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Morphology and life history of *Colacium vesiculosum* Ehrbg.

By

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(With 20 figures in the text and plate 7—9.)

Introduction.

Colacium vesiculosum, a small flagellate belonging to the family Euglenidae, was first described by Ehrenberg (1838). Stein (1878) redescribed it and added two new species, *C. arbuscula* and *C. calvum*, to the genus. The species studied by these workers were represented as without a distinct flagellum. The presence of a single flagellum was pointed out later by Kent (1880) in the forms described previously and also in a new species which he described as *C. steinii*. Dangeard (1902) stated that during its free living phase *C. vesiculosum* resembles *Euglena viridis*, but differs from the latter in the distribution and in the structure of its chloroplasts which are discoid. Lemmermann (1913) described reproduction by longitudinal fission in the stalked colonial stage, and stated also that cysts were unknown. Schiller (1924) described division in both the free-swimming flagellated stage and in the nonflagellated condition, but not in the colonial attached stages as described by Kent (1880), Dangeard (1902) and Lemmermann (1913). Schiller objected to the erection of the genus *Colacium* because the organism, in his opinion, is a typical *Euglena*. Pascher (1925) listed the characteristics of *C. ovale*, *C. arculata* and *C. elongatum*, previously described by Playfair (1921).

Since little is known about the cytology of *Colacium* and the complete life cycle has not been traced for any species in the genus, further study was accordingly undertaken with reference to the detailed morphology, nuclear division and successive stages in the life history of *C. vesiculosum*. The writer wishes to express his deep appreciation to Dr. R. P. HALL for his suggestions during the course of this investigation.

Material and methods.

The strain of *C. vesiculosum* was obtained originally from the laboratory of Prof. Dr. E. G. PRINGSHEIM. Bacteria-free stock cultures were maintained on starch-agar slants. From these, transplants were made into 500 cc. flasks containing about 250 cc. of liquid medium. Difco "tryptone" (3 grams per liter of tap water), tryptone plus sodium acetate (0.1—0.2 %), JAHN's (1931) salt solution and modifications of it were used as culture media. Hanging-drop preparations were utilized in studying morphology and fission in living material, and the centrifuge method was used in the concentration of material in fixation and staining. The fixatives of SCHAUDINN (both hot and cold method), BOUIN, CHAMPY, ALTMANN and DA FANO were used. Among the stains tried were iron-alum hematoxylin, DELAFIELD's hematoxylin, REGAUD's hematoxylin, BORREL's stain and safranin, with the following as counterstains: Bordeaux red, eosin, light green, orange G and acid fuchsin. The method described by HALL and POWELL (1928), using Bordeaux red followed by iron-alum hematoxylin, has been most successful as a nuclear stain and for demonstration of the flagellar apparatus. The ALTMANN-REGAUD method (see Figs. 9—12) has been used to advantage in studying the gullet. The organisms were usually mounted in euparol.

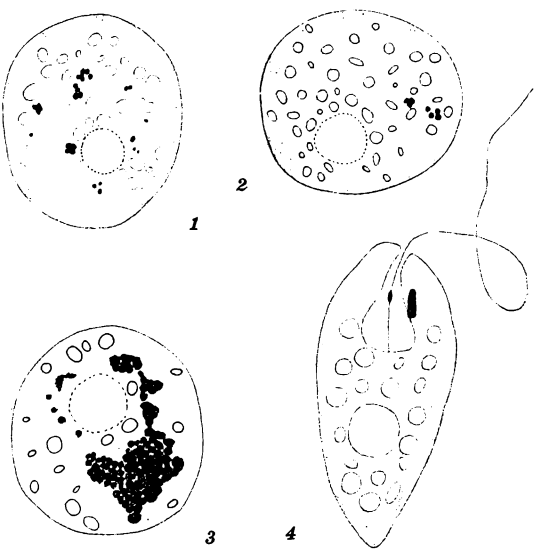
Vital staining was carried out with Janus green B and neutral red. Slides were filmed with a solution of the dye in absolute alcohol, according to the method described by HALL (1929). A drop of culture material was then placed on the slide, and a coverslip added. The edges of the coverslip were sealed with melted vaseline. In such preparations the organisms remained alive for several days. Osmic vapor was also used in studying inclusions by means of the hanging-drop method. Permanent preparations of cytoplasmic inclusions were made by the DA FANO silver impregnation method, and by fixing in CHAMPY's fluid and staining in iron-alum hematoxylin.

Morphology.

The description of *C. vesiculosum*, as given by Lemmermann (1913), is as follows:

„Zellen meist mit dünner Gallerthülle, auf einfachen oder verzweigten Gallertstielchen, ohne Gehäuse. 1 Geißel, nur im frei beweglichen Zustand vorhanden. Chromatophoren scheibenförmig mit Paramylonkernen. Augenfleck vorhanden. Vakuolensystem wie bei *Euglena*. Vermehrung durch Teilung im unbeweglichen Zustand. Dauerzellen nicht bekannt. . . . *Colacium vesiculosum* Ehrbg. — Zellen ei- bis spindelförmig, 19—20 μ lang, 9—17 μ breit, auf kurzen Gallertstielen. Bewegliche Zellen spindelförmig, 22 μ lang, 12 μ breit, mit etwas über körperlang Geißel".

The strain used in the present investigation conforms in general to this description, except that colonial stages were not found.



Figs. 1—4. Camera-lucida drawings from living material; × 2300. 1. Typical palmella stage showing chromatophores, paramylum bodies and accumulated pigment at the end of three weeks. Nucleus indicated in outline. 2. Palmella stage from young culture; normal amount and distribution of pigment. 3. Similar stage from old culture showing accumulation of pigment in large clumps which are brownish in color. 4. Typical flagellate, showing chromatophores, paramylum bodies, stigma, gullet, and flagellum with large flagellar swelling at the level of the stigma.

Flagellated stage.

The flagellate is characteristically euglenoid in appearance, spindle-shaped, anteriorly somewhat blunt, broadest in the middle, and tapering posteriorly (Text-Fig. 4). It is usually two or three times as long as broad, ranging from 9 to 12 μ in width and from

17 to 32 μ in length, the average being about 23—26 μ in length and 11 μ in width. A large flask-shaped gullet, from which a single flagellum arises, is present in the anterior part of the body. The flagellum is non-bifurcated, and arises from a small blepharoplast at the base of the reservoir. A lens-shaped swelling is present on the flagellum near the level of the stigma. From the blepharoplast, a rhizoplast extends to an extranuclear centrosome on the nuclear membrane (Pl. 7 Fig. 1). The rhizoplast and granule on the nuclear membrane are difficult to detect, and were seen only in well-stained Bordeaux red-iron hematoxylin preparations. This system of flagellum, blepharoplast and rhizoplast has been termed the neuromotor system in other euglenoids, e. g., *Menoidium incurvum* (HALL, 1923), *Euglena agilis* (BAKER, 1926) and *Peranema trichophorum* (HALL and POWELL, 1928). A similar system was described in *Phacus costata* by BRETSCHNEIDER in 1926.

