

## Investigations of the Biology of *Peranema trichophorum* (Euglenineae)

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### SUMMARY

The feeding apparatus of *Peranema trichophorum*, consisting of cytostome and rod-organ, is independent of the reservoir system; the latter is the same in structure and function as that of other Euglenineae. There are two flagella, one directed forward, the other backward and adherent to the ventral body surface. The anterior flagellum is longer and thicker than the adherent one. Both flagella are composed of a central core and an outer sheath. Electron micrographs suggest that the *core* consists of many longitudinal fibrils, and the *sheath* of many short fibrils radiating from the core, giving the whole flagellum the appearance of a test-tube brush. Treatment with certain protein-dispersing agents cause the unfixed anterior flagellum to dissociate into three fibrils.

*Peranema* multiplies freely on a diet of living yeast-cells; dead yeast is not suitable. *Euglena viridis*, *E. gracilis*, and certain other unicellular algae can also serve as food. Egg-yolk, and especially milk, can be used to maintain bacteria-free pure cultures. Casein is suitable in combination with soil-extract or beef-extract, but never as good as milk. With the latter the individuals are larger and more numerous than with yeast as food, although the cultures decline earlier. Clear liquid media of many various kinds did not support growth: particulate food seems to be essential.

*Peranema* is capable of ingesting a great variety of living organisms provided these are motionless. Small organisms are swallowed whole; larger ones are either engulfed or cut open by the rod-organ and their contents sucked out. The rod-organ can be protruded out of the cytostome and used in holding on to, and cutting, the periplast of the prey. Starch-grains, oil-droplets, and protein-particles are engulfed and digested. The main food reserves are paramylon-granules and oil-droplets.

H<sup>+</sup> ions, decomposition products of proteins, and other substances diffusing out of living, and particularly dead, organisms attract gliding *Peranema*. Chemotaxis plays an important role in leading *Peranema* to its prey.

Both gliding and swimming have been observed in normal individuals, although the latter is less frequent. While there can be no doubt that swimming results from the action of the anterior flagellum, it does not seem to play an appreciable part in gliding. Nothing is known about the function of the adherent flagellum.

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## INTRODUCTION

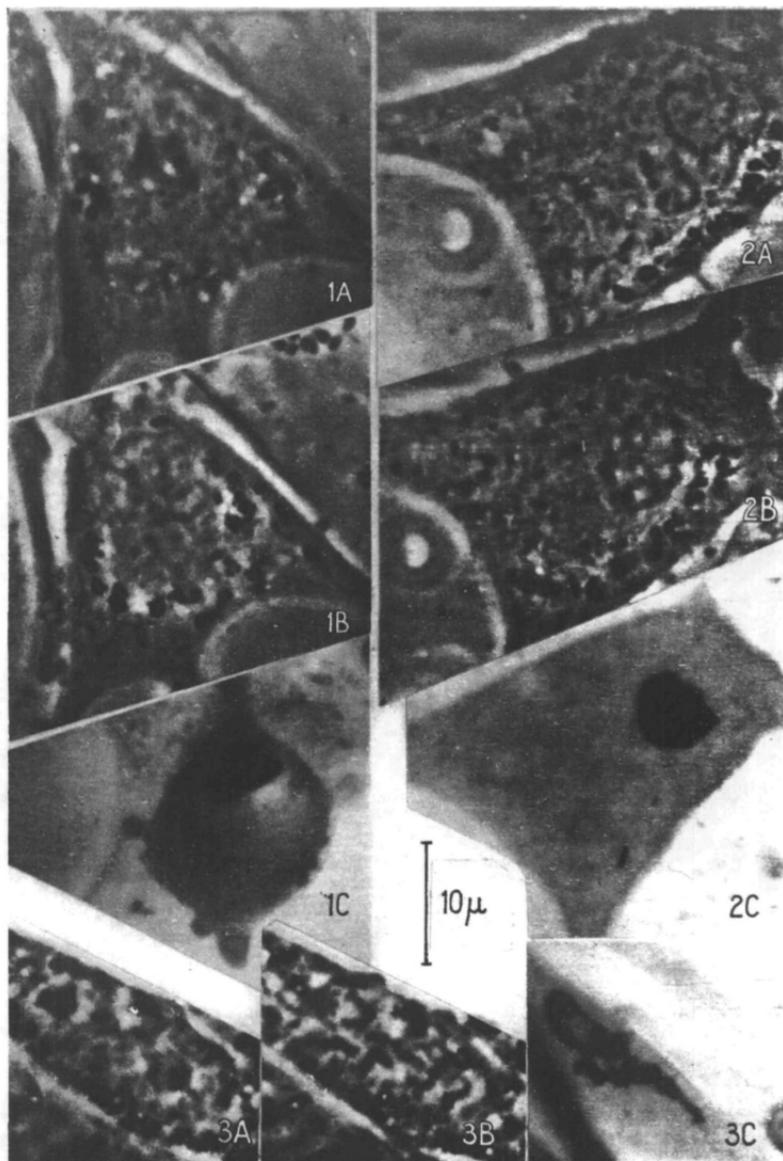
*PERANEMA trichophorum* (Ehrbg.) Stein, a colourless member of the Euglenineae, is one of the largest zootrophic flagellates and often used for demonstration on account of its conspicuous flagellum. It was, therefore, deemed useful to find reliable methods of maintaining it in the laboratory, since previous attempts at cultivation appeared to rely too much on chance or gave rise to too complex conditions. It was hoped, moreover, that *Peranema* would be found suitable for studying the zootrophic habit of nutrition, of which not much is known in flagellates. Bacteria-free cultures of *Peranema* have not previously been obtained, and the nutritional requirements of the organism were practically unknown.

The structure and function of the rod-organ and the reservoir system have long been matters of controversy. Most of the earlier writers (Stein, 1878; Bütschli, 1876, 1883-7; Saville Kent, 1880-2) believed that the rod-organ functions as a buccal tube through which food passes. This was denied by Klebs (1883, 1893) who accurately described its mode of action and recognized its function in cutting and piercing the pellicle of the prey.

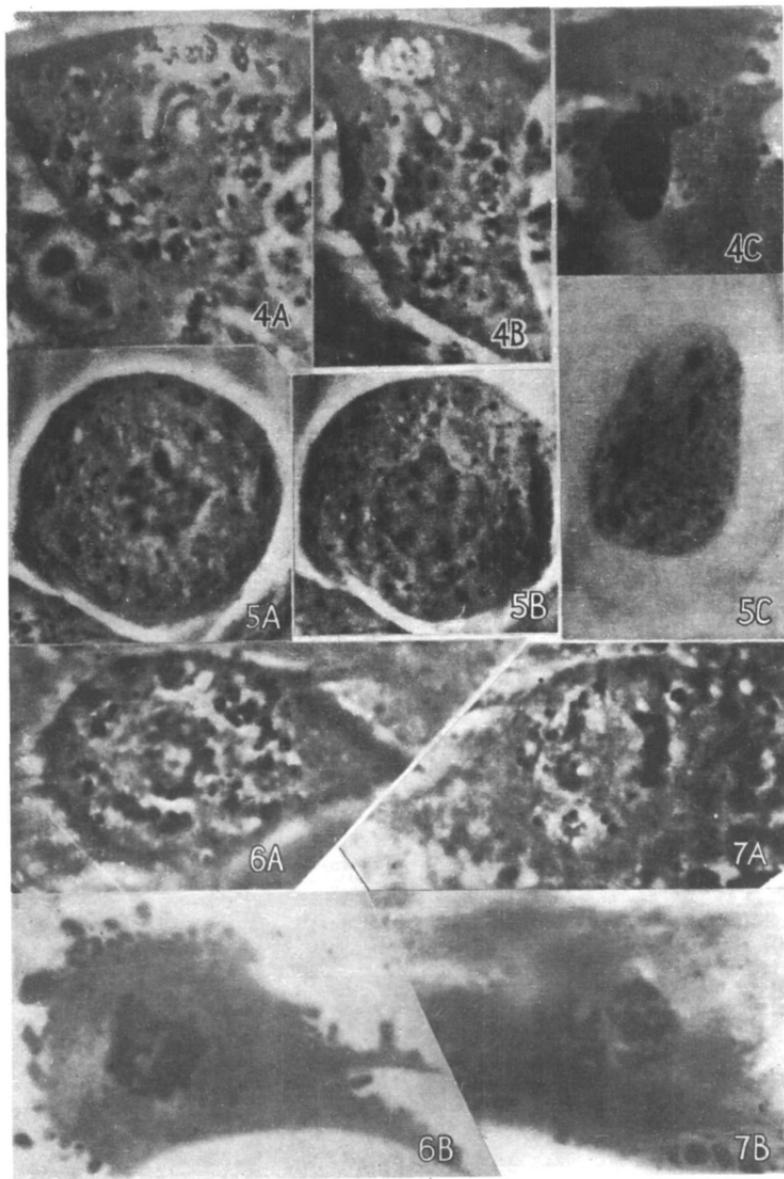
While the structure of the food-ingesting apparatus of *Peranema* was not understood by earlier writers, two different opinions have appeared in more recent publications. It is widely believed that the reservoir system, although of much the same structure as in phototrophic Euglenineae, is used in this organism for ingesting food particles, and that the rod-organ, which is attached to the reservoir, functions mainly as a mechanical support (Jollos, 1925; Hall and Powell, 1928; Hall, 1933; Lackey, 1933; see also Schaeffer, 1918, for *Jenningsia*, and Loefer, 1931, for *Heteronema*). The other opinion is that, in addition to the reservoir system (which has nothing to do with ingestion), there is another opening, connected with the rod-organ, and used for feeding (Brown, 1930, Pitelka, 1945; see also Rhodes, 1926, for *Heteronema*). Hyman (1936), although accepting the conception that the reservoir is used in feeding, confusedly described an independent rod-organ used in 'strengthening the food-ingesting passage'. It seemed necessary to observe the feeding habits of living individuals in order to clarify the position.

