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Morphology and binary fission of *Heteronema acus* (Ehrbg.) Stein.

By

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(With 3 figures in the text and plate 14—16.)

Introduction.

There are certain differences of opinion recorded in the literature concerning the details of nuclear division in euglenoid flagellates. Bělař (1916), in *Astasia levis*, has described transverse, instead of longitudinal, division of the chromosomes, and also a centriole in the endosome. Hartmann and Chagas (1910) also described transverse division of the chromosomes in *Peranema trichophorum*, and more recently Lackey (1929) reported the same phenomenon in *Entosiphon sulcatum*. Haase (1910), in *Euglena sanguinea*, reported the presence of chromosomes within the endosome. Tschenzoff (1916), Hall (1923), Baker (1926), Hall and Powell (1928), and Brown (1930), working on representatives of this same group, believed that longitudinal splitting of the chromosomes occurs. There are also variations in the accounts of flagellar behavior in binary fission. Tannreuther (1922) and Baker (1926) believed that the old flagella are thrown off in *Euglena* just prior to nuclear division, and that new ones grow out from the newly formed blepharoplasts. Ratcliffe (1927) described splitting of the old flagellum into two daughter flagella in *Euglena spirogyra*. Schüssler (1918), in his description of *Scytomonas pusilla*, stated that one of the old flagella may be retained by each daughter organism while the second flagellum arises by outgrowth. Lackey (1929) reported retention

of both flagella by one of the daughter organisms in division of *Entosiphon sulcatum*.

The present investigation was undertaken with a view to determining which of these varying accounts is applicable to *Heteronema acus*. The writer has been interested particularly in behavior of the endosome during division, the method of chromosome splitting, behavior of the flagella in division, and the structure of the pharyngeal-apparatus ('Staborgan'), as well as its fate in binary fission. This investigation was earlier reported in part in an abstract (LOEFER, 1930). The writer is greatly indebted to Doctor R. P. HALL for his many helpful suggestions during the course of this investigation.

General Morphology.

LEMMERMAN (1913, p. 168) described the genus *Heteronema* STEIN as follows: „Zellen metabolisch, mit derbem, häufig gestreiftem Periplast, manchmal tordiert. 1 Schwimm- und 1 Schleppgeißel, in einer vorderen Mundöffnung entspringend. Staborgan schwach entwickelt. 1 Haupt- und 1 Nebenvakuole, Vermehrung durch Teilung. Dauerzellen nicht bekannt. Ernährung animalisch. Bewegung rotierend mit starr nach vorn gerichteter Schwimmgeißel, deren Spitze schlängelnde Bewegungen ausführt." *Heteronema acus* (EHRBG.) STEIN is characterized in the following words: „Zellen spindelförmig, mit abgerundeten Enden, 45—50 μ lang, 8—20 μ breit. Membran zart spiralig gestreift oder glatt. Schwimmgeißel etwas über, Schleppgeißel $^1/_2$ körperlang. Mesosaprobe. In stehenden Gewässern."

In the fixed material examined, specimens vary in length and width according to the metabolic position of the animal. The length has been found to vary from 23,6 to 56 μ; the width, although generally about 15 μ, varies inversely with the length. The 'Staborgan' instead of being weakly developed, is well differentiated and is clearly visible in iron-alum hematoxylin preparations. No appreciable difference in length of the two flagella was noted. The periplast of the cell appears smooth. In numerous specimens ingested *Euglena* and *Chilomonas* (?) are present in food vacuoles. Very often the ingested organisms appear as homogeneous masses, probably in later stages of digestion (Pl. 14 Fig. 2). Small darkly stained bodies, possibly mitochondria, are often seen in the cytoplasm. Although mitochondria are usually destroyed by acetic-acid fixatives, YOUNG (1928) found that this is not always the case. This

would probably account for their presence in *Heteronema* after the use of Schaudinn's fixativeif these bodies are actually mitochondria.

Nucleus and Kinetic Elements.

The nucleus is generally somewhat ovoid, rather than spherical; its shape varies to some extent with the form of the organism, as is shown particularly in Fig. 1_{12}. Such changes in nuclear form in metabolic organisms might be expected. In a typical interphase the nucleus is located slightly posteriad of the middle of the cell, but at the beginning of division it is found in a more anterior position in close proximity to the base of the reservoir. So far as the writer's observations extend, a nuclear membrane is present in all phases of the life cycle. Within this membrane are two types of structures, the scattered chromatin granules, and the centrally located endosome (Minchin, 1922; Calkins, 1926). This term is used as the equivalent of Keuten's (1895) "Nucleocentrosome", "Karyosome" of Bělař (1916), "Binnenkörper" of Doflein (1916) and Tschenzoff (1916). The interspace is probably filled with karyolymph in life, since it is stained lightly following fixation and is thus set off sharply from the hyaline area immediately surrounding the nucleus.

The endosome, as indicated in Text-Fig. 1, varies greatly in form and appearance. It may be single or fragmented, the number of fragments often exceeding six. They may be small or large, and are usually of irregular shape. This endosome (or its fragments) is often vacuolated. A single fragment may contain one (Text-Fig. $1_{10,11}$) or several vacuoles (Text-Fig. $1_{9,14}$). When lightly stained, a dividing endosome appears to contain a number of small perforations, each surrounded by a dark ring. These vacuoles were seen at all stages of nuclear division in about half of the specimens examined, although they were relatively less abundant in telophases. This simulates the condition found in *Euglena spirogyra*, in which Ratcliffe (1927) reports the presence of a vacuole only in the interphase and prophase. Tschenzoff (1916) figured vacuoles in the endosome of *Euglena viridis*. There is no evidence in the writer's material that a centriole or other body is present in any of these vacuoles.

In *Heteronema acus* it appears that the nucleus contains fewer endosomal fragments in division than in resting stages. This suggests probable transition stages (Text-Fig. $1_{6,13}$) between frag-

mented (Text-Fig. 1_7) and single endosomes (Text-Fig. 1_1). If it is true that fusion occurs it will be noted that in many cases (Text-Fig. $1_{14, 15, 17}$) nuclear division proceeds without total fusion of all the endosomal fragments.

