Notes on the Plasmodium of Badhamia utricularis and Brefeldia maxima.

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With Plates I and II.

THE study of the plasmodium of Mycetozoa has received considerable attention on the Continent, and the account of the life-history of these remarkable organisms given by De Bary in the last edition of his 'Comparative Morphology and Biology of Fungi, Mycetozoa and Bacteria,' as well as those by Zopf and Sachs, afford an interesting view of their habits and properties; but the investigations recorded by these authorities appear to have been chiefly directed to *Fuligo varians* and various species of *Physarum*, and in following the development from spore to sporangium of *Chondrioderma difforme*.

Although the plasmodia of many Mycetozoa may be induced to crawl on a glass plate, where their rhythmic streaming may be observed, yet the comparatively short time that elapses between their emerging from hidden recesses in the substance of rotten wood, and their changing into sporangia, renders the greater number of them unsuitable for prolonged examination; none that I have met with is so favourable in this respect as that of *Badhamia utricularis*, which wanders for the most part over the surface of dead stumps, and can easily be cultivated in glass boxes or under bell-jars. Another advantage in dealing with the plasmodium of *Badhamia* is the facility with which it can be thrown into

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a sclerotium or resting stage. In this condition it may be stored away and can be brought back again into the active state by the application of water, at any time within several months. I shall have occasion to revert to this later.

The notes I now offer refer principally to this species, which I have kept in constant streaming movement on various kinds of woody fungi for more than a year.

In January, 1877, *Badhamia* was abundant in my garden at Leytonstone on some old hornbeam logs, which were also overgrown with extensive patches of *Corticium puteanum*, an effused fungus consisting of a central portion, brown and lurid from the multitude of its spores, surrounded with a white byssoid margin. The *Badhamia* advanced over the *Corticium*, entirely consuming the hyphae, or cut broad paths through the larger patches, leaving the bark to all appearance clean and bare where the plasmodium had passed on.

I allowed the plasmodium which had been thus feeding, to crawl on a glass plate, when its usual colour of rich chrome-yellow had changed to deep brown; this alteration of colour was shown by the microscope to be caused by the countless undigested brown spores of the *Corticium* held in suspension. These spores could be seen hurried along in the torrents that coursed through the branching channels, rolling over and over among the minute yellow granules and transparent vacuoles of the plasmodium.

When this had retreated from the glass plate, a map of its lace-like network was left behind, formed by the ejection on each side of the veins, of thousands of the *Corticium*-spores mixed with other refuse matter.

I placed some wet cotton-wool in front of the still dingy plasmodium; this it readily penetrated, and afterwards emerged possessing its normal yellow colour, leaving the wool charged with spores and other *débris*; it soon after changed to sporangia which became black in the course of about thirty-six hours, and as they dried assumed the blue-grey colour characteristic of the species.

The consumption of the *Corticium* was so interesting a fact that I exhibited specimens of the hornbeam bark with the *Corticium* in the act of being invaded by the plasmodium at a meeting of the Linnean Society. I also showed under the microscope the streaming plasmodium on a glass plate.

The difficulty of obtaining satisfactory observations when the plasmodium is spread over an exposed surface led me to cultivate it in glass boxes suitable for examination on the stage of the microscope (Fig. 5). The boxes are easily made, with two sides of thin glass measuring three by two inches fitted with wood ends half an inch wide, and a glass bottom, the whole fastened together with stiff glue and varnished at the points of junction and over the wood with canada balsam; a glass slip half an inch wide serves as a cover secured with an elastic band; in such boxes the plasmodium can be kept for any length of time in a damp atmosphere.

Besides the *Corticium* before mentioned, most effused fungi as well as thin species of *Daedalea* and *Polyporus*, especially *P. versicolor* and *P. adustus*, afford good nourishment to the plasmodium of *Badhamia*, though in cultivation these are apt to grow *Mucor* which leads to the decay of the plasmodium if allowed to spread; but its favourite food is *Stereum hirsutum*, a fungus that abounds on logs of oak and hornbeam, and on which *Badhamia* is constantly found during the winter months. With this we have no trouble from *Mucor*, while it is so rich a pabulum that in April and May of last year I cultivated plasmodia thickly covering an area of at least thirty inches, all of which had grown from a small quantity creeping over a piece of *Stereum* about the size of a half-crown with which I commenced operations on April 6; and in addition to this plasmodium which remained in a creeping state, an equal amount had changed into sporangia in glass boxes or under bell-jars.

Although the plasmodium grew very rapidly during the summer, and showed such vigour that it frequently spread completely over the glass shades placed over the piles of

Stereum if I omitted for two or three days to supply fresh food, yet none changed to sporangia between May 24 and September 27.

In hot weather, considerable attention is needed to keep the plasmodium in health, a fresh supply of *Stereum* must be frequently added, and the decayed bits cleared away. The new pieces are usually crawled over in the course of a few hours, and can be taken off and placed in a glass box for observation, or put under a glass shade with more *Stereum* to start a fresh colony. In the colder months no serious consequences follow if a pile is left alone for a week, the plasmodium may settle down with sluggish movement or pass into a resting stage; but in the height of summer a promising-looking colony will often fall into foul decay in twenty-four hours if it is neglected.

When plasmodium is placed in a glass box it will soon crawl up the sides, and it is then in a favourable condition for observation (see Fig. 1). The following experiments bearing on its manner of feeding have been conducted with these moist chambers.

In the first place I submit the results of a number of observations with regard to the action of the plasmodium of *Badhamia* upon starch, which has been stated on the authority of Dr. Wortmann to have been absorbed by the plasmodium of *Fuligo*[1].

In arranging for these experiments, I cut slices of raw potato and pounded them in a mortar; I then carefully washed the pulp so as to collect only the unbroken starch-grains, as an appearance of erosion is easily given by a slight bruise. Starch obtained from this source seems to be better for our purpose than any other, on account of the large size and regular form of the grains.

