Zoology. — *Observations on a special manner of feeding of a species of Difflugia (Difflugia rubescens* PENARD). By H. R. HOOGENRAAD and A. A. DE GROOT. (Communicated by Prof. J. BOEKE.)

(Communicated at the meeting of January 25, 1941.)

(*Rhizopoda* and *Heliozoa* of the Netherlands. VIII.)

The species of *Difflugia* belonging to the thecamoebe Rhizopod genus feed in general by ingesting "ready" food: all kinds of smaller organisms and particles of sapropelium, which are digested in food-vacuoles. As is known this is the usual manner of feeding of the vast majority of marine and freshwater *Rhizopoda*; by the side of it there are as variants cases of intracellular symbiosis (e.g. *Amoeba viridis, Paulinella chromatophora, Hyalosphenia papilio,* species of *Amphitrema*), in which the ingestion of ready food recedes into the back-ground or is entirely absent, and a number of representatives of the *Vampyrella* group (species of *Vampyrella, Vampyrellidium, Hyalodiscus, Leptophrys*), which feed on the living cell contents of various species of algae; they ingest these contents, after first puncturing the wall of the alga cell they have chosen for their prey.

This peculiar manner of feeding was discovered in 1865 by CIENKOWSKY in *Vampyrella lateritia* (= *Spirogyrae*), which lives on the cell contents of various *Spirogyra* species and other filamentous algae belonging to the group of the *Conjugatae*. Afterwards it has also been found in related species, which all empty in the same manner the cells of *Conjugatae*, occasionally (*Vampyrellidium*) also those of *Cyanophyceae*. In 1927 one of us (HOOGENRAAD) described a form (*Leptophrys*), which makes the cells of a species of *Desmidiaceae* (*Closterium Dianae*) its prey; a second case of feeding on the cell contents of a species of *Closterium* (*Closterium intermedium*) is mentioned by POISSON and MANGENOT (1933) in a Rhizopod, which they called *Vampyrella Closterii*. Besides, MARGARETE ZUELZER (1927) discovered an *Amoeba* (*Amoeba biddulphiae* n. sp.), which in an entirely similar way feeds on the cell contents of a marine *Diatomea, Biddulphia sinensis*. In all these cases the Rhizopods showing the aberrant manner of feeding belonged to the group of the *Gymnamoebae*, hence of the naked forms, that are not provided with a theca.

Since then it appeared, however, that this manner of feeding is not confined to this group. In 1935 STUMP discovered it in North America in a few species of thecamoebous *Rhizopoda*, belonging to the interrelated genera *Difflugia, Pontigulasia* and *Lesquereusia*: just like the species of the *Vampyrella* group these animals feed on the cell contents of the species

of *Conjugatae*. At the same time (1935) we mentioned some preliminary observations of a species of *Difflugia* (*D. pulex* PENARD), which seems to ingest the cell contents of filamentous *Cyanophyceae*; at the time we were unable, however, to state further particulars. Now, during a short stay at the Biological Station at Wijster, in July and August 1940, we collected there a number of samples of sphagnum and sapropelium; one of the latter appeared to contain a rich population of another species of *Difflugia*, viz. *D. rubescens* PENARD, which showed the same manner of feeding, with an object for its prey, however, different from the *Difflugia* species of STUMP. This case had been unknown so far; below we submit a short report of our observations.

Among the species of the genus *Difflugia D. rubescens* (Fig. 1) occupies a somewhat separate place owing to two characteristics, by which it varies from the other species of the genus. As an independent species it was described for the first time by PENARD in 1891 from material from the Rocky Mountains, which material contained exclusively encysted, and no living specimens. Previously (1879) it had probably been observed already by LEIDY, likewise in North America, but had been considered as an aberrant variant of *D. oblonga* EHRBG. (= *D. pyriformis* PERTY). Since then it has also been recognised in various places in Europe; in the Netherlands we know it from a dozen of localities, all of them in sphagnum, or at least with sphagnum contact.

Of the two characteristics differentiating *D. rubescens* from the other species of the genus one concerns the shell, the other the cytoplast. The shell is not colourless, but a lighter or darker brownish yellow and rather transparent. While the pseudo-chitinous material seems to be more strongly developed than in the other species of Difflugia, owing to which the thickness of the wall of the shell is greater than usual, the covering with xenosomata is mostly rather scarce, which, also thanks to the transparency of the shell, causes the cytoplast to be usually well perceptible through the wall of the shell. Occasionally, however, the shell bears, mostly be it in small numbers only, enormous *Diatomeae* cells and/or grains of silica. The mouth is round with a more or less crenate inner-edge.