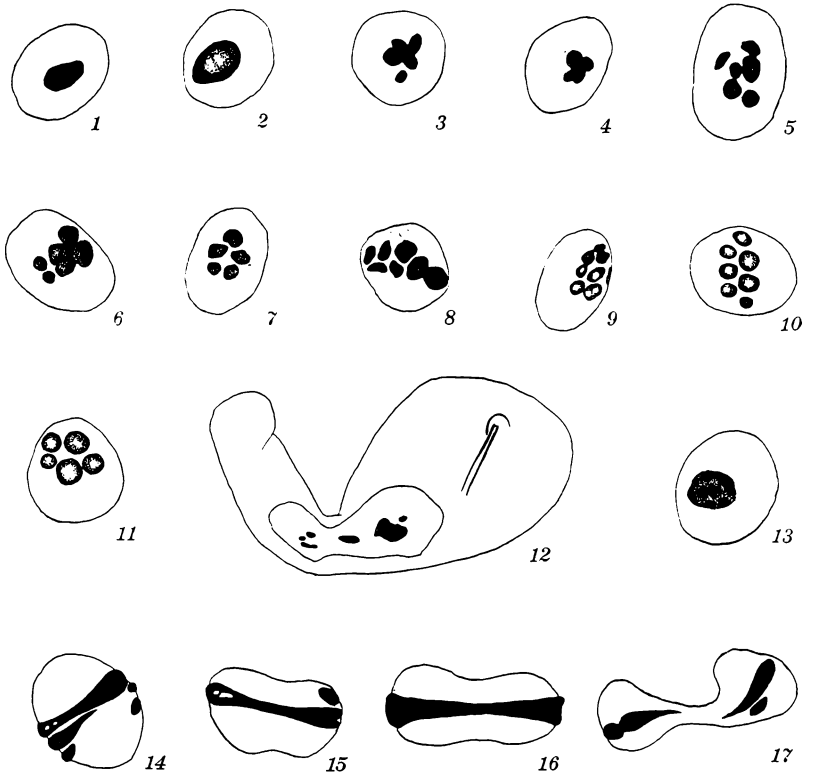

Fig. 1. *Heteronema acus.* Camera lucida drawings showing variations in form of nucleus and endosome. SCHAUDINN's fixation followed by iron-alum hematoxylin and Bordeaux red. 1467 : 1. 1—13 Nuclei in interphase. 1—2 Single endosome. 3—11 Fragmented endosome. 2, 9, 10, and 11 Vacuoles present in endosome or in fragments of same. 12 Stage in metaboly showing how the shape of the nucleus may vary with the form of the organism. 14—17 Dividing nuclei. Vacuoles present in 14 and 15; elongated endosomal fragments in 14, 15, and 17.

Chromatin is present in the form of small granules arranged irregularly around the endosome (Pl. 14 Figs. 1, 6). These granules are destined to give rise to the chromosomes in early prophase, when linear juxtaposition of the granules becomes evident.

Heteronema acus has two flagella, each originating in a blepharoplast at the base of the gullet (Text-Fig. 3_6). Fixed preparations

show little, if any, difference in their length, both being almost as long as the body. No rhizoplast was observed connecting the nucleus with the blepharoplasts.

Pharyngeal-apparatus and Gullet.

One of the structures peculiar to the family Heteronemidae (Calkins, 1926) and also to some of the Astasiidae is the pharyngeal-apparatus, termed "Mundapparat" by Bütschli (1878) and Klebs (1883); "Staborgan" by Lemmerman (1913), Doflein (1916), Rhodes (1926); "pharyngeal rods" by Schaeffer (1918); "perforatorium", Tannreuther (1922); "pharyngeal rod apparatus", Hall and Powell (1928); and "rod-organ" by Brown (1930). In *Heteronema* this structure is always located in close proximity to the gullet and consists of three principal parts, as previously pointed out by Rhodes (1926). These are the two parallel rod-like components and the falcate portion of the apparatus. All of these elements lie in the same relative position (dorsal, ventral, or lateral) with respect to the gullet. The two long 'rods' almost always lie parallel to each other and each bears an enlargement at its anterior end (Pl. 14 Figs. 2, 3). At times they may be curved or bent (Text-Fig. $2_{4, 5, 7}$). As for the falcate component, it may vary in curvature and length, its free end very often tapering off to a fine point which fades away into the cytoplasm of the gullet region (Pl. 15 Fig. 7). The basal end is usually in contact with one of the rod-like elements at a point relatively near the anterior end of the latter. When the entire apparatus is seen in a position dorsal to the gullet the free end of the falcate trichite is always curved in a counter clock-wise direction, its basal end being attached to the right longitudinal 'rod'. Reference to textfigure 2 will illustrate this point. In addition to the three 'rods' referred to above, several fibrils were observed. One of these connects the two anterior enlargements of the two parallel components; two other fibrils originate from these enlargements, both terminating at the same point in the wall of the gullet. These fibrillar connections were not always apparent, and unless the organism lies in a very favorable position they may easily be overlooked. In a few instances (Text-Fig. $2_{1, 8}$), they were observed to vary from their usual position. In the first of these figures the fibrils do not terminate in the wall of the gullet. In the second case they appear much thicker than ordinarily and are not in their usual position.

These relationships are probably anomalous since they were observed but once. It should be mentioned here that in many cases the wall of the gullet was more heavily stained on the side on which the fibrils of the apparatus terminated.

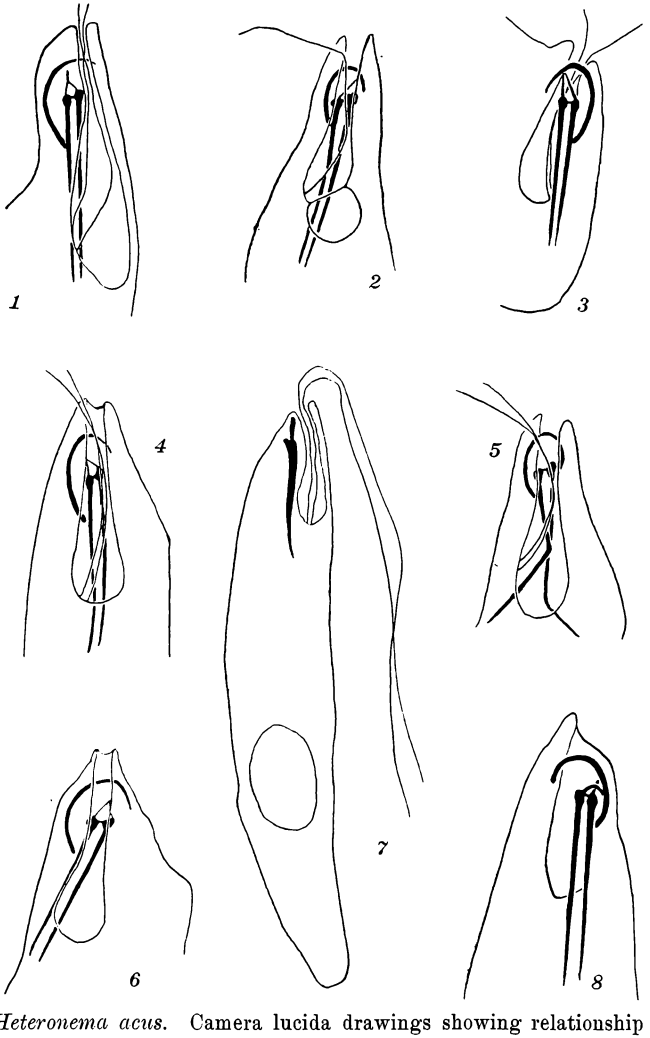

Fig. 2. *Heteronema acus*. Camera lucida drawings showing relationship of gullet and pharyngeal-apparatus. SCHAUDINN's fixative followed by iron-alum hematoxylin and Bordeaux red. 1, 2, 4, 5, and 6 Entire apparatus lies ventral to the gullet. 2 Vacuole at base of the gullet. 5 Unusual shape of the two rod-like elements which are usually parallel. 3, 8 Pharyngeal-apparatus dorsal to the gullet. All above, 1867 : 1. 7 Right lateral aspect; the fibrils shown in 2, 3, 4, and 6 were not apparent in this specimen. 1467 : 1.