If this raw starch is spread with water on the side of a glass box in front of an advancing wave of the plasmodium, it is simply incorporated without any material stimulus to

[1] See De Bary, Morphology and Biology of the Fungi, Mycetozoa and Bacteria. Engl. ed. p. 452.

the flow being set up, and as a rule no change whatever takes place in the starch-grains; they are seen to be swept over by the streaming currents, or carried along the larger veins, and after five or six days' retention, they may be cast out or left behind on the glass with no more visible alteration than if they had been grains of sand.

On one occasion I watched what I supposed at the time to be the actual absorption of starch. On May 26, 1887, I was observing plasmodium in a glass box on the side of which I had spread raw starch scraped from potato. I noticed a body having the size and general form of a starch-granule, with a slight indentation, and drew it with camera lucida (Fig. 16 *a*); a thin stream of plasmodium was then flowing over it. I left the glass box on the stage of the microscope, with the drawing below the camera; after an hour's interval I looked again and found that the object had diminished to the size *b* in Fig. 16; from that time it was under constant observation for an hour and a half, during which it passed through the forms *c*, *d*, *e*, *f*, *g*, drawn with the camera at intervals of about a quarter of an hour; the last fragment then disappeared in the film of plasmodium which had continued to stream with the regular rhythmic alternate flow during the whole of the time. From subsequent experience I can hardly suppose this to have been a grain of raw starch, for I have since watched hundreds of these grains, often for hours without intermission, and in no single instance have I been satisfied that any change has taken place, nor have I seen any appearance of erosion of raw grains left by retreated plasmodium that could not be explained by the effect of bruising.

I have frequently examined raw starch which has remained for a week and from that to ten days constantly enveloped in moving plasmodium, and not a grain has shown the slightest erosion; yet I give the above observation as possessing considerable interest as an undoubted instance of the absorption of a solid substance.

If instead of using starch in its raw state, it is first warmed

with water in a test-tube, just sufficiently to swell most of the grains, the effect is very different from what I have described. On the plasmodium reaching this swollen starch, it rapidly advances in a concentrated opaque mass, and at the same time the flow along the more distant veins is much accelerated. After some hours, when the wave of plasmodium has retired, all the completely swollen grains are found to have disappeared, while those that have been only slightly affected by the warm water have lost their softer portions and show their margins more or less eroded according to the length of time they have been subjected to the action of the plasmodium. In cases where repeated waves have passed over the starch, the erosion of such imperfectly softened grains is markedly greater, but they are not entirely consumed, and there always remains a large residuum of whole or eroded grains. Application of iodine shows the side of the box to be strewn with small fragments of starch.

It is very difficult to observe the process of absorption, because the stimulus given to the plasmodium occasions it to accumulate in a broad border, often 1 mm. in thickness, and it is only after its retreat that we can see the change that has taken place.

The plasmodium with which one of these observations was made, had crawled upon a glass shade from a pile of *Stereum* over which it had been placed. I took off the shade and half filled it with water, and with a feather gently detached the film of plasmodium, allowing it to float about; I then passed under it a piece of wet cotton wool, and in this way was able to collect it upon the wool without materially disturbing the network of veins. I placed it in a clean glass box, and with a pair of forceps took out the remaining floating pieces and added them to the rest; by these means the streaming of the plasmodium is hardly checked, and it will often begin to climb up the side of the box in the course of a few minutes. This method is useful when it is desired to make experiments with pure plasmodium free from any foreign matter.

I now proceed to relate observations on the absorption of various fungi.

On October 10, 1887, I placed a thin section of the pileus and gills of *Agaricus campestris*, measuring 7 × 4 mm., in front of an advancing wave of plasmodium, which at once concentrated in a turgid mass upon the agaric, and in the course of an hour had entirely enveloped it. On the retreat of the plasmodium after some hours, not a trace of the mushroom remained. I have repeated this again and again with always the same result; a sluggish condition of the plasmodium is invariably revived and rapid streaming in the surrounding veins towards the object is set up when this agaric is offered to it.

Several experiments were made with slices of the pileus of *Boletus flavus*, which were even more greedily devoured than the mushroom.

On October 11, I placed a section of the pileus and gills of *Agaricus melleus* before a wave of plasmodium. The action was less rapid than in the other cases, but in two hours the section was densely enveloped, and next morning, when the plasmodium had withdrawn, nothing remained on the glass but a heavy grey deposit of granular and slimy *débris*.

As the tissue of the pilei of the fungi hitherto used is composed of delicate hyphae, I next tried an experiment with harder material. On October 12, I cut a section of the stem of *Agaricus melleus* from a specimen which was tough and mature, the buff-coloured outer coat being especially firm, and the hyphae strong. I placed the section in front of a thin film of plasmodium (Fig. 1). Almost immediately on its touching the first threads of the agaric a concentration took place as in former instances. Figs. 2, 3, 4 show the manner in which the turgid border advanced. In less than two hours the whole piece was overspread, but in this case the absorption was not so rapid as it was with the softer tissue, and the section could be seen beneath the plasmodium for several hours. On the following morning the plasmodium had with-

drawn on to the *Stereum* in the box, and all that remained of the section on the glass side was a slimy deposit in which were scattered a few broken hyphae which I concluded had belonged to the tough outer bark.

On October 3, I experimented with a section of the gills and pileus of *Agaricus rubescens*. On the plasmodium reaching the section, the hyaloplasm became in some way affected, and appeared to absorb water, for it rapidly penetrated among the hyphae unaccompanied by granules and stained the section throughout gamboge-yellow. This influence on the hyaloplasm seemed to destroy its protecting power, and at the point of contact the granular plasmodium gushed out from the interior in the form of multitudes of globular bodies measuring 15 μ to 25 μ in diameter, each enclosed by a thin covering of hyaloplasm. Some of these floated into the surrounding water showing amoeboid movements, and were afterwards reabsorbed into the general mass, but many lost their vitality and fell to pieces, mixing with the grey slime of dead plasmodium. It was a considerable time before the main wave of plasmodium had covered the section, which could be detected lying beneath it for some hours without much apparent change; next morning, however, on the plasmodium having retreated, only a denser mucilage remained covering the spot where the section had been placed.

