The behavior of *Vampyrella lateritia*, with special reference to the work of Professor Chr. Gobi.

Von

F. E. Lloyd.
McGill University Montreal.

(With plate 7.)

In a recent paper (Lloyd 1926 b) I gave an account of the feeding behavior of *Vampyrella lateritia* which is at variance with that of the Russian botanist Gobi. At the time of publication of my own results, though I knew of Gobi's monograph through the advice of my colleagues Maximov and Issatchenko, I had not had the good fortune to have seen it, and this became possible only after these friends had put a copy into my possession and after a translation had been made for me by Dr. Sophie Satina. I have found in consequence that Gobi had made a very extensive study of this and related forms. In justice common to both Gobi and myself, I desire to present his views, since I could not do this in my earlier paper, and incidentally to put his observations, those of them at least which are pertinent under the above caption, into more available form than they are at present. Aside from the justice of the matter, it is a satisfaction to render recognition to an able and devoted student who passed away under melancholy conditions.

Gobi's work was published as a monograph under the title „Scripta Botanica Horti Universitatis Imperialis Petrogradensis, Fasc. XVI, 1925". It contained 463 pages of text accompanied by 12 lithographic plates in colour. Much the greater part is devoted

to the taxonomy of the group. Reference will be made in what follows only to those portions treating the structure and habits of the organism in which we are presently interested.

Vampyrella lateritia is a Rhizopod which during brief periods of its activities resembles in its movements an amoeba, that is, during a short interval after feeding, when preparing to move away from one feeding position to another; again, after having selected a new position, during its adjustment before the act of puncturing the cell which it is going to feed upon. Between these two periods it has a radiate form, being nearly spherical (but still slightly ameboid) and armed with numerous filamentous (ray) pseudopodia, the laterally placed being the longest, those in front (in the direction of movement at all events) and those behind being shorter. There are in addition "pin-head" pseudopodia which are frequently withdrawn and extended, and are possibly, when in a suitable position with reference to the substratum on which the animal is moving, used in locomotion.

Gobi's account of the feeding behavior of *Vampyrella lateritia* is as follows: When the amebule is ready to begin to feed and has settled down, it closely adheres to a filament of a *Spirogyra* (or *Mesocarpus*) and takes on a pad-like shape. Watching carefully this motionless body it is easy to observe within it near its base the appearance of a very small vacuole. In a few seconds however the size of this vacuole (the food receptive vacuole, Gobi suggests it be called) increases considerably, while the shape of the amebule becomes more convex. No change can be observed in the contents of the cell of *Spirogyra* during this period; but as soon as the above mentioned vacuole reaches its maximum the whole protoplast of the attacked cell of *Spirogyra* recedes suddenly from the cell wall. It then contracts rapidly and begins gradually to pass over or it is better to say to transfuse into the food receptive vacuole through an opening in the cell wall which is formed at the point of contact of the amebula and the filament. This vacuole becomes replete and from this time on is considered to be a digestive vacuole.

The foregoing description, in its general terms, agrees as Gobi remarks, with those previously published by Cienkowski (1865) and Hoogenraad (1907), my own description (1926 b) being largely consonent. Gobi however adds that in a number of points the description needs amplification and elucidation. The following considerations are advanced by him.

The process of ingestion. Cienkowski, he says, gives no

explanation of the process of ingestion, though remarking that it is the same in two species observed by him, *V. lateritia* (FRES.) and *V. pendula*. GOBI points out incidentally that a third species *V. vorax (Leptophrys vorax* (CNK.) ZOPF) entirely enwraps the food plant. According to J. KLEIN, who studied *V. pendula*, the process in question is a true suction, while ZOPF, P. A. DANGEARD and ROSEN take issue on this point and believe that the cell contents are fished out by means of a branched pseudopodium. GOBI denies the latter view, particularizing the behavior of the organism during attack and ingestion, briefly as follows:

After settling down on a filament of *Spirogyra* or *Mesocarpus*, the animal begins to show "strain", as a result of which the receptive vacuole is formed. This can be especially well observed in small animals just freed from the zoocarp, and which have as yet no food masses within them to impede the view of their interior. This strain is manifest also in the fact that the animal, attached to the filament, often "jerks" the latter and breaks it into fragments, or draws aside one cell from the other or even tears out some, separating them from the filament. For this reason in old cultures in which the *Vampyrella* has been feeding, many loosened and empty cells are found lying on the bottom. Their presence is so typical that one may be certainly led to find the animals in such cultures. These phenomena of jerking and breaking the filament show that the amebule has considerable strength which exceeds the mutual adhesion of two or even three adjacent cells of a living filament of *Mesocarpus*. Furthermore, this manifestation of the strength of the animal enables us to form an opinion of the degree of mutual adhesion which exists between the cells of both *Spirogyra* and *Mesocarpus*. When feeding on the latter, it always breaks of the cells or even tears them out, while it only jerks the filament of *Spirogyra* and sometimes not even that. Indirectly but quite definitely this shows that the mutual adhesion between the cells of *Mesocarpus* is weaker than that of *Spirogyra*.