The cytoplast itself, just as in other species of *Difflugia,* is colourless; it contains, however, a smaller or larger number of glossy, spherical elements, coloured a bright orange-red, which are sometimes heaped up locally, on other occasions are scattered throughout the cytoplast, and which also penetrate into the basis of the pseudopodia, occasionally even into the top of them. In spite of the fact that PENARD calls them "grains" the always rather pure spherical shape seems in our opinion to point rather to the liquid state of aggregation; we would therefore prefer to consider them as drops. Never did we see two or more of them merge, however; if, therefore, they are in a liquid condition indeed, it is possible that just as in the case of the disperse elements of an emulsion a surface layer of a somewhat different consistence will prevent them from joining.

The size of the shell seems to be very variable. PENARD (1891) states a length of 30—35 μ, but reports afterwards from the environs of Geneva two forms with a length of 58—60 μ and 83—90 μ respectively. So far we were able to ascertain for populations of very different origin also a length of 65—95 μ. Now the Wijster population seems to be the largest ever observed; the length of the shell varied from 60—140 μ, the breadth from 40—70 μ.

The scanty information about this species in the half century elapsed since its discovery refers to the morphology; little if anything was known about its manner of living. Likewise, data about the nature and the ingestion of food are wanting. Now at an earlier date already, in sphagnum of Vriezenveen, we had come across some specimens of this species with dirty-green inclusions in their cytoplast, pointing to chlorophyll feeding; to this we did not pay special attention, however, at the time. In the Wijster sapropelium sample, containing the *Difflugia rubescens* population, there were also some species of *Desmidiaceae, inter alia* some species of *Closterium*, viz., according to the determination of Dr. BEIJERINCK, Director of the Wijster Biological Station, *Closterium Ulna, intermedium* and *striolatum*, the former general, the latter two rarer. In the early part of October 1940 our attention was drawn by the fact that living specimens of *Difflugia* were not infrequently seen lying with their mouth against a sound or empty *Closterium* cell, while, when the *Closterium* cell shifted, e.g. by letting a drop of water flow under the cover glass, the *Difflugia* individual also moved, the mutual position, however, showing no change whatever.

A short time after a *Difflugia* individual was found in the same position with respect to a *Closterium* cell, which was not sound, but for the greater part empty; only in the cell tops there were two small, elliptical masses of chlorophyll, evidently remnants of the two chromatophores (Fig. 2 a). The animal was just beside the middle of the alga cell. A further examination showed that on the left and on the right in the cell there lay stretched out a *Difflugia* lobopodium, enveloping with its top the remnants of the chromatophores. At the same time it appeared that the lobopodia were not at rest, but were slowly drawn in with he remnants of the chromatophores. While the latter gradually lost their outlines in the lobopodium and faded away as it were (Fig. 2 b), they ultimately disappeared in the *Difflugia* shell; a moment after also the two lobopodia were drawn in. After some time the animal abandoned the cell. In the place where it had been attached the cell-wall showed an irregularly formed hole with fibrously frayed edges. It was not difficult to find in the same preparation another number of empty *Closterium* cells with similarly formed holes. (Fig. 3, 12—14).

The next day the ingestion of the contents of a *Closterium* cell by a *Difflugia* could be observed from beginning to end, an observation which was repeated numerous times in the course of the following weeks (Fig. 4). On the whole the proces passed off uniformly; below we are giving an

account of the case first observed as reported just now, after which we intend to give some variants and supplementary information.

When the animal was discovered (2.30 p.m.) it lay against a *Closterium* cell in the manner as described above, this time, however, not in the proximity of the middle, but close to one of the extremities, at about $^1/_7$ of the total length of the cell (400 μ) distant from it. The contents of the *Closterium* cell were, as far as we could ascertain, still completely unimpaired. At 2.40 p.m. we let a large drop of water flow under the cover glass, through which the *Difflugia-Closterium* system shifted, it is true, but the mutual position did not change.

2.45 p.m. The chromatophore nearest to the *Difflugia* is gradually beginning to recede from the wall — plasmolysis — under clear signs of disintegration and subsequent aggregation; the terminal vacuoles with their crystals are still intact, the latter still showing the normal Brownian movement. No *Difflugia* pseudopodia are perceptible in the *Closterium* cell.

3.— p.m. Under distinct signs of progressive disintegration also the second chromatophore streams into the neck of the *Difflugia*: in the neck two green, parallel streams of chlorophyll are now visible, which gradually disappear in the plasma of the *Difflugia*; the chromatophores have lost their normal, sound form and are twisted wave-like; in the *Closterium* plasma large vacuoles appear.