Usually seven bright-green chromatophores, each containing a small round or ovoid pyrenoid, are distributed regularly around the periphery of the cell (Pl. 7 Fig. 1), and their color masks the entire organism except for the colorless anterior tip of the flagellated stage. The chromatophores are elongated, thickest in the middle and tapering at the ends; in living material they are distinctly longer than in stained preparations, the smaller size in the latter probably being due to shrinkage in fixation. In cells undergoing fission the chromatophores are more numerous, 8—10 being present in prophases and anaphases (Pl. 7 Figs. 3—8), and as many as 13—14 have been observed in late telophases (Pl. 8 Figs. 13, 14). The number seems to increase progressively during the stages of nuclear division, and the characteristic number is always present in the completed daughter cell. Although actual division of the chromatophores has not been observed, the fact that they increase in number during mitosis might suggest division of these structures paralleling the division of the nucleus. The reddish-orange stigma is located lateral to the gullet, at or near the level of the flagellar swelling. Other noticeable inclusions of the endoplasm are the numerous spherical or ovoid paramylum bodies.

These organisms are very active and metabolic. The flagellum is always extended forward when the organism is in motion; movement is swift and dart-like, and involves spiral rotation as in other Euglenidae. Frequently the swimming ceases, the organism becoming very metabolic for a time, and then spiral locomotion begins anew.

Palmella stage.

The palmella, or euglenoid "division-cyst", stages are inactive, rounded or ovoid cells covered by a thin gelatinous membrane, and ranging from 11 to 19 μ in their greatest diameter, with an average of 15—16 μ (Text-Fig. 2; Pl. 7 Fig. 2). Chromatophores and paramylum bodies are as in the flagellated stage. In old cultures, or in cultures in which the food supply has been diminished, the flagellates all change to palmella stages, which either sink to the bottom or adhere to the sides of the flask and secrete a substance cementing them together in a pavement-like layer of cells (Pl. 9 Fig. 23).

Numerous pigment granules, identical in color with the stigma of the flagellated stage, are scattered throughout the endoplasm of the palmella forms. In some of the flagellated individuals the stigma appears to be made up of a number of small granules; in others this granular nature is difficult to detect. Hall and Jahn (1929) found that the stigma of *Euglena gracilis* varied in the size and number of its component granules, and in the division-cyst stages was present in the forms of dispersed granules, either loosely grouped together or widely scattered. This dispersed phase of the stigma is apparently present in the palmella stages of *Colacium vesiculosum* (Text-Fig. 2), in which the color and size of the pigment granules agree closely with the granules present in the stigma of the flagellated forms. A condensed stigma, like that of the flagellated forms has not been observed in the palmella stages. As the age of the cultures of *C. vesiculosum* increases the amount of pigment increases, and a change in color is noticeable. For the first two or three weeks the amount of pigment is apparently normal, and it possesses the bright reddish-orange color characteristic of the stigma of flagellated forms. At the end of four weeks the pigment granules appear more numerous (Text-Fig. 1), and some of them have assumed a dark brown color. At the end of six weeks all of the granules are brown, and at the end of three months the organisms are filled with large clumps of pigment which is now a brownish-black color (Text-Fig. 3).

Multinucleate stages.

In the examination of cultures of *C. vesiculosum* the attention is often attracted to very large palmella forms, measuring from 21 to 42 μ in diameter, and usually surrounded by the thin membrane which is characteristic of the palmella stage. In fixed preparations these forms are seen to possess from 2 to 8 nuclei (Pl. 9 Figs. 26, 28).

These large cells are irregular in shape, show sluggish ameboid movement, and possess from 11 to 17 chromatophores. Large flagellated stages with two or four nuclei are also occasionally found (Pl. 8 Figs. 19, 20). Further discussion of these multinucleate forms will be taken up later.

Nucleus.

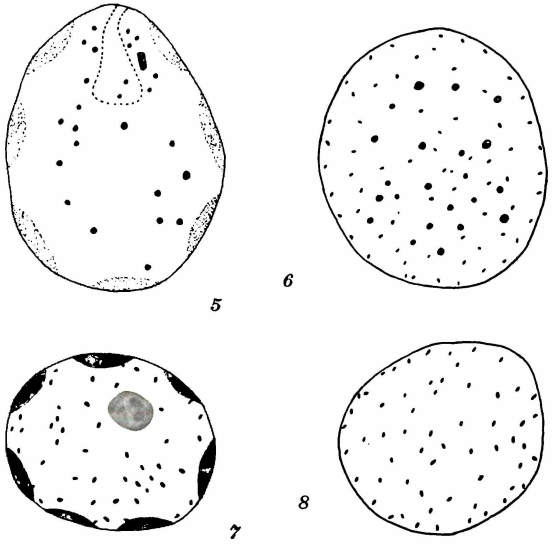
The nucleus of *Colacium vesiculosum* is situated in the posterior half of the flagellated stage (Pl. 7 Fig. 1), and is somewhat eccentrically located in the palmella stage (Pl. 7 Figs. 2, 3). Its structure is typical for euglenoids, and it is spherical or somewhat ovoid in shape. A lightly staining, but definite, nuclear membrane is present in all stages of the life cycle. Within the nuclear membrane is a heavily staining endosome surrounded by evenly scattered chromatin granules. The endosome varies greatly in shape and appearance. It may be single or fragmented into two, three or more parts. This condition is similar to that found in the nucleus of *Peranema trichophorum* (HALL and POWELL, 1928), *Heteronema acus* (LOEFER, 1931) and *Euglena leucops* (S. R. HALL, 1931). WENRICH (1924) described nuclei in *Euglenamorpha hegneri* with multiple endosomes, which he believed led to amitotic division of the cell. In *Colacium vesiculosum*, however, the fragmented endosome seems to be the natural condition of the interphase nucleus, the single or non-fragmented endosomes being common only in early division stages or in those in which division is just completed. Fragmentation of the endosome has not been observed in nuclei undergoing mitosis.

Cytoplasmic inclusions.

With the aid of vital dyes, inclusions of various sizes and shapes, irregularly distributed throughout the endoplasm, have been observed in *Colacium vesiculosum*. When using neutral red, small globules appear about three to five minutes after applying the dye, first at the anterior end and later throughout the entire organism. When first seen they are very lightly stained, but the intensity of the color increases rapidly and after 15 minutes they have reached the optimum differentiation. If these same neutral-red globules are then exposed to osmic acid they will blacken (Text-Fig. 5). A drop of the culture is placed on a coverslip filmed with dilute neutral red. When the neutral-red globules become clearly defined, the coverslip is inverted over a small drop of osmic acid in a depression slide, according to the method described by HALL (1929). Twenty to

thirty minutes later the globules show signs of darkening. Twelve hours later the globules are gray in appearance, and are completely blackened after about two days. At this time the entire organism has usually taken on a muddy-gray tinge. At the end of five days the majority of the cells are blackened completely so that the globules are no longer visible. In some, however, they are still apparent, and the green background of the chromatophores is still visible.

When Janus green B is used as a vital stain, small, bluish, rod-shaped bodies appear after a few minutes exposure to the dye (Text-Fig. 8). They are regularly distributed near the surface of the cell, and do not appear to lie deep in the cytoplasm as do the neutral-red globules; in optical section they are seen to lie just within the periplast. A similar location of the mitochondria was described by Hall (1929) in *Peranema trichophorum*. After fixation in Champy's fluid and staining in iron-alum hematoxylin, well destained specimens of *Colacium* (Text-Fig. 7) reveal small rod-shaped inclusions, similar in size and distribution to the inclusions which are stained vitally with Janus green.



