

## Recent Researches on the Saprolegnieae; a Critical Abstract of Rotherth's results.

BY

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THE study of the spore-formation of the Saprolegnieae as a most accessible type has been renewed again and again since Strasburger's 'Cell-book' gave an impetus to cytology. Büsgen (in Pringsheim's *Jahrbücher*, xiii, 1882), and a little later Marshall Ward (in *Quart. Journ. Micr. Soc. N.S.*, xxiii, 1883) elucidated the contradictory statements of older observers by showing that the zoospores were segregated in two distinct stages, interrupted by a third in which the contents of the sporangium appeared uniform and homogeneous. They regarded the clear spaces between the origins (*Anlagen*) of the spores in the first stage as transitory cell-plates (Büsgen), or nuclear-plates (Ward), and referred the homogeneous stage to the absorption of these plates. They described the appearance of shifting vacuoles in the young spores on their second and definitive separation. Finally, Büsgen expressed the view that the substance of the transitory cell-plates of the first segregation become converted into the 'expulsive substance,' which by its supposed swelling effected the dispersion of the zoospores.

In 1884 a careful examination led me to a totally different interpretation of the facts correctly observed by my predecessors. In a paper first read at the Association Française (July 1886), and printed in extenso in the *Quarterly Journal of Microscopical Science*, March 1887, I was able to prove that the hypothetical cell-plates of the first segregation are merely the optical expressions of thinnings on the parietal

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layer of protoplasm left by the aggregation into the 'origins' of zoospores, or of lacunar spaces between the latter filled with cell-sap. I interpreted the homogeneous stage as consisting 'essentially in the swelling up of the protoplasm and the loss of its resistance to osmosis,' accompanied but not caused by plasmolysis; and regarded it as 'probable that the "Hautschicht" and vacuolar walls break up at this stage as continuous layers,' and that therein was the explanation of the phenomenon. I also pointed out that a contraction of the sporangium can be observed at the homogeneous stage, accompanied by the excretion of a dissolved substance strongly attractive to certain bacteria.

A second part of my paper dealt with the liberation of the zoospores. I disproved by the use of reagents the existence of any swelling expulsive matter in the sporangium, proved the correctness of Cornu's discovery of flagella in the sporangial zoospores of *Achlya*, and referred the liberation to the automotility of the zoospores reacting to the chemical stimulus of dissolved oxygen in aerated water.

Two other accounts have now appeared; one by Berthold incidental to his work on 'Protoplasma Mechanik,' 1886; and a paper by Ladislaus Rothert which appeared in Polish in the *Proceedings of the Cracow Academy*, xvii, 1887 (it was only 'in the press' in September of that year), and in German in Cohn's *Beiträge* for 1888. As Rothert's work is fuller than Berthold's, and in no way contradicts it, I shall only deal with the former author. It is interesting to note that all of us except Berthold began, at least, our work in the Strassburg Institut, under the stimulus and aid of the same kindly master—Anton De Bary.

Rothert's paper we may greet as affording the first full and complete account of the double segregation and homogeneous stage, worked out independently, but confirming my views so far as they went, and completing them by showing what is the real cause of the solution of continuity in 'Hautschicht' and vacuolar wall. His paper however does more than this; it affords the first complete account we have of the formation

of the zoosporange, its septum, and the tubular process through which the spores escape. On these grounds I think it is well worth abstracting to show exactly what is our present knowledge of this most interesting study; and I shall supplement this abstract by criticisms of all points on which my own work has led me to take a different view to the author's.

Rothert's work was principally conducted on three forms of *Saprolegnia* belonging to the *ferax* group. A well-chosen field is half the battle; it is very difficult in working over a group to give equal attention to each; and he has shown that these species are far more favourable than *Dictyuchus* (genus) or *Achlya*<sup>1</sup>.

The sporangial formation begins by the slackening to final

<sup>1</sup> The following brief analysis of the genera may prove useful to readers:—

*Saprolegnia*.—Spores leaving the sporange and swarming freely, then encysting to swarm later in the 'second form.' Innovation usually growing through the empty sporange.

