continual twisting and writhing of the binucleate body during the process of division. Finally the posterior ends of the daughter cells are connected only by a small strand, but this is apparently the toughest part of the cell, for a veritable tug of war ensues, before the daughter cells are separated.

The parasite has been under observation in the host for more than a year and no evidence of encystation has been observed.

Nuclear Division

Prophase

The beginning of nuclear division may be recognized in four ways: (1) increase in size of the nucleus, (2) migration of the nucleus anteri-

orly, (3) changes in the endosome and (4) changes in the chromatin bodies or "chromosomes."

A considerable increase in the size of the nucleus is observed at the onset of division.

By the end of the prophase the dividing nucleus has reached a position in the anterior part of the organism, just posterior to the reservoir.

The endosome increases in size and elongates. One end can be distinguished from the other by a blunt bifurcation (Plate II, Figs. 4, 5, 6, 7, 8 and 9). Baker (1926), working with *Euglena agilis*, describes the bud arising from one of the lateral points, as forming the blepharoplast of one ramus of the new flagellum and the connection of the bud with the endosome as the rhizoplast. I find insufficient evidence from *Euglena leucops* to support this.

By the end of the prophase the endosome is distinctly dumb-bell-shaped (Plate II, Fig. 5). It would appear that at the end of the prophase there is a brief pause in mitosis since this stage was encountered most often.

The distinct, rather large chromatin bodies or "chromosomes" of the resting nucleus, keeping their identity, increase slightly in size and stain more deeply early in the prophase (Plate II, Fig. 2). As the nucleus and endosome elongate, these bodies, with one end apparently remaining attached to the nuclear membrane, are spun out into extremely long chromosomes which become arranged around the endosome (Plate II, Fig. 5). The connection between the chromatin bodies or "chromosomes" and nuclear membrane persists certainly to middle prophase and perhaps until division is completed.

Metaphase

The endosome continues to elongate, its ends increasing in size at the expense of the middle portion (Plate II, Fig. 6). One end still can be distinguished from the other. The nuclear membrane follows largely the contour of the endosome. The chromosomes, having drawn out almost the length of the elongated nucleus, now break in the middle.

There is apparently little doubt that the chromatin bodies of the resting nucleus give rise directly to the same number of elongated chromosomes that divide transversely. Twenty-two to twenty-five bodies in the resting nucleus can be followed through the metaphase when the long chromosomes produce a double number of daughter chromosomes of approximately half the length of those in the late prophase (Plate II, Fig. 7).

I have been unable to find the V-shaped chromosomes of the late prophase that have been described for euglenoids by R. P. Hall (1923, 1928), Baker (1926), and Ratcliffe (1927) which they take as evidence for a longitudinal split.

After a review of a large part of the literature on mitosis in the Protozoa, I have been able to find only one other case where a transverse division in the chromosomes has been definitely demonstrated, although others have mentioned it as a possibility [Bělař (1916) in *Astasia* and Borgert (1909, 1910) in *Aulacantha* and *Ceratium*]. Calkins (1929) describes a transverse division in the chromosomes of the ciliate *Uroleptus Halseyi* on the third meiotic spindle.

Anaphase

During this stage the ends of the endosome become quite far apart but remain connected by their narrow, middle portion. The bend is quite pronounced, the two ends of the endosome making a sharp upward turn.

The forty-six to fifty daughter chromosomes now shorten but remain radially arranged around the extreme ends of the daughter endosomes. The membrane has become distinctly constricted in the middle largely following the contour of the endosome (Plate II, Fig. 8).

Telophase

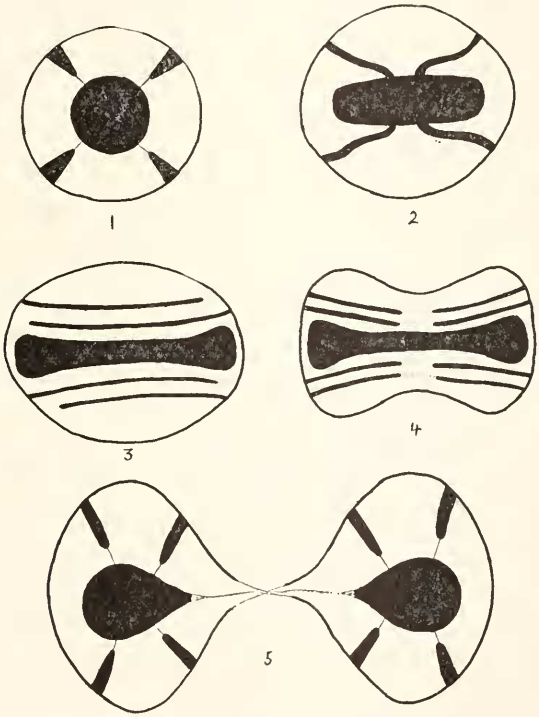
This stage begins when the ends of the daughter endosomes and daughter nuclei have pulled apart. The original endosome breaks in the center of its now narrow, lightly-stained, central portion.