This experiment was repeated with another section from the same specimen of *A. rubescens*; this was also stained yellow, but no breaking up of the plasmodium followed. I have, however, seen the same clusters of balls when a large supply of swollen starch was submitted to plasmodium.

Some days after I again tried with *A. rubescens*, but not the same specimen. The section was considerably thicker than in the former case, and, as before, the progress of consumption was slow; here, however, there was no yellow staining and no breaking up of the plasmodium, but it was not altogether a favourable diet, for on the following morning a heavy deposit of dead plasmodium was left upon the glass,

in which could be seen grey lines of undigested spores showing the remains of the gills; the hyphae had all disappeared.

On October 26, I treated plasmodium in two glass boxes with sections of the pileus and gills of *Agaricus fascicularis*. The action was very different from what had been observed with the other agarics and with *Boletus*. In one box, after the slice had remained touching the plasmodium for $3\frac{1}{4}$ hours, no advance had been made; so in order to stimulate its movement, I applied a small section of *Boletus flavus* to the same wave and about an eighth of an inch from the *A. fascicularis*: in forty minutes not only was the *Boletus* absorbed, but the plasmodium had surged in a broad fan over the *A. fascicularis* which could be seen unchanged beneath it. On the following morning the plasmodium had retreated leaving the section with no apparent alteration surrounded by a mass of mucus, and here this experiment came to an end.

In the other box with *A. fascicularis*, at 11.50 A.M. a section of pileus and ten gills was placed in contact with a strong wave of plasmodium; at 1.10 P.M. there was scarcely any advance upon it; at 3.40 I made a note, '*fascicularis* rejected.' Towards evening, however, the plasmodium advanced and enveloped the section; next morning it was left stranded and little changed. In the afternoon it was again swept over by the plasmodium, which began to prey upon the hyphae of the pileus, but it was evidently an unwholesome morsel, for it was surrounded with much grey mucus; amongst this were many isolated patches of plasmodium pushing their way in the slime with constant change of form; some appeared to have broken up into clusters of globules similar in size to the balls which escaped from the plasmodium in the experiment with *A. rubescens*; a few of these coalesced and were taken in by the larger patches, but the greater number fell to pieces and died. Again the plasmodium withdrew, and although the section had been subjected to its action for 22 hours, the gills had been so little affected that in the forks the spores could be seen arranged on their basidia in groups

of four surrounded only by water. Now, however, another wave of plasmodium advanced over it, and when again left bare on the following morning, the third day of the contest, the section was broken into small pieces and so reduced, that not a tenth of the original quantity remained. The struggle had been a tough one, for the whole lower side of the box was covered with broad bands and patches of grey slime, through which a tangle of long strings of bright orange plasmodium was twisted in strange disorder.

To restore its healthy condition, I placed in the box a piece of fresh *Stereum*, upon which the plasmodium soon concentrated itself in rich orange turgid waves, entirely withdrawing from the old pieces which were loaded with dead refuse. The following day, October 29, I cleaned out the glass box, replacing the healthy plasmodium, and added more fresh *Stereum*, on which, after rapidly increasing in volume, the plasmodium changed into sporangia on November 17.

A species of *Merulius* with small brown spores and with a white mucedinous border, somewhat resembling *Corticium puteanum*, was quickly dissolved; here again the dense accumulation of the plasmodium prevented the process being observed.

My next experiment was with the shaggy hairs scraped from the upper surface of *Stereum hirsutum*, well teased apart with needles. I placed the preparation in a glass box with pure plasmodium on cotton wool. It was at once seized upon and the plasmodium spread over a space measuring an inch and a half in circumference in the course of a couple of hours; in another hour it had nearly withdrawn, leaving the bundles of hyphae apparently little changed, but close examination showed many threads to be thinned away and broken in their continuity. After four days, when waves of plasmodium had repeatedly passed over the preparation, it had diminished to about half its original amount, and a magnifying power of 560 showed fragments in all stages of dissolution. At the same time much remained in which no alteration could be observed. Other experiments gave

the same results, showing that the consumption of coarse fibres, though it does take place, is very slow.

The effect of *Stereum* on the plasmodium is very different from that produced when it feeds upon agarics; there is comparatively little residue of slimy matter, and the flow is easy and free. With agarics, on the other hand, a heavy grey mucous deposit is left upon the glass, and the veins of the retreating plasmodium are rugged and loaded with particles, the streaming being confined to a narrow central channel. This condition, however, is frequently observed under other circumstances when the plasmodium becomes sluggish. Scrapings from the hymenial surface of *Stereum* are much more rapidly dissolved than the shaggy fibres; twelve hours immersion will often be sufficient to cause the whole to disappear with the exception of the coarse hyphae.

The most remarkable activity of plasmodium that I ever witnessed was caused by the supply of this pabulum.

Plasmodium crawling over pieces of *Stereum* had been kept for several days in a glass box, and at the time of my observation it had spread over both sides of the chamber and was slowly retreating in a widely-meshed network of narrow veins upon the clean glass. To a point on the upper edge of the network I applied a thin pulp, about the consistence of cream, of the scraped hymenial surface mixed with water. There was at first, as I have not unfrequently seen, a shrinking backwards of the margin of the network, as if notice of the presence of a food-supply had been sent off to the more distant parts; then came on a quick stream, and in a quarter of an hour the whole side was pouring up its plasmodium with astonishing rapidity. The wide meshwork was not sufficient to conduct the abundant supply, and fresh veins started off in all directions, cutting up the broad meshes; at one time the current along them all was so precipitate that I endeavoured in vain to follow the course of the particles; they rushed across the field of the microscope at a speed that was truly amazing. While the streaming was at its full height, I noticed a brown lump about the size of a large

starch-grain, and which I took to be a piece of dead sclerotium, rush along a swollen vein until it reached a fork, when it blocked the passage. Fresh streams immediately broke out on each side of the obstruction, but the main current still forced its way along the old channel, and in so doing it cut away the dark substance as a projecting point of sand is swept off by a runnel on a sandy shore. The granular constituents streamed away along one side of the vein and were dispersed in the torrent before it had passed out of the field, and all was dissolved before the reverse flow of the current set in. Meanwhile the new veins were crossing and re-crossing the wide network in every direction, and in a few minutes it was converted into a film of rapidly-moving plasmodium perforated with small openings, ending in an opaque mass which overspread the *Stereum*-pulp. The stimulus soon extended all over the glass box, and in the course of a few hours the opposite side as well as some of the pieces of pileus at the bottom were overrun with rich waves.

