The Movement of Protoplasm in Coenocytic Hyphae[1].

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With Woodcuts 8, 9, 10, 11.

THE translational movement of protoplasm varies much in the manner of its appearance, but for the most part may be considered under three headings: viz. circulation, as in the staminal hairs of *Tradescantia*; rotation, as in cells of *Chara*; and streaming, as in plasmodium of Myxogastres. There is, beside, the sliding movement seen in Desmids, and the pulsating movement of many locomotive organisms, and others less common. All these are undoubtedly spontaneous movements, and together with such induced movements as the orientation of chlorophyll-grains in leaf-cells of *Oxalis* and *Lemna* and in filaments of *Mesocarpus* and *Caulerpa*, when acted upon by light, are probably conditional upon some property of living matter not yet understood, which acting under diverse conditions brings about different manifestations of energy.

Of the several forms of movement, streaming is most pronounced, as the mass involved is larger and the speed

[1] Read before the Botanical Society of America at the Toronto Meeting, August 18, 1897.

greater. Its discovery in hyphae is therefore of considerable interest. So far as I know, literature shows but few references to the streaming movement of protoplasm in hyphae. One occurs in Vines' 'Textbook of Botany' (1895), where (p. 735) it is cited in a general way as one of the several instances of protoplasmic movement; and on the following page the direction of such movement is said to be various, except 'in plasmodia and in hyphae,' where it is a 'simple longitudinal movement.' A more definite account of the movement in hyphae is given by De Vries [1] in the Botanische Zeitung for 1885, in the case of the sporangiophores of *Phycomyces*. At the time of spore-formation the contents of the sporangiophores of this species appear to pass into the sporangia. The sporangiophores are very long, occasionally exceeding 30 cm., and unusually thick, and movement within them can be easily observed. De Vries appears to have considered this a special instance of protoplasmic movement, clearly designed for rapid supply of water and nutriment to the forming sporangia and not necessarily of the same nature as the movement in *Tradescantia* and the Myxogastres [2]. This interpretation is strengthened by the fact that he makes no reference to it in the revised edition of his *Leerboek der Plantenphysiologie*, issued a few months later, although eight pages are given to a careful presentation of protoplasmic movement.

[1] De Vries, Hugo, 'Ueber die Bedeutung der Circulation und der Rotation des Protoplasma für den Stofftransport in der Pflanze.'—Bot. Zeit., 43: 1–6, 16–26.

[2] De Vries' words are as follows:—'Betrachten wir zunächst einen besonderen Fall, in welchem die Bedeutung der Bewegung des Protoplasma für den Stofftransport klar zu Tage tritt. In den Fruchtträgern von *Phycomyces nitens* bewegt sich das Protoplasma von einem Ende bis zum anderen mit einer solchen Geschwindigkeit, dass die ganze Länge des Fruchtträgers von den einzelnen Theilchen des lebendigen Inhaltes in wenigen Stunden durchlaufen wird.'—l. c. page 4.

'Es ist klar, dass in manchen Fällen nicht nur die eigentlichen Nährstoffe, sondern auch das Wasser, nur durch die Strömung des Protoplasma in genügender Weise transportirt werden kann. Solches gilt u. A. offenbar von den Fruchtträgern von *Phycomyces* und den Wurzelhaaren der höheren Pflanzen.'—l. c. pp. 25–26.

My attention was first called to movement in hyphae in 1890, while examining bacterial cultures on plates of nutrient gelatin which had been invaded by some Mould, afterwards ascertained to be *Rhizopus nigricans*. Some account of these early observations was given before the Indiana Academy of Sciences at the meeting in December of that year[1], but nothing was recorded in print regarding the matter. The movement was so very striking, and in such very common objects, which had been favourite subjects of study by systematists, morphologists and physiologists for a century or more, that I hesitated to believe that it was unknown to science.

For a very long time the conditions under which the movement takes place were not recognized, and it was only seen at intervals, and then for so brief a space of time that serious study was impracticable. In course of time it was found out that the most important condition is a saturated, or nearly saturated, atmosphere, and all subsequent studies were made with the cultures covered so far as possible.