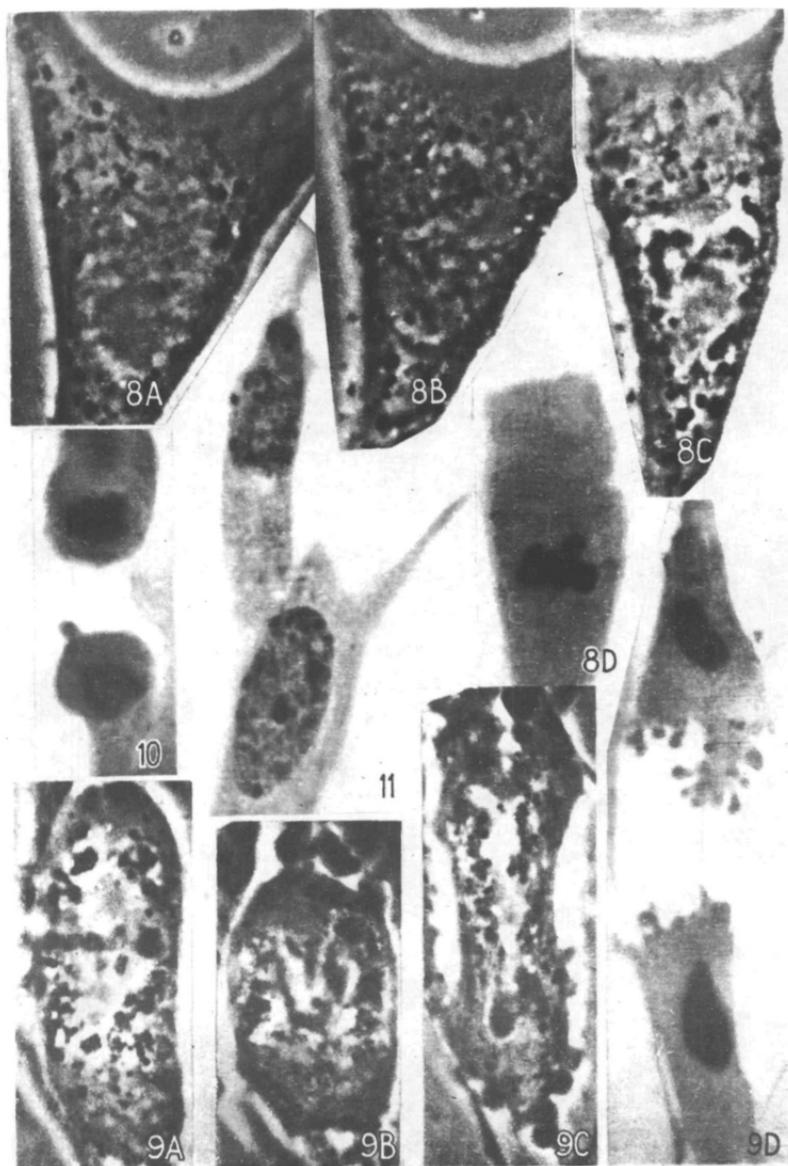
The movements of *Peranema* have been studied by Mast (1912), Lowndes (1936, 1941, 1944, &c.), and others. Lowndes, making use of high-speed cinematography, confirmed Gray's suggestion (1931) that the flagellum is an active unit and is capable of generating at least a part of its own energy. The relation between body movement and flagellar activity, the function of the flagellum, and the chemotaxis of this organism have not hitherto received attention.

*Peranema* was collected from stagnant waters containing plant residues. Thriving impure and pure cultures in various media have been obtained. With this material the morphology, growth, feeding habits, movements, and chemotaxis have been investigated in the hope of presenting a more complete picture of a common but surprisingly little-known species and contributing

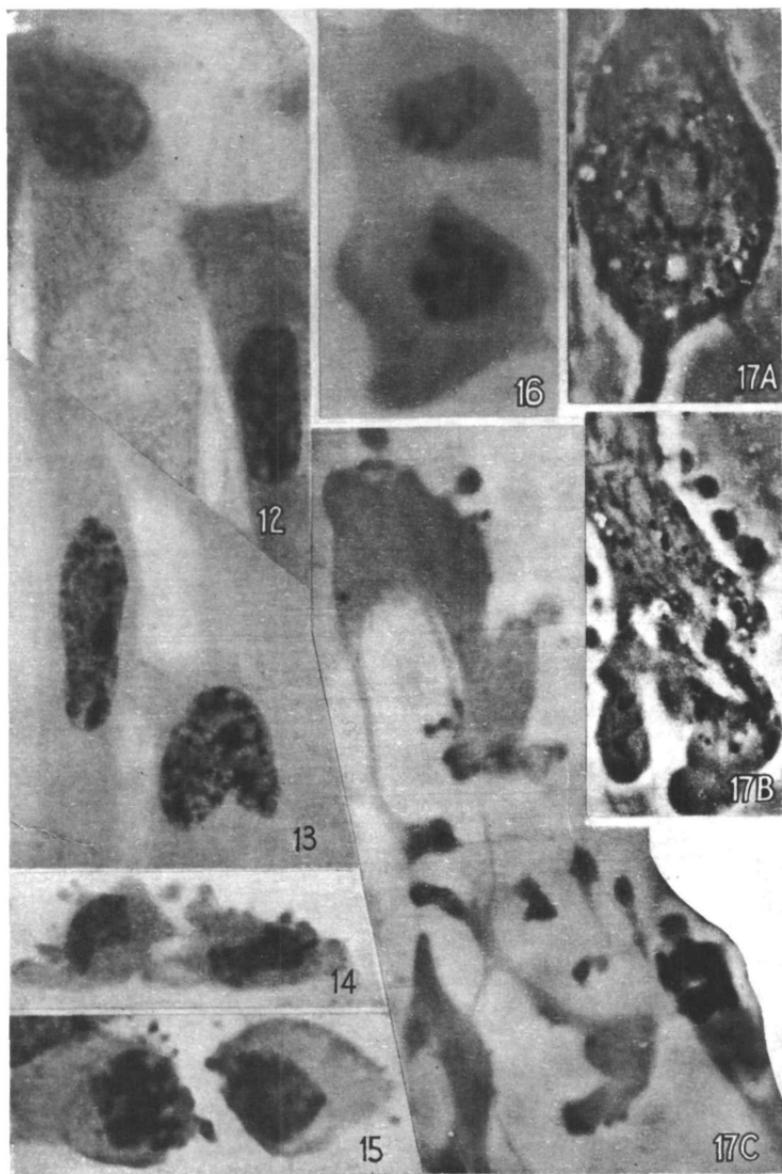


A. F. W. HUGHES—PLATE I





A. F. W. HUGHES—PLATE III

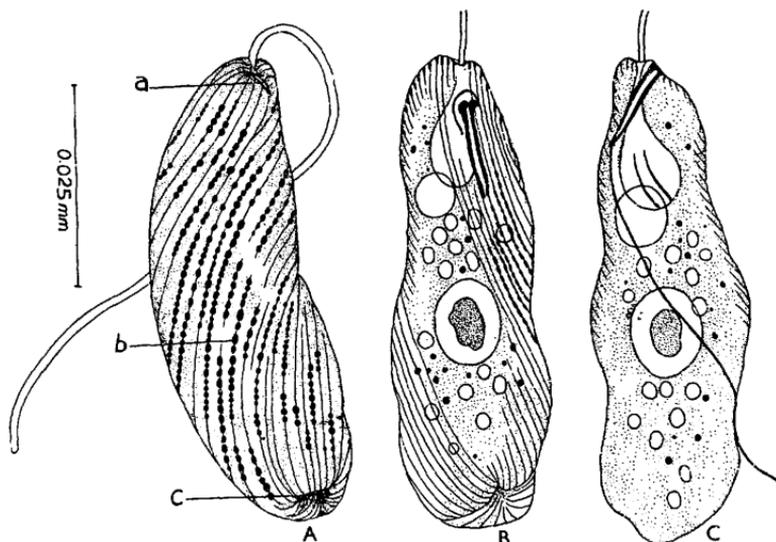


A. F. W. HUGHES—PLATE IV

to a better understanding of the relationship between coloured and colourless Euglenineae.

#### MORPHOLOGY

The body of *Peranema trichophorum* is irregularly sack-shaped, with its anterior end tapering and its posterior end rounded, truncated, or sometimes (especially when the organism is poorly nourished) prolonged on one side



TEXT-FIG. 1. *Peranema* (diagrammatic). A, B. As seen from the side, showing striation of periplast, cytostome (a), protrichocysts (b), reservoir, rod-organ, nucleus, contractile vacuole, paramylon grains, area of defecation (c). C. The same, as viewed from ventral surface, showing the adherent flagellum.

into a pointed, tail-like process mistaken by James-Clark (1868) for a rudimentary trailing flagellum and used by Skuja (1948) as a specific character.

The dimensions of the body vary with the environmental conditions and have been differently given by previous workers. Newly collected individuals are usually small, with an average size of  $42 \times 16 \mu$ . Under favourable cultural conditions, e.g. in cultures with milk as food, the size increases to  $65 \times 25 \mu$  on the average, and the individuals are visible to the naked eye as small white specks. In cultures with the yeast *Saccharomyces exiguus* as food the average size is  $52 \times 17 \mu$ .

The periplast is finely striated (Text-fig. 1, A, B). The striations are only slightly oblique. Cross-striations have not been seen. Iodine treatment, silver impregnation, night-blue staining, and dark ground illumination render the

striae more visible. Running along the striae there are many elliptical or rod-shaped granules, visible in living cells under dark ground illumination or after vital staining (Klebs, 1883; chondriome of Hall, 1926; protrichocysts of Chadefaud, 1938; corps mucifères of Hollande, 1942). In silver-impregnated preparations the striations are seen to converge near the posterior end of the body as they approach a slit-like unimpregnated region, probably the area of defaecation (Hollande, 1942).