3.15 p.m. Also the two terminal vacuoles with the crystals are beginning to move; the twisted edges of the chromatophores stretch themselves, regaining a sounder form. All the time little change is to be seen in the shell cavity of the *Difflugia*; only, it strikes us that the cytoplast, which at first filled the space almost entirely, has now receded some distance from the fundus wall. There are no epipods to be seen. In the apparently very dense *Difflugia* plasm the green colour of the *Closterium* chromatophores is becoming visible among the orange-red pigment drops of the *Difflugia*.

3.36 p.m. From one of the chromatophores a piece snaps near the end, its position, just as that of the crystal lying behind it, remaining unchanged. The movement of the two chromatophores goes on normally, at the rate of abt. 2 μ a minute. In the distal, empty part of the cell halves of the *Closterium* no pseudopodia are visible.

4.03 p.m. One chromatophore, except the isolated piece, has now disappeared entirely in the neck of the *Difflugia*; the second is still abt. 120 μ long, the distal end being still abt. 140 μ away from the terminal vacuole, which has hardly moved yet.

4.30 p.m. In the two parts of the *Closterium* cell *Difflugia* pseudopodia are becoming visible, extending distally, one of them ingesting the isolated remnant of the chromatophore.

4.39 p.m. This remnant disappears into the neck of the *Difflugia*. A part of the second chromatophore is carried along by the pseudopodium, extending distally; the latter reaches the crystal — the terminal vacuole itself is no longer discernable — envelops it and recedes with the remnant of the

chromatophore and the crystal; both parts of the cell contents disappear into the neck of the *Difflugia*.

5.— p.m. The *Difflugia* pseudopodia, which are still stretched and which fill the two cell halves almost entirely, are gradually beginning to withdraw; the entire contents of the *Closterium* cell have now been ingested in the *Difflugia* plasm.

5.25 p.m. The pseudopodia have been entirely drawn in, the *Closterium* cell being completely empty; the animal does not yet abandon the cell, however.

5.48 p.m. Again two pseudopodia are becoming visible in the empty cell, searching the wall gropingly, as it were.

6.10 p.m. Also these pseudopodia are drawn in, through which the cell is empty again.

6.25 p.m. The *Difflugia* individual abandons the *Closterium* cell; in the wall of the latter the hole made by the animal is clearly visible.

As was already observed the course of the process of food ingestion of *Difflugia* rubescens is in the main rather uniform. Of the variants the following may be mentioned.

The point of attachment of the *Difflugia* individual can be very different. Sometimes it is close to the middle of the cell, on other occasions close to one of the extremities. Also attachment exactly in the middle — the point of the least resistance — was observed occasionally, but seems to be rare. On some occasions we found an empty cell with two holes, afterwards also a few times a sound cell, which was attacked by two individuals of *Difflugia* simultaneously. In one of these cases the two animals were at almost exactly opposite points of the alga cell.

In most cases the entire contents of the *Closterium* cell will be ingested and in such a manner that the two chromatophores with the cytoplasma and the nucleus gradually disappear in the shell of the animal. In this process a disorganization of the chromatophores takes place, it is true, but they remain whole. In other cases the chromatophores break into a number of pieces, which are separately and one after another enveloped and ingested. Occasionally it was observed that one or more of these fragments remained behind in the *Closterium* cell; sometimes this was likewise the case with one of the gypsum crystals.

A peculiar variant in the treatment of the *Closterium* cell is shown in Fig. 5. When the animal with the alga was discovered the latter was still almost entirely sound. The *Difflugia* individual had attached close to the middle of the cell; at this point the cell had a crack, however, the two parts being at an angle of abt. 120°; the cracked part of the cell had partly penetrated into the neck of the *Difflugia*. The process of ingestion took place in the normal manner; in the course of it the angle formed by the two halves of the cell now became smaller (to abt. 80°), now larger (to abt. 120°). After 50 minutes ingestion was completed and the animal released the cell; the empty cell remained cracked and showed the hole in the wall made by the *Difflugia* precisely at the crack (Fig. 5 b). The *Closterium* cell in

question had a total length of abt. 320 μ by a breadth of only 10 μ; maybe, therefore, the thickness of the wall was also slight, through which the cell was apt to crack at the point where the animal attacked it.

On another occasion again, during the process of ingestion, which for the rest was on the usual lines, one of the chromatophores broke into a number of pieces, which separated. One of them remained in the distal end of the cell half, but was gradually dissolved by a pseudopodium into small elements, the green colour of which could hardly be distinguished or not distinguished at all, after which these elements were carried by the pseudopodium to the *Difflugia* individual.

In various cases the emptying of a *Closterium* cell, either entirely or partly, was observed, in which action no pseudopodium penetrated.