Some time spent at the Botanical Institute at Bonn during the summer of 1896 enabled me to prosecute my inquiry into the facts, and also to satisfy myself that no record of them had yet been made. I take the opportunity to acknowledge the kindness of Professor Strasburger in placing the resources of his laboratory at my disposal, in providing additional apparatus for my work, and of both Professor Strasburger and Dr. Noll for helpful suggestions.

The hyphae of the Mucoraceae after attaining a certain maturity, I have found, set up a movement of the protoplasm, in which nearly or apparently all the contents—that is, the cytoplasm, microsomes, food-bodies, nuclei, and vacuoles—participate. It is a streaming movement, such as a somewhat viscous, colourless liquid would produce in flowing through a pipe. There is sometimes an evident ectoplastic layer lining the cell-wall that does not take part in the movement, but often this is so thin that it is no longer visible, although one cannot suppose that it is ever entirely absent. All kinds

[1] Bot. Gaz. xvi, 36.

of granules are borne along in the current. The vacuoles, however large, are also swept along; and at the right stage of growth for movement the protoplasm is usually highly vacuolated.

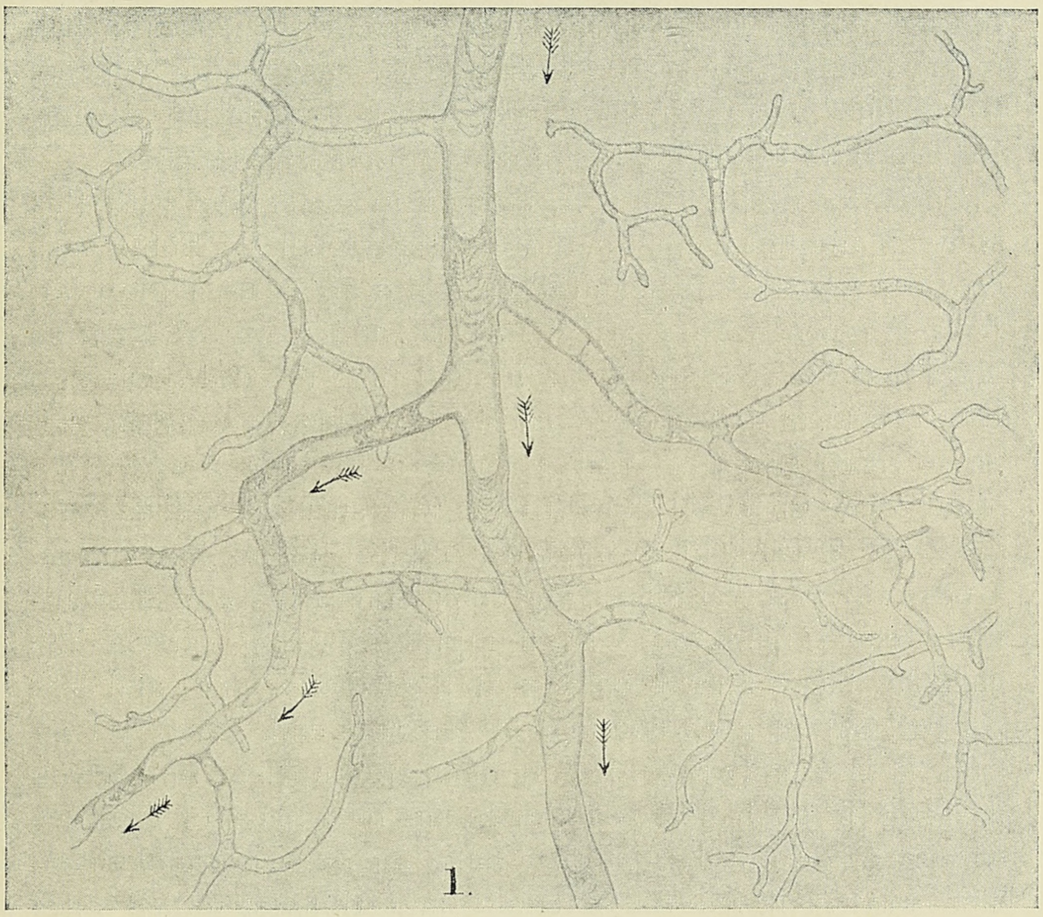
The movement is usually fitful. It does not take place in all the hyphae of an individual plant at the same time, but occurs in some of the main filaments and in part of the branches leading therefrom. It continues for a time, then without apparent reason ceases. Again it starts, either in the same direction, or more usually the opposite. The periods of movement and of rest are both indefinite. The current may flow in one direction for fifteen or twenty minutes, or possibly much longer, without the slightest check, and with brief interruptions may continue for hours.