Figs. 5—8. Camera-lucida drawings; × 2300. 5. Organism stained vitally with neutral red and then exposed to osmic acid. 6. da Fano silver impregnation. 7. Champy fixation followed by iron-alum hematoxylin. 8. Janus green B preparation.

In material impregnated by the da Fano silver method two types of inclusions are often blackened (Text-Fig. 6); these are similar to the inclusions seen in vitally stained material. Small rod-shaped bodies are distributed just beneath the periplast, much the same as the inclusions seen in Janus green preparations. Larger globules are located deeper in the endosome and resemble the neutral-red globules. The differences in size and distribution of the two types

of inclusions in DA FANO preparations make it possible to distinguish one from the other.

When a mixture of Janus green B and neutral red is used in vital staining, the small rod-shaped inclusions are the first to react, staining with Janus green, while the larger globules stain with neutral red a few minutes later. The small, rod-shaped inclusions are stainable vitally with Janus green and are demontrated by the use of CHAMPY'S fixative followed by iron-alum hematoxylin, and are also often blackened in DA FANO silver impregnation. It is concluded that these are the mitochondria. The globular inclusions may be stained vitally with neutral red and are also impregnated by osmic and silver methods; these inclusions have been termed the vacuome by certain workers. The vacuome has been demonstrated previously by GRASSÉ (1925) in *Euglena proxima*, by DANGEARD (1928) in *Euglena granulata*, by HALL (1931) in *Euglena gracilis* and *Menoidium incurvum*, and by others.

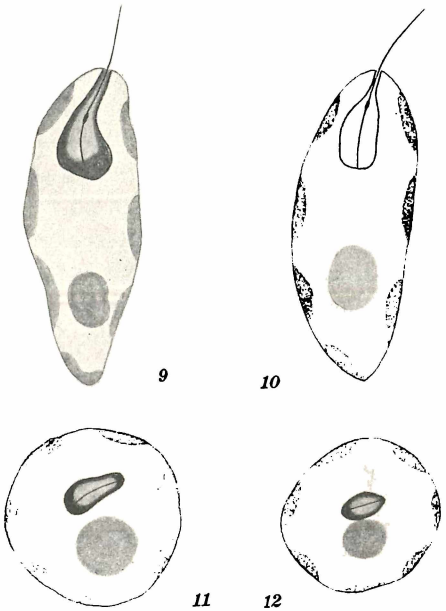
Life history.

According to KENT (1880), LEMMERMANN (1913), SCHILLER (1924), and others, *C. vesiculosum* is normally found in pond water on Cyclops, Daphnia, and other small fresh water crustacea, and occasionally on rotifers. Members of the genus *Colacium* are encountered rarely, but have been reported from England, Germany and Australia.

In *C. vesiculosum* there is a reversal of the condition usually found in Euglenida, the palmella form supplanting the flagellate stage as the dominant phase of the life cycle. The palmella stages were always the most numerous in the material used in this investigation. The palmella stages give rise to flagellated cells, multinucleate forms and other palmella stages. The daughter palmella cells resulting from binary fission are enclosed within a single membrane. Very often the cells become separated, as in *Euglena* divisioncyst stages; in other cases the cells remain within the single membrane and undergo further division. The latter are relatively numerous in cultures, and stages in which one of the daughter cells has completed fission and the other is still dividing are frequently observed (Pl. 9 Fig. 22). In young, rapidly growing cultures these stages separate into the individual daughter cells after a time, but in old cultures remain together and by further division ultimately give rise to a pavement-like layer of cells (Pl. 9 Fig. 23).

The plasmodial stages arise by a failure of the cytoplasm to divide following nuclear division in the palmella cells. Since fission

is not initiated until nuclear division is complete, it seems plausible that the two daughter nuclei might frequently divide again before cytoplasmic fission occurs. This is apparently what happens, since cells with two nuclei undergoing mitosis are frequently observed (Pl. 7 Fig. 9, Pl. 7 Fig. 2). Such a process would result in a plasmodium with four nuclei (Pl. 9 Fig. 26), and division of the four nuclei would result in a plasmodium with eight nuclei (Pl. 9 Fig. 28). Occasionally plasmodia with three, five, six or seven nuclei are observed. These may arise by the nuclei of a two- or four-nucleate plasmodium dividing at different times, or they may be plasmodia in which budding has occurred, the number of nuclei being reduced by one each time a mature bud is separated from the plasmodium. Thus a trinucleate plasmodium may arise from a binucleate stage by the division of only one of the nuclei, or it may be produced from a tetranucleate form after one bud has been pinched off. In like manner the plasmodia with five, six or seven nuclei may be accounted for.



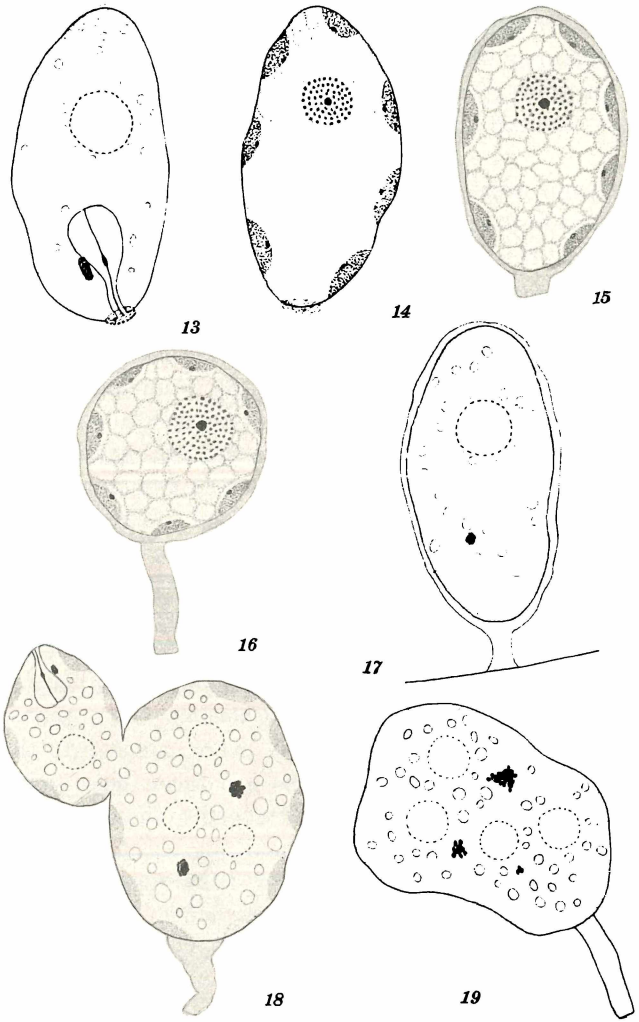
Figs. 9—12. Camera-lucida drawings from Altmann-Regaud preparations; × 2300. 9. Flagellate showing gullet and reservoir intensely blackened. 10. Same organism in optical section. 11, 12. Cells in which the gullet and reservoir have been partially resorbed. A portion of the flagellum is still visible within the blackened area.