Although the outer edge of the falcate segment generally approximates the tip of the organism, the ends of the two parallel parts ordinarily do not, except in certain instances. Other evidence seems to indicate that the latter position occurs during feeding; in such cases (Text-Fig. 3_6) the rod-like elements are very near the anterior end of the organism while the falcate trichite has modified the anterior end of the animal by widening the protoplasmic lip. Such changes would facilitate ingestion of food. In a number of specimens, similar to that shown in Text-Fig. 3_6, there is no evidence for a cytostomal opening separate from the gullet, as was suggested by Rhodes (1926) for *Heteronema*, and also by Brown (1930) for *Peranema*. In one instance (Pl. 14 Fig. 5) an ingested *Euglena* was present in a large food vacuole which was continuous with the lower end of the gullet. This stage indicates that the organism was ingested by way of the gullet, and that the food vacuole was not yet completely separated from that structure. A similar stage of ingestion was reported by Hall and Powell (1927) in *Peranema*.

The protoplasmic lip of the organism mentioned above is a funnelshaped formation terminating in the mouth of the gullet. The lower portion of the gullet is expanded to form the so-called reservoir which is believed to receive the discharge from the contractile vacuole (Text-Fig. 2_2).

Binary Fission.

In the interphase the chromatin granules are evenly distributed around the endosome (Pl. 14 Fig. 6). The endosome, as previously pointed out, may present any one of a number of appearances, and may be either single or fragmented. In the former case it often appears heterogeneous, even though no vacuoles are visible. Perhaps such appearance is due to the endosome being in a process of fusion or fragmentation.

In the prophase the first noticeable change in nuclear appearance of *Heteronema acus* is the alignment of the chromomeres in rows (Pl. 14 Fig. 3). This stage is followed by a change in position of the nucleus, which comes to lie near the base of the gullet. This anterior position of the nucleus is maintained until cytoplasmic division is almost complete, the nucleus thereafter being located more posteriad. Such a migration of the nucleus preceding division has been reported in *Euglena agilis* (Baker, 1926), and *Peranema trichophorum* (Brown, 1930), and in *Euglena spirogyra* (Ratcliffe, 1927).

In early prophase (Pl. 15 Fig. 8) the endosome is distinctly elongated and chromosomes have formed, presumably by fusion of the chromomeres. These later come to lie in an equatorial belt around the elongating endosome. The nucleus has a greater affinity for the iron-alum hematoxlyn stain in the prophases of division than in any other stages in the material examined. BAKER (1926) recorded a similar observation on *Euglena agilis* and suggests that such a staining reaction is due to an increase in bulk and a chemical transformation of the chromatin masses. Apparently a complete fusion of endosomal fragments is not necessary for nuclear division to proceed (Pl. 15 Figs. 9, 10, 12).

While the chromosomes are arranged about the endosome in the above manner they undergo longitudinal splitting. The split appears to be incomplete; that is, one end of each new chromosome thus formed remains attached to the other, giving rise to V-shaped structures (Pl. 15 Fig. 9). The arms of the V's move toward opposite ends of the elongating endosome, whose distal ends always seem to be in contact with the nuclear membrane (Pl. 15 Fig. 10). With further elongation of the endosome the chromosomes are drawn farther and farther apart. In certain instances (Pl. 15 Fig. 14) the chromosomes appear beaded, but ordinarily they are thread-like. Although the ends of the V's were readily distinguishable it was impossible to determine their exact number. An optical cross-section of a nucleus in late prophase (Pl. 15 Fig. 15) indicates that they are very numerous.

The nucleus becomes further elongated and the ends of the chromosome-V's draw apart, accompanying elongation of the endosome. This process results in a straightening out of the V's (Pl. 15 Fig. 14) to form a belt of chromosomes surrounding the endosome. Constriction of the nucleus soon follows (Pl. 16 Fig. 16) and this is accompanied by complete separation of daughter chromosomes in the equatorial zone. This stage might be considered the metaphase in the division of euglenoid nuclei.

In the anaphase (Pl. 16 Figs. 17—19) nuclear constriction continues, the chromosomes remaining parallel to the much elongated endosome. Just before its complete constriction in the formation of two daughter nuclei the chromosomes assume a granular appearance (Pl. 16 Fig. 19). During this phase of division a marked bend in the endosome is visible giving it the appearance of a large U (Pl. 16 Figs. 17—19). This may be due to an influence of the cytoplasmic division which is proceeding posteriorly.

Faint traces of the old nuclear connection still exist in the early telophase (Pl. 16 Fig. 20). The endosome of each half of the old organism has begun to thicken in the center and to take on a more or less spherical form such as appears in later telophases. The chromosomes appear no more as such, granules having been formed from the threads. These granules become scattered about the now centrally located endosome (Pl. 16 Fig 21). This is the same arrangement as is characteristic of the resting nucleus. Longitudinal cytoplasmic fission continues until two daughter organisms result.

Kinetic Elements and Gullet.

During the late prophase the base of the gullet expands (Text-Fig. 3_2). At this stage the blepharoplasts have divided and two new flagella have appeared. The writer was unable to recognize a paradesmose or rhizoplast in either resting or dividing forms. Baker (1926) found a rhizoplast in *Euglena agilis* from late prophase to telophase, and in the later stages an intranuclear rhizoplast is also evident. Hall and Powell (1928) observed a rhizoplast in some of the division stages of *Peranema trichophorum*, and Hall (1923) believed such a structure was present in *Menoidium incurvum*. *Phacus costata* exhibitis a rhizoplast in vegetative stages of its life history, according to Bretschneider (1926). The endosome of the nucleus is now approximately at right angles to the longitudinal axis of the body, but in later stages it assumes a more oblique position. The new flagella continue their growth, becoming coiled within the base of the expanded reservor (Text-Fig. 3_3). This would indicate that one of the two old flagella is retained by each of the daughter organisms, and that another one grows out from each new blepharoplast. In the late prophase growth of the new filaments is complete and separation of the two gullets has taken place, the division having proceeded anteriorly (Text-Fig. $3_{4,5}$). The origin of flagella in different euglenoids will be discussed later.