The observer is soon curious to know where so much protoplasm and cell-sap goes to. If a hypha in which the current is flowing distally be traced to the end, it will be found that the current finally becomes slower, but does not cease until the end is nearly reached. Nothing is revealed to the eye, however, that explains how the full hyphal extremity continues to receive an inflowing stream without seeming limit. We might liken it to a small lake with no outlet, into which a rapid river flows without effecting a change in the level. It would seem that the end of the hypha should be under greater stress than the part further back; but there is no clear evidence of it in change of diameter, elongation, or extrusion of water.

In tracing the filament containing the current from the free end towards the centre of the mycelium, I have always found that sooner or later it became entangled in the general mass and was lost to sight. A more ingenious method of growing the Mould may eventually enable the observer to map out the currents throughout the diversified ramifications of a whole plant.

The rate of movement varies greatly, and rarely remains at a uniform speed for many minutes at a time. I made a number of readings of rapidly flowing protoplasm in

Rhizopus nigricans at a temperature of 28° C., and found it to average 3·3 mm. per minute. This is about twice as fast as the rotation in *Nitella*, and four times that of the circulation in *Tradescantia*. It does not, however, equal the rate of streaming in the Myxogastres, as given by Hofmeister[1].



WOODCUT 8 (Fig. 1).

Part of mycelium of *Rhizopus nigricans* grown upon nutrient gelatin. The arrows indicate the direction of the currents.

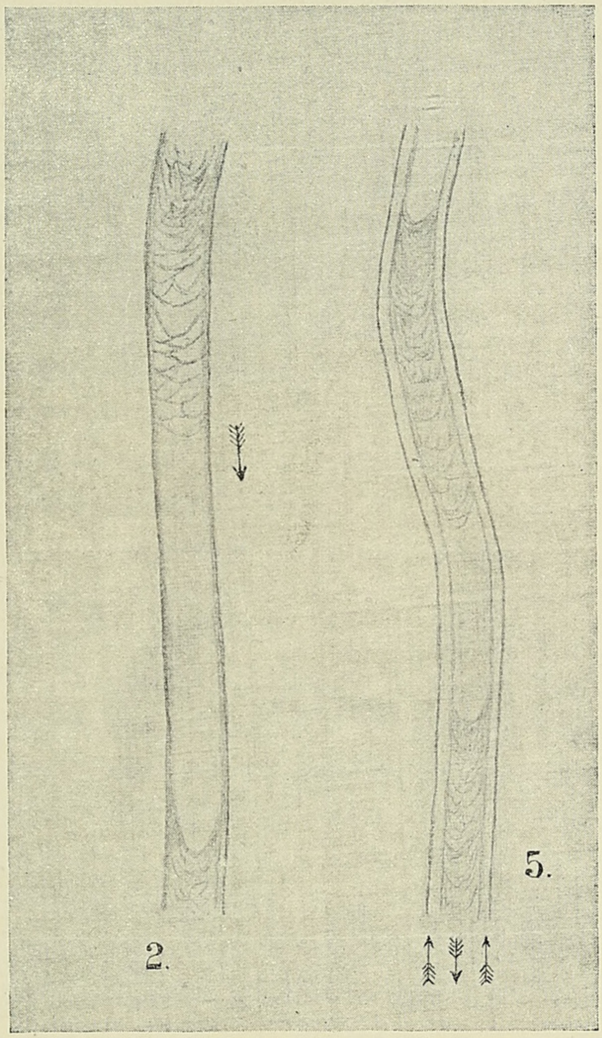
As seen under a convenient adjustment of the microscope, it has a surging precipitate flow, very striking to any observer, even to one accustomed to the diversity of microscopic life, and reminds one of the flow of blood in the capillaries of a Frog's web. (See Woodcut 8.)

[1] Lehre von der Pflanzenzelle, 48.

No direct observations were made regarding temperature, but incidentally the unusual range for rapid movement attracted attention. Cultures grown in the refrigerator, standing at about 10° C., and transferred directly to the stage of the microscope, showed as rapid movement apparently as those grown at 30° C. Not only does the organism appear less sensitive in this respect to the range of temperature than in most other known instances, but it is not readily affected by sudden change, shock, and other stimulating agents, judging from the limited number of observations made.

