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The method of ingestion in *Peranema trichophorum* and its bearing on the pharyngeal-rod („Staborgan") problem in the Euglenida.

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(With 15 figures in the text.)

Introduction.

Different opinions have been expressed in regard to the mechanism of ingestion in the Peranemidae. According to certain authors, food particles are ingested by way of the gullet (pharynx, or 'reservoir'), a structure which is characteristic of the order Euglenida. SCHAEFFER (1918), on the basis of observations on living material, came to the conclusion that ingestion takes place in this manner in *Jenningsia diatomophaga*. TANNREUTHER (1923), who examined living specimens of *Peranema*, reported that in this species also food is sucked in through the gullet, and his account was confirmed in this respect by subsequent observations of HALL and POWELL (1927, 1928). A similar view was expressed by JOLLOS (1925) in regard to Peranemidae in general: „Die Ernährung der Euglenoidinen ist teils holophytisch... oder auch tierisch (Peranemidae). Die Aufnahme geformter Nahrung erfolgt durch die schon erwähnte Mundöffnung in der Nähe des Vorderendes ..." p. 152). „Die Geißeln entspringen am Vorderende in einer Einsenkung, die bei den geformte Nahrung aufnehmenden Arten mit Mundöffnung in Verbindung steht" (p. 151).

This interpretation of the process of ingestion in Peranemidae seems to have been questioned by RHODES (1926) and BROWN (1930). Thus Rhodes states, in an abstract, that "the 'staborgan' of *Heteronema acus* functions as a true mouth ... and the opening of the

reservoir should be otherwise designated", and apparently implies that food is not ingested by way of the gullet ('reservoir'). His brief statement has not been supplemented by a detailed account of his observations, and the nature and extent of his evidence cannot be determined at present. BROWN, one of RHODES' students, has applied the same interpretation to *Peranema trichophorum* in the following statement: "... the 'staborgan' and gullet of *Peranema* are not connected in any way with the reservoir, ... the cytostome is a separate opening on the ventral side of the body". In view of the fact that BROWN did not include in his paper any description or figures of stages in ingestion, and since he has been unable to agree with the interpretation of HALL and POWELL, the writer is presenting certain additional evidence, chiefly in the form of figures, bearing on this problem.

Observations on the living organism.

Peranema trichophorum is known to feed on a variety of materials. In the writer's experience the organism has been found to ingest *Chilomonas paramecium*, *Menoidium incurvum*, small algae, portions of plant debris, and occasionally carmine particles, while BROWN (1930) has reported ingestion of *Euglena proxima*, *Entosiphon* and *Chilomonas*. Although BROWN states that a culture of *Peranema* "will not live unless it is inoculated with some euglenoid", LACKEY (1927, 1929) had previously been more successful with culture methods and was able to grow the organism in species-pure and isolation cultures.

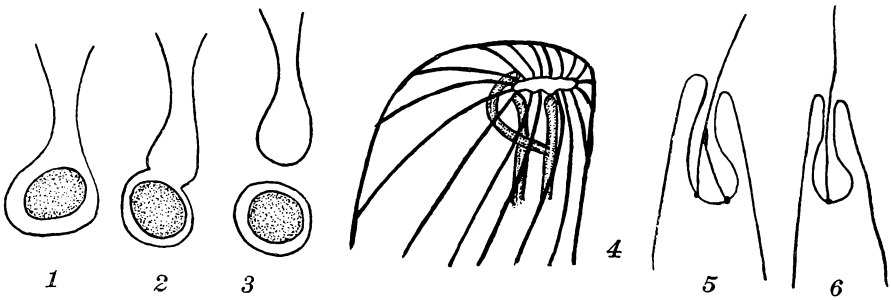


Fig. 1—6.