It is during the prophase that the old pharyngeal-apparatus migrates posteriad and is resorbed (Text-Fig. $3_{2,3}$; Pl. 15 Fig. 12). This is in agreement with the observations of Rhodes (1926). In late prophases a curved element is in evidence which is similar to, and in the position of the falcate element of the pharyngeal-apparatus (Pl. 15 Figs. 12, 14). The entire structure is completely differentiated in the anaphase (Pl. 16 Figs. 18—20). Throughout its early development the pharyngeal-apparatus is very closely associated with the region of the gullet at which its fibrillar connections later appear.

Explanation of figures S. 459.

Division of the gullet, which begins in late prophase, is completed in the anaphase. Division of the cell begins in early anaphase and proceeds from the anterior to the posterior end (Pl. 16 Figs. 17—21). Longitudinal fission occurs whether undigested food bodies are present or absent (Pl. 16).

There was no evidence for encystment in any of the fixed material; neither was there any indication of multiple fission as reported by RHODES (1928).

Discussion.

The significance of the peculiar behavior of the endosome of *Heteronema acus* and of euglenoids in general is a matter of speculation. Bělař (1916) reported a centriole within a small vacuole in the endosome of *Astasia levis*, and this structure was apparent in all stages of nuclear division. In a later paper Bělař (1926) has verified the presence of this structure in the endosome, but doubts that it should be considered a true centriole. Concerning the description by Haase (1910) of an intrakaryosomal centriole and the formation of gametes by intraendosomal mitosis in *Euglena sanguinea* Bělař (1926) states: „Die von G. Haase (1910) beschriebenen Karyosomspindeln und intrakaryosomalen Chromosomen können wohl kaum anders denn als Produkte einer auf unzureichendes Material applizierten Phantasie gedeutet werden." Berliner (1909), in *Copromonas major*, figures centrioles at the ends of the dividing endosome. In most cases, however, he states that these were not visible in the dense karyosome, in contrast to the spindle fibers which were frequently seen. Schüssler (1918) published a similar description of centrioles in *Scytomonas pusilla*. Ratcliffe (1927), in *Euglena spirogyra*, described an endosome with a vacuole containing a large, intensely stained granule. Although a constant feature of the endosome, it could not be traced through division. He described a similar granule outside the endosome; this granule, he believed, gave rise to the new kinetic elements. With the exception of the few accounts mentioned above no intrakaryosomal centriole has been found by the majority of workers on euglenoid nuclei (Blochmann,

Fig. 3. *Heteronema acus*. Camera lucida sketches of stages showing division of the gullet and origin of the new flagella. Schaudinn's fixation followed by iron-alum hematoxylin and Bordeaux red. 1. Gullet and flagella during interphase. 1567:1. 2. Division of the gullet in late prophase. Remnants of the old pharyngeal-apparatus still visible in posterior part of body. Each of the basal granules has divided and two new flagella are growing out. 1467:1. 3. Prophase, slightly later than above. The two new flagella are coiled within the expanded gullet. 2093:1. 4. Outgrowth of the two new flagella complete in late proplase. 1867:1. 5. Rudiments of the rod-like organelles are seen in a stage just before the beginning of cytoplasmic division. Flagella are omitted. 1867:1. 6. Anterior end of *Heteronema* in feeding (?) position, as seen from the left side. Regions of organism indicated as follows: d dorsal; v ventral; r right; l left. 4400:1. 7. Diagrammatic reconstruction of number 6 viewing it from the dorsal side. 4400:1. 8. Diagrammatic reconstruction of number 6 looking directly at anterior end. The circle above the pharyngeal-apparatus represents the cross section of the gullet; flagella are indicated by two dots. 4400:1.

1894; KEUTEN, 1895; TSCHENZOFF, 1916; HALL, 1923; BAKER, 1926; HALL and POWELL, 1928; BROWN, 1930).

KEUTEN (1895) introduced the term "Nucleo-centrosom" for the euglenoid endosome because he believed that the endosome might be compared with the centrosome and central spindle of certain diatoms. The fact that fragmented endosomes occur in *Heteronema acus*, as they do also in *Peranema trichophorum*, according to HALL and POWELL (1928) and BROWN (1930), would not tend to support this idea. Furthermore, no striking changes are noticed in the endosome while the chromosomes are forming in the prophase. The division of the endosome into a number of units, each with one or more vacuoles, throws doubt on the possibility of a single centriole being present in a vacuole and functioning as a division center. TSCHENZOFF (1916) has figured vacuoles in the endosome of *Euglena viridis*, although none of his illustrations show them in such large numbers as they appear in *Heteronema acus*. BĚLAŘ (1916) notes that the endosome in *Peranema trichophorum* is always alveolar in appearance. HALL and POWELL (1928) reported a similar appearance of this structure in *Peranema*, in some of their preparations, and they believe it is due to technical methods. When the endosome is lightly stained, according to the regressive method, the vacuoles are seen as light spheres with dark edges. In a more darkly stained nucleus their presence is not so evident because of the lack of contrast. They are relatively less abundant in the telophase than in other division stages. Whatever bearing this fact may have on the problem is open to conjecture. The alveolar structure of the endosome in *Peranema*, of which BĚLAŘ (1916) and HALL and POWELL (1928) speak, is apparently similar to the vacuolated condition observed in *Heteronema acus*. There is the possibility that such vacuoles may be only the result of inadequate fixation, although the writer does not believe the latter possibility to be the case.

BAKER believes that the endosome is a kinetic reserve mass which contains an endobasal body. The latter leaves the endosome and migrates to the periphery of the nucleus where, in the early stages of division, it divides and ultimately gives rise to all of the neuromotor elements. RATCLIFFE (1927) says the process differs in *Euglena spirogyra* in that the kinetic element which is budded off from the endosome arises during the period of reorganization following division, rather than in the prophase. No evidence for such a phenomenon was observed in *Heteronema acus*. BROWN'S (1930)

concept of the significance of the endosome is somewhat different, as the following quotation indicates: "I have decided that there is a relationship between the centroblepharoplast and the endosome. In other words, the action of the centrosome, or better, the centroblepharoplast, does not initiate mitosis alone; but that this kinetic force is an interaction of both the centroblepharoplast and the endosome as well as intranuclear physiological forces. If such a kinetic mass as the endosome is charged by a type of 'mitokinetism' or any type of electrostatic force, and if that force is associated with other forces which are of a physiological nature, there will be balance between all the forces which may initiate mitosis. Now if this balance is upset and a change in polarity occurs and the endosome is elongated, then this structure will split (Fig. 8 Pl. 20). In all probability the same interacting forces cause the chromosomes to split longitudinally and to 'flow' apart" (Brown, 1930, p. 414). No kinetic complex was observed in *Heteronema acus* by the writer. In numerous instances the endosome was split (Text-Fig. 1_{14}, 3_3; Pl. 15 Figs. 9, 10, 12), but it is impossible to tell whether or not there were any signs of "mitokinetism" in these cases. Lackey's (1929) view, that the endosome of *Entosiphon sulcatum* is "purely trophic . . . not giving rise to the kinetic elements, . . . not even throwing off a portion of its substance into the cytoplasm", is in accord with the present findings in *Heteronema acus*.