It was a sight not soon to be forgotten; the marvellous exhibition of such active life in so low an organism was most impressive.

In my experience with the plasmodium of *Badhamia* the flow is usually more rapid in the larger than in the smaller veins, which is what one would expect on mere hydraulic principles. The flow through the veins continues for about a minute and a half to two minutes in one direction, when it comes to a stand and immediately reverses its course; it gradually increases in rapidity for about half a minute and retains the maximum of speed for a varying length of time, when the rate again gradually diminishes. The continuance of the flow is longer when in the direction in which the plasmodium is moving; sometimes when the advance is rapid it will go on for three minutes before the return current sets in. When a wave is spreading over the glass in ordinary conditions, the maximum flow through the veins is at the rate

of about half an inch in a minute; it is often of course slower, but in the case just referred to the speed was very much greater [1].

With regard to the digestion of food-material, there is no doubt that it goes on to a large extent in the inner and streaming part of the plasmodium; but that the hyaloplasm has also an absorbing power was beautifully shown in the following instance.

On February 16, 1886, I was engaged in watching *Badhamia*-plasmodium in a glass box, where it had remained for several days in a moist atmosphere, when I noticed on the side a dark object, probably a cluster of spores of some fungus, from which mycelium was spreading in diverging threads (Fig. 17). I saw the plasmodium advance with a clear margin of hyaloplasm from the line *a* in Fig. 17 to the line *b*, and as it encroached upon the hyphae, they instantly melted away in its transparent substance like sugar in boiling water. They left no trace beyond two small fragments of the cellulose-wall (Fig. 18, *e*) which remained in the hyaline medium and were never mixed up with the granular part. In this case the stimulus of the food was not powerful enough to occasion an opaque concentration of the plasmodium, which spread over the clean glass in an almost transparent film (Fig. 17, *b*).

The wave was arrested at the line *b* in the figure and soon retreated. It was then interesting to note the effect produced on the parts of the threads which had not been immersed; in the course of half an hour, there was observed a breaking-up of the cell-wall with its contents into a string of bead-like fragments for a considerable distance from the point reached by the plasmodium, and this process continued for some hours, until the chain attained the length marked in the figure (Fig. 18, *c''*).

[1] The question of light has nothing to do with the movements of the plasmodium of *Badhamia*; waves will spread over the sides of a glass box or a glass shade, quite indifferently whether in day or night, whether on the part exposed to full daylight or that turned to a dark corner.

I have repeatedly examined cotton wool to see if there was any appearance of absorption, but find that no change takes place, even when it has been penetrated by the plasmodium for many weeks together.

To sum up these experiments,—they indicate a remarkable power possessed by the plasmodium of *Badhamia* of discriminating between different foods. We find that it can be raised from a sluggish and scarcely moving condition to one of great activity by supplying it with *Agaricus campestris*, *Boletus flavus*, or with the prepared hymenial surface of *Stereum hirsutum*; that the coarser fibres of the latter fungus are more slowly absorbed, but that this plant is so nutritious to the plasmodium that it grows rapidly and healthily upon it.

We find that *Agaricus melleus* and *A. rubescens*, though quickly overspread, are less freely assimilated and afford doubtful nourishment; while with *A. fascicularis* we see that for three hours the plasmodium refused it altogether; and when at last invaded, in one instance the section was rejected and never touched again, and in the other, like a hungry man with an unwholesome meal, the creature fed, but almost died of indigestion.

We find that starch, when swollen by moderate heat, is absorbed, which is proved, not only by the manner in which the grains are eroded or disappear, but by its stimulating influence on the plasmodium, while raw starch and cotton wool are not affected[1].

Again, these experiments show, that whatever may be the digestive principle of plasmodium (possibly a peptonising ferment as suggested by Krukenberg[2]), it is not confined to

[1] The spores of fungi also appear to be protected by their firm walls, and an *Oidium* or small pullulating fungus (Fig. 11, *c*, *d*) which always accompanies the plasmodium of *Badhamia* is not only uninjured, but would seem to thrive within its substance; it forms a considerable proportion of the refuse matter thrown out by retreating waves upon the sides of the moist chamber, where it multiplies with great rapidity.

[2] See De Bary, Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria, p. 452.

any special part of the mass. With starch and the sections of agarics the absorption took place in the streaming interior, while in the case last related, it occurred in the hyaloplasm alone; the threads were completely dissolved in the hyaline margin, with the exception of the small fragments referred to, which were kept under constant observation until they were almost ejected by the far-retreating plasmodium.

While I have thus endeavoured to summarise the principal facts brought out by earlier observations as well as by the experience of the last twelve months, during which time the organism has remained under daily notice without a break in its constant rhythmic motion, it may not be out of place to refer to some of the negative results that have attended these investigations. I need hardly say that they afford no clue to the mystery of this rhythmic streaming any more than they explain why, at uncertain intervals of hours or days, the plasmodium will rouse up without provocation from a quiescent condition, and flow over a glass shade and then return to its former state. We may suppose that it is searching for food, but this is far from accounting for the unity of action that appears to pervade the creature.

At the risk of being tedious, I give the following note taken in February, 1887:—Plasmodium under a glass bell four inches high by four wide, crawled up the sides, completely clothing the shade with the most exquisite yellow tracery; on the following day this had changed to a loose reticulation of thicker orange-coloured veins; on adding water upon the plate beneath, the whole of the glass was in a short time covered with delicate little fans of yellow plasmodium starting from the orange veins, as it were, clothing the bare stems with leaves. I then introduced two pieces of *Stereum*, and in five hours the plasmodium, which for two days had overspread the shade, had almost entirely retreated and concentrated upon the *Stereum*.