A matter of considerable interest in connexion with this study is the behaviour of the moving vacuoles. Sometimes they are few and small, and when at rest are as usual globular. More frequently they are numerous, and many of them so large that the confines of the hyphal tube distorts them into long cylinders with convex ends. Occasionally a hypha will be so filled in places with vacuoles that only a very thin lining of protoplasm clings to the wall-surfaces, and at long intervals a plate of protoplasm extends across the lumen. Under all these variations movement takes place with seemingly equal readiness. In a rapidly moving current the vacuoles become more convex at the anterior end, and less convex, flat or even concave, at the rear end. A very long vacuole is usually preceded by dense protoplasm, into which it seems to push, and is followed up by a mass of vacuoles with such thin walls that they have the appearance of foam. The way in which the foremost and thinnest-walled ones change position, vary their form, and coalesce, as they are swept along, reminds one forcibly of the behaviour of soap bubbles (Fig. 2).

Very interesting changes occur in the form and individuality of the vacuoles when the stream passes through a bent and tortuous filament, or over an obstruction such as may be produced by doubling the hypha upon itself so sharply as to nearly close the passage, or when two streams flow together, as may happen at any angle from a very acute to a very obtuse one, or when a stream sends part of its contents into a side branch, or when a rapid stream forces itself upon and merges into a slower one. I shall, however, content myself at this time by saying that these appearances are such as would be produced by a viscous fluid, holding

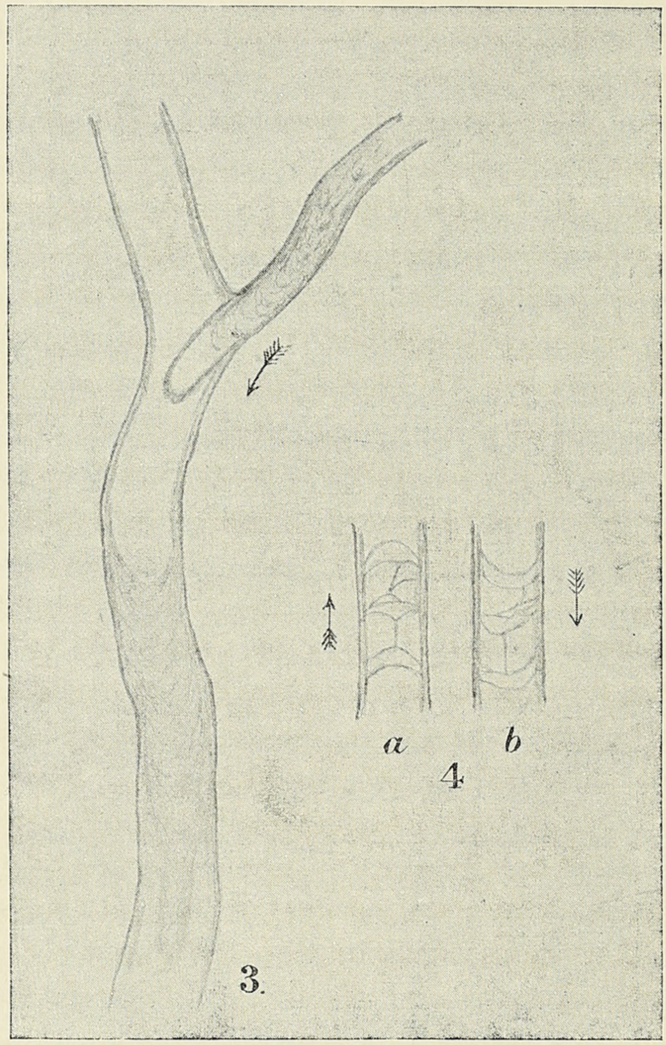


WOODCUT 9.

Fig. 2. A long vacuole is pushing forward into dense protoplasm, followed by highly vacuolated protoplasm.

Fig. 5. Hypha with axial stream of protoplasm, and a peripheral return current of non-vacuolated protoplasm.

drops of a less viscous fluid, when forced through tubes of like construction to the mycelial filaments. When, for instance, the stream impinges squarely upon the angle of a wall separating two branches, the larger vacuoles are generally bisected, a part of each going to the right and a part to the left. When one stream flows into another, it frequently



WOODCUT 10.