Chromosomes.

It was noted that the chromosomes in the late prophase were beaded in some cases, although they were usually of uniform, thread-like appearance. Such appearance is attributed to different degrees of staining. The nuclear membrane was observed to persist throughout division even though it appeared very thin. Tschenzoff (1916) did not find this to be true in *Euglena viridis*, in which form the membrane was not visible during division.

With the exception of Steuer's (1904) account of amitosis in *Eutreptia viridis*, and Dobell's (1908) account of *Copromonas subtilis*, mitotic division has been described for all the forms of the group investigated. In a later account (Dobell and O'Connor, 1921) Dobell's original view appears somewhat modified, and the possibility of a simple mitosis is admitted. Doubling of the chromosome number in euglenoid nuclei takes place either by a transverse or by a longitudinal splitting of the chromosomes during the meta-

phase. HARTMANN and CHAGAS (1910), BĚLAŘ (1916), and LACKEY (1929), in *Peranema trichophorum*, *Astasia levis*, and *Entosiphon sulcatum*, respectively, report the first method. TSCHENZOFF (1916), in *Euglena viridis*; HALL (1923), in *Menoidium incurvum*; BAKER (1926), in *Euglena agilis*; RATCLIFFE (1927), in *Euglena spirogyra*; HALL and POWELL (1928), in *Peranema trichophorum*; and BROWN (1930), in the same species, report longitudinal splitting of the chromosomes. Perhaps certain critical stages which show longitudinal splitting have been overlooked by the investigators who report transverse division, since 'transverse division' in *Heteronema acus* merely represents the end process of an earlier longitudinal split. With omission of such critical stages (Pl. 15 Figs. 9, 10) it would be quite logical to conclude that transverse chromosome splitting is characteristic of *Heteronema acus*. It also appears that the longitudinal splitting occurs during the prophase, as has been described for most of the species of the order Euglenida. TSCHENZOFF'S (1916) description of chromosome splitting is as follows: „bei *Euglena viridis* tritt die Spaltung der Chromosomen in der Anaphase oder Telophase der vorherigen Teilung auf. Die gespaltenen Chromosomen bewahren ihre Individualität durch den Ruhekern hindurch bis zur Metaphase, wo sie paarweise sich lagern und dann auseinander wandern". WILSON (1925, p. 138) is doubtful as to whether or not an anaphasic or telophasic split actually occurs even in metazoan nuclei. RATCLIFFE (1927), however, finds support for TSCHENZOFF'S view in *Euglena spirogyra*.

Origin of New Flagella.

Accounts of the origin of new flagella in fission of euglenoids vary widely. In some cases the old axial filaments are said to be discarded and later replaced by outgrowths of new ones from blepharoplasts. Certain workers believe that the original flagellum is retained by one of the daughter organisms and that additional flagella arise by outgrowth; others report that the old axial filament splits, each half thus being retained by a daughter cell.

DOBELL (1908) says the new flagella grow out from the centrioles of *Copromonas subtilis* following a resorption of the old one. BERLINER (1909) reports the same process taking place in *Copromonas major* after the old flagella are thrown off. A similar origin of flagella has been described by SCHÜSSLER (1918) in *Scytomonas pusilla*. SCHAEFFER (1918), in *Jenningsia diatomophaga*, reports that

in one instance of reproduction one of the daughter organisms inherited the old flagellum. Hall (1923) states that in *Menoidium incurvum* the semblance of splitting of the old flagellum is more probably due to outgrowth of a new filament in close conjunction with the old, rather than to actual splitting of the old flagellum. Baker (1926) reports the formation of new filaments in *Euglena agilis* following loss of the original flagellum. Hall and Powell (1928) report the retention of the old flagellum by one of the daughter organisms in *Peranema trichophorum*. Lackey (1929) remarks that in division of *Peranema* the old filament is resorbed or thrown off and new flagella are developed. The case of *Entosiphon sulcatum* (Lackey, 1929) is unique, in that one of the daughter organisms retains both old flagella, while two new ones grow out from the basal bodies of the other. Another point to be noted is that new flagella of *Entosiphon* do not attain their full length until just prior to cell division, whereas, in *Heteronema acus* they have attained full length at the onset of the metaphase.

Steuer (1904) was the first investigator to report splitting of the two flagella in division. His figures of *Eutreptia viridis* afford inadequate evidence that new flagella arise in this fashion rather than by outgrowth. Ratcliffe's (1927) concept of the origin of new flagella in *Euglena spirogyra* is as follows: "The flagellum shortens until it is drawn into the exterior opening of the reservoir. The mass at the bifurcation disappears and the blepharoplasts move apart so that the axial filaments form an inverted V. The intranuclear bodies bud off masses which pass through the nuclear membrane to the base of the reservoir where they become the blepharoplasts of the daughter organism. New axial filaments grow out from these to unite with the original ones, and the flagellum splits longitudinally, thus forming the new flagella." A shortening of the flagellum might indicate that resorption of that structure was taking place. Ratcliffe's account is interesting, since no other investigators have reported partial resorption of the flagellum prior to splitting or other method of flagellar formation in fission. It should be pointed out that the figures which he uses as evidence for flagellar splitting might also be interpreted as illustrating outgrowth of new flagella. On account of the possibility of two different interpretations of the same evidence, it is obvious that Ratcliffe's figures do not afford definite proof of the splitting of the flagellum. In *Heteronema acus* the outgrowing filaments are seen attached to blepharoplasts in the prophase. In earlier stages

of their formation (Text-Fig. 3_2) the new outgrowths could possibly be interpreted as originating by a split of the old flagellum, but a later stage (Text-Fig. 3_3) definitely shows them to be coiled within the expanding reservoir. Such a coiled position could not be accounted for on the basis of a 'split-fibril' theory.

The Pharyngeal-apparatus.

It was mentioned above that members of the family Heteronemidae and some of the Astasiidae are characterized by a peculiar structure. BÜTSCHLI (1878) and KLEBS (1883) refer to it as the "Mundapparat". LEMMERMAN (1913), DOFLEIN (1916), and RHODES (1926), call it the "Staborgan"; SCHAEFFER (1918) designates it as the "pharyngeal rods". TANNREUTHER (1922) applies the term "perforatorium". HALL and POWELL (1928) refer to it as a "pharyngeal rod apparatus", but BROWN (1930) objects to the use of this term and suggests that the term 'Staborgan' or "rod-organ" be applied. In the present discussion the term pharyngeal-apparatus will be used to designate this structure for reasons which will be made clear presently.