Figs. 1—3. Stages in formation of a food vacuole in *Peranema trichophorum;* free-hand drawings based on darkfield observations of living specimens. The pharyngeal-rods, observed to end at the rim of the cytostome, are omitted from the drawings. Fig. 4. Relation of the pharyngeal-rod apparatus to the cytostome *Jenningsia diatomophaga* (after SCHAEFFER, 1918). Figs. 5 and 6. Cytostome, gullet and flagellum in *Euglena* and *Astasia*, respectively (after HALL and JAHN, 1929).

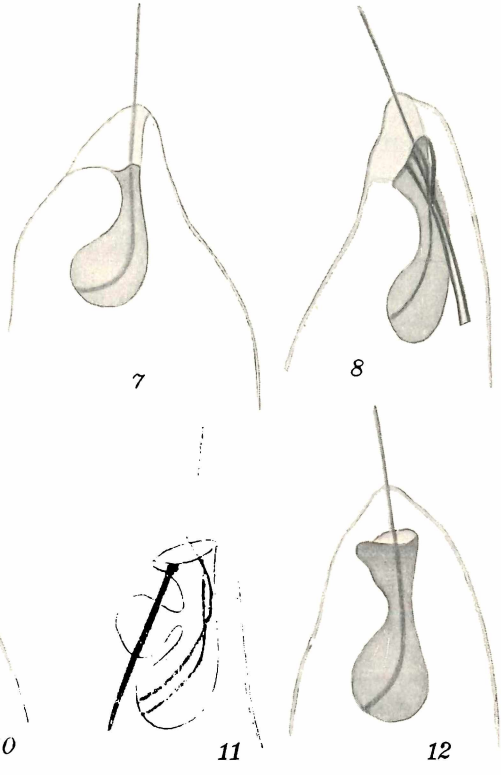
In attempting to follow the actual process of ingestion, the writer has experienced difficulty in that the flagellates are usually active and prolonged observations are rarely possible. As noted in living material the organism, on coming in contact with a food particle, contracts suddenly and undergoes violent metabolic movements. Shortly afterward, the flagellate usually elongates and begins to swim away; at this stage the food particle may be seen in the gullet. Partly on account of the sudden increase in thickness of the flagellate and also because of its increased activity, it has been impossible for the writer to observe the actual passage of food through the cytostome into the gullet. In a few instances, however, the formation of food vacuoles has been observed with the darkfield microscope. In this process (Figs. 1—3) the food particle passes into the lower portion of the gullet, which is enlarged to accommodate it, and this portion of the gullet is then gradually constricted from the rest to form a definite food vacuole. On separation from the gullet, the food vacuole usually passes into the posterior half of the body.

Although the actual entrance of food particles through the cytostome has not been seen, the writer has seen such particles in the gullet shortly after ingestion has occurred, and has also followed the formation of food vacuoles from the posterior region of the gullet. It seems only logical, therefore, to conclude that such food particles must have been ingested by way of the cytostome in order to reach the gullet. As noted below, this conclusion is confirmed by the writer's findings in permanent preparations (Figs. 13—15).

Observations on permanent preparations.

Preparations of *Peranema trichophorum* have been made with the fixatives of Champy, Schaudinn, Mann-Kopsch, and the osmic-chromic-acetic mixture of Grassé (1926), followed by iron-alum hematoxylin both with and without a counterstain (eosin, Bordeaux red, acid fuchsin, orange G). Material fixed in Altmann's fluid was stained in Regaud's hematoxylin; in some cases the organisms were bleached in hydrogen-peroxide before staining, and in other cases left unbleached. In addition, the writer has examined material impregnated by the Mann-Kopsch (Weigl) and Kolatchev osmic methods and the Da Fano silver method, although such preparations have been of little value with respect to the problem under discussion. For demonstration of the pharyngeal-rod apparatus and

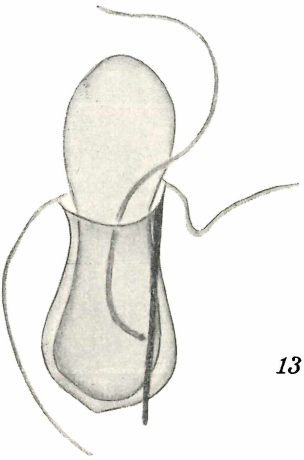
flagellum, SCHAUDINN fixation followed by Bordeaux red and iron-alum hematoxylin has been somewhat more useful than other methods. The ALTMANN-REGAUD method is particularly advantageous for demonstration of the gullet, since the surface of this structure is usually blackened to some extent and its outlines can be traced without the least difficulty. BROWN (1930) apparently did not try this method which has given such good results in the writer's preparations.