Here we had an area of about forty square inches, covered with two or three hundred little advancing fans of plasmodium, springing from a network of branches, which was

simultaneously influenced to withdraw to the food placed upon the glass plate below.

Then again I have not been able to obtain any light on the impulse that occasions the change to sporangia. I have had a large supply of plasmodium spreading over a pile of *Stereum* under a bell-jar, and have removed portions into glass boxes and under glass shades, so that I have sometimes had seventeen separate colonies at one time, where the conditions of food and moisture have been apparently the same; one after another of these colonies have undergone the change, while others continued to stream. Again, the whole of the main supply has suddenly formed into sporangia, some of them suspended in clusters by yellow threads or bands, and others formed into sessile plasmodiocarps upon the plate or pieces of *Stereum*; the portions transferred to the smaller receptacles have meanwhile remained unaltered. On the other hand, so long as the plasmodium has been continuous, however extensive, the change to sporangia has taken place simultaneously throughout the whole[1]. There is no doubt that hot weather is unfavourable to the development of sporangia, but it is remarkable that for four months not a single colony went into its final stage; though I should say that I lost a number from want of proper attention, they died and decomposed with a strong ammoniacal smell, having been poisoned by the products of the rapidly decaying *Stereum*. Many of the surviving colonies went into sporangia in the month of October; one which changed on the 25th seems worthy of special mention.

This was in part revived sclerotium which had dried in July. I wetted it on October 1, and it returned to active movement in the course of an hour or two; to this I added about an equal quantity of plasmodium from the store under

[1] Since writing the above, a large growth of plasmodium has formed into sporangia under a bell-jar; more than half changed on March 13, 1888, the rest on March 15. On March 11, when an appearance of sporangia occurred, I had added water, which checked the development. This plasmodium was a part of the continuous cultivation begun on Jan. 22, 1887.

a bell-jar, and the two quickly coalesced; on October 13, I fed this mixed plasmodium with *Merulius*, which it devoured, and all went into sporangia together eleven days after.

In connection with the change to sporangia I here refer to a beautiful exhibition of spore-formation which came under my notice in a specimen of *Brefeldia maxima*.

On November 27, 1887, a large mass of opaque white plasmodium was found emerging from the ground at the foot of an old fir stump. I cut off a part which had spread over some dead oak leaves, and placed it in a glass box with the cut surface against the side (Fig. 5). The piece was cushion-like in form, apparently homogeneous in substance, and closely studded with papillae. The face resting against the glass measured an inch and a quarter in length, and half an inch in height.

At ten o'clock on the following morning, the base and central part of the cut surface assumed a loose spongy texture, which, as the day advanced, became filled with air and occupied about half the area of the section.

At 11 A.M. a flush of pale purple appeared along the upper edge of the spongy tissue; upon this rested the broad white mass of the aethalium, composed of narrow and somewhat branching sporangia, closely cohering together and spreading radially towards the surface, where they terminated in the papillae before mentioned.

About 2 P.M. the papillae lying against the glass began to push upwards irregular and broad extensions sufficiently thin to be examined by transmitted light, and filled with remarkably large colourless granules measuring from 2 μ to 4 μ in diameter; the movement among these granules was extremely slow and difficult to follow, except when they poured into the pseudopodia which were here and there thrown out; no vacuoles were visible. At about 3.30 the large granules broke up into very minute bodies and numerous vacuoles made their appearance; and now for the first time the streaming movement was observed with alternate flow at intervals of about two minutes. This condition of things

continued for about half an hour. At 4 P.M. the broad extensions of plasmodium suddenly branched out from centres into clusters of short diverging branchlets (Fig. 6, *a*), most of which, if not all of them, contained a vacuole. At 4.30 each branchlet had constricted itself from its neighbour and taken a spherical form, the vacuoles disappeared, and the whole substance was divided into a multitude of spores (Fig. 6, *d*). In a few hours these had developed their spore-walls, and on the following morning had become purple-brown in colour, and in every respect resembled those which filled the sporangia. The capillitium with its strange many-chambered vesicles had already formed in the lower parts of the sporangia before any apparent change had taken place in the plasmodium at the extremities.

Brefeldia may be an especially favourable species for showing this phenomenon, on account of the extremely thin membrane which covers the sporangia, for I have never observed this free spore-formation, unconfined by any enclosing wall, in any other of the Mycetozoa.

I now return to the consideration of the resting condition referred to in the earlier part of these notes.

If plasmodium of *Badhamia*, spread on the side of a glass box, falls into an inactive state, a mottled appearance of the film is very frequently observed, which is caused by a tendency of the granules to draw together in loose groups: this passes off if the streaming revives; but if causes arise which produce greater stagnation, the concentration becomes more marked, and in process of time the aggregations are separated from each other by more or less defined hyaline spaces. When this has taken place all streaming has ceased, the plasmodium contracts to a thicker mass, and the surface is observed to be partitioned into slightly convex areas corresponding to the superficial layer of so-called sclerotium-cells which have now taken definite form[1].

[1] See De Bary, Comparative Morphology and Biology of Fungi, Mycetozoa and Bacteria, p. 428.

When the process is going on slowly we may sometimes notice all these stages in one lobe of plasmodium; at one extremity there may be a thin film showing streaming movement with no aggregations; then follows the mottled appearance, and further on the definite thin-walled cells may be traced, densely crowded and constituting the thick sclerotium. Sometimes a piece of plasmodium will become detached on the glass plate, and be left behind by a retreating wave, and will form into cells while all the rest continues its streaming movement.

I have had plasmodium change to the sclerotium form on wet cotton wool, but this was probably from want of nourishment. As the supply of moisture was here abundant, the margin of the sclerotium was not so abrupt as usual, and in a narrow border the cells were spread in a thin layer; many of the outermost were quite detached from the rest, and showed slow amoeboid movement.

The chief cause of the resting condition in Mycetozoa, as is well known, is lack of moisture.