*Leptomitus*.—Resembling *Saprolegnia*, but with frequent strangulations on hyphæ and sporangia. As these occur also according to Cornu, in forms otherwise referable to *Achlya* (*Achlyogeton* Schenk,) and *Pythium* (*Myzocytium* Schenk), I regard them as mere habit characters, of less worth probably than the septa of *Saprolegnia torulosa*, which De Bary regards nevertheless as scarcely more than a form of *S. ferax*. Hence it is that I described ('fälschlich,' as Rothert writes) as a *Saprolegnia*, a form with constricted hyphæ, but with the innovations growing into the empty sporangia, and with the sexual reproduction of *S. ferax*.

*Achlya*.—Spores on their liberation assembling to encyst in a hollow sphere at the mouth of the sporange, then swarming in the second form; innovation growing out laterally at the base of the empty sporange.

*Aphanomyces*.—Like *Achlya*, but with linear sporangia containing only a single file of zoospores; innovation growing into the empty sporange.

*Dictyuchus* (genus).—Spores do not leave the sporange but encyst in situ, emerging only in the second form. The sporange wall often deliquesces at the maturity of the spores.

'*Dictyuchus*-form.'—When the spores of *Achlya* or *Saprolegnia* fail to leave it at maturity they encyst within, constituting this form or dictyosporange. They either swarm ultimately in the second form or germinate in situ by emission of a hypha.

The 'first form' of zoospore is ovoid with a pair of flagella from the front (narrow end). The 'second form' is uniform with an anterior and a posterior flagellum diverging from the hilum. The existence of these two forms constitutes the phenomenon of 'diplanetism.'

arrest of the apical growth of a hypha, while protoplasm continues to stream in from the base, usually determining an ovoid enlargement; at first the thick protoplasm of the sporangial part of the hypha passes gradually into the thinner investment of the basal part; but soon the contrast is sudden and sharp. Then at this junction the granules disappear or migrate from the protoplasm so as to form a longish ring of hyaloplasma which grows at its inner circumference to finally form a transverse disk extending across the hypha from wall to wall, sharply bounded towards the basal hypha but on the sporangial side gradually passing into the granular protoplasm.

In about half-a-minute the transverse septum appears (simultaneously) at the base of the disk, at first pale ('verwaschen'), soon sharp-contoured. In favourable cases we may see that a rather broad basal section of the disk of hyaloplasma assumes a higher refraction; this gradually thins off and becomes more clearly defined and finally condenses ('sich verdichtet') into the septum; which is clearly not formed from a granular cell-plate as Strasburger states. Before this, however, a number of Pringsheim's cellulose corpuscles had accumulated about the limiting area, and fell, by the appearance of the hyaloplasma disk, into an upper and a lower group. As the upper group disappears on the completion of the hyaloplasma disk, Rothert thinks it probable that the granules, consisting of a very soluble form of cellulose, are absorbed into the disk and by their solution afford materials for the septum.

Slight modifications occur in the formation of the hyaloplasma disk according to the relative fulness or emptiness of the sporange.

We now come to the formation of the zoospores, and first of all their partial segregation, in which stage we may call them 'spore-origins' (Sporen - Anlagen). We can distinguish between (1) full sporangia which at first contain no central cavity or to which class usually belong the smaller sized ones; (2) ordinary or normal sporangia with a fairly thick parietal

investment surrounding the cell cavity or lumen [often two or three vacuoles in *Achlya*]; and (3) starved sporangia, as I have elsewhere named them, the 'inhaltsarme' of Rothert, with only a thin parietal investment of protoplasm and an immense vacuole. These differ in the processes of segregation.