Fig. 3. The current from a branch is rushing into a long vacuole in the main hypha. Further along the main hypha, the protoplasm is pushing into and through a large vacuole (only partly shown), owing to an acceleration of the current.

Fig. 4. Portion of a highly vacuolated hypha with the protoplasm in motion, *a* just before, *b* just after, a change in direction of the current.

plunges into the midst of large passing vacuoles, and disruptures them. When a part increases its speed over the part immediately preceding, better progress is made by pushing through the centre of a long vacuole, as this lightens the friction at the sides (Fig. 3).

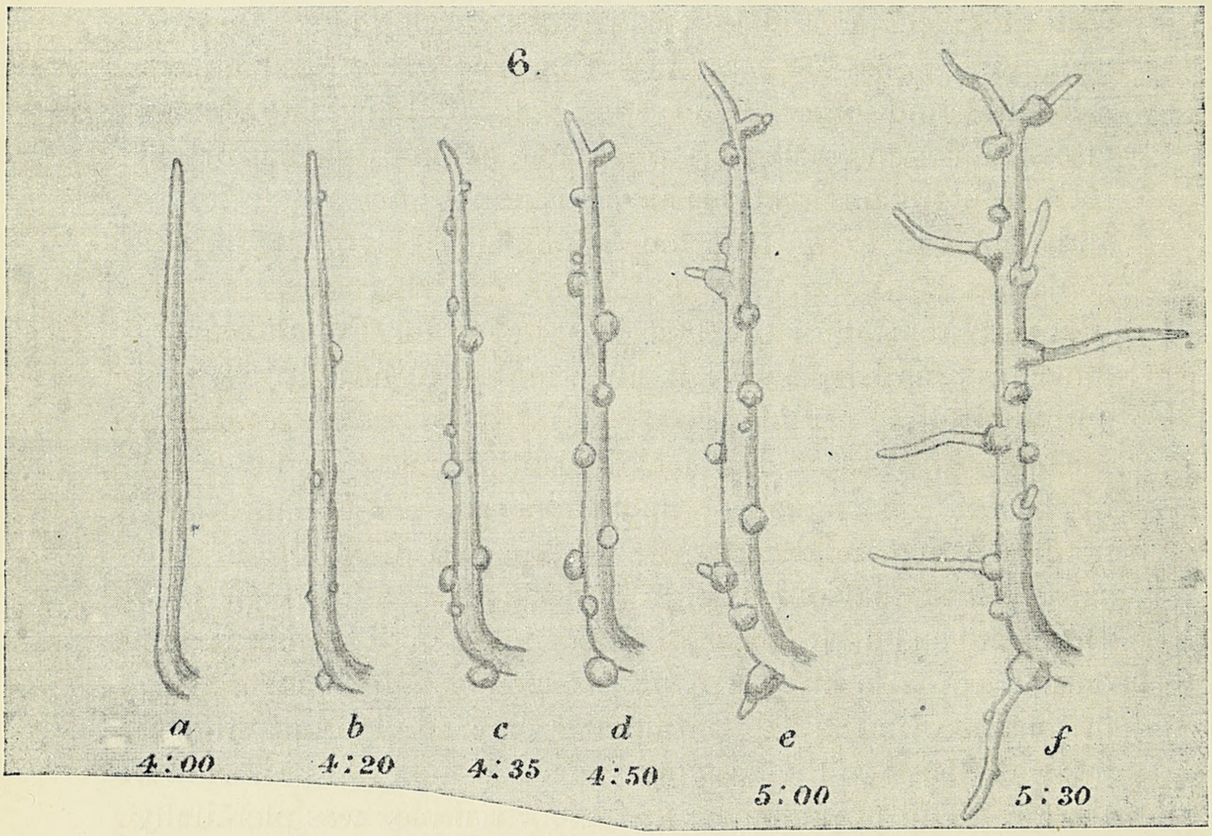
In fact I cannot see in the variety of catastrophes which overtake the moving vacuoles any ground of support for the supposed autonomy of the vacuoles and a special vacuolar membrane, advocated by De Vries[1], Went[2], Wakker[3], Bokorny[4] and others. These theories appear to me to be little in accord with the phenomena that attend the streaming in coenocytes, while much can be seen to favour the views of Pfeffer[5], Bütschli[6] and others, who appeal to the laws governing viscous fluids in which surface tension assumes an important rôle. But my observations have not been sufficiently intimate and complete to warrant me in pursuing this phase of the subject.