BÜTSCHLI (1878) described and figured an organelle of *Astasia tricophora* (now known as *Peranema trichophorum*) consisting of two closely placed lines. These, he believed, are the walls of an associated gullet tube, the opening of which is at times marked by a bright circle. At the anterior end of this double streak he figured a curved element which runs toward the base of the flagellum. In this picture the three rod-like elements are clearly visible. He mentioned a gullet tube closely associated with the pharyngeal-apparatus, but he figured no additional anterior tube from which the flagella are known to originate. If the other figure of his (number 19 b), in which a food body is being ingested, is studied, it will be noted that the food body is being taken in (apparently into the opening from which the flagellum emerges) by an anterior modified lip which is similar to some of the anterior lip modifications of *Heteronema acus* which were figured above. What BÜTSCHLI designates "the elongated pharyngeal-tube for the taking up of nourishment", described in close association with the pharyngeal-apparatus, might very well be what is ordinarily termed the gullet, which leads into the reservoir.

According to the observations of KLEBS (1883), „stehen die Stäbe nicht mit einer Schlundröhre in Verbindung, noch bilden sie

eine solche, sondern stellen ein Organ für sich dar, das der Innenfläche der Membran anliegt und als Hilfsapparat bei dem Hineinschaffen der Nahrung in den Mund und von da direkt in den Körper dient." The description of this organelle would indicate that the pharyngeal-apparatus lies on the inner surface of the gullet — the only tubular structure present. It was seen in the record of observations that *Heteronema acus* possessed only one anterior opening — that leading into the reservoir. Except for the fact that the pharyngeal-apparatus of *Heteronema* lies in the cytoplasm outside the gullet (with its anterior fibrillar connections leading to the wall of the gullet) there is no striking dissimilarity between the present account and Klebs' description of this apparatus in *Peranema*. Klebs did not, however, observe any fibrillar connections.

Both Bütschli and Klebs referred to this structure as the "Mundapparat", because of its close relationship to the mouth, or pharynx, in feeding. The word 'rod' is not altogether disignatory since these longitudinal elements vary from the usual appearance of rods in that each has a knob-like enlargement at one end and tapers off to a fine point posteriorly. Also, they are often sharply bent or curved. Furthermore, both fibrillar and rod-like elements make up this apparatus. The term 'organ' is likewise misleading in protozoan terminology. Tannreuther (1922) applied the term "perforatorium" because he believed that in *Peranema* this structure pushes the cuticle out with it and actually perforates the prey. Remarking on the feeding habits of *Jenningsia diatomophaga* Schaeffer (1918) says: "Although I saw a number of instances of feeding and took particular pains to see whether the pharyngeal rods were actually protruded, or were used merely in distending the mouth, I was unable to determine their exact function. I incline to think however, that the rods were not protruded beyond the mouth opening". Obviously the basis for the term "perforatorium" is doubtful. It is for these reasons that the writer has preferred the use of the term pharyngeal-apparatus (= "Mundapparat" of Bütschli and Klebs).

Rhodes (1926) states: "The 'Staborgan' of *Heteronema acus* functions as a true mouth or cytostome. It is separate from the reservoir . . . the opening of the reservoir in all *Euglenoidina*, Blochmann (Euglenida, Stein), is quite generally called the mouth or cytostome. This is an error, for the Peranemidae, characterized by the 'staborgan', which is a true cytostome, and the opening of

the reservoir should be otherwise designated." The detailed evidence upon which the statements in the above abstract are based has not yet been published; hence a comparison of his evidence with the findings here reported is impossible. HALL and POWELL (1927, p. 159) make this comment concerning the pharyngeal-apparatus — "while it always seems to lie outside the gullet as observed by RHODES in *Heteronema*, ends anteriorly in the margin of the cytostome in *Peranema*. Our figure 4 (Pl. 2) shows an ingested *Chilomonas* (?) lying in a large vacuole continuous with the gullet. . . . This figure is so suggestive of the process of food vacuole formation from the base of the gullet, already described by TANNREUTHER, that we believe we are justifiably skeptical in regard to RHODES' interpretation, insofar as he applies it to the Peranemidae as a group". One of the figures cited in this paper (Pl. 14 Fig. 5) would indicate a method of food-intake in *Heteronema acus* similar to that described by HALL and POWELL (1927) for *Peranema trichophorum*.

BROWN'S (1930) account contains this quotation: "I agree with KLEBS (1883) and RHODES (1926) that the 'staborgan' and gullet of *Peranema* are not connected in any way with the reservoir, but that the cytostome is a separate opening on the ventral side of the body." According to the present investigation there is no evidence to indicate that there is a 'cytostome' separate from the opening which leads to the reservoir in *Heteronema acus*. Moreover, KLEBS' (1883) account is not at all in accord with BROWN'S description, and it would appear that the latter has misquoted the earlier paper.

During the prophase the old pharyngeal-apparatus is seen in the posterior region of the cell. The anlage of the new organelle appears as a curved element, similar to the falcate trichite, in the late prophase. According to RHODES (1926) each pharyngeal apparatus differentiates from the cytoplasm in early telophase. BROWN (1930) finds that in *Peranema* new 'rods' are formed in the early anaphase and during the telophase the falcate 'rod' is formed by outgrowth from one of the longitudinal components. It is to be noted that in some of BROWN'S figures (Pl. 20 Fig. 11; Pl. 21 Fig. 13) the new elements are in very close relationship to the neck of the gullet. Evidence cited in this paper shows a similar close relationship in *Heteronema acus*, in which the present investigation revealed no 'cytostome' apart from the opening which eads to the reservoir.

Summary.

The morphology of *Heteronema acus*, a colorless, biflagellate euglenoid is compared to that of other *Euglenida* in both resting and division stages. A nuclear membrane is present in all phases of the life cycle observed. The endosome of the nucleus may be single or fragmented and very often it is vacuolated. Fusion of the endosomal fragments is believed to occur in the early prophase. There is no evidence that the endosome gives rise to an endobasal body which in turn gives rise to new kinetic elements.

The chromomeres of the resting nucleus surrounding the endosome give rise to chromosomes in the prophase. They become arranged in a belt about the elongating endosome and undergo an incomplete longitudinal splitting. The arms of the V's thus formed are drawn toward opposite poles of the elongating endosome until a final separation occurs at the apex of each V in the metaphase. Chromatin granules reappear in the anaphase and telophase.

The pharyngeal-apparatus is located entirely on one side of the gullet. It consists of three main 'rods', earlier described by Rhodes (1926), and several additional fibrillar connections. When the entire apparatus is seen in a position dorsal to the gullet, the free end of the falcate trichite is always curved in a counter clock-wise direction while the basal end is usually in contact with the right longitudinal component. One of the fibrils connects the knob-like anterior enlargements of the two longitudinal 'rods'. Two other fibrils originate from these enlargements and both terminate at the same point in the wall of the gullet. There is no evidence for a cytostomal opening existing separate from the gullet. On the contrary it appears that the pharyngeal-apparatus is instrumental in modifying the lip of the organism to facilitate food ingestion through the mouth and gullet, and thence into the body of the protozoan. During division the old pharyngeal-apparatus is resorbed and new ones have begun to form in late prophase.