CASH and HOPKINSON (1905) seem to have held a similar idea. "Our own observations prove that the organism will first anchor itself to an alga — usually to the terminal cell — by means of its longer and more mobile pseudopodia, which have a remarkable power of concentration. They will gather in a bundle on that side of the body where, for the purpose, they are most required. By an exertion of force difficult to understand in so tiny a creature, the filament is snapped at a joint and access to the interior is thus

gained, the contents being rapidly absorbed by the introduction of two or more digitate, pseudopodial processes". In their figures (their plate 10) they represent masses of pseudopodia extended along the filament, as if capable of and actually exerting pulling strain on the filament. I have observed such pseudopodia (Lloyd, 1926 b, fig. 14) but they were withdrawn before the filament fragmented. Furthermore, according to their own figure (3, plate 10) the filament broke in the wrong direction!

Gobi then remarks that, since as Benecke showed the turgor pressure in the cells of these plants amounts to about 6,5 atm., the animal must apply the same force to plasmolyse the cells; but that, since *Vampyrella* plasmolyses the cells of *Mesocarpus* much more easily than those of *Spirogyra*, it is unlikely that the former has so great a turgor pressure as the latter, for it can break up the filament of *Mesocarpus* [1]), but only separate a single cell in *Spirogyra*.

And again, the formation of the receptive vacuole is an indispensable preparatory stage which immediately precedes the ingestion of solid matter from the attacked cell, namely, the contracted protoplast. This stage was overlooked by all previous investigators. The present of this gradually increasing receptive vacuole makes it easy to understand why the green protoplast of the cell to which the amebule has attached itself remains unchanged at the beginning of ingestion and why after a while it suddenly recedes from the cell wall and contracts rapidly. The rapid expansion of the receptive vacuole in the body of the amebule shows that at the beginning the animal sucks out sap only; i. e. it plasmolyses the cell. The protoplast contracts as soon as the turgor in the cell is gone. It is only then and only in this manner that the amebule can ingest the protoplast, i. e. the entire plasmatic content of the living cell. Without this plasmolysis which it had previously produced the animal would be unable to absorb the protoplast. This also explains the necessity of the food receptive vacuole, which is found also in *V. velata* and, according to Klein's figures of *V. pendula*, in this also. Indeed the presence of a food receptive vacuole which soon becomes a digestive vacuole is common to all the *Vampyrellae* and is to be considered a generic character.

This vacuole was first reported by Gobi in April 1887. A little

[1]) The food plant represented by Cash and Hopkinson (1905) is, no doubt, a *Mesocarpus* (they call it *Conferva*) with short cells. It would appear that in such forms abjection may involve pairs of cells with fair regularity, hence the attack of the *Vampyrella* on "alternate cells".

later and independently it was observed by Penard, who described it as follows:

"Un processus tout exceptionnel est celui qu'on trouve dans la *Vampyrella Spirogyae*, qui se nourrit, presque exclusivement, peut-être, du contenu des cellules des *Spirogyra*. J'ai décrit ailleurs (Arch. d. Sc. phys. et nat. décemb. 1889) le procédé dout je l'ai vue se servir pour s'incorporer cette nourriture, et sans le répéter en detail, je me bornerai à dire qu'on y peut, à mon avis, reconnaître un véritable phénomène de succion; la *Vampyrella* collée a l'algue fait le vide dans son propre corps (fig. 6) de manière à prendre la forme d'une coupe ou d'une ventouse, puis la paroi de l'algue éclate en un petit trou par lequel l'interieur de la cellule passe rapidement dans la vampyrella; l'échatement, ou le choc est si brusque que souvent la cellule tout entière de la *Spirogyra* se détache de la tige dont elle formait un des articles."

From the preceding details reported by Gobi he concluded that the amebule of *Vampyrella* literally sucks in its food. In this he agrees with Cienkowsky, but points out that the latter did not see the food receptive vacuole. Zopf, P. A. Dangeard and Rosen, as already pointed out, believed that the cell is pierced by a pseudopodium which then grasps and withdraws the protoplast. Zopf thought that the entering pseudopodium branched and spread throughout the cell, and then, contracting, drew out the protoplast with it, admitting however, that is was difficult to see these branching pseudopodia. Gobi points out that Zopf's figures show that the animal he observed was attached to dead *Spirogyra* cells. Now as *Vampyrella* never attacks any but living cells, Zopf was probably looking at *Nuclearia*, for which *Vampyrella* could easily be mistaken. Zopf's ideas are scarcely distinguishable from those of Dangeard and of Rosen, and it is unnecessary to repeat Gobi's criticisms.

Returning to the views of Klein, it appears according to Gobi that that author tried to explain the ingestion of food matter into the body of the animal by means of a loss of the hydrostatic pressure of the attached cell. The disappearance of the pressure is due to the sudden collapse of the somewhat expanded cell wall at the very moment when it is pierced by the amebule. In collapsing the cell wall squeezes out a certain portion of the cell contents. It is obvious that the collapsing takes place as a result of the contraction of the protoplast, the latter, having lost its cell sap does not press any more on the cell wall. But it is rather doubtful,