The daughter endosomes and nuclei now round off and the chromosomes arrange themselves at the periphery of the nucleus. The connection between chromosomes and endosome is established early in the telophase (Plate II, Figs. 9 and 10).

The accompanying diagram (Text figure 3) illustrates the history of four chromosomes, in the resting nucleus and through mitosis. Kater (1926, 1927, 1928) brings forth evidence that even in the higher plants and animals, reconstruction of the daughter nuclei is by vesicle formation of contiguous chromosomes, that may be traced well into the succeeding prophase. He considers this as "good evidence for genetic chromosomal continuity." The evidence for this continuity is still stronger in *Euglena leucops*.

Calkins (1929) has the following to say concerning the chromosomes of the ciliate *Uroleptus Halseyi:* "In *Uroleptus Halseyi* the chromosomes are divided transversely at each vegetative division. On the theory of the gene these facts are possible only on the assumption of

a single type of gene for each chromosome and on this assumption there would be not more than twelve types of genes in *Uroleptus Halseyi*. Here there are twelve chromosomes in the third meiotic spindle and twenty-four in the amphinucleus. There are forty-eight in the first meiotic spindle and twenty-four in the second. We conclude that each of the twenty-four chromosomes found in the amphinucleus divides once to form the forty-eight of the first meiotic spindle; that two of these are separated from two by this division, and that one is separated from one at the second meiotic division, thus leaving twelve in the third meiotic spindle in which each chromosome represents a single type of gene. It is immaterial, therefore, whether division of each chromosome is transverse or longitudinal,



TEXT FIG. 3. Diagrammatic representation of the resting nucleus of *Euglena leucops* sp. nov. and the history of four of its chromosomes during division.

Fig. 1—the resting nucleus.

Fig. 2—the middle prophase.

Fig. 3—the beginning of the metaphase.

Fig. 4—the end of the metaphase and the beginning of the anaphase.

Fig. 5—the late telophase wherein begins the reconstruction of the nucleus of the interphase.

See the text and Plate II for a more detailed description.

for it would be equational in either case." Carrying this theory a step further, since the chromosomes are distinct even in the resting nucleus in the case of *Euglena leucops*, a single type of gene can be considered as recognizable at any stage in the life of the organism.

Experiments on Retardation in Growth of the Flagellum

As already stated, the flagellum of organisms liberated into tap or spring water will make its appearance outside the gullet in five to ten minutes. In 12 per cent Locke's solution, the organisms have been carried fourteen days without ever elaborating a flagellum. In a pure glucose solution with a molecular concentration approximately equal to the Locke's solution they have been carried more than a day or until death without showing a flagellum. The same thing is true for creatine, a mixture of different amino acids and other soluble protein derivatives. By varying the concentration of the molecular or ionic substances it is possible to vary the time required for the flagellum to be elaborated and for the appearance of the changes in body form that accompany it. The hydrogen ion concentration apparently has nothing to do with the retardation in the development of the flagellum. Viscous substances such as thin starch paste, as well as aqueous agar mixtures, which of course do not alter the molecular concentration of the media appreciably, have little or no effect on retarding the growth of the flagellum.

By increasing the osmotic pressure of the medium in which *Euglena leucops* elaborates and retains the flagellum, the organisms have been made to settle down and again move only by metabolic movement, the flagellum apparently being broken off. But if the osmotic pressure be once more decreased, the flagellum will reappear. This process has been repeated four times with the same individuals and the organelle grew out as readily the fourth time as it did the first.

It would appear therefore, that molecular or ionic concentration is the main factor in retardation of flagellar growth and that this organelle is capable of continued or repeated growth.

The osmotic pressure of the plasma of the host is apparently great enough to prevent the elaboration of a flagellum.

Results of Attempts to Cultivate the Parasite

All attempts to cultivate the parasite *in vitro* failed. They were carried fourteen days in Locke's solution diluted to 12 per cent at pH 7.6. At the end of this period practically all the paramylum was consumed.

They may be kept alive three or four days in spring water.

Methods of Infection

The parasite may enter the host in three ways:

(1) By the ingestion of liberated parasites. The *Stenostoma* that the writer has been able to infect, feed extensively upon Protozoa. The parasites, either swimming actively or moving on the bottom of the container by euglenoid movement, are ingested by the host and enter the space between the gut and body wall, presumably, directly through the wall of the enteron.

(2) The *Stenostoma* susceptible to infection are predatory and cannabalistic. On a number of occasions I have observed the transmission of the infection by an infected flatworm serving as food.

(3) Vegetative zoöids are infected from the parent.