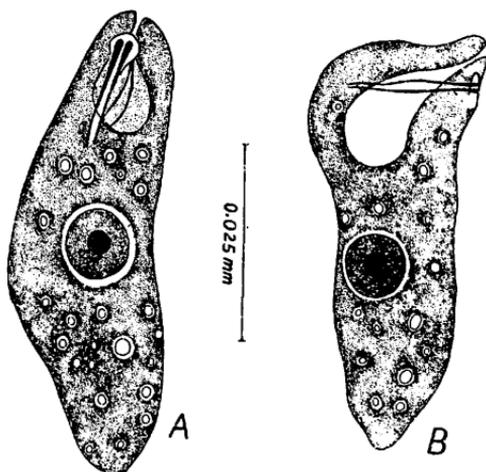
Scattered in the cytoplasm are many paramylon granules. They are disk-shaped with a thick rim. When observed from the side they are ovoid. The number and size of these granules vary considerably: they are fewer and smaller (with a diameter of about  $1.5\mu$ ) in individuals fed with *S. exiguus*, more numerous and larger (diameter about  $3\mu$ ) in individuals fed with starch, casein, or milk.

The reservoir, situated in the anterior quarter of the body, is connected by a canal with an opening lying slightly to the right of the anterior end. It divides the latter, as seen in longitudinal optical sections, into a larger and a smaller lip (Text-fig. 1). Posterior to the reservoir is a simple contractile vacuole, the pulsation of which can easily be observed.

The rod-organ is adjacent to the reservoir. It is composed of two parallel rods each of which is hook-shaped with a thickened and slightly curved anterior end and a tapering posterior end (Text-fig. 1). According to Chadefaud (1938) the rods are semicircular in cross-section, i.e. with a furrow on the inner side. They have been mistaken by many workers for the wall of a canal, called the siphon. In agreement with Brown (1930) and Hyman (1936) it was found that the rod-organ is not connected with any part of the reservoir system. The anterior end of the rod-organ is attached to a hyaline, roughly semicircular zone, the position of which is lateral and ventral to, but not connected with, the reservoir canal (Text-fig. 1B). As this zone usually overlaps the reservoir canal, the attached rod-organ also appears to be connected with the reservoir. However, normally gliding individuals have repeatedly been observed with the hyaline zone and the rod-organ completely separated from any part of the reservoir system (Text-fig. 1, B, C). A lateral view in fixed and stained specimens shows the rod-organ again to be ventral to, but independent of, the reservoir. The clear zone in front of the rod-organ is regarded by Brown (1930) and Pitelka (1945), but not by most other authors, as the true ingestive opening. Hyman (1936) has seen this structure too but does not describe it as an opening. The results of my feeding experiments (p. 292) are in favour of Brown's and Pitelka's conclusion, and the term cytostome will therefore be used to denote this opening. The cytostome has a thickened anterior margin described by Brown (1930), Chadefaud (1938), and Hollande (1942) as a third, the falcate rod. As it is not connected with the two parallel rods and does act together with them during feeding, it is perhaps better regarded as a stiffened rim of the cytostome than as part of the rod-organ (Text-fig. 2, A, B).

Hall and Powell (1928) and Hall (1934) deny the existence of a second

opening, but some of their figures (1928, pl. I, 1, 2, 3, 4) indicate the presence of two openings in the same individual. In their figures an opening at the anterior end of the body is connected by a canal to a second opening, labelled 'cytostome'. I also saw two openings but found that at the anterior end to lead into the reservoir from which the flagella protrude, while the other (posterior and ventral to the reservoir opening) is the true cytostome. In the figures of Lackey (1933) the cytostome is connected with the part of the reservoir canal in front of it, thus giving the appearance of a 'tuning-fork shaped cytostome'.



TEXT-FIG. 2. *Peranema*, Schaudinn/Heidenhain. A, viewed from ventral surface. B, twisted cell, viewed from the side.

Neither in living nor in stained specimens could a canal leading from the cytostome into the body be traced. It seems that there is not a permanent gullet, and that food enters through a temporary passage.

From the oblique position of the rod-organ it appears that bilateral symmetry, so often given as a diagnostic character of the Peranemaceae, does not, in fact, exist in *Peranema*. Klebs (1883) seems to be the first who has used the term 'bilateral', but not in the correct sense: he meant 'dorsoventral'. Lemmermann (1913), Doflein (1916), and others followed him without being aware of the inaccuracy. Dorsoventrality is very distinct in *Peranema* which glides only on one surface, the ventral side of the body (cf. p. 301). When individuals glide along the bottom of the container, they are observed through the dorsal surface with the posterior end of the rod-organ pointing towards the right as seen under a compound microscope (Text-fig. 9A, p. 299). When they glide along the upper surface of the water, however, it is the ventral

surface of the body which is observed, and the posterior end of the rod-organ points to the left (Text-figs. 1C, 2A).

There are two flagella. The one directed forward is the one known alone to most of the previous authors. It is wider than the second one and up to twice the length of the extended body. The second flagellum is much narrower and only two-thirds the length of the body. It turns back immediately after protruding from the reservoir opening and adheres to the ventral body surface, simulating a streak on the periplast (Korshikoff, 1923; Lackey, 1929, 1933; Hall, 1934). This second flagellum is visible in living specimens under dark ground illumination or under the phase-contrast microscope. By gentle pressure or by treatment with osmium tetroxide it can be detached. Skuja (1948) doubts the existence of the second flagellum and believes that what is considered as a second flagellum may only be a daughter flagellum of a dividing individual. It seems unlikely, however, that a newly grown flagellum should adhere to the periplast or that it should be narrower than the primary flagellum.

In stained specimens the two flagella are seen to originate independently from two basal granules which lie side by side in the wall of the reservoir, as in other Euglenineae. There is no bifurcation of flagellar roots. What Doflein (1916) and Brown (1930) describe as bifurcation seems to be a misinterpretation due to failure to observe the second flagellum. The structure of the flagellar apparatus of *Peranema* therefore accords with the views of Pringsheim (1948) regarding the flagellar apparatus of the Euglenineae.

Save for the difference in size, the anterior and posterior flagella have the same structure. Each is differentiated into an axial core and an outer sheath which can be observed under the light microscope, particularly after treatment with Loeffler's stain, under dark ground illumination, or under the electron microscope. The latter reveals still finer structures (Text-fig. 3A, a). Contrary to Lackey's statement, but in accordance with Hollande (1942), the sheath covers the whole length of the anterior flagellum except for the part within the reservoir (the root) which, when squeezed out by the contraction of the body during the preparation of specimens, is seen to be naked (Text-fig. 3B a). The sheath seems to consist of numerous fibrils radiating from the axis, thus giving the whole flagellum the appearance of a test-tube brush. It is in this respect different from the flagellum of *Euglena* where the sheath consists of longer fibrils wound spirally round the axis (Brown, 1945, and own electron-micrographs).

The axial core shows in gold-shadowed specimens longitudinal striation, indicating fibrillar differentiation. The number of fibrils, as revealed by the electron-microscope, is considerably higher than the two of Brown (1945, 1946) or the four of Dellinger (1909). The same has been found in several other Euglenineae, including *Euglena gracilis* (Brown's material). My conclusions are in agreement with Korshikoff's (1923) and those of Schmitt, Hall, and Jakus (1943) that the axis consists of a relatively large number of thin fibrillae. The two thick fibrils, found by Brown in *Euglena gracilis* and

'*Astasia Klebsii*' (in fact the colourless race of *E. gracilis*), may be bundles of fibrillae situated in the edges of the ribbon-shaped flagella, as suggested by my electron-micrographs.

By the application of protein-dispersing reagents an attempt was made to render the inner structure of the flagellum better recognizable. With previously fixed material no results were obtained, but the treatment of unfixed individuals with chemicals, such as urea, known to disperse fibrillar proteins stabilized by electro-valencies, constantly leads to the separation of the anterior flagellum into three fibrils, possibly as a result of the dissolution of the sheath. While in some of these reagents, e.g. urea (from saturated to 3 M solution), NaOH (0.1-0.2 N), &c., the fibrils ultimately dissolve, in  $\text{Na}_2\text{SO}_3$  (2-8 per cent.), dissolution is only partial even after 24 hours. In HCl (0.2-5 N), on the other hand, the flagellum remains intact, even after treatment for 24 hours.

Like Deflandre (1934a, 1934b), who used nigrosin, and Hollande (1942), I could not find in *Peranema* the lash-like processes which, according to Vlk (1938), should be present on the surface of the flagella of all members of the Euglenineae. Neither Loeffler's mordant staining technique, nor electron-microphotographs showed any trace of such appendices, although both methods were successful in various species of *Euglena*, *Trachelomonas*, and *Phacus* (Pringsheim and Hovasse, 1950).

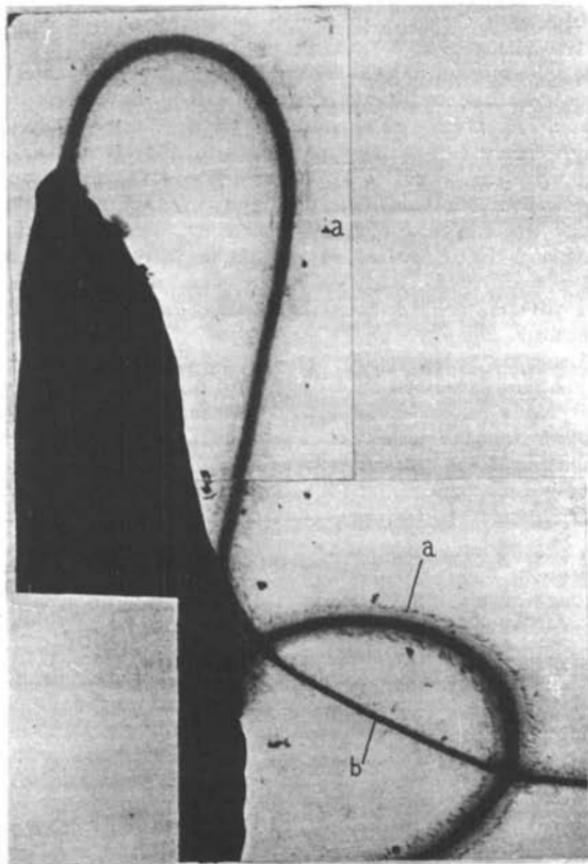
#### CULTIVATION AND GROWTH

*Peranema* was cultivated by Lackey (1927) in a wheat-water medium and by Hall and Powell (1927, 1928) and Mast and Hawk (1938) in a beef-suet infusion, all with bacteria as food. Brown (1930) maintained that a culture of *Peranema* will not live unless inoculated with some euglenoid flagellate, but Shettles (1937) used a balanced salt solution with rice grains and *Chilomonas* as food.