In the immediate proximity of a *Difflugia* individual that was discovered after it had emptied a *Closterium* cell and had already abandoned it, there lay a number (abt. 20) of dark-brown grains of 10—15 μ diameter, which gave one the impression as if they had been excreted by the animal. Afterwards these particles were also more than once seen in the cytoplast of the animal itself, and finally it was observed — so far but once — that an individual disposed of a clump of these grains by excretion. We intend to return to the significance of this later on.

Occasionally an animal was taken in the act of attacking a *Closterium* cell (Fig. 6 a, 7, 11). Then a plug of animal plasm was clearly visible between the mouth and the wall of the plant cell. Afterwards, when contact became closer this plug disappeared again, but a mass of plasm became visible on the other side of the cell, which was evidently held from below.

The time required for the process of ingestion seems to vary a good deal. One case was over in 40 minutes; in another case a *Difflugia* was found in contact with an entirely sound *Closterium* cell at 6 o'clock p.m., only about half of the latter's contents being ingested the next morning at 10.30, while the ingestion was not completed until noon.

As was observed before, the place of the hole made in the wall of the *Closterium* cell by the *Difflugia* individual, varies. During ingestion it is not visible, but all the more visible in empty cells. The form, too, varies; it is usually irregularly round, elliptic or quadrangular; the size (i.e. the greatest measurement) is mostly 10—20 μ. (Fig. 3, 12—14). The edge is never completely smooth, but always more or less irregularly crenate or corrugate, or drawn out in fibrous frays. Occasionally (Fig. 3 c) there hung beside the hole a shred of the cell wall, probably the fragment of the wall that had formerly been in the place where there was now a hole (vide p. 223).

A few times empty cells with two holes were found, and afterwards also a sound cell, to which two *Difflugia* individuals had attached themselves at the same time; this cell was found back the next day with two holes in the wall. Finally, in a similar case, the attack on a cell by two individuals simultaneously could also be observed from beginning to end.

These individuals had attached themselves to the cell wall almost exactly opposite each other. The result was that one of them secured the whole contents of the cell but for some remnants, which remained in the cell, whereas the other did not get anything.

About the form of the holes made by *Vampyrella* and related species in the cell wall of the algae attacked, only incomplete and contradictory data are available.

CIENKOWSKY speaks of a large, not sharply outlined, hole. KLEIN is altogether silent about it, but in one of his figures pictures an alga with a small, broad, elliptical hole with smooth edges. According to SCHERFFEL the animal makes "a beautiful round hole"; LLOYD calls the hole "clean cut"; MIHAÉLOFF describes it as a star-shaped form. The holes made by *Difflugia* c.s. in the wall of the *Spirogyra* cells always have, according to STUMP, the form of tears or rents, and never the round form of the holes made by *Vampyrella*. Finally, POISSON and MANGENOT are quite positive when they say that the wall of the *Closterium* cell is cut open by their *Vampyrella Closterii* according to a semi-circular line; from the picture it appears that in this process a fragment of the cell wall is loosened and turned back like a sort of lid. (Fig. 3*g*).

The nature of the process by which *Difflugia* punctures the wall of the *Closterium* cell is still uncertain. Originally (CIENKOWSKY and others) the opinion was held that in the *Vampyrella* group this process was of a typically chemical nature, i.e. that by the excretion of matter of a perhaps enzymatous nature the animal dissolves the cell wall locally. Later observers (PENARD, ENTZ, ZUELZER, LLOYD, GOBI, POISSON et MANGENOT, STUMP) have made it probable, on the ground of observations of various objects, that partly, at least, also mechanical factors may play a part in it. This is very evident indeed in *Leptophrys elegans* (HOOGENRAAD 1927), which literally cracks and breaks in two the cells of *Closterium Dianae*, ingests the contents, subsequently to eject the two halves of the cell wall. Also the observations in the case of *Difflugia rubescens* seem to give support to this view. Above it was already pointed out that the holes made by this animal have edges, the appearance of which suggests indeed a tearing open of the cell wall. Occasionally it was observed, as is also shown by POISSON and MANGENOT in a picture of the *Closterium* attacked by their *Vampyrella Closterii*, how as it were a piece of the cell wall is detached, though not quite separated from it, so that it hangs down from it as a rag. Particularly the case described on p. 221 of the specimen of the *Closterium* cell that was cracked in the ingestion, points to the probability that mechanical factors also play a part; it may be imagined that during the sucking action the cell cracks at the point of the hole owing to suction.