In the full sporange granules gradually wander into the hyaloplasma disk which thus becomes indistinguishable. In the other forms the granular protoplasm first retracts from the disk with which it is only connected by a thin hyaline layer investing the wall and a few delicate plasmatic threads, so that the lumen is widest at the base of the sporange. Vacuoles then appear in the disk, soon enlarging and communicating with the main vacuole of the sporange. The disk then thins in the centre, and rises peripherally up the walls. The granular protoplasm again stretches down towards the septum, and finally by the wandering of granules into the hyaloplasma the latter loses its character. At the beginning of this process, the septum usually bulges towards the basal hypha, thus indicating an increase in the turgescence of the sporange. At the end of these processes the protoplasm usually shows more or less striation or flockiness, due to the uneven distribution of granules, and, in unfilled sporangia, has an uneven surface towards the lumen. The distribution of granules in the protoplasm, and of protoplasm in the sporange, gradually becomes uniform. During these stages after the formation of the septum the sporange never elongates by more than half per cent., irrespective of the concavity of the septum and the formation of the 'process,' except in Rothert's *Saprolegnia*, sp. 2.

The 'process' may be formed even before the septum, at the same time with the differentiation of the spore-origins, or most frequently between these two formations. It usually occupies the apex of the sporange, but may develop at any point [except the septum]. Here again hyaloplasma accumulates at a spot, bulging out the membrane; the bulging of the membrane continues with the accumulation of hyaloplasma, until a short cylinder with a nearly hemispherical top

is formed, filled with this substance. The convex terminal wall or 'cap' is duller and less sharp-contoured than the rest of the sporangial wall; its boundary not being distinct from the protoplasm on its inner side. The hyaloplasma plug soon becomes granular, except a thin layer lining the cap of the process. [The protoplasm of the apex of all growing hyphæ is hyaline; in all cases this 'hyaloplasma' shows granules on treatment with iodine.]

The segregation of the zoospores proceeds thus. In normal sporangia appear numerous splits in the protoplasmic investment, stopping just short of the cell-wall and opening into the vacuole; these appear and disappear, and finally become constant forming a honeycomb network. At first numerous plasmatic bridges connect the origins so mapped out; but most of these soon disappear; it is to the optical expression of these bridges that we must refer Büsgen's 'Körnerplatten;' this is especially clear in *Achlya*. Some protoplasm may remain long distinct from the 'origins,' apart from the continuous wall.

In full sporangia the appearance of a zigzag slit indicates the segregation of the origins in the smaller sporangia; in the larger the segregation is produced by the appearance of linear lacunæ (Spalten) which form a connected system.

In poor sporangia the segregation rather takes place by the aggregation of protoplasm in heaps, at the expense and by the thinning of the intervening part of the parietal layer. Here also plasmatic bridges may occur, and some fragments of protoplasm are left out of the schema. (In *Aphanomyces* the spore-origins appear as bulgings of the parietal layer of protoplasm, which meet and form transverse disks, joined by the intervening thin annular portions of the parietal layer.) Rothert describes these elevations as shifting, rising and flattening out for some time before becoming stable; but I feel sure that this is a misinterpretation of the gradual 'rotation' of the protoplasmic lining of the sporangium as a whole, carrying the origins with it, which may also be well observed in thin 'full' sporangia of my *Saprolegnia* (*Lep-*

*tomitus*) *corcagiensis*, as in other species of *Saprolegnia* and *Achlya*.

At the period of this 'rotation' (as I hold it) there appear clear spots free from granules in the centre of each origin, near the sporangial wall; these Rothert interprets as nuclei, though he has failed to stain them. I have succeeded once in so doing in *Achlya* with Draper's dichroic ink, a logwood stain. In a long discussion Rothert insists on these origins being simply 'Anlagen,' and united by the *uninterrupted* 'Wandbeleg' of granular protoplasm; and discusses Büsgen's 'Körnerplatten,' which he shows rest on a confusion between the plasmatic threads often uniting the origins, and the fact that there is usually an accumulation of coarse granules on the whole of the convex half of the origins, a point to which I have also drawn attention.

He ascribes the errors of his predecessors to unsuitable objects for research, to the use of insufficient powers, and the influence of preconceived ideas derived from the consideration of the embryo-sac. I may mention that Dr. Büsgen has written to me that this last was actually the case with himself. Of course these facts and considerations do away with the hypothetical gelatinous 'Zwischen-substanz,' which is only the expression of the 'Wandbeleg' between the origins.

This description of the stage of preliminary segregation is essentially the same as mine, completed however by the observation of the plasmatic threads joining the 'origins,' which I have verified and accept. I have adverted to one error of interpretation in these preliminary processes.