##### 1. Preliminary experiments

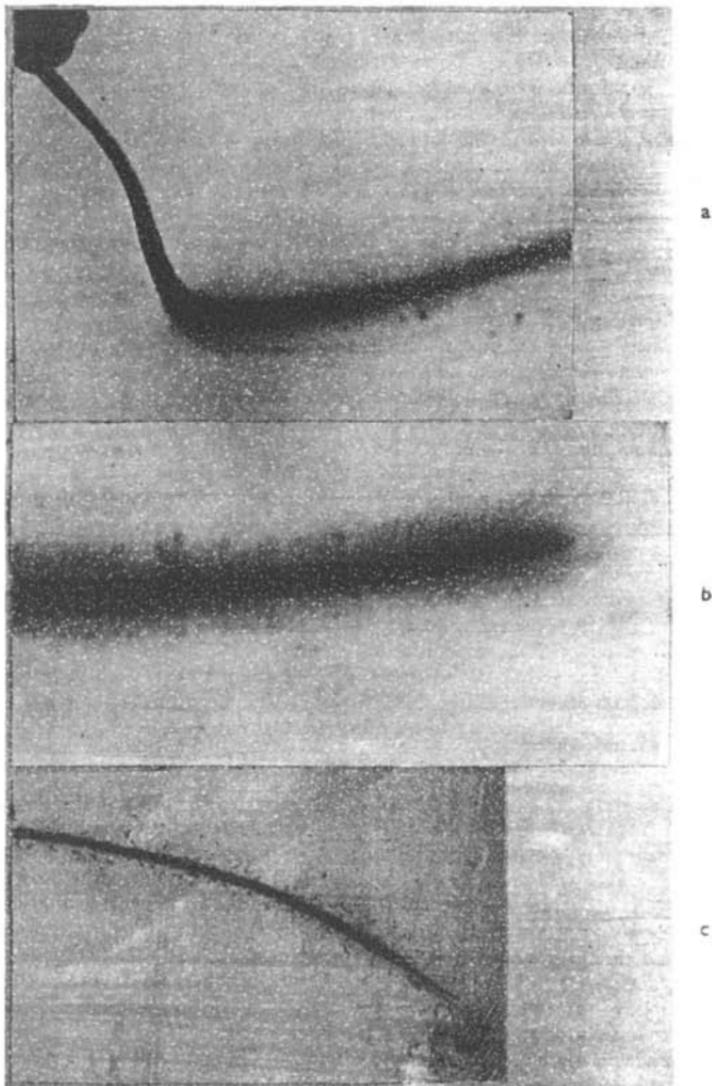
The material with which I started had been grown for some time with *Saccharomyces exiguus* as food suspended in half-saturated calcium sulphate solution, a method which had previously been devised for *Paramecium* (Pringsheim, 1928). Cultures were fed daily during the first week, and less frequently later on. Flourishing cultures were obtained after about a month. Wheat and hay infusions and various bacteria from agar slopes did not support satisfactory growth.

With *Saccharomyces exiguus* as food the following solutions, apart from calcium sulphate, were tried: half-saturated aqueous calcium carbonate; diluted Benecke solution; one-tenth diluted natural and artificial sea-water; soil extract and glass-distilled water. *Peranema* multiplies in all these solutions: least well in distilled water, best in soil extract and almost as well in Benecke solution, which both accelerate the rate of multiplication compared with calcium sulphate solution, although they do not give a denser peak population.



TEXT-FIG. 3A. Electron-micrographs of the flagella of *Peranema*. *a*, curved anterior flagellum; *b*, posterior flagellum; osmium tetroxide.  $\times 12,000$ .

That *Peranema* can grow in distilled water does not imply that it needs no mineral salts, since yeast present in the culture supplies a certain amount. This is borne out by the facts that the health of the culture is poor in the beginning but improves after repeated feeding and that without feeding *Peranema* soon dies in distilled water but can live several days in solutions with calcium (cf. Pringsheim, 1928, for *Paramecium*). Accordingly addition of very low concentrations of calcium salts greatly improves the cultures,



TEXT-FIG. 3B. (a) root of anterior flagellum squeezed out of reservoir; (b) end of anterior flagellum showing core and sheath; (c) end of posterior flagellum; sheath much less dense.  $\times 16,000$ .

one-sixteenth to one-sixty-fourth being better than more concentrated solutions.

Apart from *Saccharomyces exiguus* the following organisms from pure cultures were tested as food: *Polytoma uvella* Ehrenb., *Chlorella pyrenoidosa* Chick, *Prototheca Zopfii* Krüger, *Stichococcus bacillaris* Naeg., *Chilomonas paramecium* Ehrenb., *Euglena gracilis* Klebs, *Astasia longa* Pringsheim, *Saccharomyces cerevisiae* Hansen, and *Rhodotorula glutinis* Fresenius. Only *S. cerevisiae* is as good as *S. exiguus* in maintaining *Peranema*. *Rhodotorula*, taxonomically near to it, may be inferior because it cannot be suspended in water. *Chlorella* in the light grows so fast that *Peranema* is eventually suppressed. The two species of Euglenineae as well as *Stichococcus* only support moderate growth. Between a green *Euglena* and *Peranema* there is a complicated balance in multiplication, the former first growing quicker, then *Peranema* catching up, until eventually, when the food organism declines, the latter also gradually decreases until after about a year only a few individuals remain.

The influence of the pH has been investigated by using a series of Clark's buffer solutions ranging from pH 4 to 9. With *S. exiguus* as food *Peranema* was found to multiply between 5.2 and 8.4, with an optimum at neutrality. In order to see whether *Peranema* is capable of becoming adapted to higher concentrations of mineral salts, dilutions of artificial sea-water ranging from 1/20 to 1/4 were inoculated with *Peranema*. The growth was normal in the lowest concentration, and there was none in 1/5 and 1/4. If inoculated from 1/20, growth occurred up to 1/5. Even if the increase of concentration was effected by gradual evaporation of a culture in 1/20 sea-water, *Peranema* could tolerate only a concentration of 1/4.2 (24 per cent. sea-water). Compared with other flagellates (Finlay, 1930; Hardin, 1942) *Peranema* has only a very restricted capacity of adapting itself to salt-water.

## 2. Bacteria-free cultures

*Peranema* purified by washing (Pringsheim, 1946) could be grown with *Saccharomyces* as food in two-member pure cultures. Healthy cultures were, however, only obtained with soil extract as the medium. No growth occurred in dilute sea-water or calcium sulphate solutions, even after repeated inoculation. As both these media support growth when bacteria are present, bacteria evidently play some part in the nutrition of *Peranema*, although they alone do not suffice to maintain the cultures. Butterfield (1929), Lilly (1942), and Hardin (1944) had similar results with various other protozoa. Since soil-extract works well, whether bacteria are present or not, it seems that some substances produced by bacteria and beneficial to the growth of *Peranema* can adequately be supplied by soil extract. Attempts to culture *Peranema* on yeast killed by heat, even as low as 80°, failed.

Really pure and thriving cultures were obtained with cow's milk. One drop of milk was added to 8 c.c. of either distilled water, calcium sulphate solution, or 1/10 sea-water. The resulting mixtures were heated in a steam-chamber

for an hour on three consecutive days, and inoculated with single, washed individuals. Multiplication in all three media was so intense that after 3 weeks the inner surface was densely covered with *Peranema*—large, healthy individuals containing numerous food vacuoles. Tests proved the sterility of all the cultures. No difference in the rate of multiplication in the three media has been observed. For maintaining the cultures, however, that with calcium sulphate is preferable to the others because during sterilization the milk is coagulated in this medium, and the fluid is then clear, permitting better observation of the organisms. Otherwise the milk emulsion remains turbid until the suspended milk droplets have been ingested by the *Peranema*.

Milk cultures decline earlier than those with yeast as food, ceasing to flourish after 3 months owing mainly to the accumulation of waste products. The decline could not be prevented by adding more milk. Autoclaved milk gave good results initially but not in successive subcultures. Something essential for *Peranema* seems to be destroyed by excessive heating. The optimum concentration of milk is 0.5–1 per cent. Concentrations higher or lower than these do not support appreciable growth. Bacto peptone, Bacto tryptone, yeast extract, glucose, starch, and almond-oil have no effect when added to milk cultures, whereas 0.1–0.2 per cent. beef-extract accelerates growth, although it does not increase the final density of the population.

With milk as food *Peranema* is not only bigger than with yeast (cf. p. 282) but also contains more and larger paramylon grains and oil-droplets, and it multiplies more quickly. In milk cultures the population reaches its maximum of about 17,000 individuals in 1 c.c. after about 1 month. This density is maintained for about 10 days and then falls steadily to a level of about 12,000 individuals which lasts about 50 days; subsequently the population declines rapidly. Yeast cultures reach only a maximum of about 7,000–8,000 in 1 c.c. but last longer, and extinction does not occur until after 10 months or more, provided food is added repeatedly.

Fairly good results were also obtained with egg-yolk as food, although the density of the cultures was never as high as in milk cultures. Mixtures of casein and butter have been tested in an attempt to analyse the effect of the ingredients of milk. Fat-free casein was kindly supplied by Dr. E. Kodicek (Dunn Nutritional Laboratory, Cambridge). To 10 c.c. of distilled water, calcium sulphate solution, soil-extract or 0.1 per cent. beef-extract was added 0.1 per cent. butter plus 0.02 gm. casein. Culturing and subculturing was possible, particularly in the last two media, but none of the cultures was nearly as good as those with milk. Addition of whey, prepared by clotting fresh milk with commercial rennet, gave but little improvement. Butter or an ether extract of milk, both in 0.1 per cent. beef-extract or yeast-extract, as well as casein in soil-extract, beef-extract, or yeast-extract all support moderate growth. Addition of glucose does not improve the results. The optimum concentrations of butter and casein are 0.36 and 0.2 per cent. respectively. On the whole none of these media is satisfactory. Since butter alone does not support growth it seems that *Peranema* can multiply well only

when some proteinic substance is present which is digested inside the body. No evidence of proteolytic enzymes being excreted could be found by placing drops of a dense *Peranema* culture on gelatine slopes under aseptic conditions.