A requirement of the first importance for the display of movement, as I have said, is an atmosphere heavily charged with moisture.

Unless extravasated drops are plentifully scattered over the hyphae, movement is not likely to occur. It will probably be interesting to describe an instance of their origin and subsequent behaviour as seen under a Zeiss microscope with D objective and No. 4 ocular (Woodcut 11). The culture was on nutrient gelatin with *Rhizopus elegans*. A filament lying in contact with the substratum and showing vigorous streaming was traced to the growing apex, and found to project into the air. The proximal half of the aerial part was plentifully supplied with drops. The distal part was free from drops. The observations began at 4 p.m., and twenty minutes afterwards drops began to appear at some distance apart. These grew larger, and intermediate ones appeared. At 5 o'clock branches began to protrude through the larger drops. In half an hour the branches had grown quite long, and they

[1] Pringsh. Jahr. f. wiss. Bot. xvi, 463. 1885.
[2] Ibid. xix, 295. 1888. [3] Ibid. xix, 423. 1888.
[4] Biolog. Centr. xiii, 271. 1893.
[5] Abh. d. Sächs. Ges. d. Wiss. xvi, 185. 1890.
[6] Investigations on Protoplasm, 227. 1894. (German text 1892.)

themselves began to show drops, which by 6 o'clock became plentiful. In the meantime the proximal half had increased and enlarged its drops, but no branches had appeared. The protoplasm had streamed into the portion under observation almost continuously for a time, then at intervals it stood still, the intervals of rest becoming longer and longer, but no reverse



WOODCUT 11 (Fig. 6).

Changes observed in the distal part of a free hypha during an hour and a half. Drops of water successively appeared on the surface, and through some of them branches eventually protruded: *a*, appearance at 4 p.m.; *b*, at 4.20; *c*, at 4.35; *d*, at 4.50; *e*, at 5; *f*, at 5.30.

movement occurred. Observations were continued until 7 p.m., three hours in all. During this time the branches grew at an apparently uniform rate, and without noticeable synchronous relation to the protoplasmic movements. The next morning observations were resumed, and from 10.30 to 11 a.m. streaming in the same direction as on the previous day was seen, with occasional interruptions, and one backward movement for a distance of about twice the diameter of the filament. At 3.30 p.m. the observations were renewed, and for half an hour streaming in the same distal direction with occasional periods of quiescence was noted. At 4 o'clock a reverse movement of full strength continued for several minutes, then changed to the former distal course. In this way the reverse or proximal movement alternated several times with the distal movement during the next half hour. The observations were now abruptly terminated by an unfortunate displacement of the microscope.

It is possible that the drops upon the hyphae, which appear to precede streaming, are formed by the action of some excreted osmotic substance, as suggested by Wilson[1] in case of *Pilobolus crystallinus*; but it seems to me more probable that the position of the drops is determined by the localization of a cytohydrolytic ferment, which acting upon the wall renders it more permeable, and the internal pressure then forces out the water. This hypothesis would also explain the extension of this particular watery part of the cell-wall into a branch. A similar suggestion has been made by Marshall Ward[2] in connexion with the study of a species of *Botrytis*, in which the extrusion of enzym-drops was observed. But the drops upon the Mucoraceae are undoubtedly of a different nature from those seen by Marshall Ward, for in *Botrytis* they appear usually at the tips of the hyphae[3] and remain there as growth continues; in the Mucoraceae they are lateral, and the branches grow through and beyond them. An enzym has already been separated, in fact, from *Rhizopus nigricans* by Kean[4], which is capable of softening cellulose walls, and probably is produced by other members of the Mucoraceae; but in what part of the fungus it resides has not been determined.

[1] Unters. a. d. bot. Inst. zu Tübingen, i, 15. 1881.

[2] Ann. Bot. ii, 331. 1888.

[3] l. c. 339.

[4] Bot. Gaz. xv, 173. 1890.