If one asks oneself how such a deviating manner of feeding as the ingestion of the cell contents, after the cell wall has been punctured, may have arisen, the following seems possible. At any rate our starting point must be the usual manner of feeding by envelopment and ingestion in

vacuoles of the particles of food. Also in some forms of the *Vampyrella* group (*Vampyrella* spec. div., *Leptophrys*) this occurs by way of exception, whereas this is the rule in the species of the genera *Amoeba, Difflugia, Pontigulasia* and *Lesquereusia*. Also *Leptophrys elegans* ingests the *Closterium* cells in its cytoplast, before they are broken in two; at the same time the living contents are then drawn out. To this may correspond as an extreme case that the isolated cells (*Closterium*) or the cells living in cell groups (*Spirogyra* etc.) are no longer ingested, but opened and emptied. In this connection an observation made by STUMP is of importance. For he saw that, when filaments of *Mougeotia* were attacked by *Lesquereusia spiralis* at the end, entire cell groups would be taken in through the mouth in the animal plasm and there both wall and contents were consumed. This observation affords at the same time an argument for the presence of cellulases, which, if present, might act locally in the normal manner also in the puncturing of the cells and thus might prepare the play of mechanical forces.

A striking characteristic of many *Sarcodina* which feed in the way we have described is the colour of their plasm, in which red tints, scattered diffusely or bound to granules, dominate, and are only mixed with green colours after an abundant ingestion of chlorophyllous food. When other elements take the place of chlorophyll, such as *Diatomeae, Cyanophyceae, Ciliata,* grains of starch, the red colour is lacking or at any rate less strongly developed. It is generally assumed that this pigment is caused by carotinoid components of the chlorophyll. According to POISSON and MANGENOT the pigment in the *Vampyrella Closterii* described by them is bound to globular elements — drops — of abt. 1,5 μ diameter, which reduce osmic acid and dissolve in strong sulphuric acid, the orange-red colour of the pigment passing into a bluish green during the process. This red colouring of the plasm is nearly always lacking in *Difflugia rubescens*. In nearly all cases we have been able to ascertain, quite in agreement with PENARD, that the plasm itself of the animal is colourless, or rather shows the peculiar greyish blue tint, which it has in most *Rhizopoda*. Only, in a very single case, we think we have seen, diffusely scattered in the plasm, very small reddish granules, imparting a faint red tint to the plasm body in question. As we have already observed above the plasm of *Difflugia rubescens* surrounds a varying number of bright orange-red coloured drops, which are either more evenly scattered, or heaped up more locally and often penetrate into the pseudopodia to a certain distance, occasionally even to the top of them. As regards their habitus they strongly remind one of the globular, usually pale yellow, sometimes also orange coloured elements, occurring in the plasm of a problematic Rhizopod (*Diplophrys*) and an equally problematic *Heliozoon* (*Elaeorhanis*), which, though on doubtful grounds, are taken for bodies of a fatty nature. At any rate these plasm elements in *Difflugia rubescens* do not seem to be directly connected with the chlorophyll nature of the food ingested.

We have never observed "ready food" in the plasm of these *Difflugia*, nor is it mentioned by other observers. Only the green inclusions originating from the chlorophyll we had seen before already, without being able then to account for their nature. The fact that the drops of pigment, which are so characteristic of *Difflugia rubescens*, are lacking in the species of the genera *Difflugia*, *Pontigulasia* and *Lesquereusia*, which feed in an entirely similar way on chlorophyllous food, also tells in favour of the independence of these drops of possible chlorophyllous components.

The observations made of *Difflugia rubescens* corroborate the existing view that the *Rhizopoda* which feed on the cell contents of various kinds of algae are on the whole strong specialists. In the sapropelium sample which contained the population various groups of algae were represented more or less generally, among which *Diatomeae*, *Protococcales*, *Conjugatae* and *Desmidiaceae*. *Difflugia rubescens* confined itself in its ingestion to the three species of the genus *Closterium* just mentioned, without, as far as we could ascertain, showing any preference, however, for any of these three species. In the same material we very regularly came across individuals of *Difflugia acuminata*, which feed only on the cell filaments of a filamentous alga of very frequent occurrence. If this specialization corresponds to a similar one of the enzymes that are perhaps produced, and the latter again to a difference in the chemical nature of the wall of the alga cells, is unknown to us.

This does not imply that *Difflugia rubescens* might not show adaptation to other food in a different environment, as this also occurs with other Protozoa — cf. e.g. *Dinamoeba mirabilis* (DE GROOT, 1936); this was, however, not the case in our material.

Many representatives of the *Vampyrella* group encyst after ingestion, in order to digest the food taken in the nutritive cyst that has been formed. Often a division into 2—4 daughter animals takes place in the cyst, which leave the cyst as active individuals, while a number of brownish red remnants — partly digested chlorophyll? — are left behind. Something like it was not observed in *Difflugia*; there are, however, also cysts known of this species and in some samples even fairly numerous; so far it has not appeared, however, that they are in any way connected with the ingestion, while the division, as far as it is known, is an ordinary binary division in an active state. As it was already stated above, however, brownish-red grains appear in the protoplasm of *Difflugia* after ingestion, which grains are ejected after some time. Obviously, in these bodies analogues are seen of the remnants left behind in the cyst of *Vampyrella* and others, and the bodies are considered as waste products of the chlorophyll that cannot be digested any further.