Many attempts to cultivate *Peranema* in monophasic liquid media have been made. Of the 45 mixtures tested—various combinations of beef-extract, yeast-extract, liver-extract, tryptone and a number of other peptones in various degrees of hydrolysis, glucose, alkali caseinate, and sterile filtrates of casein solutions, egg-albumin and blood-serum—none supported growth. So far as these results go, *Peranema* seems to be strictly zootrophic, as it requires particulate protein food.

#### FEEDING HABITS

While the feeding habits of ciliates and rhizopods have been extensively studied, little is known about those of zootrophic flagellates. *Peranema* is relatively large in size and slow in motion, and ingests readily a great variety of food particles. It seemed therefore suitable for observations on behaviour towards food substances.

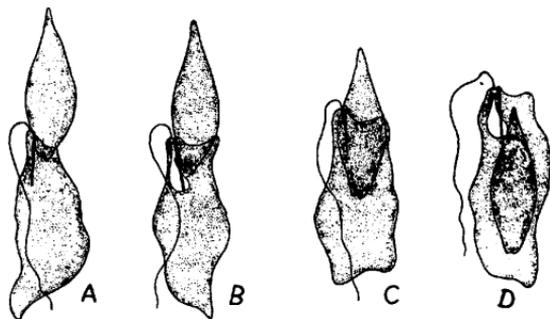
In the first place an answer was sought to the question whether there is any food selection, and experiments were begun in which they were fed: (1) with different kinds of substances separately, to see if there is variation in the frequency of ingestion; (2) with a mixed diet, to see if *Peranema* has the ability to select one and reject the other component; and (3) with non-nutritive coloured substances, continuously for a longer period, to see if *Peranema* can reject these.

A few drops of a *Peranema* culture, containing about 4,000 individuals, were transferred to watch-glasses with a suspension of food-particles or of organisms. At intervals samples were counted in order to determine the ratio of the number of individuals *with*, to that of individuals *without*, the food in question. The result obtained was that, among the substances used, casein was ingested most frequently, while sand-grains and calcium carbonate powder were not taken at all. Between these two extremes, arranged in order from higher to lower frequency of ingestion, were: carmine, *Chlorella pyrenoidosa*, *Stichococcus bacillaris*, *Saccharomyces exiguus*, Chinese ink, *Euglena gracilis*, *E. viridis*, and starch-grains. Thus carmine and Chinese ink, both of no food value, are more frequently ingested than both species of *Euglena*, on which *Peranema* can live.

Carmine was ingested also in the presence of a food substance, for instance casein, *Stichococcus*, or *Saccharomyces*. Like carmine, casein was ingested more readily than any of the organisms tested in mixture with it. Carmine particles are ingested even when the individuals have already taken a considerable amount of real food, e.g. *Euglena* or casein. Satiety does not cause *Peranema* to reject carmine, unlike *Stentor* (according to Schaeffer, 1918). Even by prolonging the supply of carmine, *Peranema* cannot be 'trained' to reject it, as Metalnikow believed to have proved in *Paramecium*.

What there remains of food-selection in *Peranema* has nothing to do with the food-value of the substances, but is determined by physical and chemical properties and, in the case of living organisms, by the motility of the prey.

The controversy regarding the mechanism and the place of ingestion has already been mentioned (p. 280). Previous observers had mostly drawn their conclusions from morphological observations (Hall and Powell, 1928; Brown, 1930; Lackey, 1933). It was hoped that feeding experiments would help to clear up both these points. In observing the process of ingestion, hanging-drop preparations were used containing individuals which had been without



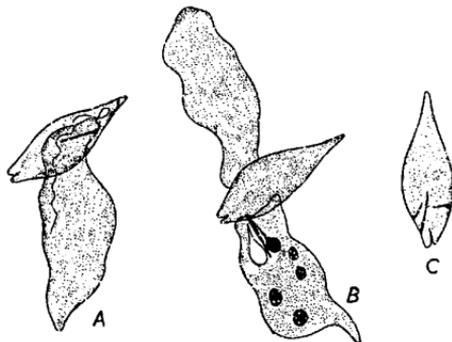
TEXT-FIG. 4. Successive stages (A-D) of *Peranema* swallowing a *Euglena viridis*. The wide extension of the anterior end is seen, and also the independence of the food entrance from the reservoir.

food for a day. Of the thirteen species used as food Euglenineae of larger size gave the most valuable results. In no case were actively moving forms ingested.

When 10-20 individuals of *Euglena viridis* were added to a hanging drop, some soon became motionless and were then attacked by *Peranema*. The moment the tip of the undulating anterior flagellum of a *Peranema* touched a *Euglena*, the whole flagellum began to beat violently; whereupon the cell-body contracted and the flagellum drew back. Soon the cell relaxed and the flagellum resumed normal movement. The process of contraction and recovery was repeated several times until the body came into contact with the *Euglena*, when ingestion began. It always started at the anterior end of the prey (Text-fig. 4). When a *Peranema* touches the front end of a *Euglena*, the rod-organ together with the adjacent region of the ventral body-surface moves forward from its original position, becoming level with the anterior end, until it touches the *Euglena*. The rod-organ then protrudes a little and becomes attached to the surface of the *Euglena*. The body of the *Peranema* starts to move forward around the prey, while the rod-organ remains attached to the periplast and begins to push the *Euglena* through the widely expanded anterior end of the *Peranema* into the interior. The rod-organ is then detached from the

periplast, moves over the surface of the *Euglena* and is re-attached on another part of the periplast, while the body of the *Peranema* follows. By repeatedly attaching, pushing, detaching and re-attaching the rod-organ, with forward movement of the body, the *Euglena* is swallowed. The time required for the whole ingestion varies from 2 to 15 minutes, with an average of 8 minutes. During ingestion the two rods always move together as if connected.

It is not uncommon for a *Peranema* to ingest two *Euglenae* at a time, although its size is scarcely larger than that of the prey. A *Peranema* was seen,



TEXT-FIG. 5. *Peranema* cutting open the periplast of *Euglena viridis* and ingesting its protoplasm. A, *Peranema* protrudes into the cell of *Euglena* after perforating its periplast with its rod-organ. B, Two individuals attack the same *Euglena*. The protoplasm of the *Euglena* is sucked in by one of the *Peranemae* through a way separate from the reservoir, which remains clear. C, *Euglena*, after being attacked, shows three splits on the body-surface.

2 minutes after taking in a *Euglena*, to meet another which was then attacked and swallowed immediately. Both of the two ingested *Euglenae* were soon digested. In some instances the second *Euglena* was only partly swallowed and, perhaps because there was no room to spare for it, eventually egested.

Sometimes a *Peranema*, instead of ingesting a *Euglena* as a whole, cuts open the periplast and sucks out the contents (Text-fig. 5). When the cytostome is applied to the attacked *Euglena*, the rod-organ is protruded and rasps up and down over the periplast of the prey. After 6-10 minutes the protoplasm of the *Euglena* with the green chromatophores begins to flow into the body of the *Peranema*, not through the reservoir but through a passage lateral and nearly parallel to it (Text-fig. 5B). This passage can only be seen when food is passing and may, therefore, be only a temporary, rather than a permanent, structure. The reservoir and its canal, during this process of ingestion, remain clear and free from food particles. Ingested food is accumulated at the posterior end of the passage to form digestive vacuoles which are detached apparently by contractions of the body. The shape of these vacuoles is usually that of the food enclosed, i.e. the food appears to be in direct contact with the surrounding cytoplasm.

Sometimes *Peranema* sucks in only part of the contents of a *Euglena*; in other cases it consumes the whole, leaving only the periplast. As the contents of the prey decrease during the sucking, the anterior end of the *Peranema* may penetrate through a hole in the periplast into the interior and continue to suck there (Text-fig. 5A). The rod-organ, protruding slightly into the cytoplasm of the *Euglena*, seems to help in pushing the food into the body. Sometimes the rod-organ moves slowly along the boundary between the cytoplasm and the periplast, separating the one from the other. The remaining empty periplast shows regularly one or more splits where the rod-organ has been applied (Text-fig. 5C).

The frequencies of the two types of ingestion, engulfing and sucking, vary with the circumstances. When a *Euglena viridis* is attacked by a single *Peranema*, the whole cell will be swallowed. When it is attacked by two or three, it will be either shared by the predators, each sucking a portion of it, or engulfed by one of them. When it is attacked by many *Peranemae*, it is always pierced and sucked by a number of individuals; swallowing by a single individual has not been observed under such conditions. Towards *Euglena gracilis* and its apochlorotic race ('*Astasia longa*'), *Peranema* behaves in the same way as towards *E. viridis*.

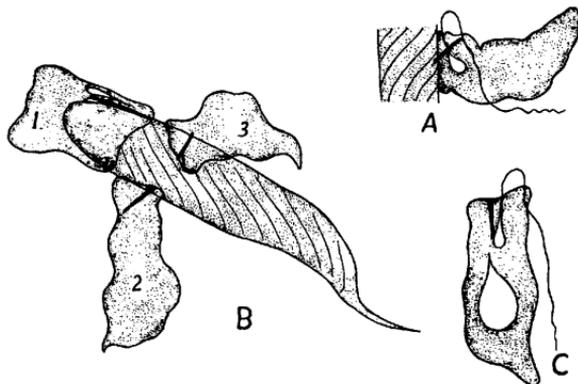
*Euglena spirogyra*, although much larger than *Peranema* itself, is also attacked, but only when it has been killed, e.g. by pressure or by heat. It is easily killed by pressing the coverslip, and cytoplasm flows out through lesions in the periplast. If then *Peranema* individuals are introduced, they soon attack the wounded *Euglena*. Those which arrive first approach the wounds whence cytoplasm is flowing. Only when such sites are occupied do they begin to cut new openings with the rod-organ. Heat-killed *E. spirogyra* is preferentially attacked at the anterior end of the cell. The front part of *Peranema*, truncated and widely expanded, is applied firmly to the surface of the prey, while the rod-organ moves to cut the periplast. It was observed that, while the cytostome and its attached rod-organ were working in contact with the periplast of the *Euglena*, the anterior end of the *Peranema* bearing the flagellum and the reservoir opening was shifted to one side, i.e. out of contact with the *Euglena* (Text-fig. 6A). The cytoplasm of the prey could be seen flowing through the cytostome into the body while the reservoir remained empty.