Gobi observes, whether the collapse of the cell wall could help the process of squeezing out the protoplast, for the jerking of the cell of *Spirogyra* on which the animal feeds, the straightening out of the folds of the cell wall of the emptied cell and the tearing out of the cells from the filaments of *Mesocarpus* disprove the possibility of such an interpretation. "It also disagrees with the process of feeding of *V. velata* on the cell contents of *Staurastrum muticum*. In this case after the turgor of the cell is gone the collapsing of the cell wall can not even take place. Thus the phenomenon of collapsing of the cell wall has absolutely no influence on the squeezing out of the cell contents. This squeezing out process actually does not exist and the cell content is just sucked in by the *Vampyrella*. Now let us suppose that the collapse of the cell wall plays some part in the process of ingestion. What factor might cause the collapse? Evidently the contraction of the cell content. But why should this happen? I have already explained the cause of this phenomenon on p. 64, discussing this problem in regard to *V. lateritia*. Such a contraction of the cell content occurs as I shall show later in all *Vampyrella* species. In every case the amebulae when beginning to feed first plasmolyses the attacked living cell. The loss of the cell sap due to which the digestive vacuole is formed causes the contraction of the protoplast. Not one of the authors who studied *V. pendula* Cnk. (Cienkowsky, Klein, Dangeard, Zopf) reports anything concerning the fact of the formation of the food receptive vacuole prior to the ingestion of the food material. But from the following description given by Klein of *V. pendula* Cnk., we can conclude that such a vacuole is present and therefore also the above mentioned preparative stage for feeding. After the amebula or plasmodium has settled down on the attacked green cell, writes Klein (for instance on a filament of *Oedogonium*), it retracts all its pseudopodia and remains immobile for a time while boring an opening in the cell wall. This immobile condition of the animal is followed by a sudden contraction of the protoplast in the attacked cell and the cell content gradually begins to pass (i. e. is drawn in) into the body of *Vampyrella*. At first the protoplast settles in the very middle part of the animal's body as a lump (Klein, l. c. 201). We can conclude from these statements the following: Such a sudden contraction of the protoplast can happen only because the living cell loses its sap, i. e. because plasmolysis is produced. It is obvious that the animal plasmo-

lysed the cell while it was settled on it in an immobile state. The position of the ingested protoplast in the middle of the body in the form of a lump obviously shows that in this particular place, i. e., in the middle of the body, the food receptive vacuole is to be found, and that this is where it previously develops".

The fate of the food receptive vacuole is as follows: "From the very moment that the ingested food fills the large receptive vacuole in the body of the amebula and until the disintegration of the food material into a quantity of undigested residue (egesta) which is scattered throughout in the body of the zoocarp, the whole digestive process indicates that the large receptive vacuole which later becomes a digestive one does not remain unchanged in the body of the amebula. When the disintegration of the food material (i. e. digestion) takes place and the latter breaks up into pieces this vacuole also gradually breaks up into a number of comparatively smaller digestive vacuoles. Their size decreases as the food is further disintegrated and digested. Finally when the zoocarp becomes mature a number of such vacuoles remains in the zoocarp. They contain only the brown undigested residue (egesta) from the disintegrated lumps of the food material and thus change into "excreting" vacuoles. Their position and number in the ripe zoocarp is variable."

Summary of Gobi's views. From a perusal of Gobi's discussion above presented, one needs not be uncertain about what he meant in regard to the points at issue between himself and other observers. His remarks may be summarized as follows: (1) After the position of attack is assumed, a food receptive vacuole is formed in the cytoplasm of the animal. (2) The function of this vacuole is to induce plasmolysis of the attacked *Spirogyra* (or *Mesocarpus*) cell, whereby the protoplast is caused to shrink from the cell wall. (3) At this moment the attacking animal "jerks" [1]) the filament, with the result that, in the case of *Spirogyra* a cell is broken off, in *Mesocarpus* even more than one is separated. It is clear that Gobi regards the fragmentation of the plant filament as the result of sheer brute strength exerted by the amebule. (4) The amebule then sucks out the contracted protoplast. (5) During the ingestion of food the body of the animal becomes „strained" and seems „to grow

[1]) I have the assurance of my colleague Professor Babkin that this is the correct equivalent of the Russian verb used.

stiff". The ray-like pseudopodia contract considerably or are even completely retracted.

In regard to these recapitulated points on which Gobi lays emphasis, there is a wide difference of position between him and myself as to the observable facts. I would say that, once the objective facts are agreed upon, the difficulties of interpretation are removed. I shall therefore present the facts as they stand revealed to me. The evidence was obtained by the study of living animals and the repeated study of motion pictures (photomicrographic) made by myself. I may say incidentally that my experience with this technique has proven to me the very great value of it as a means for repeated study of the behavior of the same animal under the same conditions and has led me to see some inadequacies in my own previous account (Lloyd, 1926 b) which must therefore receive emendation now. We shall consider the above points raised by Gobi seriatim.