When I changed my place of residence last spring, and wished to take with me the store of plasmodium which was in active state under a number of covers and in glass boxes, I removed the glass shades from those not required to be retained in the streaming condition. They at once began to form into sclerotia, and in three days were dry and ready to be packed away. After the lapse of five months, on adding water to parts of these sclerotia, the thin hyaline walls of the cells broke down and were dissolved, and in three or four hours the streaming movement returned.

On October 16, I had a rich plasmodium covering a pile of *Stereum* under a large bell-jar, which was inadvertently exposed for about three hours to hot sunshine. At the end of that time the whole of it had changed to fine rugged sclerotium, which I removed from the bell-jar and set aside. When portions of this were wetted again within a few days they returned to the active state in about half an hour; when

moistened, after three or four weeks, they took a longer time to recover the movement.

The dry sclerotium of *Badhamia utricularis* is dark brick-red in colour, of brittle, horny texture (Fig. 7), and consolidates in irregular effused masses, which are usually made up of cord-like convolutions and knobs; the cells of which it is composed vary in size from 10 to 20 μ in diameter.

When a thin section of dry sclerotium is placed in water and examined under the microscope, the cells are seen to swell from absorption of moisture, and in a short time a slow change of position takes place in the contained granules, among which may be observed from 5 to 20 nuclei, according to the size of the cell (Fig. 8). These are not easy to detect, but if the swollen cells are carefully separated and crushed on a cover-slip, then dried and stained with magenta, the nuclei, with their nucleoli, are brought out with beautiful distinctness, especially when mounted in Canada balsam (Fig. 10).

The sclerotium is often formed with free rods connecting one part with another (Fig. 7, *a*); a section of one of these, when softened in water, shows very well how the refuse matter is discharged by encysting plasmodium; the outer wall of the rod is composed of mucus charged with spores of Fungi and cells of Algæ, together with other rubbish, while the enclosed cells contain only pure plasmodium.

The sharpest definition of nuclei and nucleoli which I have succeeded in obtaining has been when the cover-slips on which the plasmodium of *Badhamia* had been thinly smeared, were instantly dropped into absolute alcohol; the preparations were then stained with magenta and mounted in balsam. Stainings of hyaloplasm taken from turgid plasmodium show very few nuclei, which appear to be confined to the interior substance, for the scattered individuals that are met with were probably introduced through imperfect manipulation.

If the streaming plasmodium is examined without having resort to staining, and a morsel is placed under a cover-slip with slight pressure, the nuclei cannot at first be recognised;

but as disintegration takes place they gradually become visible (Fig. 11, *a*).

They are rendered more conspicuous if a bit of plasmodium is torn with needles in a drop of water, care being taken that the cover-slip does not press too closely; viewed under a magnifying power of about 1200, the globular vesicles of finely granular plasma which ooze out from the mass are seen to contain nuclei, which are colourless and faint, but at the same time perfectly distinct in outline. They measure, as a rule, a little over 3μ in diameter, and show a clearly defined nucleolus. Occasionally a nucleus may be seen to be shot into an expanding vesicle in which none had previously appeared, as if it had formed an obstruction in the narrow passage through which the plasma issued; some globular vesicles that have become detached contain nuclei in great abundance, while others again have few or none.

When an object such as is here described has remained for half an hour or so under the cover-slip, the yellow granules will be found to have mostly disappeared, the minute granules will have more or less dispersed or congregated together, while the colourless plasma is seen crowded with nuclei throughout the preparation.

The most successful arrangement for minute observation of the streaming plasmodium is obtained when we happen to have it climbing freely over the sides of a glass shade. If this has been going on for a day or two, we frequently see little buddings out from the larger veins of delicate fans of network measuring perhaps an eighth of an inch across, or less. A drop of water is placed over one of these, and it is gently detached, with precaution that no branches are injured except the main stem, which connected it with the vein. If the little fan be placed in water on a glass slide, and the cover-slip supported at one side by a piece of blotting-paper to prevent its pressing the plasmodium, it will continue its streaming movement without interruption, and will remain in healthy condition for some days. If the fan lies in a small bubble of air surrounded by water, it will confine its movements very

much within the limits of the bubble, and will spread out in the most delicate reticulation. I have watched such an object for hours with a $\frac{1}{10}$ immersion lens, when every granule and particle of food-matter was brilliantly defined, but I have not been able to distinguish with certainty any trace of the nuclei; though when I took away the bit of blotting-paper and allowed the pressure of the cover-slip to kill the plasmodium, and water at the same time to mix with it, almost immediately the whole field was seen closely beset with well-defined nuclei (Fig. 11).

From the fact that the nuclei are invisible when surrounded with living protoplasm, it is not surprising that the process of their multiplication is difficult and perhaps impossible to observe. That the nuclei multiply with the increase of the plasmodium, there is no question. As before stated, I have cultivated large quantities of *Badhamia* plasmodium from a very small centre, and stainings taken at any time, whether on the eve of the change to sporangia, or many weeks before, are invariably found to swarm with nuclei.

In the stainings we find that, as a rule, the nuclei are of the same size, and each possesses a single nucleolus; at the same time, we not unfrequently meet with forms which suggest that division was taking place. This appearance was especially frequent in the plasmodium of *Brefeldia*, before referred to, the stainings of which were taken several days before the spore-formation occurred in the part remaining at the foot of the fir-stump. We notice in these forms the presence of two nucleoli taking a relative position in the two halves of the nucleus, and occasionally we meet with three nucleoli in the same nucleus (Fig. 12).

In the figure taken from stainings of *Brefeldia*, the difference in size is partly owing to the stretching of the nuclei in the thin film of plasmodium.

In concluding these notes I would just refer to the last office we see performed by the nuclei.

If a sporangium of *Badhamia*, taken about twenty-four hours after it has assumed its ultimate shape, but before the spores

have ripened, is thinly spread upon a glass slide, we find that each nucleus has collected round itself the proportion of protoplasm which would finally constitute a spore in which it stands as a centre, and each such portion has separated from those around it, or is in the act of so doing, and has become a distinct organism; for if examined at the right maturing moment these young spores may be seen in slow movement, and throwing out hyaline pseudopodia which fill with granular plasma, with the same amoeboid character which they would again exhibit when the ripe spores burst, and the new swarm-cells began the circle of development afresh (Fig. 13).