When a sucking *Peranema* becomes full of food vacuoles it detaches itself from the *Euglena* and its place is taken by another individual. As the cytoplasm of the prey gradually decreases in volume by continuous sucking, the *Peranema* penetrates more and more deeply into the interior and may come to lie completely within the almost empty body of the prey. In one instance three individuals were seen within the periplast of a single *E. spirogyra*.

If not more than four individuals attack a *E. spirogyra* the one nearest the front end usually tries to engulf it, although this can never be successful owing to the smaller size of *Peranema*. The attack proceeds in the same manner as that on *E. viridis* and, after a few minutes, the anterior end of the *E. spirogyra* sinks into the body of the *Peranema* through its tremendously expanded

anterior end (Text-fig. 6B). When the *Peranema* has eaten to capacity, only about one-fifth of the prey is swallowed. As the *Peranema* attempts to engulf more, the rod-organ moves here and there attempting to anchor itself to the periplast, and the body rotates about the axis of the *Euglena*.

It is at this stage that the ingested part of the *Euglena*, sunk deeply into the body of the *Peranema*, is clearly seen to be outside the reservoir, which remains empty and transparent and is pressed to one side of the body (Text-fig. 6B). Eventually the *Euglena* is egested through the cytostome. Immediately



TEXT-FIG. 6. *Peranema* feeding on heat-killed *Euglena spirogyra*. A, during the attack; reservoir and flagellum shifted away from the prey; rod-organ seen to be independent of the reservoir. B, Three individuals attacking the same *Euglena*; the one in front is trying to engulf the whole prey, the reservoir remains empty; the other two begin to apply their rod-organs. C, The same individual (1 in B), immediately after egesting the partly swallowed *Euglena*; note the independence of the rod-organ and the cytostome in front of it, from the reservoir; the space, which was previously occupied by the prey, is separated from the reservoir.

after this, while the anterior end of the *Peranema* remains for a short time widely expanded, the independence of the feeding apparatus from the reservoir and its canal is especially clear (Text-fig. 6C). The cytostome, since it has not yet contracted to its normal size, can be observed near the distal end of the rod-organ as a crescent-shaped opening lateral to the reservoir. The space in the body of the *Peranema* previously occupied by the ingested part of the *Euglena* remains as an empty vacuole not connected with the reservoir system. Soon the anterior end is restored to its normal tapering shape, and the cytostome with its attached rods returns to its previous position, ventral and posterior to the reservoir opening, and regains its former appearance as a transparent region near the end of the rod-organ.

*Cyclidiopsis acus* Korschikoff (= *Astasia linealis* Pringsheim) is much less attractive to *Peranema* than *Euglena spirogyra*, but otherwise affects it in a similar way.

Cannibalism occurs in *Peranema*. When transferred from a culture to a

hanging drop, a certain number of individuals are usually killed or injured and shed their flagella. They are soon eaten by the active individuals, their periplasts being perforated and the contents sucked out. No swallowing of the whole organism has been observed.

*Chilomonas paramecium* is only ingested when motionless, and is then ingested as a whole, without its surface being perforated. Contrary to the statement of Hall (1933) *Chilomonas* was never found to pass through the reservoir system. Hall relied on the observation that in his stained preparations the food vacuole containing the ingested *Chilomonas* appeared to be connected with the reservoir. This, however, does not necessarily mean that the prey is ingested through the reservoir. A food-vacuole with *Chilomonas* occupies a relatively large space, and when the cytoplasm contracts during fixation, the vacuole may seem to be connected with the reservoir.

Cells of *Saccharomyces exiguus* and *S. cerevisiae* are always swallowed whole. For the purpose of observation yeast cells were made more visible by staining with 0.2 per cent. Congo-red solution. When a gliding *Peranema* touches a yeast cell with its flagellum it either contracts and moves away or continues gliding, so that the yeast cell 'flows' along the flagellum to the anterior end of the body. When it arrives there *Peranema* reacts in various ways. It either ingests it or keeps gliding normally, leaving it behind. When ingestion occurs, *Peranema* usually contracts considerably so that, by the time it recovers to its normal shape, the yeast cell is already inside the body. Occasionally, however, the yeast cell is ingested while the *Peranema* maintains normal gliding, without contortion of the body. In the latter case, the passage of the yeast cell into the body can be observed. The observation confirms that it passes from the cytostome into the cytoplasm not through the reservoir system, but through a way near to but not connected with this. Sometimes, however, when the ingested cell lies ventral to the reservoir it seems to be within it, and when it passes posteriorly, it may give the impression that it comes out from the end of the reservoir. A side view will reveal the true position of the yeast cell, ventral to and outside the reservoir. The rod-organ appears to be of little importance in the ingestion of such small organisms. From one to six yeast cells have been observed in a single food-vacuole.

These observations confirm the conclusions drawn from morphological studies described above and the statements by Brown (1930) and Pitelka (1945). They are also in agreement with what is known in other flagellates. In no case have particles been seen to enter the reservoir of any of the Euglenineae, nor indeed of any of the other flagellates. Kent's (1880-2) and Tannreuther's (1923) claims that *Euglena* ingests particles through its reservoir have been proved to be wrong (Mainx, 1928; Hall, 1933*b*). Apparently the reservoir system has some other function than feeding (e.g. respiration or drainage) and when, in the evolution of the Peranemaceae, the mode of nutrition changed to zootrophy, a new opening was developed for the purpose of ingestion. If that be the case, the terms 'cytostome', 'gullet', and 'pharynx' should not be used for the parts of the reservoir system.

## DIGESTION

Almost nothing is known of the digestive powers of non-parasitic flagellates, although proteolytic enzymes have been found in cultures of certain species of *Euglena* (Mainx, 1928; Jahn, 1931; Hall, 1937).

Which of the substances present in the food eaten by *Peranema* are indeed digested can only be ascertained by feeding experiments with different kinds of food. For this purpose *Peranema* from cultures with yeast as food has been used. Since such individuals contain only a moderate amount of reserve substances, observation is relatively easy. The food substances used were: lipid-free casein, egg-albumin, almond-oil, and rice-starch. To one of two watch-glasses with 2 c.c. of the culture-fluid food was added, while the other one was used as control. At intervals samples were taken for microscopic examination. Hanging drops containing single individuals, which were isolated after having ingested a number of food-particles, were also used. These gave a clearer picture of the changes in the ingested food, because the uptake of new food-particles was prevented. Simple histochemical tests (such as iodine for starch; sudan III, sudan black, and chlorophyll-extract for fats; potassium hydroxide for paramylon) were made.

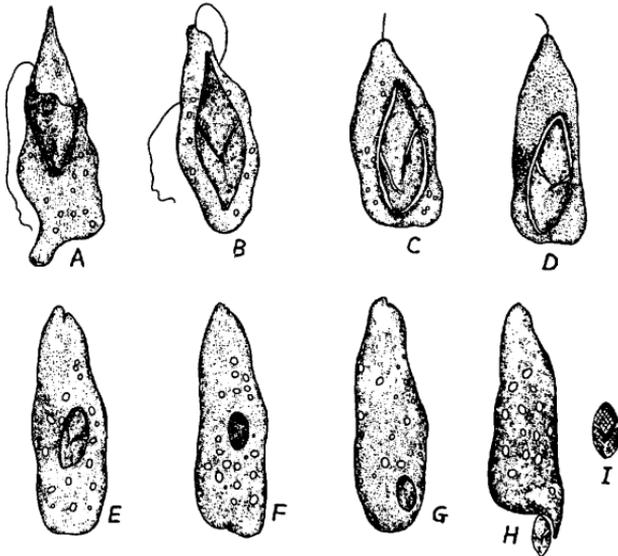
The food-vacuoles of *Peranema* assume the shape of the ingested food and do not undergo translocation along a constant path within the cytoplasm. They either remain at the same place or are moved at random by body-movements. They are largest when newly formed and decrease in size gradually until they are entirely absorbed; or, more usually, the remains, much reduced in bulk, are discharged through the area of defaecation. Although the digestible parts of the food must have been liquefied in the course of digestion, an enlargement of the food-vacuoles, such as commonly occurs in ciliates and rhizopods, has never been seen.

The digestion of *Euglena viridis* by *Peranema* may be taken as an example (Text-fig. 7). Newly ingested cells preserve their colour for about half an hour and then fade to yellow; no change of size is as yet observable. At the same time a very thin layer of the cytoplasm of the *Peranema* around the *Euglena* becomes transparent. After 1 hour the size of the *Euglena* has decreased to about two-thirds. The transparent layer is still visible. By the next day the *Euglena* has been reduced to about one-fifth its original size. Its shape has become ovoid, its colour brown, and it is no longer recognizable as a *Euglena*. The transparent layer has disappeared, and many paramylon grains are seen in the cytoplasm of the *Peranema*. The remains of the *Euglena* are usually discharged, but sometimes they may remain in the body for another day or two, although no further decrease in size can be detected during this time. The discharged remains seem to consist mainly of the periplast. Striations are still visible, as well as splits, apparently those made by the rod-organ.

By feeding *Peranema* with yeast cells stained with indicators (Shapiro, 1927, &c.; Pringsheim 1928), the pH of the food-vacuoles was found to lie

between 5.8 and 6.8. This is very different from those in ciliates and rhizopods, where the pH can be as low as 3 (Shibley and de Garis, 1925; Shapiro, 1927; Pringsheim, 1928; Mast, 1942; Mast and Bowen, 1944).