The flagellated stages arise either by fission of the palmella cells or by budding of the plasmodial forms. A few hours after division has occurred, the transition from the flagellated stage to the palmella stage may be observed. The flagellates cease their swimming and become very metabolic. Close observation of these forms shows that after a short time the external portion of the flagellum is discarded, the organisms become less active, and later round up. In reference to this process Schiller (1924) made the following statement: „Öfters wird auch zwischen das *Euglena*- und

Colacium-Stadium ein in der Dauer nach nicht festgestellten, amöboides Stadium eingeschaltet." These ameboid stages of SCHILLER are evidently the metabolic transition stages described above. The gullet can be detected for several minutes after the flagellum has been discharged, and appears to lie deep in the cytoplasm without an external opening. A similar stage in this transition was observed in material prepared by the ALTMANN-REGAUD method. In such preparations the gullet is intensely blackened and its relation to the rest of the organism is clearly seen. Text-Fig. 9 shows the normal flagellate with the gullet blackened, and figure 10 an optical section of such a stage. Text-Figs. 11 and 12 show two rounded individuals in which the gullet has been partially resorbed, stages similar to those observed in living material. In a later stage the gullet disappears completely, and the quiescent rounded palmella stage results. SCHILLER (1924) observed in part the transition from the flagellate to the palmella stage, and concerning this process made the following statement: „Wenngleich die Vorgänge beim Übergang aus einem in das andere Stadium, wie oben gesagt wurde, gut gesehen werden konnten, gelang es doch nicht, die Aufgaben des Eug-Stadiums und des Eintrittes ins Col-Stadium zu erkennen." The transition from the discarding of the flagellum, through the gradual resorption and disappearance of the gullet, to the palmella stage has been followed in living material and has been confirmed in stained preparations. In this transition it would be difficult to determine the point at which the organism ceases to be a "*Euglena*-Stadium" (SCHILLER) and becomes a "*Colacium*-Stadium" or palmella stage. During this change into the palmella stage the organism secretes a thin, gelatinous covering which does not become noticeable until the forms are rounded.

In a few cultures the transition as described above was accompanied by the formation of almost transparent stalks, apparently continuous with the gelatinous membrane surrounding the cell. In an individual which has recently discharged its flagellum a ring of small globules is present around the cytostome (Text-Fig. 13). This is interpreted by the writer as the beginning of stalk formation, and similar forms were described and figured by SCHILLER. In a later stage the globules lose their identity and a small mass of clear substance appears at the anterior end, while the gullet is diminished in size (Text-Fig. 14). Sometimes gelatinous membranes surround the organisms and short stalks of 1.5—2.0 μ are present at the anterior end (Text-Fig. 15). In some of these forms the gullet and

reservoir can be detected with difficulty, but in the majority are entirely absent. This would suggest that stalk formation, when it



Figs. 13—19. Camera-lucida drawings of stages in formation of the stalk; 14 and 15 from Bordeaux red-iron hematoxylin preparations; 13 and 16—19 from living material; × 2300. 13. Specimen which has discharged the external portion of the flagellum; a ring of small granules is present around the cytostome. 14. Gullet and reservoir disappearing; a mass of clear substance is present around the anterior tip of the organism. 15. Short stalk and membrane present; gullet no longer visible. 16. Rounded specimen with a long stalk. 17. Stalked form as found attached to the shell of a crustacean. 18 and 19. Stalked plasmodial forms, one with a mature bud ready for separation.

occurs, parallels the resorption and disappearance of the gullet and reservoir during the transition from the flagellate to the palmella stage. Stalked forms were found in only a few cultures and these contained a considerable amount of solid material in the medium when the cultures were inoculated. This observation suggests that the solid particles present in the media provided a stimulus for stalk formation, since stalks were never observed in the clear salt or peptone solutions.

An attempt was made to establish the normal association between *C. vesiculosum* and the small crustacea to which it is normally attached. For this purpose *Daphnia* and *Cyclops* were placed in rapidly dividing cultures of *Colacium*, and the flagellates were introduced into a balanced aquarium containing numerous *Daphnia*, *Cyclops* and other small crustacea. Specimens of *Colacium* were placed also in stender dishes with crustacea from the aquarium. In the aquarium and in the stender dishes the environmental conditions approximated the normal habitat of *Colacium*. An examination of the culture flasks at the end of 48 hours showed numerous *C. vesiculosum* attached by short transparent stalks to the shells of the crustacea, and others in the process of attachment. At the end of 72 hours the cultures became so contaminated with bacteria that the crustacea died. In the aquarium fewer forms were observed to attach to the crustacea, presumably due to the relative scarcity of the protozoa. The stender dishes proved more satisfactory than larger aquaria for establishing the association of *Calacium* with the crustacea. Heavy inoculations from agar slants were made into the stender dishes. At the end of four days the crustacea were colored green due to the large number of *Colacium* attached to their shells; numerous forms were also found attached to small pieces of debris present in the culture. Examination of crustacea from these cultures showed that, although many *Colacium* were attached by short stalks, the majority were non-stalked palmella stages which were attached by means of the slimy covering which encloses these forms. This was a constant feature for all the crustacea examined. The attachment of *C. vesiculosum* to the shell of the crustacean is a condition of symbiosis according to SCHILLER, *Colacium* being brought in contact with a changing food supply by movement of the crustacea and the latter using the protozoa to some extent as food.

The stalks of the forms attached to the crustacea were very short, 2—3 μ in length, and often difficult to detect. This is in agreement with the observations of SCHILLER (1924), who made the

following statement: „Die von mir beobachteten Stadien ... seltener bildeten sie kurze Gallertstiele, und nie sah ich in diesem Teiche so lange Gallertstiele wie sie Lemmermann in der Süßwasserflora abbildet.“ However, the stalks of *Colacium* appearing in the culture flasks varied from 2 to 12 μ in length. The longer stalks were small in diameter while the short stalks were thick. Stalks were observed frequently on plasmodial stages (Text-Fig. 19) as well as on the palmella and transition stages. Stalked, budding plasmodial forms (Text-Fig. 18) were occasionally observed on the shells of the crustacea and were more numerous in the cultures. Often two buds, almost as large as the mother cell, were observed on these forms, a condition suggestive of the stalked colonial stages described by other investigators. However, the flagellum develops in the distal end of the bud, while Kent (1880), Dangeard (1902) and Lemmermann (1913) described colonies attached at the anterior end.

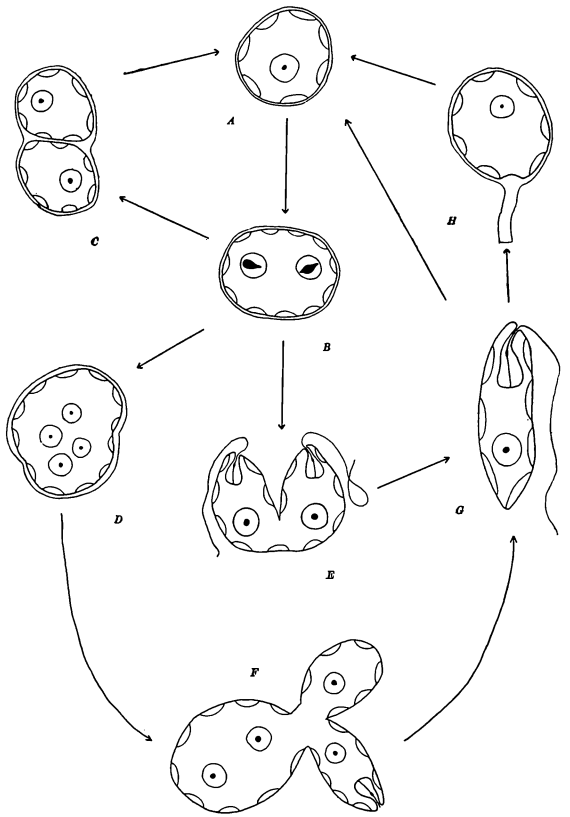


Fig. 20. Diagram of the life cycle of *Colacium vesiculosum*. A Palmella stage. B Telophase stage; daughter nuclei completely separated. C Two daughter cells enclosed within a single membrane and arising from B by binary fission. D Plasmodium arising by multiplication of nuclei in B. E Daughter flagellated cells before separation, arising by binary fission from B. F A budding plasmodium. G Flagellated cell arising by binary fission from E or by budding from F. H Stalked stage; may be interposed before the flagellates (G) transform into the palmella stage (A)