A staining taken at this stage presents a beautiful object, resembling a tesselated pavement, each polygonal area being dotted with its nucleus.

EXPLANATION OF FIGURES IN PLATES I AND II,

Illustrating Mr. Arthur Lister's Notes on the Plasmodium of *Badhamia utricularis* and *Brefeldia maxima.*

Figs. 1–4. *Badhamia utricularis.* Advance of plasmodium on section of stem of *Agaricus melleus.* 1 drawn at 12.25 p.m., 2 at 12.40 p.m., 3 at 1 p.m., and 4 at 2 p.m. × 3½.

Fig. 5. *Brefeldia maxima.* Twenty-four hours after it was placed in glass box: spore-formation just commencing. Natural size.

Fig. 6. *Brefeldia maxima.* Branchings from papillae lying against side of box. *a* and *b* taken at 4.10 p.m., *c* at 4.20, *d* at 4.30, when the spore division was completed. × 250.

Fig. 7. *Badhamia utricularis.* Sclerotium. × 4.

Fig. 8. *Badhamia utricularis.* Sclerotium-cells. × 565.

Fig. 9. *Badhamia utricularis.* Sclerotium-cell, stained with magenta, nuclei just discernible.

Fig. 10. *Badhamia utricularis.* Sclerotium-cell crushed. *a* part of cell-wall. *b* nuclei. × 1200.

Fig. 11. *Badhamia utricularis.* Creeping plasmodium, pressed with water under cover-glass. *a* nuclei. *b* oil-globules. *c* pullulating fungus. × 1200.

Fig. 12. *Brefeldia maxima.* Nuclei with nucleoli of plasmodium stained with magenta. × 1200.

Fig. 13. *Badhamia utricularis.* Young spores showing amoeboid movement, the spore *a* changing its form to *a'''* in the course of a few minutes. × 565.

Figs. 14 and 15. Starch grains swollen in warm water, drawn from the side of glass box in which *Badhamia utricularis* was spreading. The grains in Fig. 15 have been overspread and eroded by plasmodium; those in Fig. 14 have not been reached by plasmodium. × 160.

Fig. 16. Substance dissolved by plasmodium of *Badhamia utricularis* diminishing in size as by letters, taken at intervals of about a quarter of an hour. × 250.

Fig. 17. Mycelium growing on side of glass box. *a* edge of advancing plasmodium of *Badhamia utricularis* when it first reached the mycelium. *b* line reached by plasmodium before it retreated. *c*, *d* threads of mycelium, which were dissolved by plasmodium between the lines *a* and *b*. × 66.

Fig. 18. *c'* the thread *c* drawn half an hour after the plasmodium had retreated, showing the extremity breaking into bead-like fragments from action of plasmodium. *c''* the same some hours after, showing extent of injury beyond the point reached by plasmodium. *e* fragments of mycelium, showing all that remained of the threads *c* and *d* between the lines *a* and *b*. × 250.

Fig. 1.

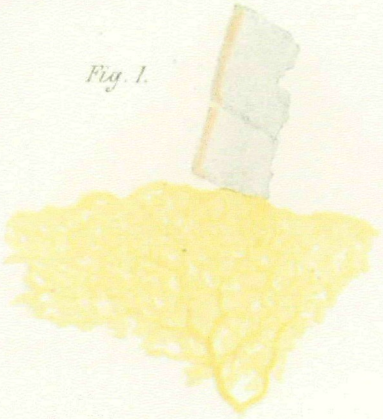


Fig. 2.

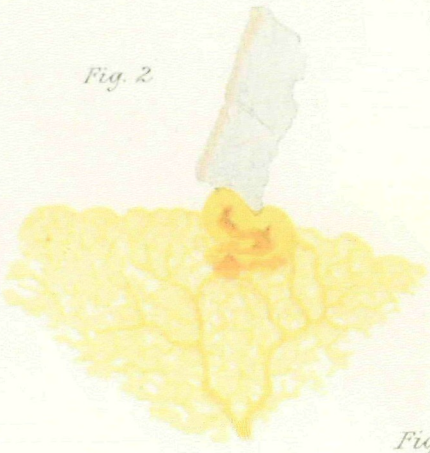
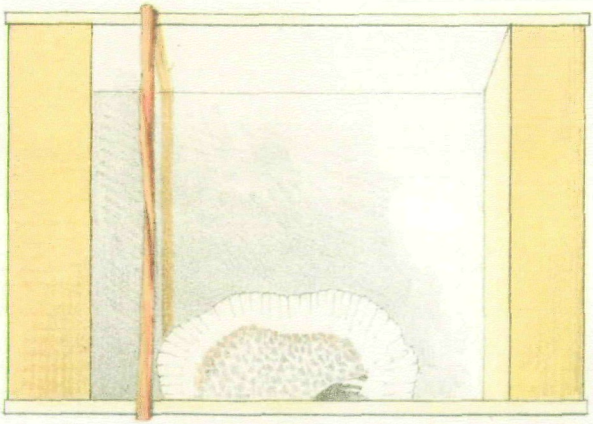


Fig. 5.