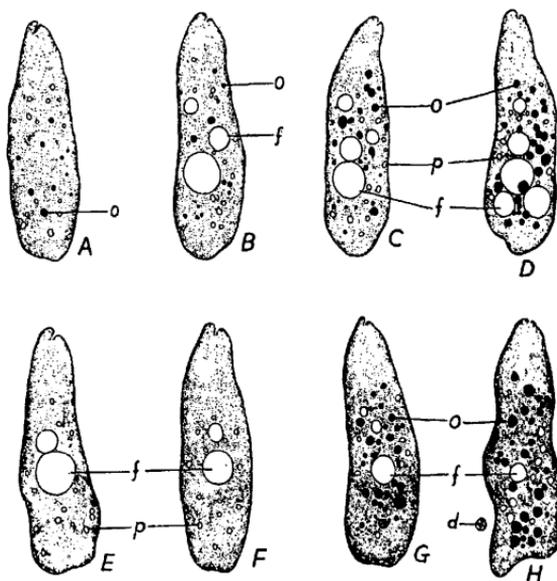
While rhizopods and ciliates are known to digest fat (Lund, 1914; Dawson and Belkin, 1928, 1929; Mast, 1938; Wilber, 1942) nothing so far is



TEXT-FIG. 7. Digestion of *Euglena viridis* by *Peranema*. A, *Peranema* engulfing a *Euglena*. B, The *Euglena* is ingested; note the two splits on the surface of the *Euglena* made by the rod-organ. C, After an hour, the size of the *Euglena* has diminished and a thin clear layer appears around it. D, After 2 hours of ingestion. E, The next day, the size of the prey is much reduced, and the clear layer has disappeared; paramylon-granules appear in the cytoplasm of the *Peranema*. F-H, Successive stages on the third day. H, The remains of the *Euglena* are discharged. I, Enlarged view of the discharged *Euglena* with periplast-striation easily visible.

known in this respect about flagellates. For the relevant experiments almond-oil, stained dark blue with sudan black, was used as food, in order to distinguish the ingested fat from that formed by resynthesis. *Peranema* ingested these stained oil-droplets without visible ill effects (Text-fig. 8). After 2 hours of feeding almost every individual contained a number of blue food-vacuoles. The sudan III test did not reveal any increase in the number of oil-droplets in the cytoplasm. After 5 hours, however, in addition to the large, spherical, blue oil-drops, many droplets, staining red with sudan III, were present in the cytoplasm of most individuals. Five hours later the size and number of colourless droplets stainable with sudan III had markedly increased. In individuals isolated in hanging drops it was found that, while the secondary

droplets increased gradually, the ingested oil-drops decreased in size. Evidently these colourless droplets are fat-substances resynthesized from products of digestion, rather than formed by splitting up the ingested oil into smaller droplets.

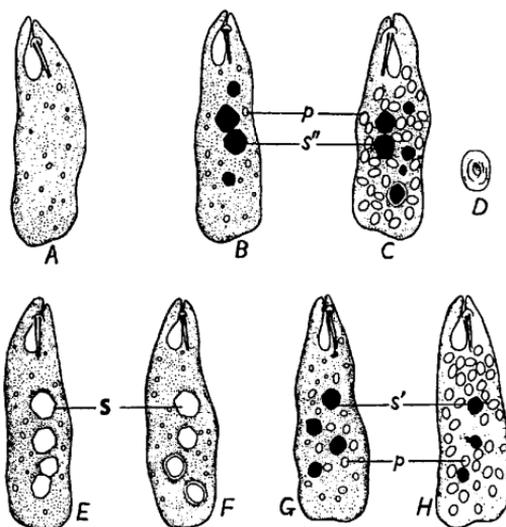


TEXT-FIG. 8. Digestion of almond-oil. A, Control individual not fed with almond-oil. B-D, Individuals which have taken up almond-oil stained blue with sudan black supplied in a watch-glass. B, After 2 hours of feeding. C, After 5 hours. D, After 24 hours of feeding. E-G, Successive stages of individual isolated in hanging drop and digesting ingested oil. E, Two drops of almond-oil have just been ingested. F, After 3 hours; the drops have already decreased in size. G, After 5 hours of ingestion; many oil-droplets have appeared in the cytoplasm. H, Individual after 24 hours of digestion, with a further increase in the number of oil-droplets in the cytoplasm; the remains of one of the ingested oil-drops (*d*) have been discharged into the hanging drop. *f* = food-vacuoles, containing dark-blue oil-drops; *o* = colourless oil-droplets stainable with sudan III; *p* = paramylon granules.

Starch is also digested by *Peranema*, though but slowly (Text-fig. 9). After 1 day's feeding with rice-starch many ovoid paramylon granules appear in individuals which before contained only a few small granules. The newly formed granules are of the same size and shape as those in individuals from milk cultures. After some time, only the centre of the ingested starch-grains stains blue with iodine, while the outer layers are faintly brown. This indicates that the starch is chemically attacked. The decrease in size of the starch grains is clearly observed in hanging-drop preparations. The correlation between increase in number and size of paramylon granules and the decrease in size

of the ingested starch shows that the former result from digestion of the latter.

For observations on the digestion of protein, casein was used, mainly because it is easy to handle and has proved a suitable food (Text-fig. 10). It was found that after 24 hours, when the ingested casein particles have

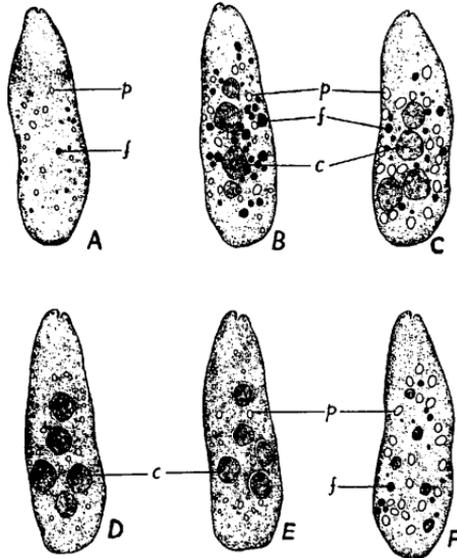


TEXT-FIG. 9. Digestion of rice-starch. A, Control individual without starch. B and C, Individuals which have taken up rice-starch in a watch-glass. B, After 1 hour of feeding; starch-grains stain dark blue with iodine. C, After 24 hours of feeding, showing the appearance of numerous paramylon granules; though the starch-grains are represented black in the figure, the peripheral layer in fact only stains faintly, indicating that it is being digested. D, Enlarged view of a starch-grain. E-G, Successive stages of an individual isolated in a hanging drop after ingesting four starch-grains. E, Immediately after feeding. F, After 2 hours of digestion, showing clear layers around two food-vacuoles. G, After 8 hours: paramylon-grains have augmented; one starch-grain stained only faintly in its peripheral layer while the centre was dark blue. H, After 24 hours: many paramylon-grains, starch gradually disappearing. *p* = paramylon-grains; *s* = ingested starch; *s'* = starch blue by iodine; *s''* = faintly stained outer layer.

decreased to about one-half or one-third of their original size, many oil-droplets of various size appeared; the quantity of paramylon only increased subsequently. In order to decide whether the oil is derived from fatty impurities in the casein, specially re-purified casein was used in repeating the experiment. The result was almost the same. It is concluded, therefore, that *Peranema* is capable of digesting protein and of converting it to oil and paramylon.

Both oil and paramylon are to be considered as reserve food, because in flourishing cultures, individuals contain more of them, and when the cultures are declining, both decrease in quantity until very little is left, while the size

of the cells decreases also. The same changes were observed in individuals specially starved. During one day of starvation the number of oil-droplets increases considerably, but after that both oil-droplets and paramylon granules decrease steadily in number and size until the individuals die.



TEXT-FIG. 10. Digestion of casein. A, Control individual not provided with casein. B, Individual fed with heat-denatured but otherwise untreated casein for 24 hours in a watch-glass, showing the appearance of paramylon-granules and many oil-droplets. C, Individual supplied with alcohol-ether extracted casein, after 24 hours, showing a lesser increase in oil-droplets. D-F, Successive stages of an individual digesting extracted casein after isolation in a hanging drop. D, Five food-vacuoles with casein. E, After 2 hours of digestion, showing a thin, clear layer around some of the food-vacuoles. F, After 24 hours, showing decrease in size of casein-particles and increase of paramylon-granules; scattered between them are oil-droplets staining with sudan III. *c* = ingested casein; *f* = oil-droplets; *p* = paramylon-granules.

diminution of the *Peranema* cells is considerable, from the average size of individuals in yeast cultures,  $50-55 \times 16-18 \mu$ , at the beginning, to  $42-46 \times 9-10 \mu$  on the seventh day of starvation. Shortly after that they all perish.

#### MOVEMENT AND CHEMOTAXIS

##### *Movement*

It has long been known that *Peranema* is capable of performing two kinds of movement, gliding along the substratum or the water surface, and swimming freely in the water (Kent, 1880-2; Mast, 1912; Massart, 1920). Although Mast has observed both gliding ('crawling') and swimming movement, he

believes that the latter occurs only exceptionally. I have regularly observed free-swimming individuals in undisturbed cultures, however, and can testify that swimming occurs under natural conditions although not as frequently as gliding.

The mechanism of the movement of the *Peranema* flagellum has often been studied (Bütschli, 1883-7; Gray, 1922, 1931; Krijgsman, 1925; Lowndes, 1936, 1941, 1944a, b; Brown, 1945). But the nature of the gliding movement has not attracted much attention. In the following the term 'flagellum' is used for the long, free flagellum. The function, if any, of the posterior flagellum, attached to the ventral body surface, is unknown. As stated by Massart (1920), *Peranema* always glides on the same (ventral) surface, whether over solid bodies or the water-air interface. During gliding the flagellum stretches out in a straight line and appears rigid and motionless save at the distal end, which beats incessantly to the right as seen under the microscope, its tip describing roughly an ellipse. The change of direction of movement seems not to be determined by the action of the flagellum. In most of my observations the body turned first to the new direction while the flagellum remained behind; only in a few cases did the flagellum bend first. The same conclusion has been reached by Mast (1912).

Mast holds that *Peranema* glides more quickly when the undulation of the flagellum extends over a greater length as a result of irritation. This was not confirmed. On the contrary, when the individuals are stimulated, as Verworn and Lowndes (1944b) have already pointed out, though the flagellum beats then throughout its entire length, the body contracts and no longer moves forward. Maximum speed of gliding can only be observed when both the flagellum and the body are in a state of full extension. Measurements at room temperature have given an average speed of  $15.6\mu/\text{sec}$ . when individuals are moving along the bottom of the container, and  $22.9\mu/\text{sec}$ . when they are gliding over the water-air interface. Mast (1912) recorded a speed of  $21.7-43.4\mu/\text{sec}$ ., Shortess (1942)  $26.17\mu/\text{sec}$ ., and Lowndes (1944b)  $20\mu/\text{sec}$ ., all without mentioning the substratum over which the individuals moved. No correlation has been found between the size and speed of the individuals.