The life history of *C. vesiculosum* is rather involved, including as is does the formation of free-swimming flagellates, quiescent palmella stages, large plasmodial forms, and stalked individuals (Text-Fig. 20). Reproduction is rapid and may be accomplished by (1) simple binary fission, giving rise to flagellates and palmella stages, or (2) budding, giving rise to flagellates. All the stages involved in the life cycle were present in the culture flasks and were also found attached to the shells of the crustacea and in the media of the stender dishes. Cysts were not observed.

Nuclear division.

With the initiation of nuclear division the fragments of the endosome fuse to form a heavily-staining mass in the center of the nucleus. The chromatin granules fuse to form short thread-like chromosomes, situated first around the periphery of the nucleus (Pl. 7 Fig. 3); later the threads become arranged irregularly around the slightly elongated endosome (Pl. 7 Fig. 4). The endosome elongates further until its ends come in contact with the nuclear membrane. With the chromosomes arranged around the endosome in this manner they undergo longitudinal splitting. The split is apparently not complete, one end of each daughter chromosome thus formed remaining attached to the other, giving rise to V-shaped structures (Pl. 7 Fig. 5). Following this split the free ends of the chromosomes migrate toward the opposite poles of the endosome. In the next stage observed the chromosomes are arranged parallel to the long axis of the endosome which extends beyond the ends of the chromosomes (Pl. 7 Fig. 6). The nucleus becomes further elongated and the ends of the chromosomes draw further apart, resulting in a straightening of the V's to form a belt of chromosomes around the endosome (Pl. 7 Figs. 7, 8). In some of the nuclei at this stage the chromosomes appear as homogeneous threads (Pl. 7 Fig. 8), and in others they appear to be made up of a number of chromomeres (Pl. 7 Fig. 7). The granular nature of the chromosomes at the end of the prophase agrees with that reported by LOEFER (1931) in *Heteronema acus.*

In the metaphase a slight constriction appears in the elongated nucleus, accompanied by a complete separation of the daughter chromosomes in the equatorial zone (Pl. 7 Fig. 10). This process is apparently very rapid, for in preparations containing numerous stages of division, such a phase is rarely seen.

In the anaphases (Pl. 7 Figs 9, 11) the chromosomes migrate toward the ends of the much elongated endosome and remain parallel

to it. At this stage the endosome becomes definitely thinned in the middle, and the chromosomes are massed around the ends of the endosome which have become enlarged. During the late anaphase the endosome is bent, giving the appearance of an S-curve.

In the early telophases (Pl. 8 Fig. 12) faint traces of the old nuclear connections still exist. The endosome of the daughter nucleus has begun to thicken in the center, while the chromosomes are no longer present as such, granules having been formed from the threads. The endosome next contracts and becomes centrally located (Pl. 8 Figs. 13, 14), and the daughter nuclei assume the appearance of the interphase nucleus.

Cytoplasmic division.

The first indication of cytoplasmic division is the appearance of a longitudinal split occurring in the telophase after the two daughter nuclei are completely separated. In preparations containing dividing forms these telophases are always numerous. Fission takes place in two different ways, the first giving rise to palmella stages and the second to flagellated forms. In some individuals the longitudinal split appears to start from opposite sides of the cell (Pl. 8 Fig. 15); in these forms the constriction ultimately gives rise to two daughter palmella cells enclosed within a single membrane (Pl. 8 Fig. 16). Similar stages were observed by Schiller (1924).

In others the split appears only at one end (Pl. 8 Fig. 17), the end destined to become the anterior end of the daughter organisms, and the plane of fission is more or less perpendicular to the old axis of nuclear division. As the separation continues a clear space appears in the cytoplasm at the anterior end of the daughter cells, and as this space increases in size a developing flagellum appears within it. In a later stage the gullet and flagellum have developed completely (Pl. 8 Fig. 18), and separation of the two daughter cells is almost complete, the nuclei having assumed the characteristic interphase appearance. Schiller (1924) described what he believed to be division in the flagellated stage in living material. From his description and from his figures it is evident that what he really observed were late telophase stages as described above. In the writer's material examination of living forms from 8 to 12 P. M. showed many late telophases; in such stages the split is apparant at the anterior end, and as separation continues a flagellum is developed in each of the daughter organisms. The forms become very metabolic; in a short time separation is complete and the daughter

cells swim rapidly away. On first consideration of this evidence it appears that division is taking place in the flagellated stage as SCHILLER (1924) concluded. However, thorough examination of stained preparations shows that only the nuclei of the palmella stages are undergoing mitosis, and that the flagellum appears only in the late telophase after nuclear division is complete. Nuclear division in the flagellated stage has never been observed.

Budding.

The large multinucleate or plasmodial forms, as previously stated, give rise to flagellated stages by a process of budding. These plasmodia have from two to eight nuclei, the majority having four, and apparently arise from the palmella stage by a failure of the cytoplasm to divide after nuclear division. Cells in which two nuclei are undergoing mitosis are not uncommon (Pl. 8 Fig. 21). At certain periods, small evaginations or buds appear on the surface of the plasmodium (Pl. 9 Fig. 24). A nucleus migrates into the bud, which grows larger; as the bud increases in size the gelatinous membrane surrounding the organism is ruptured, being no longer visible in stages with large buds (Pl. 9 Fig. 25). Two buds are frequently observed on the same plasmodium. In a later stage a gullet and flagellum are developed in the distal end of the bud (Pl. 9 Fig. 27). The bud is now elongated and resembles the typical flagellated stage in appearance and in morphological details; separation from the mother cell soon follows. This process has been observed throughout its entirety in living material and this evidence has been confirmed in stained preparations. The plasmodial forms are numerous in all cultures and are evidently a natural phase of the life cycle of *C. vesiculosum*.

Occasionally multinucleate forms possessing a gullet and flagellum are encountered (Pl. 8 Figs. 19, 20). These may possibly arise by division of the nucleus in the flagellated stage without cytoplasmic fission, or by the development of a gullet and flagellum after the multinucleate condition is attained. Since nuclear division in the flagellated stage has never been observed, the first hypothesis seems less plausible. In budding and in binary fission resulting in flagellated forms, the daughter cells develop a gullet and flagellum, and it is possible that in a plasmodium ready for budding the formation of gullet and flagellum might occasionally precede the actual budding process. Whether or not this is what actually happens is difficult to determine, since these forms occur rarely and are perhaps abnormalities.

Discussion.