It is probable, if we consider the facts now in our possession, that in order for streaming to take place the filaments must have a high internal pressure. It is doubtless the pressure which forces water through places of less resistance in the walls, although the walls throughout are highly permeable, as shown by the rapidity with which the mycelium collapses upon removal from a moist to a dry atmosphere. The ready permeability of the walls will account for Wilson's results in obtaining drops by placing particles of sugar on the surface of *Pilobolus*. Internal pressure, as we know, is secured through osmotic action, and altogether it seems probable that the necessity for a very moist atmosphere to bring about streaming lies in the fact that the part of the mycelium in contact with the moist substratum cannot take up water fast enough to secure pressure throughout the whole structure so long as extravasated moisture is rapidly removed from the aerial surface of the very permeable hyphal walls.

By admitting the existence of a high osmotic pressure, we shall be able to explain many of the phenomena connected with the streaming. In the first place we must premise that although osmotic pressure originates in the movement of the molecules of the osmotic substance and varies directly as the density of the solution, yet it is converted into, and is manifested as, hydrostatic pressure which is uniformly distributed per unit area throughout the enclosing wall. The more tensely the cell-wall is stretched by the internal pressure, the more sensitive every part becomes to any variation in the pressure. If we imagine a hyphal tube some millimetres in length, under strong pressure, with one end in a watery substratum and the other in air, it is easy to see that any water taken up osmotically at the submerged end must instantly expand some part of the tube (growth), or force water out through the walls of the aerial part (extravasation). The water taken in at one end and discharged at the other displaces the whole column and moves it along toward the end where the water is escaping. But in the hyphal tube the water is an interrupted column lying in the more viscous protoplasm, and the movement will therefore be noticeably greater along the axis of the tube where there is least friction. For this reason every transverse surface tends to become more and more convex in the direction of the movement, which accounts for the heightened convexity of the forward end of the vacuoles and the flattening or introversion of the rear end.

There is, furthermore, less resistance to axial movement just in proportion to the thinness of the transverse layers of protoplasm ; therefore a thin-walled vacuole will tend to move faster than a thick-walled one; and this action, coupled with the tendency of the transverse layers to become thinner as movement progresses, dependent upon density, viscosity, elasticity and other factors, brings about the frothy appearance in the rear of a long vacuole.

It is evident that if the movement is due to the change in water-content at the extremities, streaming toward the free aerial parts should be more frequently seen than the opposite movement; and this is the case, the observation cited in detail being almost typical. It would also follow that streaming would be more constant toward and into growing branches and developing sporangia than toward other parts, for at these places both metabolism and extension of the walls are demanding material and removing resistance from the advancing column. This also agrees with observation, and in fact is the particular case noticed by De Vries, mentioned at the outset. The same greater proportion of movement toward advancing extremities and into sporangia has been observed by De Bary[1] in the streaming of the Myxogastres.

The number of conditions which may have an influence upon the movement is considerable, and their interaction very complex. But foremost among them, after sufficient turgidity is attained, is undoubtedly the facility of absorbing water in one part and its release in a distant part. Some attention was given to cases where this was minimized, and a specific instance or two may be cited. A culture of *Mucor racemosus*,

[1] Morph. and Biol. of Fungi and Myc., 426.

a species much given to the early formation of cross-walls in the hyphae, was grown upon neutral nutritive gelatin. This species branches acropetally in a most uniform manner, the branches making an angle of about sixty degrees with the main axis, and both growing quite straight. The advancing hyphae all remained appressed to the moist substratum, giving a regular featherlike appearance. On the second day movement was to be seen. At this time the central part of the mycelium bore plenty of sporangiophores, and was furthermore much septated. The protoplasm was crowded with vacuoles, but quite free from granules. The movement was not especially lively; in all cases it proceeded toward the growing apex, and although the whole branch-system was in normal growing condition, the stream mostly kept to the main axial hypha, only now and then entering a side branch. On the day following the movement still continued in the same direction. In two cases I was able to trace the stream back from the growing apex into the central mass of mycelium until a cross-wall was reached where all movement ceased. In these cases the whole length of the effective hypha was in contact with the moist substratum, although the proximal end was overlaid with growing mycelium, while the distal end, into which the stream poured, was both extending and half exposed to the air. I assume that in this case the small difference in osmotic conditions between the two ends of the active hyphae was the cause of the sluggishness of the current.