There also seems to exist some uncertainty about the part played by the pseudopodia in ingestion. Earlier observers (ZOPF, DANGEARD, ROSEN) are of opinion that in *Spirogyra* the cell contents are as it were fished out from the cell by ramified pseudopodia. Other observers deny this and

in this form it is indeed not correct. But also the view of LLOYD, viz. that *Vampyrella* does not send a single protoplasm element into the cell is not likely in this case and in other cases surely not true. The most striking case of pseudopodial activity is certainly the one described by HOOGENRAAD (1907 b) of *Hyalodiscus rubicundus*, which, after opening and emptying a cell in the normal manner by means of a pseudopodium put out through the space of this cell, which pseudopodium has a form which normally never occurs in this species, often punctures the transverse walls of the neighbouring cells and thus also ingests their contents. Likewise, it is said by STUMP that *Lesquereusia* sometimes puts out pseudopods into the cells of the *Conjugatae* and thus ingests isolated pieces of the chromatophores which had remained fixed to the cell wall. The same phenomenon was observed by us in *Difflugia rubescens* (vide p. 220). However, as we remarked before, we have also been able to observe various cases of ingestion in which no pseudopodium of *Difflugia* penetrated into the cell and remnants of chromatophores were left behind in the *Closterium* cell. With *Difflugia rubescens* the activity of pseudopods in ingestion therefore does not seem to be essential, which may be connected with the peculiar processes that take place in the interior of the *Closterium* cell.

Indeed, in many cases we were able to observe in the *Closterium* cell, attacked by *Difflugia rubescens*, phenomena, which, as far as we can ascertain, are not reported by any of the earlier observers, and which may throw somewhat more light on the mechanism of ingestion of the cell contents (Fig. 8). Whereas in a sound *Closterium* cell the chromatophores lie very close to the cell wall, they will soon begin to recede from the wall in a cell that is being attacked, in which process the rather straight outline of the chromatophores passes into a corrugated line. Soon vacuoles appear to develop in the sinuous curves, which, growing in size constrict the chromatophores at various points; the chromatophores then give one the impression of a spiral or net-work. During further disorganization the chromatophores are as it were crushed to pieces by the growing vacuoles, which pieces are mostly mutually connected only by a narrow (plasm?-) bridge. The cause of this vacuolization of the plasm and degeneration of the chromatophores must be found in the attack of the *Difflugia*, since the phenomenon always appeared in that half of the cell which was attacked by the *Difflugia*, from here to spread throughout the cell. In this process the chromatophore would often be found already in an advanced state of disorganization in one half of the cell, whereas the other half of the cell showed no signs yet of vacuolization and the chromatophore in it still seemed completely sound. In one case the first chromatophore was already partly ingested by the *Difflugia*, when the second showed only a slight shrivelling up, which soon progressed from the middle towards the top of the cell; when the first signs of degeneration began to show at the top a strong vacuolization and aggregation was meanwhile visible already at

the middle. At the same time a stretching of this chromatophore took place, so that it reached over the middle of the cell — possibly it was pushed up — and approached the mouth of the *Difflugia* (vide fig. 8). The influence radiating from the attacking *Difflugia* and giving rise to the process is already perceptible a long time before the cell wall is punctured. In one case illustrating this fact particularly strongly a *Closterium* cell was found at 8.10 p.m., which had been attacked by a *Difflugia* at about $^1/_3$ from the end of the cell; then the two chromatophores were still completely sound (Fig. 6 a). At 9.10 p.m. the chromatophore in the cell half, which had been attacked, appeared to recede from the wall, which process proceeded at a slow rate till 10.30 p.m. (Fig. 6 b). Meanwhile, however, the *Difflugia* appeared to move very slowly towards the middle of the cell. At 7.30 the next morning the *Difflugia* was exactly before the middle of the cell; in the cell half that had been attacked first the chromatophore was shrivelled up strongly and was aggregated in front of the mouth of the *Difflugia*; the other chromatophore shows only a slight shrivelling yet (Fig. 6 c). At 1.30 p.m. the cell is emptied and shows in the middle the typical hole. The whole course of the process strongly suggests that the chromatophores are being squeezed out of the cell, maybe as a result of strong internal tension caused by the vacuolization; in this process the degeneration of the chromatophores seems to us a secondary, the plasm vacuolization, on the other hand, the primary phenomenon.

It is worth observing that similar vacuolization phenomena also occur in alga cells during degeneration processes which were not caused by an attack of a *Difflugia*.