Kent (1880), Dangeard (1902) and Lemmermann (1913) reported division by longitudinal fission in the attached colonial stages of *C. vesiculosum*. Schiller (1924) did not observe this type of division; nor did he observe two individuals on a common stalk, the necessary result of such a division. However, he described division of the "*Euglena*-Stadium", the common flagellated form of *Colacium*, as well as the division of non-flagellated stages resulting in daughter flagellated cells and also daughter palmella cells enclosed in a single membrane. This latter process is similar to the division observed in the writer's material. Binary fission, according to Schiller, is completed in from 40 seconds to five minutes. In the writer's material division required one and a half hours or more; however, the actual fission of the cell, from the first appearance of the longitudinal split to the complete separation of the daughter cells, required only a few minutes. Since Schiller's conclusions were based entirely on living material, and since such a short division period as he reported has not been described in other euglenoids, it is possible that he has interpreted the late telophase stages as the entire process, and that his supposed division of the "*Euglena*-Stadium" is the end product of division of the non-flagellated palmella stages.

Binary fission in Euglenida occurs usually in the flagellated stage and evidence of constriction of the cell body is present in prophases. In such forms fission of the cell continues, paralleling the division of the nucleus. In *C. vesiculosum* binary fission occurs only in the palmella stage, and cleavage is not initiated until the late telophase, at which time the daughter nuclei are completely separated and are approaching the normal interphase condition. Although fission of *C. vesiculosum* is delayed beyond the usual time, the division of the nucleus is typically euglenoid and agrees in general with that reported in other forms: *Euglena viridis* (Tschenzoff, 1916), *Menoidium incurvum* (Hall, 1923); *Euglena gracilis* (Tannreuther, 1923), *Euglena agilis* (Baker, 1926), *Peranema trichophorum* (Hall and Powell, 1928), *Heteronema acus* (Loefer, 1931), *Euglena leucops* (S. R. Hall, 1931), and others. Binary fission of the stalked forms, as reported by previous investigators in *C. vesiculosum*, has not been observed.

According to Kent, Dangeard and Lemmermann the stalked colonial stages are the most prevalent forms of *C. vesiculosum*, and in their opinion such forms constitute the dominant phase of the

life cycle. SCHILLER (1924) maintained that this was incorrect, and that the flagellate stage is the typical vegetative form of *Colacium*. Concerning this he made the following statement: „Wenn man bisher das *Colacium* als die eigentliche vegetative Form des Flagellaten ansah, so kommt die Schuld dem Planktonnetz zu, das die Krebse mit der *Colacium* fängt, das *Euglena*-Stadium jedoch durchschlüpfen läßt." In the writer's material, which is a pure line, the colonial stages reported by former investigators have not been observed; however, single stalked individuals were present in some of the material examined. These forms occurred infrequently, and were never as numerous as the other stages present in the culture. This suggests that stalk formation is facultative and not a constant feature of *Colacium* as previously supposed. The difficulty that SCHILLER pointed out, namely, that the flagellated stages escape when material is collected, is overcome by the writer's method. The palmella stages are always the most numerous, and in older cultures the flagellated forms are entirely absent. Since binary fission occurs only in the palmella forms, it is evident that they are the dominant and typical vegetative stage of the life cycle. The brief flagellated stage is transitory, lasting only a few hours, or one or two days at the most, and then transforms directly into the palmella condition, in which some of the organisms are stalked. This transient nature and the fact that during the transition to the palmella stage specimens may become attached to the shells of crustacea, suggests that the flagellated forms may serve as a means of distribution in changing from one crustacean host to another.

HALL and JAHN (1929 a) pointed out that in a typical uniflagellate chlorophyll-bearing euglenoid, the flagellum bifurcates near the level of the stigma, and that at or near the point of bifurcation there is a flagellar swelling which is usually directly opposite the stigma. The structure of the flagellar apparatus of *C. vesiculosum* differs from that of other uniflagellate Euglenidae in that the flagellum is not bifurcated; however, it does have the flagellar swelling opposite the stigma. This swelling, like the gullet, is very large in comparison to the size of the organism.

The occurrence of plasmodial forms which reproduce by budding, a constant feature of the writer's cultures, has not been reported in other Euglenida. In the Mastigophora budding is uncommon, but has been reported in a few species. PASCHER (1913), for example, described budding in *Palatinella cyrtophora*, a chrysomonad, as the

normal method of reproduction. In this form budding involves the formation of small daughter cells unlike the mature individual in size and appearance. Kofoid (1920) described budding in *Noctiluca milaris*, a dinoflagellate, in which the parent organism dies after giving rise to numerous daughter cells. In this species many nuclei are formed by repeated mitotic division, following which several hundred buds are formed as protuberances on the surface of the cell. Elliott (1934) described stages with two, three and four nuclei in *Haematococcus pluvialis*; these cells divide into as many daughter cells as there are nuclei, and the process sometimes involves budding with the formation of flagella. He stated that such forms occurred in old cultures only and were apparently abnormal. The buds of *Colacium*, like those in *Noctiluca*, develop into complete flagellated individuals before separation from the parent cell; in the former, however, not more than two buds are formed at once, and the buds approach the size of the normal flagellated stage. Although the process is unusual in Mastigophora, budding appears to be a normal feature of one phase of the life cycle of *C. vesiculosum*.

There has been a tendency on the part of some investigators, Schiller (1924) in particular, to regard *Calacium* as a typical *Euglena*. Kent (1880) stated that in all essential details *Colacium* agrees with simple free-swimming species of *Euglena*. Dangeard (1902) maintained that the structure of *C. arbuscula* is similar to that of *Euglena gracilis*; that *C. calvum* possessed discoid chloroplasts with paramylum as in *E. velata* and related species, and that *C. vesiculosum* possessed the organization of *E. viridis*. Schiller (1924) made the following statement concerning the systematic position of *Colacium*: „Vom ökologischen und phylogenetischen Standpunkte hat die Gattung *Colacium* kaum Berechtigung, denn sie ist eine typische *Euglena*, die sich eben bequem handhabe." On the other hand, observations on the morphology and life history of *C. vesiculosum* indicate that it differs from other Euglenidae in four major respects. First, the flagellum is non-bifurcated, whereas a bifurcated flagellum is characteristic of species of *Euglena*. Second, the palmella stage supplants the common flagellated form of euglenoids as the vegetative stage of the life cycle. Third, the plasmodial stage involving budding, and fourth, the stalked forms are unparalleled in members of the genus *Euglena*. This evidence indicates that *C. vesiculosum* is not a typical *Euglena* as previously supposed, and also that Schiller's conclusions were based on insufficient evidence. It is concluded, therefore, that the genus *Colacium* is justifiable.

The specific description of *C. vesiculosum*, as given by LEMMERMANN (1913), may be emended by the following additions:

(1) seven to ten (commonly seven) elongated chromatophores regularly distributed around the periphery of the cell, and each with a small spherical or ovoid pyrenoid near the internal surface;

(2) non-bifurcated flagellum, one and a half to two times the length of the body, and possessing a large flagellar swelling at the level of the stigma;

(3) rounded or slightly ovate paramylum bodies, rather large and numerous:

(4) large gullet, curved at anterior end;

(5) occurrence of large multinucleate stages which give rise to flagellates by budding.

Summary.

The life history *C. vesiculosum* includes free-swimming flagellates, quiescent palmella stages, large plasmodial forms and stalked forms. Reproduction is rapid and may be accomplished by (1) simple binary fission, giving rise to flagellates and palmella stages, or (2) by budding, giving rise to flagellates. Binary fission occurs only in the palmella forms, which supplant the flagellate type as the dominant vegetative phase of the life cycle. Plasmodial forms arise by a failure of the cytoplasm to divide following mitosis. Cells with 2—8 nuclei were described, giving rise to flagellates by a process of budding. Single stalked individuals and stalk formation were described, but stalked colonial stages were not observed. No cysts were seen. The non-bifurcated flagellum was pointed out as an exception to the condition usually found in uniflagellate Euglenidae.