Figs. 7—12. *Peranema trichophorum*; × 2090. Figs. 7, 9 and 12. Different views of cytostome, gullet ('reservoir') and flagellum; pharyngeal-rod apparatus omitted from drawings. ALTMANN-REGAUD preparations. Fig. 8. Relation of pharyngeal-rod apparatus to cytostome and gullet. ALTMANN-REGAUD preparation. Fig. 10. Pharyngeal-rod apparatus; SCHAUDINN's fixative followed by Bordeaux red and iron-alum hematoxylin. Fig. 11. This specimen showed a connection between the gullet and what appears to be a contractile vacuole. One pharyngeal rod is shown; the other rod, lying to the right of the first, and the curved element are omitted from the drawing. The flagellar situation depicted was interpreted by HALL and POWELL (1928) as a stage in outgrowth of a second flagellum. SCHAUDINN-iron hematoxylin method.

The gullet of *Peranema* (Figs. 7—12) is a sac-like structure with a relatively narrow anterior portion and an expanded posterior portion, the anterior end opening to the outside through a cytostome lying a short distance from the anterior tip of the organism. In a

few instances an apparent connection between the contractile vacuole and the gullet has been detected in fixed preparations (Fig. 11); this confirms observations on living specimens that the contractile vacuole empties into the gullet in *Peranema*, and agrees with descriptions of this organelle in other Euglenida



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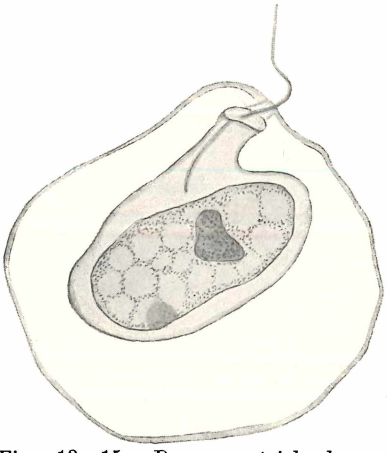

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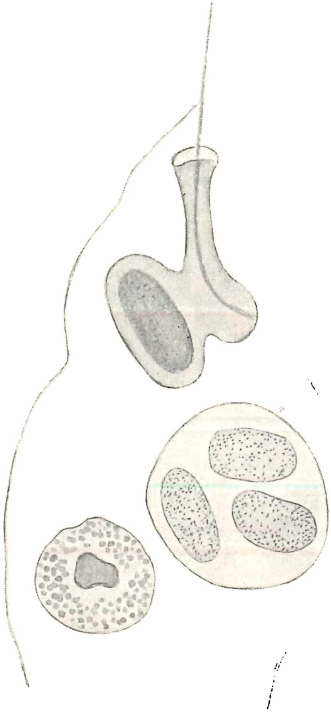

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Figs. 13—15. *Peranema trichophorum*; × 2090. Fig. 13. Early stage in ingestion; Altmann-Regaud preparation. The flagellum emerges from the cytostome which encircles the partially ingested food. One pharyngeal-rod is shown parallel to the gullet and ending in the rim of the cytostome; the other, not shown in the drawing, lies to the left of the first. Fig. 14. An ingested *Chilomonas* lies within the gullet. The flagellum was seen inside the gullet and emerging from the cytostome. The pharyngeal-rods, omitted from the drawing, lie above the gullet and end at the rim of the cytostome. Schaudinn's fixative followed by Bordeaux red and iron-alum hematoxylin. Fig. 15. A stage in the separation of a food vacuole from the gullet. The flagellum lies within the gullet and extends through the cytostome; pharyngeal-rods, omitted from the drawing, showed the typical relation to the cytostome. A second food vacuole is shown anterior to and to the right of the nucleus. Altmann-Regaud method, with bleaching in hydrogen-peroxide.