The origins now contract, widening the interspaces and breaking most of the plasmatic threads, and at the same time become smooth on their free surfaces which before were rough and granular. In this stage Rothert has seen the 'rotation' and change of place I have before adverted to. This stage lasts at most one or two minutes, to give place to Büsgen's 'homogeneous stage,' which Rothert calls the stage of swelling up of the spores. The origins swell up, touch, and apparently

fuse, the sporange becoming clear and brighter; the septum, previously concave, becomes convex, bulging into the sporange, and the rounded cap of the process becomes flat; the sporange has lost its turgescence. Directly afterwards vacuoles appear in the protoplasm; they come and go for some time.

Closer observation of a favourable object like *S. Thuretii* shows that the larger granules have disappeared leaving the protoplasm finely granular; and that the fusion of the spores is not complete, they are only in contact, polyhedral and separated by fine plane spaces. In many cases however it is difficult, in some impossible to see any separation even in this species<sup>1</sup>. In others the apparently complete fusion may be the rule, the demonstration of separation the exception. The interspaces now extend to the wall of the sporange, which has now ceased to be a single cell; the 'origins' have become *spores*.

Accompanying this stage is often seen a swarming of Bacteria from all parts to execute a lively dance round the wall of the sporange and at its expiration to scatter anew. On one occasion zoospores of *Saprolegnia* [In which period of their diplanetism? probably the second] behaved in the same way. Everything seems in favour of its being some nutritive substance that attracts the Bacteria rather than oxygen. This can only be cell-sap; and if it passes out in sufficient quantities to attract Bacteria, there must be a diminution of the volume of the sporange; probably greater than that due to the inbulging of the septum and the flattening of the process. Measurements gave a *shortening* of from 1 to 4 per cent. Taking the latter figure the reduction in volume would be 11.5 per cent., or with that due to the two septa 13 per cent. The wall, previously turgescient, now contracts with expulsion of cell-sap, and the cause of this

<sup>1</sup> Yet Rothert wrote in the Botanische Zeitung, 'lässt die Quellung bis zur völligen Verschmelzung gehen, was nicht richtig ist.' He sees now that I was right in my observation; and that it needed other favourable objects to obtain the correct interpretation; and I had noticed, as he admits, the incompleteness of the homogeneity in *S. corcagiensis*.



contraction is the complete rupture of the continuous protoplasmic investment of the walls into segments belonging to each origin. This is easily seen in poor sporangia—in optical section the protoplasmic investment can be seen to rise and divide between two origins, and go half to each. At this moment the origins (now spores) become full of minute vacuoles, which diminish in number and enlarge as the spores swell and the protoplasm becomes finely granular. 'It is here quite clear that the two processes, the vacuolation and the swelling up of the spores, go hand in hand; the phenomenon of swelling is easy to understand if we make the really obvious supposition that the protoplasm of the spores has a tendency to take up water. This could not hitherto have full play so long as the "Hautschicht," which must be regarded as continuous, hindered endosmose: but when the investment of the wall is ruptured, the "Hautschicht" is interrupted at the points of severance, and before its reconstitution cell-sap is taken up into the protoplasm of the spores and determines their swelling. The cell-sap so absorbed, or a part of it, is at once excreted in the form of the shifting vacuoles<sup>1</sup>. I must here note that in my paper in 1886 I had pointed out that 'probably the "Hautschicht" and vacuolar wall break up at this stage and become reconstituted later on, and that herein is the true essence of the homogeneous stage,' which I had written a page above 'consists essentially in the swelling up of the protoplasm, and the loss of its resistance to osmosis.' I thus had perceived and demonstrated the essence of the homogeneity; but owing to the unfavourable type I had chiefly worked over I had failed to discover the rupture of the continuous 'Wandbeleg,' which is undoubtedly its proximate cause. The demonstration of this belongs to Rothert and Berthold. I also demonstrated to Prof. De Bary and Dr. Büsgen in 1884 the loss of turgescence of the sporange, and its marked contraction, accompanied by the bacterial dance.

<sup>1</sup> In fact the vacuolation is really paralleled by such cases as the vacuolation of the protoplasm of a torn *Vaucheria* filament.