Nuclear division is typically euglenoid and involves longitudinal splitting of the chromosomes. A nuclear membrane is present in all phases of the life cycle. Cytoplasmic division does not occur until late telophase, and may start from both ends of the cell and result in daughter palmella cells, or it may start from only one end resulting in daughter flagellated individuals.

It was pointed out that the morphology and life history of *C. vesiculosum* differ from those of other Euglenidae in four major respects, and on the basis of this evidence it was concluded that the genus *Colacium* is justifiable.

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Description of plates.

Plate 7—9.

Colacium vesiculosum: camera-lucida drawings from material fixed in SCHAUDINN's fluid and stained with iron-alum hematoxylin and Bordeaux red; ×2300, unless otherwise stated.

Plate 7.

Fig. 1. Flagellated stage. Nucleus in interphase; flagellum with swelling and ending in blepharoplast at the base of the gullet; rhizoplast extending to extranuclear centrosome on nuclear membrane.

Fig. 2. Palmella stage. Nucleus in interphase.

Fig. 3. Early prophase. Beginning of fusion of chromatin granules to form chromosomes.

Fig. 4. Later prophase, with endosome elongated and chromosomes formed; eight chromatophores present.

Fig. 5. Chromosomes are split longitudinally and separation of the arms of the V's has begun; endosome further elongated.

Fig. 6. Further separation of chromosomes which are becoming arranged parallel to the elongated endosome; nine chromatophores present.

Figs. 7, 8. Late prophases. Chromosomes further separated and extending to the ends of the endosome; granular nature of chromosomes evident in fig. 7.

Fig. 9. Large cell with two dividing nuclei; one nucleus in early anaphase and the other at a 45° angle to it; only one end of the latter is visible in the figure.

Fig. 10. Metaphase. Chromosomes are completely separated.

Fig. 11. Anaphase. Chromosomes migrating to the ends of the endosome which is S-shaped and with enlarged ends.

Plate 8.

Fig. 12. Early telophase. Chromosomes replaced by chromatin granules; traces of old nuclear connection still present.

Fig. 13. Later telophase. Endosome completely divided.

Fig. 14. Later stage. Endosomes of daughter nuclei approaching resting appearance.

Fig. 15. Cytoplasmic division beginning at both ends of the cell.

Fig. 16. Division completed; the two daughter palmella cells enclosed within a single membrane.

Fig. 17. Telophase, with cytoplasmic fission beginning at one end only; clear spaces, within which are developing flagella, have apeared in the daughter cells.

Fig. 18. Later stage, with gullet and flagellum completely formed in each daughter cell.

Fig. 19. Large flagellated cell with four nuclei.

Fig. 20. Flagellated cell with two nuclei.

Fig. 21. Large cell with two nuclei undergoing mitosis. One nucleus in late prophase and other in early anaphase.

Plate 9.

Fig. 22. Three cells within a single membrane; condition resulting from division of two-cell palmella stage.

Fig. 23. Pavement-like layer of cells as found on the sides and botton of culture flask; $\times 1100$.

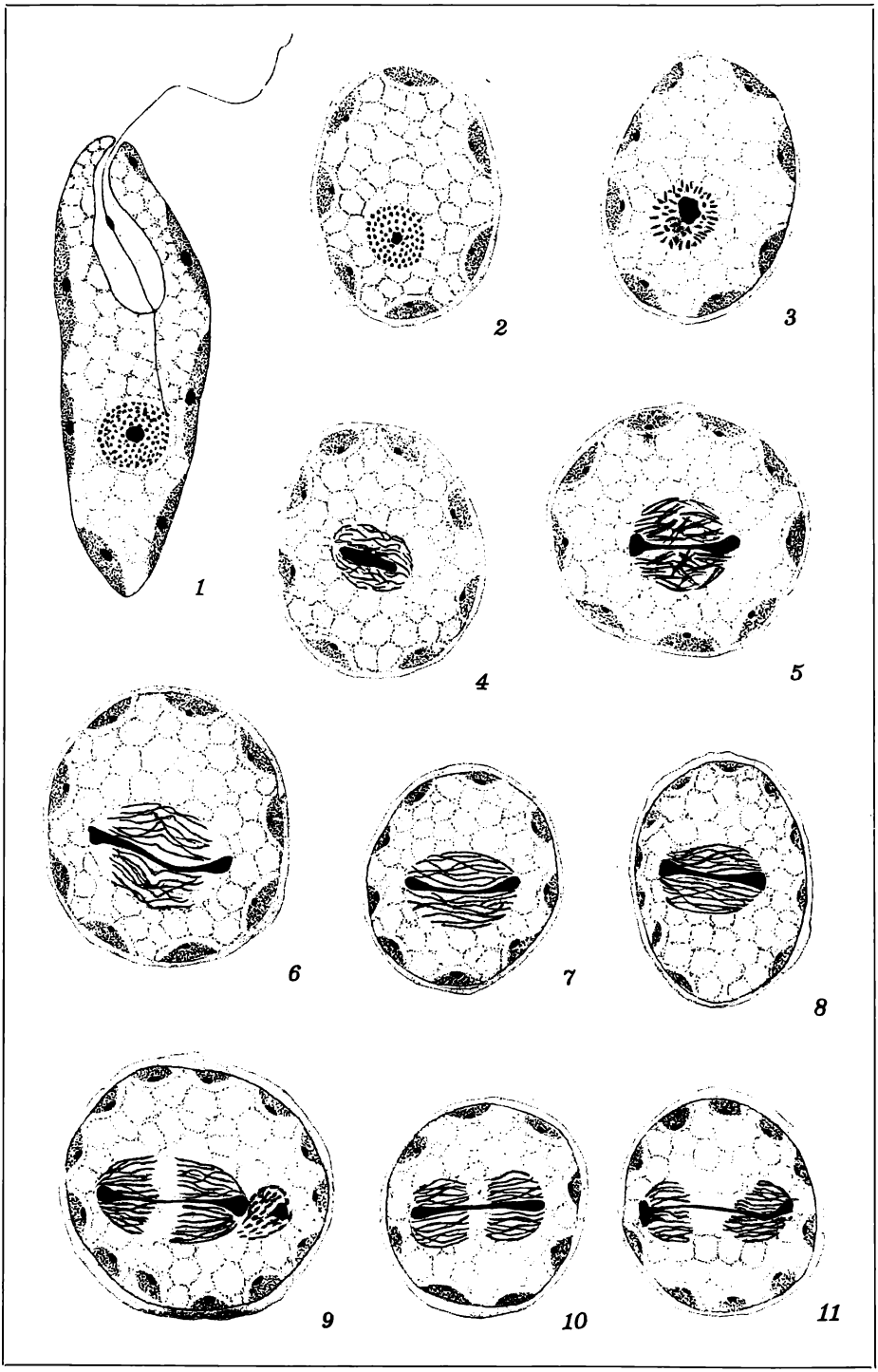
Fig. 24. Plasmodium with two nuclei; small bud developing.

Fig. 25. Tetranucleate plasmodium, with large bud.

Fig. 26. Plasmodium with four nuclei, showing fragmentation of endosome.