(HAYE, 1930). The flagellum of *Peranema*, as in members of the families Euglenidae and Astasiidae (Figs. 5, 6), is inserted near the base of the gullet and extends to the outside through the cytostome (Figs. 7—12).

The pharyngeal-rod apparatus (Figs. 8, 10), as described previously in detail (HALL and POWELL, 1928), has been found to consist of two rod-like structures, which lie parallel to the gullet as a rule and end at the rim of the cytostome, and a third curved element which extends along one side of the cytostome. The apparatus is thus quite similar to that described by SCHAEFFER (1918) in *Jenningsia diatomophaga* (Fig. 4) and to that of *Heteronema acus* (LOEFER, 1931).

Observations on fixed and stained material (as evidenced by Figs. 13—15) confirm the observations on living material and demonstrate that food is taken in through the cytostome and on into the gullet (or 'reservoir'), the structure in which the flagellum is inserted. Fig. 13, for example, shows an early stage in ingestion, in which it may be noted that the partially ingested organism extends through the cytostome into the gullet, and also that the flagellum extends to the outside through the same cytostome. The phayngeal-rods, one of which is shown in the drawing, lie parallel to the gullet and end at the rim of the cytostome. In fig. 14, an ingested *Chilomonas* is contained in the greatly expanded gullet, and separation of the food vacuole has not yet begun. A later stage, which appears to represent the separation of a food vacuole from the gullet, is shown in Fig. 15. Although such stages are rare in the writer's preparations (only a few others were observed), they present convincing evidence that the typical euglenoid cytostome and gullet function in the ingestion of solid food in *Peranema trichophorum*.

Observations on permanent preparations of *Peranema* have thus confirmed the earlier interpretations of HALL and POWELL in the following respects: (1) The flagellum is inserted in the gullet ('reservoir'), and the external portion of the flagellum emerges through the cytostome. (2) Food particles are ingested through the same cytostome and pass into the lower portion of the gullet. (3) Partially formed food vacuoles are continuous with the gullet. (4) The pharyngeal-rods end at the rim of the cytostome and, as a rule, lie parallel to the gullet. (5) No other mouth opening, and no other gullet or pharynx is present in the specimens examined.

Discussion.

Since it involves a question of structural organization, the method of food-taking in *Peranema* bears a direct relation to problems of classification in Euglenida. Schaeffer (1918) has pointed out that the transition from holophytic to holozoic nutrition in the Euglenida has been accompanied by a development of the pharynx which, in the holozoic Peranemidae, has become "provided with special rods which make it possible to open and close the pharynx, and also to act somewhat like a suction apparatus by means of which solid matter may be eaten with despatch . . ." According to Schaeffer, therefore, the gullet (or pharynx) is a structure characteristic of all three families of Euglenida, and is utilized in the Peranemidae for the ingestion of solid food, thus obviating the necessity for the development of a new mouth opening with the assumption of the holozoic habit. This interpretation has since been supported by Tannreuther (1923) and Hall and Powell (1927, 1928) for *Peranema trichophorum*, by Jollos (1925) for the Peranemidae in general, and by Loefer (1931) for *Heteronema acus*.