The Effect of the Infection upon the Host

Upon one occasion the writer observed an uninfected *Stenostomum* ingest a portion of an infected one of the same species. About eight parasites entered the enteron of the uninfected rhabdocoel. In less than an hour approximately all of the parasites were seen in the mesenchyme of the new host. The next day they had about doubled in number. The host divided on the second day and the (approximately thirty) parasites were distributed about equally between the two. At the end of a week the infection had become well established.

Stenostoma lightly infected show no ill effects whatever and appear normal in all their reactions. No signs of "nervousness" are exhibited even though the parasites can be seen passing over, under and in contact with the cephalic ganglia. Kepner and Carroll (1923) found this to be true in the case of *Stenostomum leucops* infected with the ciliate, *Holophyra virginia*.

Since the parasites evidently absorb their food from the plasma in the mesenchyme, one would expect that rather heavily infected animals would grow and reproduce slowly; but specimens infected with as many as fifty or sixty parasites have been seen to divide every second day under favorable conditions. This is as fast as normal individuals reproduce.

Frequently the flatworms become infected with two or three hundred parasites which causes them to appear bloated, due to the ectoderm being lifted to make room for the increased contents of the mesenchyme. Animals in this condition become sluggish, the ectoderm breaks in one or more places, the parasites are liberated and death generally follows in a day or so.

Means for freeing the flatworms of the parasites have not been found.

Infection Experiments

Even though species of *Stenostoma* other than the two mentioned above ingest the parasite, only one, *S. grande*, has been infected experimentally. All attempts to infect *Catenula lemna*, the host of *Astasia captiva*, failed, even though it ingested *Euglena leucops* in large numbers.

Systematic Position of Parasite

The apparent specificity of this parasite excludes the probability of its being found in hosts other than rhabdocoels. The only euglenoid described as a parasite of this group is *Astasia captiva*, from *Catenula lemna*, and this rhabdocoel apparently is not capable of becoming infected with *Euglena leucops*. *A. captiva* differs further from *E. leucops* in the following respects:

(1) *A. captiva*, according to Beauchamp (1911) is without a stigma, save possibly a *colorless* rudiment.
(2) It has a flagellum while in the host.
(3) It does not change in shape or otherwise when freed into spring water.
(4) *A. captiva* dies in a few hours after liberation.
(5) The paramylum grains of Beauchamp's parasite are elliptical in outline.

The *Euglena*-like form that Haswell (1907) mentioned but did not describe, resembles very closely *Euglena leucops*. Nevertheless it was intracellular and a parasite of a mesostomid, the most highly specialized of the Rhabdocoelida, and hence the farthermost removed from *Stenostomum*.

Wenrich (1924) has shown that the colorless variety, *pellucida* of *Euglenamorpha hegneri* may arise from the green variety, simply by loss of color. More conclusively Zumstein (1900) and Ternitz (1912) have shown that *Euglena gracilis* will lose its chlorophyll and become colorless when supplied with rich nourishment. They point out that their results do away with the boundary line between the genera *Euglena* and *Astasia* which have been separated on the basis of color (chlorophyll and stigma). Color, therefore, is not a sound character on which to base a classification of *Euglena*-like forms.

R. P. Hall and Jahn (1929*a*) say "A bifurcation of the flagellum is characteristic of the different species of uniflagellate Euglenidae examined and the flagellum in such species always shows a 'flagellar swelling' at the level of the stigma (which is always present) in vegetative stages. . . . Such structural features, however, have not

been observed in the vegetative stages of any of the non-chlorophyll-bearing species examined by us or by other workers."

Further, they believe that certain stigma-bearing but chlorophyll-free flagellates, described in the literature as *Astasia*, should be put in the genus *Euglena*. They made no observations upon the conditions of the flagellum in these forms.

Bělař (1916) was of the opinion that *Astasia captiva* was an *Euglena*. He possibly was influenced by the presence of a stigma in this form, even though Beauchamp states that it was a colorless rudiment.

Because of the presence of a definite stigma and the bifurcation of the flagellum, the writer is of the opinion that this new form should be placed in the family Euglenidae Stein. If it is placed in this family, obviously it belongs in the genus *Euglena* Ehrbg. because of its morphological features, which would preclude its belonging to any other genus of this family.

Summary

A new species of chlorophyll-free *Euglena*, *E. leucops*, is described, and though it lacks color, the presence of a stigma and the bifurcation of the flagellum place it in the family Euglenidae.

A flagellum is not present while the parasite is in the host but is elaborated quickly outside the host if favorable conditions of osmotic pressure prevail. This organelle is capable of repeated growth.

The stigma has been followed through binary fission and found to divide, but not always equally.

Nuclear division is described. The "chromosomes," which remain distinct, even in the resting nucleus, divide transversely during the metaphase.

Osmotic pressure apparently may initiate and accelerate cell division.