Fig. 27. Plasmodium with two large buds; one has developed a gullet and flagellum and is ready to separate from the mother cell.

Fig. 28. Large plasmodium with eight nuclei. Two chromatophores are seen free in the cytoplasm and are not arranged around the periphery of the cell as usual.



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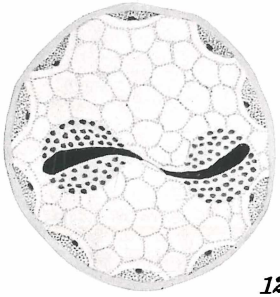

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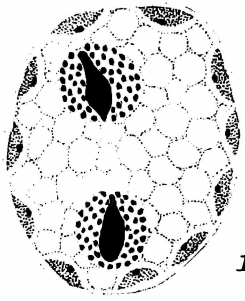

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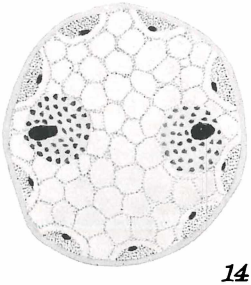

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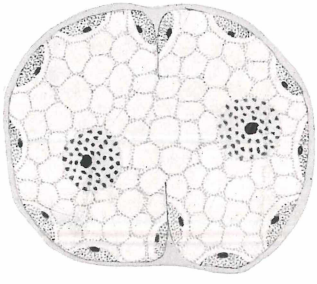

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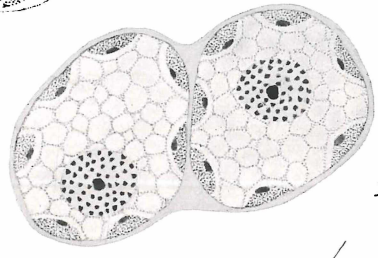

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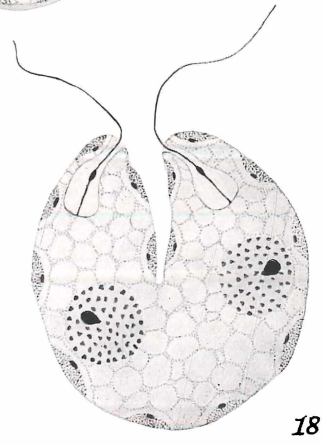

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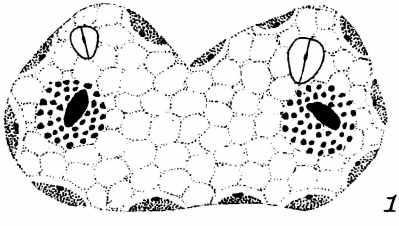

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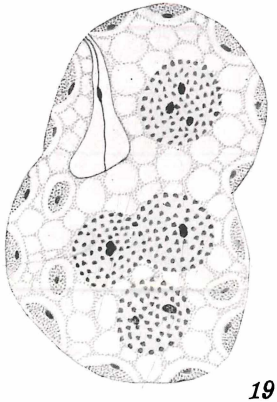

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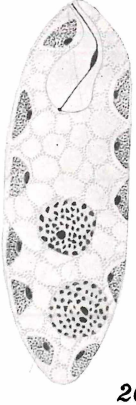

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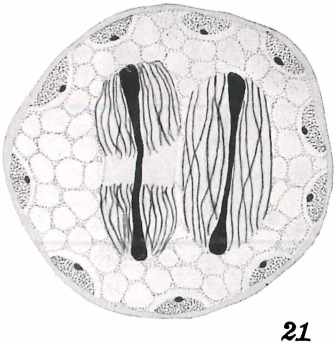

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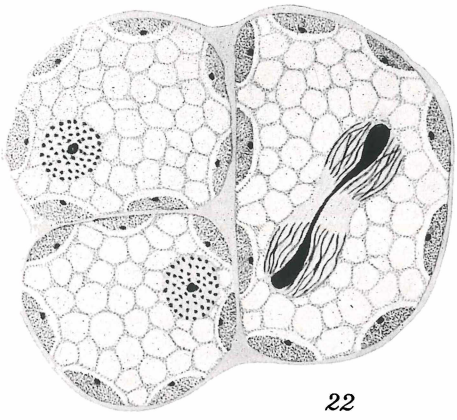

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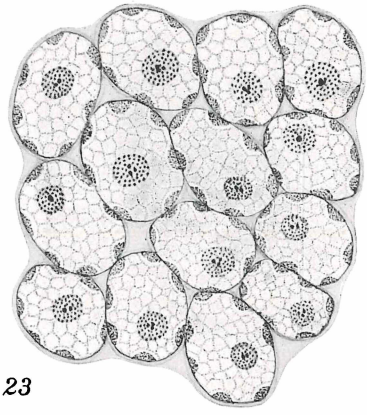

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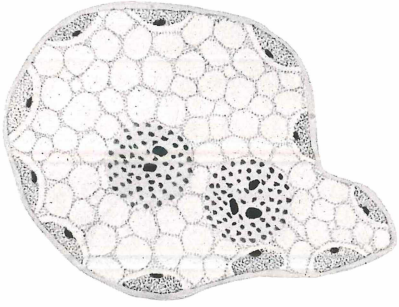

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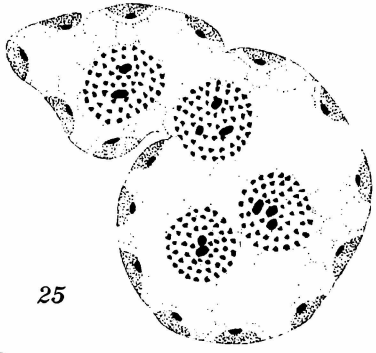

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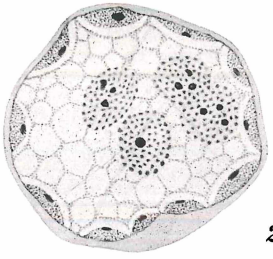

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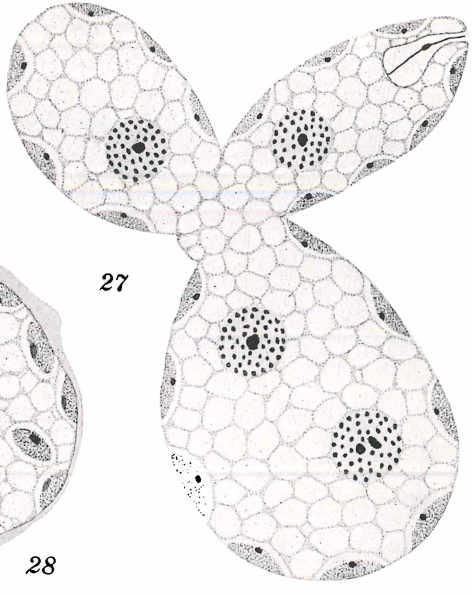

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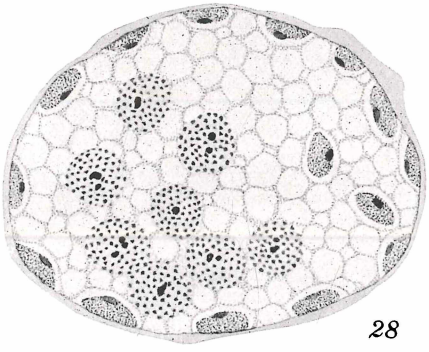

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