In certain Peranemidae (*Jenningsia*, *Peranema* and *Heteronema*, for example), a well developed pharyngeal-rod apparatus is present and presumably aids in ingestion, as was suggested by Schaeffer. Other Peranemidae, however, apparently take in solid food without the aid of such an organelle. Lemmermann (1913) stated that the genus *Euglenopsis* lacks this apparatus, and the writer has since examined preparations of *Euglenopsis vorax* which showed food vacuoles but no trace of any pharyngeal-rods. Likewise, *Scytomonas* ingests solid particles and apparently has no pharyngeal-rod apparatus, since Berliner (1909), in *Scytomonas major*, and both Schüssler (1917) and Wenyon (1926), in *Scytomonas pusilla*, have failed to find such an organelle. Although details of the feeding process in *Scytomonas* are apparently unknown, Lemmermann has stated that food is ingested by way of the cytostome in *Euglenopsis*. While Schaeffer's generalization concerning the pharyngeal-rods is applicable to a number of genera, the available evidence indicates that in *Scytomonas* and *Euglenopsis* solid food is ingested in the absence of such an accessory apparatus and that the pharyngeal-rod apparatus is not essential to the ingestion of solid food in Peranemidae.

The contention of Rhodes and Brown implies that in *Heteronema* and *Peranema* there is a phylogenetically new mouth opening, the position of which is marked by the pharyngeal-rod apparatus, and

that this new mouth is entirely independent of the characteristic euglenoid gullet, or pharynx ('reservoir').

So far as *Peranema* is concerned, the writer has shown that the pharyngeal-rods end at the rim of the cytostome (Figs. 8, 10), as was reported by HALL and POWELL, and peculiarly enough some of BROWN's figures (Pl. 19, Figs. 1, 4; Pl. 20, Fig. 11; Pl. 21, Figs. 13—15) apparently show the same situation. Furthermore, in his description of differentiation in later stages of fission, BROWN states: "... the new cytostomes are formed ... On the edge of this newly formed cytostome a group of four granules grows in size and later they collect into two distinct rod-like structures (Pl. 20, Fig. 9). These form the lateral rods of the rod-organ. The daughter reservoirs pull farther apart ..." In view of BROWN's conclusion as to the "mouth" opening of *Peranema*, it is interesting to note that the "cytostome" to which he refers opens into the gullet (in which the flagellum is inserted, and which BROWN terms the 'reservoir'), and that the "lateral rods", as shown in his figures, end at the rim of the opening (cytostome) through which the flagellum emerges. BROWN has neglected to explain just how the pharyngeal-rod apparatus, which he both describes and figures as being associated with the cytostome (opening of the gullet) in anaphases and telophases, later becomes separated completely from the typical euglenoid cytostome and gullet and associated with a new "mouth". In view of BROWN's apparent lack of observations on the actual process of ingestion and formation of food vacuoles, and the conflicting pictures presented by his own figures and descriptions, it would seem that he was not justified in questioning the earlier interpretation of HALL and POWELL (1927, 1928).

As for *Heteronema*, RHODES' abstract has not been supported by a detailed account of his observations, and it is at present impossible to determine whether his evidence is any more conclusive than that of BROWN. It might be mentioned, however, that LOEFER (1931) has been unable to find in *Heteronema acus* any evidence which might support RHODES' statements. Thus, in spite of the contradictory opinions of RHODES and BROWN which are unsupported by detailed published evidence, it appears highly probable that the feeding method previously described by SCHAEFFER is that which actually occurs in Peranemidae, and that the cytostome and gullet of these flagellates are merely the typical euglenoid structures which have become adapted to the ingestion of solid food.

Summary.

The writer has presented additional evidence which supports the view of Schaeffer (1918), Tannreuther (1923), Jollos (1925), Hall and Powell (1927, 1928) and Loefer (1931) that, in the Peranemidae, food particles are ingested by way of the gullet ('reservoir') which is homologous with the gullet found in species of Astasiidae and Euglenidae. Repeated observations on living specimens and on fixed and stained preparations of *Peranema* have confirmed this interpretation of Schaeffer and others, and have failed to reveal any evidence which supports the contention of Rhodes (1926) and Brown (1930) that there exists in the Peranemidae a new mouth opening which is entirely separate from the gullet ('reservoir') characteristic of the Euglenida as a group.

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