It is thinkable that as a common cause of these phenomena variations of the turgor can be assumed, which in their turn can be determined again by various factors. It also strikes one that in most cases during the emptying action of a *Closterium* cell a bright grey plug of plasm can be observed in the neck of *Difflugia*, round which the typical orange-red drops will often be grouped archwise (Fig. 9). Probably this plug must be considered as a mass of *Closterium* plasm that has been squeezed out. For, in one case we were in a position to observe that during the emptying process of a cell the plasm of the *Difflugia* withdrew to within the fundus of the shell, the grey plug mentioned being left behind in the opening of the neck and in contact with the *Closterium* cell. After some minutes the *Difflugia* plasm lay down again against this neck plug, enclosing it like a hood; for a moment a clear line of demarcation was visible between the two masses of plasm. This phenomenon was repeated a few times, during which, while the *Difflugia* plasm withdrew, some parts of the grey plasm with elements of the chromatophore were carried along and were transported to the interior of the *Difflugia* body by a kind of invagination, as in the ingestion by *Amoeba verrucosa*. — For the microphotographs we are greatly indebted to our friend, Mr. Drs. E. C. H. KOLVOORT, Bussum, for the translation into English to Mr. J. B. POLAK, Deventer.

Samenvatting.

1. *Difflugia rubescens* Penard voedt zich met den levenden inhoud van *Closterium*-cellen, die opgenomen wordt, nadat de celwand door het dier geopend is.

2. De eigenschappen der door het dier gemaakte opening maken waarschijnlijk, dat het ontstaan der opening een proces van in hoofdzaak mechanischen aard is; een voorafgaande gedeeltelijke oplossing van den celwand langs chemischen weg is mogelijk.

3. Tijdens de opname treedt plasmolyse in de *Closterium*-cel op; daarop volgt een sterke vacuolisatie, gepaard gaande met fragmentatie en aggregatie der chromatophoren.

4. Onverteerde resten der chromatophoren worden in den vorm van roodbruine, bolronde lichamen uitgestooten.

5. Enkysteering staat met de voedselopname niet in onmiddellijk verband.

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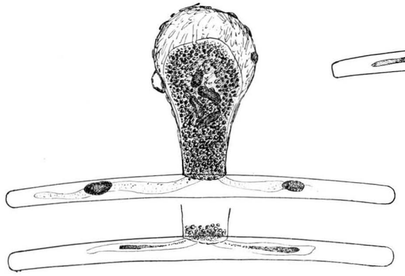
Fig. 1.



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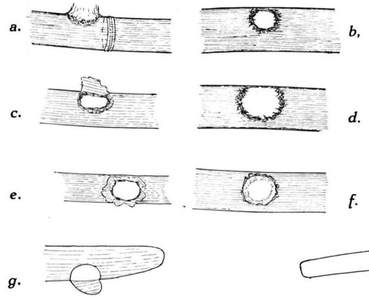

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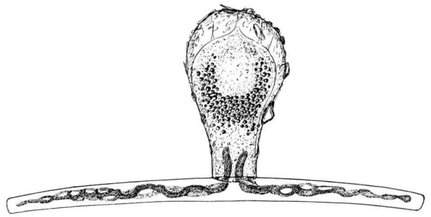

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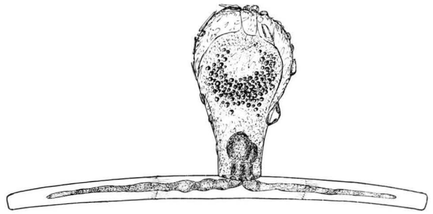

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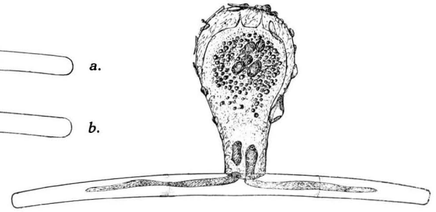

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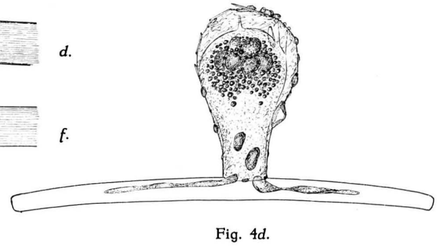

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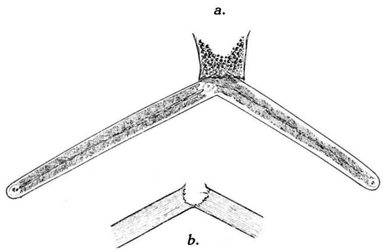