(1) Concerning the food receptive vacuole. That a food receptive vacuole occurs there is no doubt. This vacuole receives the whole mass of food material when it is sucked in by the animal. As to the method of origin, however, I must differ from Gobi. After the position of attack is assumed by the animal, in a few minutes one may observe that the transverse walls of the attacked cell begin to collapse inwardly until they become distinctly concave (plate fig. 1) and in giving this account I have *Spirogyra weberi*, which has replicate end walls, particularly in mind. It is evident therefore that the turgidity of the cell has been reduced. The question now is what has happened to bring this about. It could happen because the cell has been plasmolysed, that is, by reducing the volume of the sap. This is Gobi's view, and the mechanism as he conceives it is a vacuole, formed in the body of the animal containing, it must be, a sap of sufficiently high concentration of solution to be hypertonic to the plant sap. The same result could be attained if the animal could cause a change in permeability of the protoplast so that it could no more retain its sap, when it would become relaxed. Conceivably this could happen, but it would be idle to speculate as to how in the light of positive evidence in another direction. In the third place, the collapse of the transverse septa could result if the longitudinal wall could be extended, thus enlarging the internal volume. This is precisely what happens. As Cienkowsky believed, the early period of attack on the *Spirogyra* cell by the animal is occupied, after its stance is fully achieved, by the digestion

of the cell wall in an oval area lying against the body of the animal. This means that there is a local chemical alteration of the cell wall of the attacked cell from the normal cellulose to a hydrocellulose which is soft and yielding and can be stretched. As this change overtakes the area of the wall under course of digestion, the turgor pressure of the cell causes the softened wall to bulge outwardly, forming a blister projecting into the body of the amebule, and it is this blister which was taken by GOBI for a food receptive vacuole. Strictly speaking, it is a bulging in of the body of the animal by the bulging area of the *Spirogyra* cell [1]). Eventually the blister bursts and thus is initiated a food vacuole, properly speaking, accompanied by a sudden enlargement of the *Vampyrella.* We must note, however, the happenings to the cell as a whole at about this moment, when it is invariably separated from its neighbors. It is well known that this cell abjection takes place in the replicate-walled species of *Spirogyra* and in *Mesocarpus* whenever a cell is damaged to the extent of lowering the turgor pressure beyond a certain amount (COHN, BENECKE). The particulars of the process in both these types were made a subject of inquiry by myself (1926 a) being prompted thereto by observations of *Vampyrella.* As BENECKE showed for *Mougeotia,* any means of procuring the damage of a cell accompanied by the lowering of its turgor pressure sufficiently results in abjection. Toxic stains, or the rapidly local killing of cells by metallic iodine or from other causes, suffice to produce the result. If therefore the *Vampyrella* does in fact pierce the wall, unless the animal opposes a pressure on the opening equal to, or, as GOBI asserted, greater than that exerted by the cell attacked, the cell will be thrown off. GOBI objected that the abjected cell always had bulged out ends, and therefore must have been

[1]) It is evident that PENARD observed the inwardly arching surface. He says, "The *Vampyrella* attaches itself to a cell of *Spirogyra,* retracts its pseudopodia except a few by which it adheres to the alga, then moulds itself to the cell upon a portion of its surface, and becomes motionless. For a moment nothing happens. Then we see the attacked zone rise up into an arch in the interior, the margins remaining firmly attached and formed into a ring; the arch gradually rises, and suddenly the wall of the alga bursts, the cell juice of the *Spirogyra* passes into a violent stream into the *Vampyrella;* the greyish plasma of the cell passes, in its turn, more slowly, with the green chromatophore, which is seen to glide in a mass; the cell is completely emptied; the *Vampyrella* emits pseudopodia, becomes detached and moves away, leaving a very visible rupture in the empty cell (wall)". Were it not for the interpretation which PENARD applied, viz: that the arching is produced by true suction, his description would have been complete.

under a condition of high turgor. The fact is, however, and this is a matter of photographic record, that Gobi was mistaken. Sometimes the ends of the cells bulge and sometimes they do not; again, in the same cell, one end wall may be left bulging and the other not (see fig. 8, plate 26, Lloyd, 1926 b). Whether or not this is correct depends upon the degree of synchronization of two moments, the piercing of the wall — or one would better say the bursting of the lateral wall as the result of local digestion — and the adjection of the injured cell. It must be understood that the degree of deturgidity sufficient to insure the abjection of the injured cell may be reached before actual bursting, in which case its end wall may be evoluted. If the bursting occurs first, an end wall may remain infolded. The difference in time may be a small fraction of a second, and would escape the unaided eye. We know from the motion picture record, however, that this must certainly be the case. It should also be pointed out that very frequently one end of the loosened cell will be found to bulge and the other not. This is usually the case when the animal attacks a cell at the end of a filament. The free end of this cell will be in a state of extension, a cell having been thrown off already; its inner wall, however, will generally remain unfolded when in turn it, the now terminal cell, becomes cast off.

If the digestion of a small area of the cell wall results in bursting, there must be a more or less sudden explosion of sap from the injured cell into the body of the animal, which in consequence must show a correspondingly rapid enlargement. This is actually the case, and the sudden engorgement of the animal and the abjection of the injured cell generally take place within a fraction of a second of time though the injection of the animal may be prolonged to 2—3 seconds. There is no doubt that the sudden violent movement often displayed at this moment is merely the result of the abjection of the injured cell by the neighboring uninjured one and that the animal is carried away willy-nilly astride its prey like a rider on a bucking horse. We see therefore that the energy required to procure abjection of the injured cell resides in the plant itself, and the *Vampyrella* only sets this energy free by injuring a cell in such fashion that the turgor pressure is reduced to null. It must be apparent upon reflection that a small soft bodied animal without appendages, resting wholly upon a *Spirogyra* filament could not exert a convulsive movement sufficient to break the filament since it has no fulcrum to work with, and