After this stage the lines of separation become clear, contract, and gradually round off, beginning at the angles; and as they contract they retreat from the sporang wall, which now shows a double outline. The front spore, as it retreats from the process, leaves the layer of hyaloplasma at the apex, and is only connected with it by one or two strings which are finally retracted into the spore, as the hyaloplasma from which they are drawn disappears or becomes confounded with the end wall. [My description would state that one or more vacuoles appear at the base of the hyaloplasma disk, and by their enlargement separate a terminal portion from the front spore, leaving one or two strings along which the hyaloplasma is retracted into the front spore.] Next appear the cilia, as slow outgrowths, at first short straight bristles, with simple oscillations. The front spore has its cilia always at the front end next the process; but there appears no polarity about the others. At the same time the spores manifest shaking (wackelnde) movements, increasing in strength till their discharge.

During this contraction and development of the spores, they become warty, and some of the processes are abstricted. These lumps of protoplasm after independent movements are mostly absorbed (probably always) by the very spores from which they were separated; a few may be unabsorbed, pass out with the spores and undergo diffuence; but this makes no difference to the spores themselves. Rothert recalls similar processes described by De Bary in the formation of the oospheres. The mature zoospores now contain three vacuoles, of which at least one, that at the front end, contracts rhythmically. [I have seen in *Achlya* at this stage all three vacuoles rhythmically contractile.]

On treatment with iodine about a quarter of the protoplasm turns dark brown, and contains black granules just below the surface. Nothing of this shows in the fresh state, nor is there any polar relation of the dark portion to the axis of the spore. Rothert suggests no explanation; it seems to me that we may fairly refer the browning to glycogenic contents to be used

up in the formation of the cyst-wall when the spores come to rest.

The discharge of the spores occurs thus. The end wall may open in various ways. (1) The front spore presses into the process and against the cap, pushing it up into a hemisphere. The end wall gets paler and lost to view a little before it disappears. (2) The end wall disappears before the spore reaches it; discharge at once ensues. (3) In a few cases it lifted like a lid, and only disappeared after discharge was completed. (4) In cases where the end wall was unusually stiff and clearly outlined, the front spore pressed through an invisible opening, tearing to pieces in the passage; a few others followed, undergoing the same fate; but these gradually enlarged the hole so that the rest could pass through normally, but very slowly, leaving part of the end wall in situ, which probably never disappears. In my paper I have described the first two modes of discharge; the third I have not seen; the fourth I have since observed in *S. ferax* (*monoica*).

In discharge the front zoospore, which had retreated from the process, now moves up into it; and as soon as it opens, presses out and goes on its own spontaneous motion. The others follow, at first 'stormily,' the front ones close pressed against one another; and this is sometimes the case with all; quite as often, however, the later ones move to and fro, without haste, and only find the exit after much hesitation; not infrequently do the last fail to find it, and encyst within the sporangium. No change in calibre or length takes place in the sporangium during this process.

While this description of the formation of zoospores and the opening of the sporangium is chiefly taken from *Saprolegnia*, it applies on the whole to the other species examined, including the *Achlya polyandra* of the Strassburg Laboratory.

Rothert admits that his observations on *Achlya* were less complete and numerous than on *Saprolegnia*. Here apparently he has never seen the stage of swelling result in complete homogeneity; the planes of separation persisted throughout; after this the spores, instead of contracting from

one another, retreat from the cell-wall, and cease to be clearly separated. In liberation the spore-mass forms a cylinder and presses out, becoming thinner at the outer end, and only later at the base; sometimes this column breaks up transversely into several, and finally these break up into separate spores often united by plasmatic threads; as the spores pass out they group into a hollow sphere at the mouth of the sporange.