In another case still more uniform osmotic conditions were secured. Spores of *Rhizopus elegans* were grown in bread-broth entirely submerged. On the fifth day neither vacuoles nor movement were yet to be seen. On the tenth day vacuoles were abundant, and two or three instances of faint streaming were detected, but the development was no longer entirely beneath the surface, for a few sporangiophores had risen into the air.

In the last case another phenomenon of protoplasmic movement appeared, which had also been seen a number of times under less favourable conditions. The microsomes were in quite active locomotion through the protoplasm, exhibiting a kind of indefinite circulation. Far finer examples of this movement were obtained, however, in sowings of *Pilobolus* spores upon the surface of bread-broth. In twelve hours after sowing (overnight), they had germinated freely with long branching hyphae. Each spore contained a large vacuole, and a few small ones appeared in the hyphae. The microsomes were especially distinct, and kept up a lively coursing in all directions, but more especially lengthwise the hyphae and into and out of the spores. They appeared to follow no defined routes, which seems to indicate that they were not dependent upon circulating currents in the liquid. Moreover, sometimes collisions occurred between microsomes moving in opposite directions. I have no explanation of this phenomenon to offer; but do not think that it is connected in any direct way with the streaming movements in hyphae. It was necessary, however, to mention it, in order to distinguish it from the floating of microsomes in currents, which I now desire to describe.

I have so far said nothing about return currents in the hyphae, but they are occasionally to be seen. When the return current exists it is generally well defined, and always occupies the periphery of the hyphal cavity. It carries no vacuoles, and can only be detected by the movement of the microsomes. As the hypha is seen in optical section, there appears to be the usual surging, vacuolated stream moving through the centre, and on either side next to the walls a narrow uniform layer of clear protoplasm, in which minute particles are moving in opposite direction to the central stream. Between the two streams is a quiescent partition of protoplasm of about the same thickness as that lining the cell-walls. These quiescent layers vary much in thickness, and are at times so thin as to be scarcely discernible. It is obvious that whenever such return currents exist, their volume is ample to bring back all the protoplasm borne forward by the central stream, especially as it has become freed from all vacuolar sap, which has probably been used in growth or extravasated (Fig. 5).

It has been impossible in this presentation of my studies to touch upon a number of questions which have arisen during their progress, or to discuss fully those brought forward. I have attempted to give as briefly as possible the main facts regarding the streaming movement of the contents of hyphae where the coenocytic structure furnishes an uninterrupted passage of considerable length. The passage is usually some millimetres, and often many centimetres, in length, and often equals one-fourth millimetre, or even one-half millimetre, in diameter. I have brought together a number of observations which seem to me to clearly indicate that the movement is in the main a physical one, dependent upon osmotic conditions; although there can be little question that lying back of the physical factors the living protoplasm functions as a strong inciting and controlling agent. I have found, in fact, although not before mentioned, that streaming can be set up in a purely artificial manner that does not differ in any observable particular from the natural streaming. In a culture of *Rhizopus nigricans*, the application of a drop of a 20 per cent. solution of potassium nitrate caused a vigorous movement for a time through the hyphae toward the place of application. It is evident that this plasmolytic agent extracted the water from the filaments, thus causing the flow. After a time normal conditions prevailed in the hyphae experimented with. A 15 per cent. solution produced but slight movement. I need not recapitulate the conditions which, under normal relations, effect the plasmolytic changes.

I have observed the streaming movement in the following eight species of Mucoraceae: *Mucor Mucedo*, L.; *M. racemosus*, Fries; *Rhizopus nigricans*, Ehr.; *R. elegans* (Eidam), Ber. and De T.; *Phycomyces nitens* (Ag.), Kze.; *Sporodinia Aspergillus* (Scop.), Schröt.; *Thamnidium elegans*, Lk.; and *Pilobolus crystallinus* (Wigg.), Tode. I do not doubt but that it occurs normally in many other coenocytic forms, where the conditions are favourable.

In conclusion I may express the opinion that the movement is an incidental feature in the life of the plant. It does, without doubt, aid in the transfer of nutrient material to points where growth is taking place, as De Vries has pointed out; but I believe that growth is not dependent upon it, and that full and normal development takes place without the movement coming into action.