Cash and Hopkinson recognized this difficulty. If so, we should expect that it would be just as able to break the filament of delicate walled nonreplicate species. This I believe it does not do (plate 7). I have observed the attack upon *S. longata*, but this species does not break off since abjection does not take place so far as I have observed. It is well known that the two juxtaposed end walls, commonly referred to collectively as the transverse wall or septum, are not, in *Spirogyra*, cemented together, for on plasmolysis, they separate, the pectic material of the middle lamella having become altered into a gelatinous — or at least plastic — inelastic material which contributes nothing toward holding the cells together. This is accomplished solely by the common sheathing cell wall. There is therefore nothing more to break in *S. longata* than in *S. Weberi*, but the latter breaks while the former does not. The greater readiness of *Mesocarpus* to fragment lies in its structure described by Benecke and by myself, and similarly the greater readiness of *S. Weberi* over *S. longata* to fragment too lies in its structure. Dead or injured cells are disposed of, in *S. longata*, by the slower process of abscission.

Gobi describes an interesting case when he observed that an individual of *Vampyrella* emptied two *Spirogyra* cells at once. His drawing of the filament and the attached animal (his plate 1, figs. 4—6) show that the area of the wall digested must have been partly on one and partly on the other cell, and that the bursting of the softened membrane followed the abjection, and not preceded it, since the end walls are all evolved. Since the animal was spread out over the adjoining ends of the two adjacent cells, it is difficult to see how it could at once "jerk" loose two cells together from the third, and the adjacent cells from each other. But it is simple enough when the part played by the turgor of the cells is understood.

The origin of the food receptive vacuole is thus seen to be due to the sudden expulsion of sap accompanied possibly by some fragments of protoplasm into the body of the amebule previously occupied by the blister, but enlarged by that volume represented by the original volume of the sap less that volume which can be contained after the cell wall and protoplast have become relaxed. The blister itself, recognized as such by Penard, previous to its bursting, could by mistake be thought to be a vacuole in the body of the amebule, and this, I think, is what Gobi saw, at least in part. He may however have observed a true vacuole in the imme-

diate vicinity of the blister. I have a photograph showing such a vacuole (plate 7 fig. 1), but whether this is a constant feature I am unable to say. If a constant feature, it may be inferred that the blister bursts into the vacuole, which would therefore be properly regarded as the food receptive vacuole of Gobi.

The volume of the sap thus injected into the animal may be so great that he immediately throws off a part of it by contraction, squeezing the sap out into the surrounding medium (plate 7, figs. 10—11). The outward flow takes place either (a) between the edges of the animal's body and the surface of the cell wall, or (b) backwards into the cell cavity, between the shrinking protoplast and the cell wall. I have no proof of either.

This is an opportunity to say that hitherto I have not sufficiently appreciated this behavior which, as a matter of fact, is indulged in two or three times, or sometimes oftener, during the process of ingesting a cell. That is, the animal does not get progressively bigger from the beginning to the end of the process, but readjusts its volume by expelling sap from time to time. Nevertheless, at the close of ingestion, the volume of the animal is several times that at the beginning, partly due to the ingested protoplasm including of course the chloroplast, and partly to retained sap.

We now come to the question of the way in which the *Vampyrella* extracts the protoplast from the *Spirogyra* or *Mesocarpus* cell. The two views which have grown up historically are (a) that the protoplast is fished out by means of a probing pseudopodium (Zopf, P. A. Dangeard, Rosen); and (b) that it is displaced by suction (Cienkowsky, Klein, Penard) and with the latter Gobi agrees, and I deem rightly, for I also have taken the same view. Hoogenraad appears to take the same position though tacitly, for he offers no evidence on the point. The matter seems clear enough, particularly when the point is conceded that the attacked cell opens by bursting into the amebule. Soon after this moment the chloroplast begins to swell and become beaded, and the protoplast begins to slide forward towards the opening from either end of the cell and proceeds uninterruptedly (when the whole procedure is normal) till it has become entirely ensconced within the food receptive vacuole. This being said, however, we are yet ignorant of how it is accomplished and no one so far as I am aware has offered any explanation adequate to the case. Observation has convinced us that there is no pseudopodial mechanism which is protruded into the cell. In one of my motion pictures, incidents from which have been publi-

shed as plate 27 of the cited publication (LLOYD, 1926) the whole movement of the chloroplast into the food vacuole is shown with the greatest clearness. The protoplast with its inclusions has all the appearance of a gelatinous mass oozing through an opening by displacement, the pressure being lower within the animal's body than on the material outside. If for the moment we assume this to be the case, another difficulty at once forces itself on our attention, for we have to ask how such reduction of pressure can be procured within a protoplasmic body without any framework, that is, without any obvious framework. My own view is that there is such a framework. It has been supposed — at least no one has raised a question about it — that the food receptive vacuole has the shape which would without further reflection be assigned to it, a simple smooth-walled vacuole. I think, however, that this is not the case, but that the food receptive vacuole is provided with buttress-like ridges which support the dome of protoplasm and by flattening out increase the volume of the interior. I have thought that the force of surface tension would thus be permitted to come into play on the interior surface in such a manner as to enlarge the vacuole. If true, it is probably not the whole truth. PENARD seems to have got close to the idea when he thinks of the body of the animal sticking to the cell and acting like a cupping glass, but he though that this (faisant "la vide") caused the bursting of the cell wall. It would indeed be a vigorous action if this were the case, which as a matter of fact is not. The evidence that ridges are present is again the direct observation, not alone of the motion picture, but of a number of individual animals during attack. That this is really an adequate explanation, however, must await further study.