In his 'Nachtrag' he insists strongly that my description of the liberation of the zoospores in *Achlya polyandra* is incorrect, and that they are not biflagellate as Cornu and I describe. This involves two points; first of all the identity of my species with *A. polyandra* of Hildebrand (who founded the species in Pringsheim's Jahrbücher, vii. 1867-8), and next whether I am justified in extending my observations to other species of *Achlya*. As to the first point, my species was identical in all characters with Hildebrand's careful diagnosis; while De Bary expresses grave doubts as to the identity of his<sup>1</sup>. As to the second point, the behaviour of the zoospores at and after liberation in another species, which I identify with *Achlya recurva*, Cornu, is exactly the same as in *A. polyandra*. Cornu ascribes flagella to the zoospores of *Achlya* generally, without particularising the species; and a positive assertion of a trustworthy observer is worth all the negative evidence in the world. I have always failed to see the flagella without iodine staining; and Rothert has never definitely looked for them by staining at the stage of liberation<sup>2</sup>. We shall see later that there is independent ground for believing in their presence.

*Dictyuchus clavatus* was also observed by Rothert. Its processes are essentially the same as in the other genera, except that the liberation is effected by the deliquescence of the sporangial wall when the spores slowly separate a little and at once encyst. *Leptomitius lacteus* shows the relations of *Saprolegnia* in the main.

The oogonia, as seen in *Achlya*, show exactly the same

<sup>1</sup> Beiträge zur Morph. u. Phys. d. Pilze, Ser. IV. p. 49.

<sup>2</sup> As he has informed me by letter.

processes as the sporangia : formation of oosphere 'anlagen'; development of septum ; rupture of the connecting layer ; swelling of the oospheres ; excretion of cell-sap, as shown by a contraction of the oogonium and the assemblage of swarming Bacteria. After this swelling the oospheres (oospores Rothert terms them) contract and round off, excreting lumps of protoplasm and taking them up again. They also show the same dark granules on treatment with iodine. In position as in development zoosporangia and oogonia are homologous; which is to be developed seems rather a matter of date than anything else : a hypha cut off to-day produces the former ; to-morrow or the next day it would have produced the latter ; but on the whole it appears that cultures from successive generations of zoospores tend to produce oospores more readily, and recent cultures from oospores produce especially abundant crops of zoosporangia. To this I may add that cold, and drying up of the water (to a less degree), both tend to induce the early formation of the sexual fruit.

I have found in a *Saprolegnia*, which I believe to be De Bary's *S. ferax*, form *torulosa*, that small cultures drying up tended to produce spheroidal dilatations at the ends of fine hyphæ which were cut off by septa. On moistening, the contents became ordinary zoospores, and these were freed by deliquescence of the cell-wall.

In his supplement or 'Nachtrag,' Rothert first gives an abstract of Berthold's confirmatory work : and then proceeds to investigate my theory of liberation, which I ascribe in my paper 'not to any such expulsive matter as has been assumed, but to the chemical stimulus of the oxygen in the medium acting on the automotile zoospores.' He asserts that I have founded this on insufficient data, and have pushed it too far, as it cannot apply to *Aphanomyces*, *Achlya* (other than those species which Cornu and I have examined), and to *Dictyuchus*, for that these have no cilia. I have already shown that the probability is that flagella will everywhere be found when properly looked for in the escaping zoospores of *Achlya* and *Aphanomyces*. The genus *Dictyuchus*, in which the spores

only slowly roll a little apart on the deliquescence of the cell-wall, has nothing to do with the case at all; and I cannot conceive why he refers to it in this connection.

Rothert denies that in ill-aerated cultures *Dictyuchus*-forms occur owing to the zoospores failing to escape. I thought the fact notorious, and did not adduce details. Here, however, is a crucial case. The cover of a culture was luted to an air-tight cell of wood-pulp saturated with paraffin, fixed to the slide while warm. The first two zoosporangia to open discharged all their zoospores; the third discharged half, and one remained sticking in the passage; many more opened, but all their zoospores encysted in the sporange, constituting the *Dictyuchus*-form. The inference is obvious that they escape to get into purer conditions than inside the sporange; but that I pushed this evidence somewhat far in ascribing the stimulus to free oxygen is rendered probable by some experiments Rothert publishes as conclusive; they are, however, very imperfect. I shall now discuss these.