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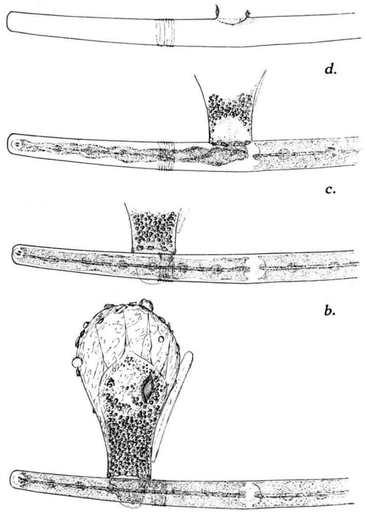

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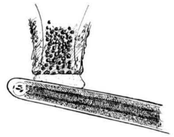

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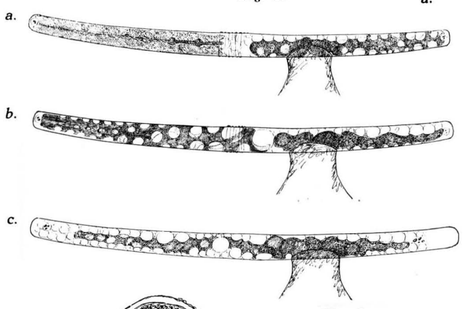

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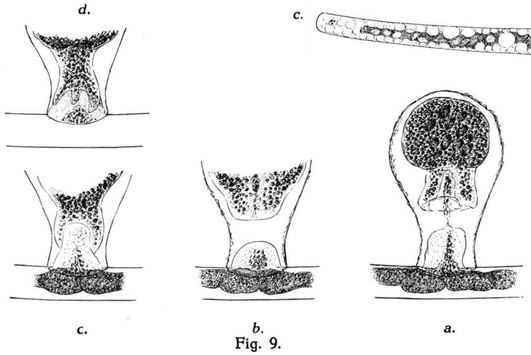

Fig. 9.

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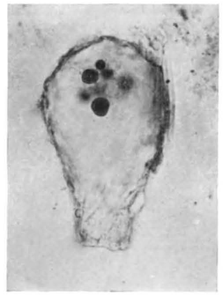

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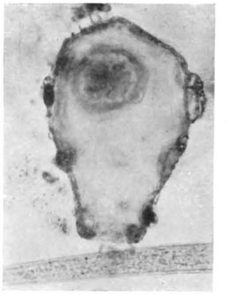

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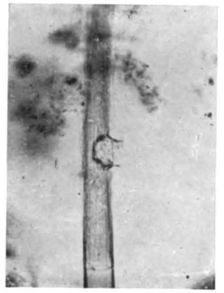

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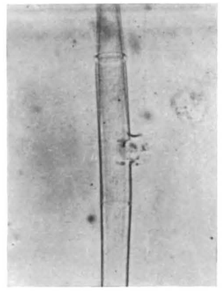

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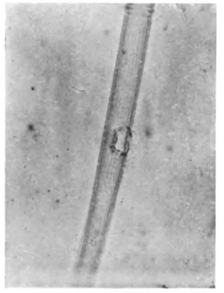

Fig. 14.

Fig. 1. *Difflugia rubescens* PENARD. Living, active individual, with pseudopodia, epipodia, drops of pigment and remnants of food.

Fig. 2. Two stadia of ingestion of the remnants of the chromatophores by the pseudopodia.

Fig. 3. The holes in the *Closterium* cell wall, *a.* from aside, the others more or less from above. *c.* A piece of the cell wall is cut out and turned back. *g.* (after POISSON and MANGENOT). The hole in the cell wall made by *Vampyrella Closterii*.

Fig. 4. Four successive stadia in the ingestion process of the contents of a *Closterium* cell; only the two chromatophores are shown.

Fig. 5. *a. Closterium* cell cracked by the sucking action of *Difflugia*. *b.* The same cell after ingestion of the contents, with the hole in the cell wall.

Fig. 6. Four stadia of the ingestion process. *a.* First contact. *b.* The cell held from below by a lump of plasm. *c.* Ingestion of the chromatophores. *d.* The hole in the cell wall.

Fig. 7. First contact of *Difflugia* with the *Closterium* cell. Pseudopodia drawn in; a lump of plasm touches the cell wall (cf. fig. 11).

Fig. 8. Disorganization of the *Closterium* chromatophores; strong vacuolization of the *Closterium* plasm.

Fig. 9. Details of the ingestion process.

Fig. 10. *Difflugia rubescens*. Shell in longitudinal optical section.

Fig. 11. First contact (cf. fig. 7).

Fig. 12—14. Holes in the *Closterium* cell wall; 12 and 13 from aside, 14 aslant from above.

The figures 1—9 are from pen drawings, 10—14 from microphotographs. 1—9 magnified 300 ×, 10—14 320 ×.