New flagella arise during division as outgrowths from blepharoplasts. The origin of new flagella in other *Euglenida* is discussed and it is pointed out that a splitting of the old flagellum in this group is a very improbable occurrence.

No cysts were observed, nor was there any evidence of multiple fission.

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Explanation of Plates.

Plate 14—16.

Heteronema acus: Camera lucida drawings of specimens fixed in Schaudinn's fluid and stained with iron-alum hematoxylin and Bordeaux red. Nuclei are shown in optical section. Unless otherwise stated, magnification 2800 : 1.

Plate 14.

Fig. 1. Nucleus in interphase. Pharyngeal-apparatus anteriorly located. A darkly stained line visible below gullet. Falcate segment of the apparatus almost at right angles to the long parts of the apparatus.

Fig. 2. Interphase. Endosome heterogeneous. Seven food vacuoles present. Pharyngeal-apparatus in normal position close to the neck of the reservoir.

Fig. 3. First indications of prophase in the alignment of the chromatin granules. Two food vacuoles present. 2200 : 1.

Fig. 4. Nucleus in interphase. Funnel-shaped protoplasmic lip terminates in the gullet. Rod-like portions of the apparatus extend to tip of the organism. 2200 : 1.

Fig. 5. Optical section of an organism which has ingested a Euglena. Vacuole surrounding the contained organism is continuous with the reservoir of the gullet.

Fig. 6. Interphase nucleus, lightly stained, showing scattered chromatin granules and a vacuolated endosome.

Plate 15.

Fig. 7. Early prophase. Endosomal fragments almost completely fused. Fusion of chromatin granules to form chromosomes has begun.

Fig. 8. Endosome elongated. Chromosomes completely formed. Base of gullet slightly enlarged. Nucleus lies near gullet. This position is maintained until fission is complete.

Fig. 9. Chromosomes are split longitudinally; separation of arms of V.'s has begun. Endosome fragments not entirely fused.

Fig. 10. Further separation of the split chromosomes, with the apices of the V.'s perpendicular to and pointing away from the longitudinal axis of the endosome.

Figs. 11 and 13. Later stages showing the elongation of the endosome and the further drawing apart of the chromosomes.

Fig. 12. Same as above. Endosome in two fragments. Old pharyngeal-apparatus visible in posterior region of cytoplasm. 2200 : 1.

Fig. 14. Late prophase with belt of chromosomes surrounding elongated endosome. Chromosomes present a beaded appearance. Formation of the new gullets complete. New trichites evident. One food vacuole present.

Fig. 15. Optical cross section of nucleus in stage similar to Fig. 14. Approximate chromosome number indicated not counted.

Plate 16.

Fig. 16. Metaphase. Chromosomes have separated and endosome of nucleus is elongating. Constriction of the nucleus is taking place. 2200 : 1.

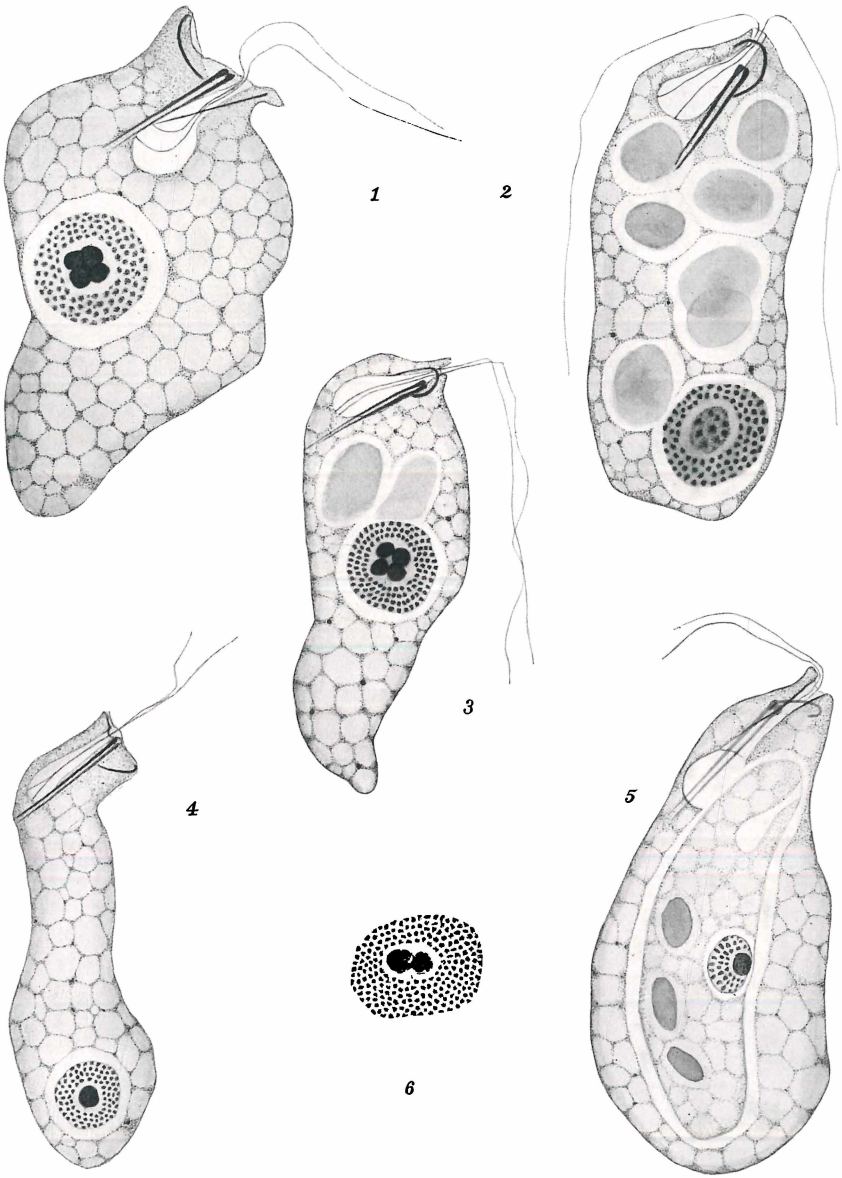
Fig. 17. Anaphase. Cytoplasmic division has begun. Old pharyngeal-apparatus lies posterior to the nucleus and is almost completely resorbed. A new one is developing in close contact with the neck of each reservoir. 2200 : 1.

Fig. 18. Middle anaphase, showing further constriction of the nucleus. New pharyngeal-apparatus developing; old apparatus no longer visible.