I. Water is boiled in a test-tube and quickly cooled to 24° C. by pouring cold water on the outside; a square paper-cell is put on the slide, filled with the boiled water, and covered with a well-fitting cover, so that the water, very poor in air, was almost completely shut off from the atmosphere; and we have reason to assume that during the observation it remained approximately free from air. Before covering, excised *Saprolegnia*-material with sporangia in various stages was introduced, and observed. Both development and liberation of the spores was normal, though both were much slackened; no spore remained in the sporange though they soon came to rest.

In answer to this we may, note four distinct points. (1) The air is very imperfectly expelled from water by a single boil up. (2) Air is taken up on cooling, and especially in placing on the slide. (3) Slide, cover, and especially paper-cell, are coated with an air film which they give up to the water. (4) This poor solution of air in water is probably infinitely richer than the inside of the sporange with the active

metabolism that from all analogy we must infer goes on in the maturation of the zoospores. The experiment is not conclusive.

II. The zoospores on emission in a similar experiment are not attracted when a cleft for air is left, nor when there are air bubbles. This is quite possible according to Fechner's law; from zero to a very small quantity of air the attraction may be more marked than from a small quantity to saturation.

But I admit that my reasoning went too far in definitely ascribing the exit of the zoospores to *positive aerotaxy*. The facts are equally ascribable to what I may term '*negative pneumatotaxy*,' or the escaping from the products of their own metabolism. Some preliminary experiments lead me to think however that carbon dioxide is not the stimulating substance.

Rothert has repeated Walz's experiments, which tended to show that liberation was due to an expulsive substance, treating the zoosporangia at the moment of liberation with syrup (twenty-five per cent. cane sugar) or glycerine. He found first that the motion of the free zoospores is arrested by these, but does not recommence on dilution, though they retained their power of germination. On the sporangium the effect was peculiar and apparently irreconcilable with either theory. On adding a drop of the reagent, liberation stopped and soon recommenced; the same sequence occurred on adding a second drop, &c.; finally it stopped, not to recommence even on dilution. This is certainly conclusive against the expulsive substance; but I fail to see how it tells at all against my views, as the free zoospores are also arrested in the reagent.

Rothert has confirmed my absolute disproof of the existence of an expulsive substance; for after the arrest of liberation by the action of iodine or alcohol, on dilution no further liberation takes place. He concludes that on the whole his experiments tell rather in favour of spontaneous liberation. But he is met by the difficulty that all the reagents behave in the same way to *Achlya* as to *Saprolegnia*, while for *Achlya* he cannot admit the possibility of any but an expulsive mechanism.

This is obviously due to his having overlooked the cilia, and cannot weigh at all in the matter.

I may here point out that the aggregation of the spores in *Achlya* into a hollow head at the mouth of the sporange they have just left, appears to be due to the mutual attraction of the spores and the tendency to place themselves with their axes parallel. This is visible even in the sporange, and induces the aggregation into a cylinder or gut-shaped mass in poor sporangia, and materially interferes with their final separation. When they leave the sporange this is counter-balanced by that peculiar irritability ('negative pneumotaxy'?) which determines their exit. This mutual attraction, which I may term *adelphotaxy*, can only act at a short distance; when the sporange is discharged near the margin of the hanging drop, or in a thin layer of water on a slide, we constantly see single spores escape from the mass, swim away, and encyst apart. Cases of *adelphotaxy* are not so rare as we might think; in the embryology of animals this form of irritability is implicitly assumed by every one. In the vegetable kingdom we find it most obvious in the *Pediasireae*.

This paper is not final; it is obvious that while I have shown that the liberation is due to irritability of the zoospores, and is probably induced by a chemical stimulus, we are still in the dark as to whether this stimulus is really the positive one of oxygen in the medium (aerotactic), or the negative one of the soluble products of the metabolism of the zoospores in the sporangia themselves (pneumatotactic). Moreover there are numerous processes of differentiation in *Achlya* which I am now studying, and which will, with the completion of my researches on the nature of the liberative stimulus, form the subject of a fresh publication.

We are indebted to Rothert for the discovery that fragments of a healthy culture of *Saprolegnia* may be cut off and will continue to thrive in the hanging drop, and are much more normal than the fly-leg cultures usually worked with. I have found garden-centipedes far more suitable for large cultures than meal-worms.