Gray (1931) and Lowndes (1936, 1941, 1944a, b) believe that the flagellum is an active unit capable of generating its mechanical energy. The latter author stated that the undulation wave starts from the base and is transmitted to the tip. This was confirmed by studying the movement in a soft 0.25 per cent. agar jelly. The fact that only the tip of the flagellum beats strongly suggests also that the flagellum generates at least part of its energy. However that may be, the role of the flagellum in gliding is by no means established. During gliding *Peranema* is attached to the substratum so firmly that it is not readily detached by the sucking force of a capillary tube. Furthermore, when *Peranema* is transferred to a new medium, it is usual for a number of individuals to lose part of their flagellum. Normal gliding was observed in individuals with only two-thirds of the flagellum left, so that the active tip was no longer

present. In individuals with still shorter flagella, about one-third of the body-length, normal gliding movement no longer occurs, although they can proceed slowly by wave-like contractions passing from the anterior to the posterior end of the body. Such wave-like contractions can occasionally be observed in normal individuals also. Evidently the cell-body is capable of moving by itself.

The results were confirmed by observations on individuals with the flagellum removed with the aid of a pair of glass needles under a dissecting microscope. When the whole flagellum was removed the individual immediately rounded up and remained motionless, as if dead. After not more than 2 minutes it recovered and, while the body remained in the same place, the anterior end was extended from the mass and then withdrawn by contraction. This was repeated again and again. After 17 minutes a very short flagellum, not longer than one-fifteenth the body-length, had appeared; it could be only seen under high magnification. This short flagellum was for the most part inactive, but occasionally gave a few feeble beats. The individual was still unable to glide. After about half an hour the flagellum had grown longer, to about one-fifth of the body-length. By this time the body was capable of being extended to its full length but never kept this shape for longer than a minute. After 50 minutes the flagellum had grown to one-third of the body-length and was beating forcibly, but stiffly, like a brandished stick rather than like a whip; the body now progressed apparently by peristalsis, waves of contraction being seen to pass from the anterior to the posterior end of the body.

Only after 2 hours, when the flagellum had attained a length equal to that of the body, its end began to undulate in the normal way, and gliding movement was resumed. The results obtained from individuals with only part of the flagellum removed were practically the same; i.e., the body is capable of advancing by peristaltic contractions while the flagellum is still short and seemingly functionless. Gliding, however, only begins when the flagellum has reached almost its normal length, earlier after shedding the flagellum than after its removal by an operation.

All these observations, especially that with half the flagellum shed, support the suggestion that the body is moving independently of the flagellum, which is not essential for gliding. This is comparable to the behaviour of other Euglenineae, e.g. *Euglena mutabilis* Schmitz, which are capable of gliding although devoid of actively functional flagella, only short stumps, not sticking out of the reservoir opening, being present. It is possible that there is in *Peranema* a correlation between the activity of the flagellum and the maintenance of the normal shape of the body. Like other gliding organisms *Peranema* discharges mucilaginous matter which tends to cover the surface of the container of old cultures, even if free from other organisms. What connexion this mucilage may have with the gliding movement is not known in *Peranema* any more than in other instances.

Lowndes's assertion (1944*b*) that the flagellum may have a sensory function is in agreement with my observations. When a gliding *Peranema* hits an

obstacle with the tip of its flagellum the latter undulates strongly, and the body contracts spasmodically before it has itself touched the obstacle. Whether this is a grain of sand or suitable prey does not at first make any difference. We conclude that the tip of the flagellum is mechanically irritable, and that the difference between inert particles and food only affects behaviour if the latter produce chemotactically active substances which prevent the gliding individual from turning away. When beginning to glide again, an individual that has hit an indifferent object moves in any direction, without preference; one that has come near to a nutritive object, however, glides nearer and nearer to it. The flagellum is therefore sensitive to touch, while chemical sense may be located in the cell-body.

Whereas the role of the flagellum in the gliding movement is doubtful, swimming is definitely performed by its action. By using a compound microscope with the tube horizontal and fastening a test-tube culture to the vertical stage, the swimming movement can be watched without agitating the culture. As the individuals proceed in all directions at random, the translocation is certainly not due to streaming of the medium. During swimming the body rotates continually, usually counter-clockwise but sometimes also clockwise. Generally the posterior end moves almost steadily in a straight line while the anterior end describes a spiral, the body pivoting about the posterior end, but sometimes the body describes a double cone. The greater, basal part of the flagellum rotates, describing a funnel, also counter-clockwise or clockwise, while its tip is bent sideways and undulates as in gliding. *Peranema* does not swim steadily over a long distance. At short intervals of time the body contracts without apparent cause and, after re-extending, the individual starts swimming again, either in the previous or more frequently in another direction. The speed of swimming of different individuals varies between 20.3 and 42  $\mu$ /sec. with an average of 31  $\mu$ /sec. (faster than when gliding).

#### *Chemotaxis*

The existence of chemotactic irritability in *Peranema* is revealed by the tendency to assemble around sources of food. When for instance a dead *Euglena spirogyra* is introduced into a drop of a *Peranema* culture, many individuals soon assemble around it, and when a piece of casein is hung in a test-tube culture of *Peranema*, it is densely covered with individuals after a few hours. Chemotaxis is, however, not restricted to substances suitable as food. If coarse carmine lumps of the size of about half a wheat grain are added to some drops of *Peranema* culture in a watch-glass, individuals soon assemble around them and proceed to ingest them, piece by piece. It is evident that carmine is not ingested by chance.

For testing chemotaxis the capillary method of Pfeffer (1888) was adapted to the special needs of this organism. Pringsheim and Mainx (1926) in their work on *Polytoma* filled capillaries, left open at both ends, by their own suction force, instead of under the air-pump; but for the present experiments it was necessary to seal one end after filling, by quickly dipping into melted

paraffin, because during the relatively slow movement of the organisms the fluid column in the capillary began to move. Another innovation connected with the larger size of the organisms was the use of watch-glasses instead of drops between slide and slip. About 0.2 c.c. of the stock-culture was poured into the watch-glass and left on the stage of the microscope for at least an hour before the open end of the capillary was dipped into its centre.

Very distinct reactions are exhibited towards acetic, nitric, hydrochloric, and sulphuric acids. At certain concentrations, for instance 1/100 N HCl, individuals assemble around the opening of the capillary, forming a ring with a clear space within, owing to equilibrium in this zone between attractive and repulsive action. The higher the concentration, the larger the diameter of the empty space. When the concentration of the acid is decreased, the empty space becomes smaller and eventually, at proper concentrations, the individuals react by directly coming to the opening of the capillary, forming a cluster instead of a ring. Such concentrations are considered to be those to which *Peranema* reacts positively. They have been found to be:

Acetic acid . . . . .	1/200-1/500 N
Nitric acid . . . . .	1/750-1/1000 N
Hydrochloric acid . . . . .	1/650-1/800 N
Sulphuric acid . . . . .	1/550-1/800 N

The fact that the thresholds of the three strong acids are of the same order and are more dilute than that of the weak acid, and that corresponding salts of these acids do not provoke any reaction of *Peranema*, indicates that the chemotaxis is caused by H<sup>+</sup> ions rather than by the anions. A similar phenomenon is known for *Paramecium caudatum* (Jennings, 1906), *Polytoma uvella* (Pringsheim and Mainx, 1926), *Euglena gracilis* (Mainx, 1928), and *Astasia ocellata* (ibid.).

To alkalis no clear reaction was observed. Protein-decomposition products, such as yeast-extract, beef-extract, bacto-tryptone, and bacto-peptone, have a strong attractive effect. The threshold for yeast-extract (dry, Difco) is at 0.1 per cent.; for bacto-peptone at 0.05 per cent.; for the other two substances at 0.001 per cent.; while 0.1-0.2 per cent. of these causes very strong positive chemotaxis.

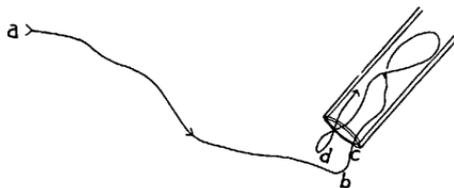
Starch grains do not exhibit an attractive action when presented in a capillary tube, while carmine, which is engulfed so plentifully, causes strong attraction in these conditions. Even very dilute suspensions of carmine in capillaries cause positive chemotaxis.

The impression that dead individuals of *Euglena* are sought out by *Peranema* with the help of a chemical sense was also tested by the capillary method. *Euglena spirogyra* or *E. viridis*, killed by moderate heating in CaSO<sub>4</sub> solution, were sucked into capillaries so that they lay at a short distance from the open end, and the *Peranema* individuals in the watch-glass could not touch them. Many *Peranemae* approached the opening of the capillary, some of them gliding forward until they came near to the dead *Euglena*. Even old culture fluid, freed from *Euglena* by centrifuging, caused positive reaction. As this

medium is slightly alkaline, the positive reaction cannot be caused by carbonic acid discharged by *Euglena*. The fresh, unused culture medium is not attractive.

In a similar way it was shown that living or heat-killed *Saccharomyces exiguus* exude chemotactically attractive substances which can be washed out and tested with a positive result.

The mode of reaction can easily be watched, owing to the slowness of movement (Text-fig. 11). It is clear that *Peranema* turns back when about to



TEXT-FIG. 11. Diagram of the path of a *Peranema* gliding into a capillary tube containing water-extract of carmine. (a) starting point from which the individual moved steadily to (b), then it turned to the left and approached the opening of the capillary; (c) after a few minutes it entered the capillary; when it came out to point (d) it turned sharply and went back into the capillary.

leave a diffusion zone into which it has penetrated by chance, exhibiting phobochemotaxis. Whether the chemotaxis of *Peranema* could be described as klino-kinesis can only be decided by special investigations.

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