I have remarked that during ingestion the animal may contract and thus expel an overplus of sap. I mention this again because it occurs to me that this sort of contraction is what GOBI may be referring to when he speaks of "straining". Such contractions and an ameboid changing of shape during the early period of attack, have such suggestion of straining, of getting a good hold on the food plant. Beyond this I cannot follow GOBI.

In regard to the number of cells which can be devoured during one feeding. GOBI records the number of nine, the highest which has been observed. I have seen five cells completely devoured. The large volume of water which is taken in, or which appears to be taken in, is so vast as compared with the size of the animal

after he has finished that it cannot be otherwise than that the water is disposed of in the course of feeding as well as afterwards. I have already pointed out that contractions occur which can account for this during feeding, when the mouth of the receptive vacuole is open, if against the hole in the cell wall. The lax condition of the cell wall would permit the regurgitation of the sap into the cavity of the cell in replacement of that drawn out, one of two possibilities above mentioned. The difficulty here is that the protoplast might also be regurgitated, and this certainly does not occur.

After the animal has withdrawn from the now emptied cell his volume is greatly in excess of that at the beginning (in a particular case three times the original volume) and this is due not alone to the solid ingesta, but to a considerable volume of water. This water is thrown off quite rapidly during the ensuing period. In the course of a few minutes the volume of the animal becomes markedly reduced, not by any sudden contraction but by the activity of contractile vacuoles (in the case just mentioned the volume was reduced to one third that at close of feeding). Concerning this Gobi says, "The contractile vacuoles are very rarely found in the body of the amebulae of *V. lateritia*. They are not constant formations and have not fixed position in the body. They appear occasionally in various parts of the body, are of various sizes and contract irregularly without any definite rhythm. The rhythmically contracting vacuoles are entirely absent in *Vampyrella lateritia* (Fres.). This is in agreement with the reports of Cienkowsky (1865, p. 219), who noticed the absence of pulsating vacuoles in this species. Zopf claims to have found 1—4 contracting vacuoles in the amebulae of this organism, but this is based on an error, as he confused two different organisms and was evidently dealing with those of *Nuclearia*. In this connection Hoogenraad says, "The body is only moderately vacuolated, the vacuoles are small and only to be found near the surface of the body, and never protrude beyond. Pulsating vacuoles are absent". In my own account I did not insist on a distinction between pulsating vacuoles, i. e. those with constant position and true pulsation as in *Paramecium*, and the occasional roving contractile vacuoles such as occur in *Ameba*. When this distinction is insisted upon, one agrees with Gobi. It is evident, however, that he saw superficial vacuoles which burst outwardly, as did I. They are of importance, especially during the excretion of water after feeding, and their activity accounts for the rapid

loss of volume between the recession from one cell and the attack of another, as observed by me. Such vacuoles are to be seen during the early period of encystment. These I figured in my paper. In a word, *Vampyrella* is similar to *Amoeba* in this regard, but has many more contractile vacuoles (of the *Amoeba* sort) than that animal usually has. The number and their activity varies however with the volume of the animal.

Concerning the behavior of the pseudopodia, GOBI observed that the ray-like pseudopodia retract considerably or even completely during attack, accompanying the "straining" of the animal. CIENKOWSKY remarks that they remain unchanged or may disappear entirely. Of the considerable number of animals which I have studied during the feeding process, I have never seen the ray pseudopodia retained during the whole process, although some ray pseudopodia may be used as anchors. Even the anchoring rays are usually withdrawn as soon as the animal has got his close attachment to the surface of the cell accomplished though CASH & HOPKINSON thought otherwise. The animal can, however, quite readily send out long ray pseudopodia when he finds it necessary to change his stance, as may occur if the material of the protoplast which he is withdrawing becomes refractory and does not move with normal ease into the food receptive vacuole. Under such circumstances the animal has been seen by me to send out new pseudopodia, attaching himself to a distant filament, and to draw away from the opening through which he is dragging the protoplast and still keep on sucking it into himself. A case like this was seen, apparently, by GOBI who remarked: "After many efforts the amebule overcame the attached cell, having previously anchored his body to another filament". Occasional behavior such as this shows us very clearly that the process is accomplished by suction. The pin- head pseudopodia on the other hand frequently remain in evidence during most of the time of feeding, but tend to disappear toward the last. They may however, disappear very soon, and the animal then appears quite smooth for most of the time of feeding.

Ejection of waste. I have seen no account of this process. On several occasions I observed that during feeding the animal pauses to contract, and quite appreciably does so. At the moment of contraction a cloud of particles is seen to arise from the whole free surface and remains suspended above it for some time (fig. 9, plate 25, LLOYD, 1926 b). In one case I was able to photograph this cloud of material. It seems reasonable to infer that this is ejected waste.