Attempts to cultivate the parasite *in vitro* failed.

The host may be easily cultivated and the infection readily continued.

No evidence of encystation has been observed.

Observations upon the flagellates for more than a year have given no evidence of a method of division other than binary fission involving mitosis.

BIBLIOGRAPHY

Baker, W. B., 1926. Studies in the Life Cycle of Euglena. I. Euglena agilis, Carter. *Biol. Bull.*, **51**: 321.

Beauchamp, P. de, 1911. Astasia captiva, N. Sp. Euglenien parasite de Catenula lemnae Ant. Dug. *Arch. de Zool., Expér. et Gén.*, Ser. 5, T. 6.

Bělař, K., 1916. Protozoenstudien I. Amoeba diplogena, Astasia levis, Rhynochomonas nasuta. *Arch. f. Protist.*, **36**: 14.

Borgert, A., 1909. Untersuchungen über die Fortpflanzung der tripyleen Radiolarien, speziell von Aulacantha scolymantha H. II. Teil. *Arch. f. Protist.*, **14**: 134.

Borgert, A., 1910. Kern-und Zellteilung bei marinen Ceratium-Arten. *Arch. f. Protist.*, **20**: 1.

Calkins, G. N., 1929. The Chromosomes of Uroleptus Halseyi. *Science*, **70**: 562.

Hall, R. P., 1923. Morphology and Binary Fission of Menoidium incurvum (Fres.) Klebs. *Univ. Calif. Publ. Zool.*, **20**: 447.

Hall, R. P., 1925. Binary Fission in Oxyrrhis marina Dujardin. *Univ. Calif. Publ. Zool.*, **26**: 281.

Hall, R. P., and T. L. Jahn, 1929*a*. On the Comparative Cytology of Certain Euglenoid Flagellates and the Systematic Position of the Families Euglenidae Stein and Astasiidae Bütschli. *Trans. Am. Mic. Soc.*, **48**: 388.

Hall, R. P., and T. L. Jahn, 1929*b*. Dispersed Stages of the Stigma in Euglena. *Science*, **69**: 522.

Hall, R. P., and W. N. Powell, 1928. Morphology and Binary Fission of Peranema Trichophorum (Ehrbg.) Stein. *Biol. Bull.*, **54**: 36.

Hartmann, M., and Chagas, C., 1910. Flagellaten studien. *Mem. Inst. Osu. Cruz*, **2**: 64.

Haswell, W. A., 1892. Note on the Occurrence of a Flagellate Infusorian as an Intracellular Parasite. *Proc. Linn. Soc., N.S.W.*, 2d Ser., Vol. 7.

Haswell, W. A., 1907. Parasitic Euglenae. *Zool. Anz.*, **31**: 296.

Kater, J. McA., 1926. Chromosomal Vesicles and the Structure of the Resting Nucleus in Phaseolus. *Biol. Bull.*, **51**: 209.

Kater, J. McA., 1927. A Cytological Study of Dormancy in the Seed of Phaseolus vulgaris. *Ann. Bot.*, **41**: 629.

Kater, J. McA., 1928. Nuclear Structure and Chromosomal Individuality. Somatic and Germ Nuclei of the Rat. *Zeitschr. f. Zell. u. mik. Anat.*, **6**: 578.

Kepner, W. A., and Jenette S. Carter, 1930. Ten New Species of Stenostomum. *Zool. Anz.*, in manuscript.

Kepner, W. A., and R. P. Carroll, 1923. A Ciliate Endoparasitic in Stenostomum leucops. *Jour. Parasit.*, **10**: 99.

Keuten, J., 1895. Die Kerntheilung von Euglena viridis Ehrbg. *Zeitschr. f. wiss. Zool.*, **60**: 215.

Kofoid, C. A., 1923. The Life Cycle of the Protozoa. *Science*, 57: 397.

Ratcliffe, H. L., 1927. Mitosis and Cell Division in Euglena spirogyra Ehrenberg. *Biol. Bull.*, **53**: 109.

Reynolds, B. D., and W. L. Threlkeld, 1929. Nuclear Division in Hydramoeba hydroxena. *Arch. f. Protist.*, **68**: 305.

Ternetz, C., 1912. Beiträge zur Morphology und Physiology von Euglena gracilis Klebs. *Jahrb. f. wiss. Bot.*, 51.

Tschenzoff, B., 1916. Die Kernteilung bei Euglena viridis Ehrbg. *Arch. f. Protist.*, **36**: 137.

Wenrich, D. H., 1924. Studies on Euglenamorpha hegneri n. g., N. Sp., a Euglenoid Flagellate found in Tadpoles. *Biol. Bull.*, **47**: 149.

Zumstein, Hans, 1900. Zur Morphology und Physiology der Euglena gracilis Klebs. *Jahrb. f. wiss. Bot.*, 34.