Fig. 19. Late anaphase. Chromosomes no longer visible as such. Granules present. Endosome vacuolated. Nuclear constriction almost complete. Food vacuoles present.

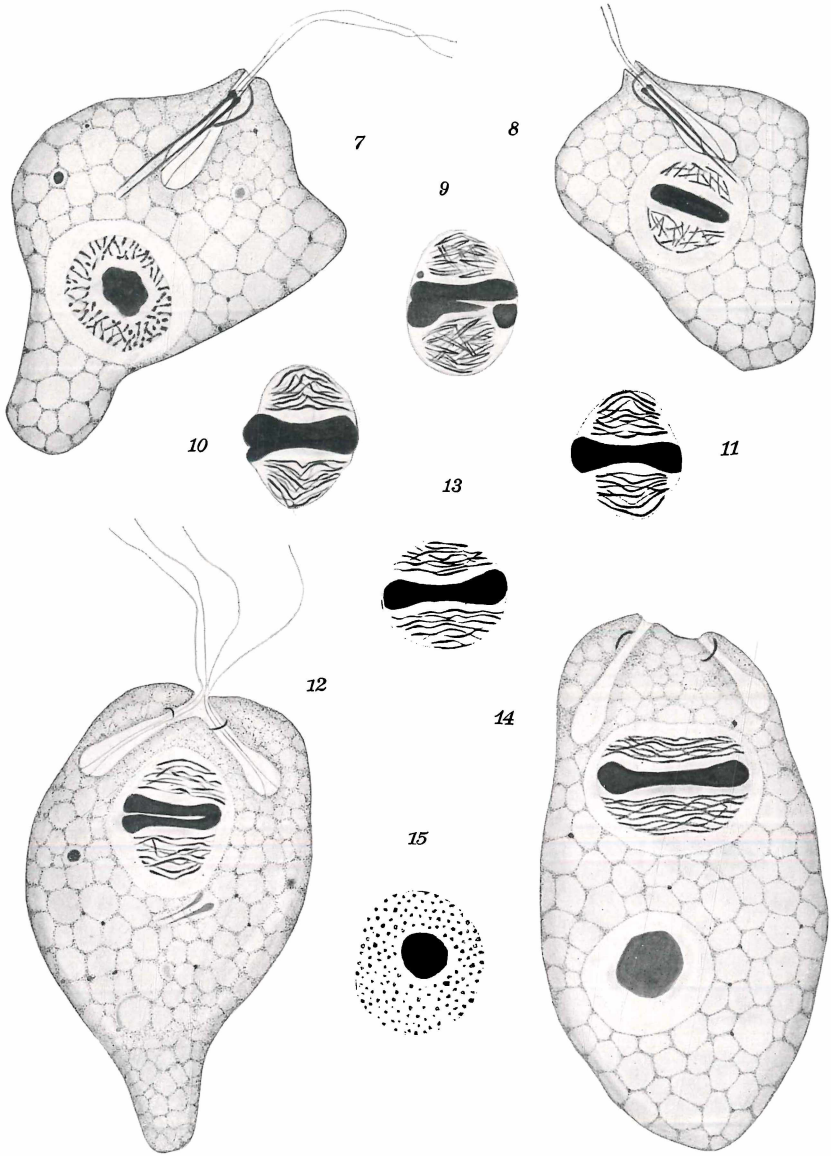
Fig. 20. Telophase. Endosome completely divided. Cytoplasmic division more advanced. Food vacuole present. 2200 : 1.

Fig. 21. Late telophase. Cytoplasmic division almost complete. Daughter nuclei have become spherical. Chromatin granules show arrangement somewhat similar to that of interphase.



Loefer.

VERLAG VON GUSTAV FISCHER IN JENA



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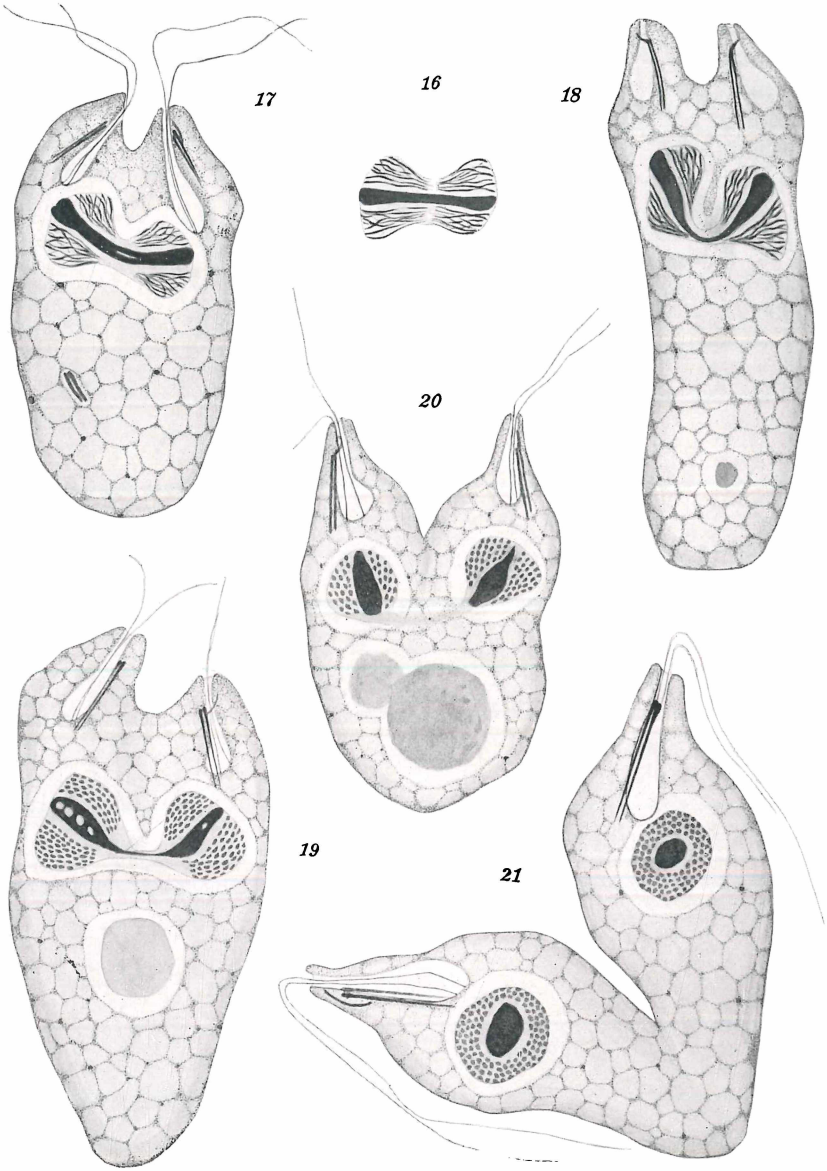
VERLAG VON GUSTAV FISCHER IN JENA



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