Whatever it is, it appears that the particles are expelled from many vacuoles simultaneously. These vacuoles are small and numerous and it is to these that the general opacity of the animal is due, at least in large part. I did not observe that the ejected particles carried any pigment, and, if this is true, it would be inferred that the carotinoid pigment, as I have shown it to be, is held as a pigment proper to the animal and not discarded.

Digestion. After the process of feeding is at an end, the protoplast including the chloroplast of the destroyed cell lies within the food receptive vacuole. By this time the chloroplast becomes fragmented, a process which begins spontaneously during even the early period of withdrawal, and beads off into a number of rounded particles as Gobi observed. One cannot see the cytoplasm easily, but it is to be supposed that it behaves similarly. At all events in the course of a short time the cytoplasm of the contracting animal encroaches on the engulfed food which after a few minutes may be seen enconced in a number of food vacuoles. These at first are bright green, lying surrounded by the orange colored cytoplasm of the animal. This condition Gobi truly remarked to have a very attractive appearance, the rich orange of the pigment and the green chlorophyll offering a pleasing contrast. As feeding continues, the food masses become so numerous as to form a mulberry-like mass, which gradually loses its fresh green color and becomes a dirty green. It is only after encystment and some period of days during which digestion proceeds that the animal becomes again transparent enough to transmit light. Gobi evidently took this view for he points out that the peculiar shade of color seen after digestion depends on the masking effect of the color of the egesta on the pigment proper.

Encystment. Concerning the membranes which envelop the encysted animal, Cienkowsky maintained that the velum is a nitrogenous substance (Stickstoffhaltig), while the membrane proper of the zoocarp is cellulose. Gobi, by means of the Chlorzinc-iodine test found conclusively that both membranes are cellulosic, saying however, that because of the greater density of the inner membrane its staining reaction is more intense. I have repeated the test (also the sulfuric-acid-iodine test) and can verify Gobi's conclusion. There is no doubt that he was correct. The same occurs according to Gobi for other species which he studied *(V. veluta, Leptophrys kuetzingii*, which Gobi thinks is really *L. vorax)*.

The origin of the velum was a matter of concern to Gobi and in this connection he criticized Cienkowsky, who described a stellate

configuration (presumably of the basal attached portion of the velum) due to the coagulation of the excreted material before the pseudopodia were entirely withdrawn. To this Gobi asserts that the pseudopodia are always withdrawn and a contraction of the body occurs first. Then a translucent lamella, the velum, appears, and becomes progressively more and more loosened from the surface as a result of further contraction, when the second membrane becomes evident but remains closely investing the body.

It is evident, I think, that the velum receives whatever configuration it may have from the shape of the animal at the time of secretion. The body of the amebule is then more or less contracted but remains attached to a filament by a somewhat outspread base. So far as my own observation is concerned, there are no radial elongations from the margin such as Cienkowsky appears to have seen. When further contraction followed by the secretion of the second membrane occurs, the velum is left free and then constitutes an irregular delicate veil anchoring the zoocarp to the surface on which it came to rest. The irregularities appear to me to arise partly from any irregularities of form of the base or attached surface of the amebule, which may have extending pseudopodia with accompanying shallow furrows which may be present extending meridionally on the body, and partly to partial collapse when the further contraction takes place. It may very well be that on occasions the shape diverges sufficiently from the usual to have justified Cienkowsky's adjective, but the matter is not of much moment.

Citations.

An extensive bibliography is furnished by Gobi in his monograph. The citations given below are only those pertinent to the present paper.

Benecke, W.: Mechanismus und Biologie des Zerfalles der Conjugatenfaden in die einzelnen Zellen. Jahrb. wiss. Bot. Bd. 32 p. 453—476 1898.

Cash, J. and J. Hopkinson: The British Freshwater Rhizopoda. Vol. 1 part 1. London: The Ray Society 1905.

Cienkowski, L.: Beiträge zur Kenntnis der Monaden. Arch. f. mik. Anat. Bd. 1 p. 218—223 1865.

Note. Scherffel (1926, p. 231) records the observation that *Vampyrella pendula* feeds upon *Oedogonium,* in the wall of which a „beautiful, round hole was bored in the membrane" by the animal.

Dangeard, P. A.: Recherches sur les organisms inférieures. Ann. Sci. Nat. VII. Bot. T. 4 p. 241—341 1886.
Gobi, Chr.: Scripta Botanica Horti Universitatis Petrogradensis. Fasc. 16 1925. Petrograd.
Hoogenraad. H. R.: Einige Beobachtungen an Vampyrella lateritia Leidy. Arch. f. Protistenk. Bd. 8 p. 216—224 1907.
Klein, J.: Über Vampyrella. Bot. Zeit. 1882.
Lloyd, F. E. (a): Cell disjunction in Spirogyra. Papers Mich. Acad. Sci. Arts and Letters Vol. 6 p. 275—287 1926.
— (b): Some behaviors of Vampyrella lateritia and the response of Spirogyra to its attack. Ibid. Vol. 7 p. 395—416 1926.
Penard, E.: Etudes sur les Rhizopodes d'eau douce. Mem. Soc. phys. et d'hist. nat. Geneve T. 31 No. 2 1890.
Rosen, F.: Studien über das natürliche System der Pflanzen. Beiträge zur Biol. der Pflanzen Bd 8 1901.
Scherffel, A.: Einiges über neue oder ungenügend bekannte Chitridineen. Arch. f. Protistenk. Bd. 54 p. 167—260 1926.
Zopf, W.: Zur Morphologie und Biologie der niederen Pilzthiere (Monadinen). Leipzig 1885.

Explanation of plate.

Plate 7.

All the figures in this plate were obtained by the author by motion picture photography, one picture per half-second.

Fig. 1. *Vampyrella lateritia* in position of attack on a filament of *Spirogyra weberi*, within one second of abjection. The blister like swelling of the *Spirogyra* cell-wall protruding into the body of the animal, observed by Penard, may be seen. A vacuole in the amebule may be seen just above and in contact with the blister.

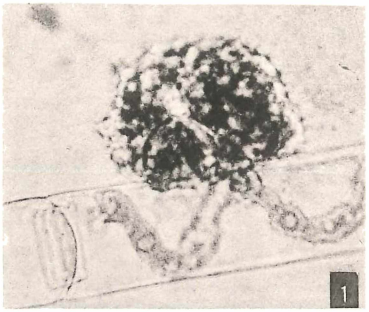
Fig. 2, 3, 4. Three successive pictures of the animal; fig. 2, just before abjection (observe the form of the transverse septum) of the *Spirogyra* cell; fig. 3, just after (note the bulged septum), and fig. 4 after the first contraction of the animal, expelling water.

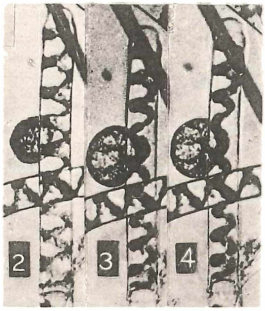
Fig. 5. An animal in early attack showing vacuolated structure. Pin-head pseudopodia can be seen in this figure and in fig. 1.

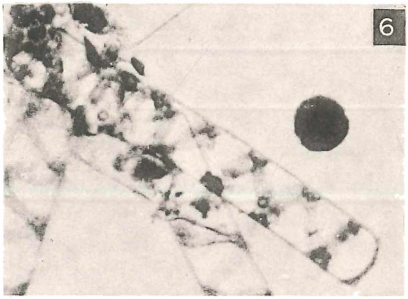
Fig. 6—11 incl. Successive pictures showing various episodes in the attack on *Spirogyra longata:* fig. 6, approach; fig. 7, touching and beginning to flatten; fig. 8, flattening to take a pillow-like form: fig. 9, definitive position of attack, just before bursting of the *Spirogyra* cell; fig. 10, just after bursting of the attacked cell; fig. 11, a few second later, just after the first contraction of the animal, expelling water.

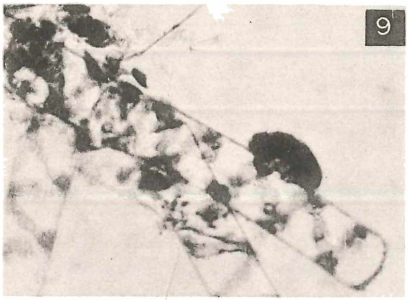
The proof sheets of this paper have been lost during mailing and the coppy has been send to my laboratory where we are expecting prof. Lloyd's visit this autumn. We looked over the proofsheets and corrected them as far as possible. But as the manuscript isn't in our hands we can't guarantee possible errors.

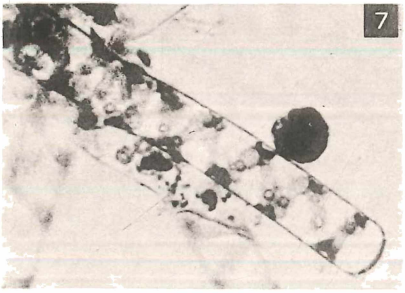
Laboratory for Plant Physiology
Masaryk University Brno Prof V. Úlehla.

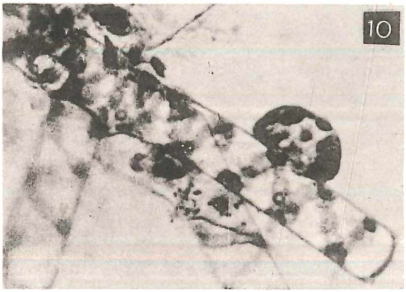


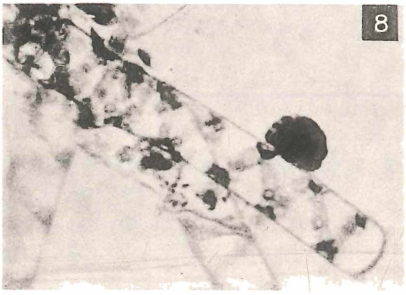


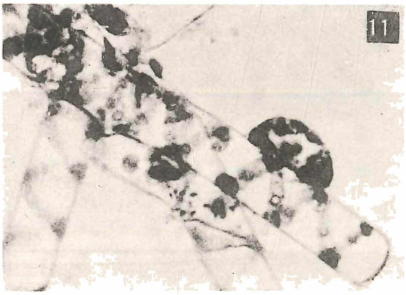












Lloyd.

VERLAG VON GUSTAV FISCHER IN JENA