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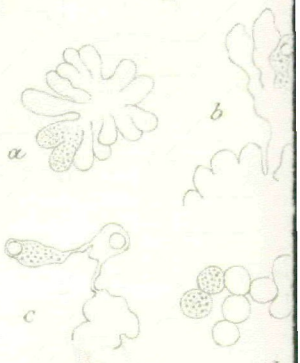

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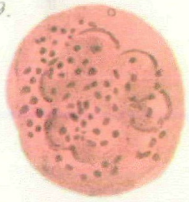
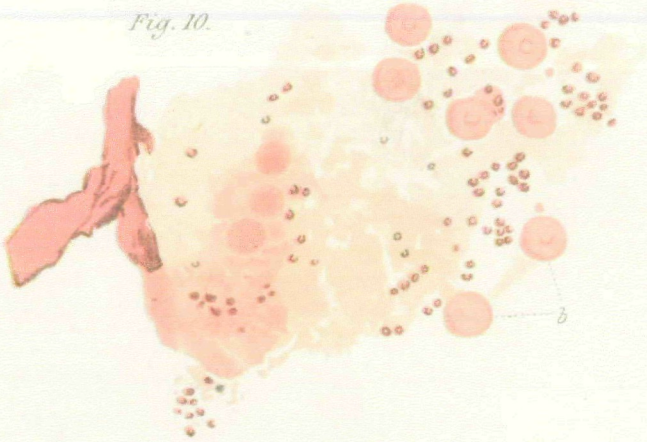


Fig. 10.



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LISTER.- PLASMODIUM OF BADHAMIA UTRICULARIS & BREFELDIA MAXIMA.

Fig. 4.

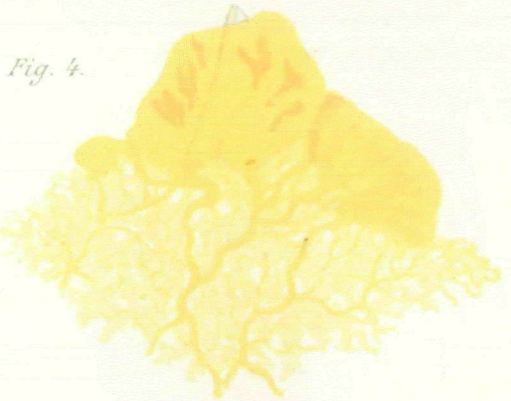
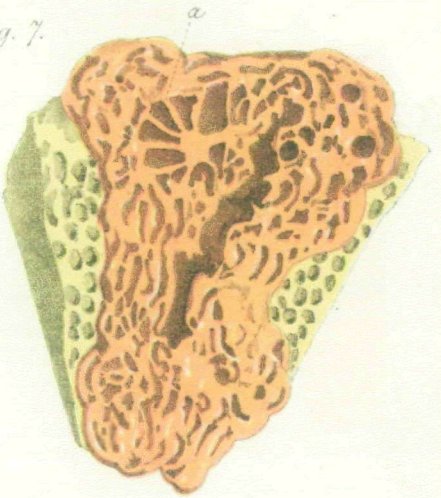


Fig. 7.



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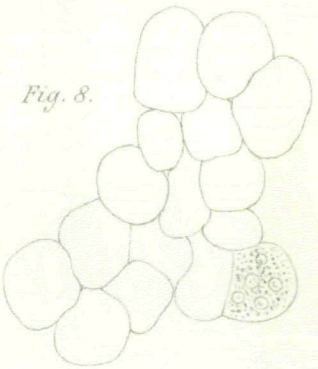

Fig. 13.

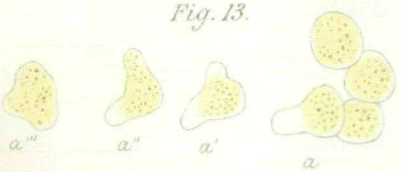

Fig. 12.

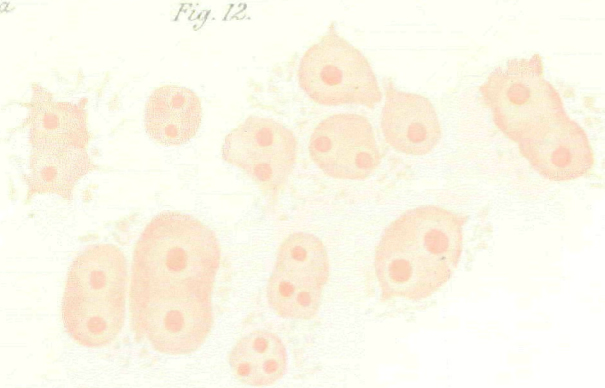

Fig. 11.

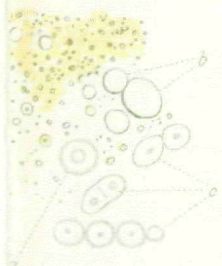

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Fig. 14.

Fig. 15.

Fig. 17.

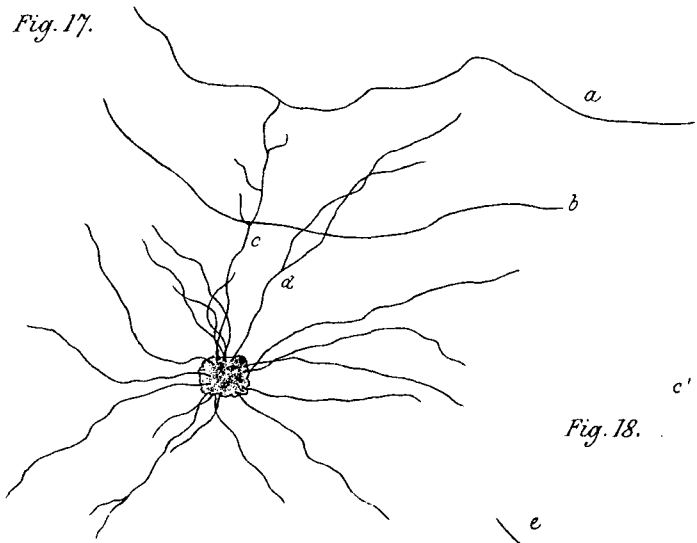
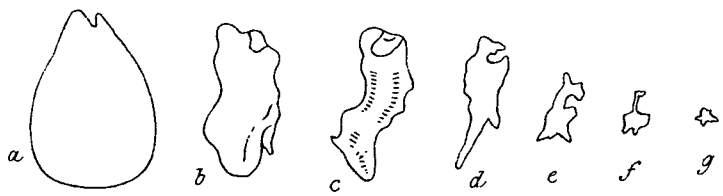


Fig. 18.

Fig. 16.



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LISTER.–PLASMODIUM OF BADHAMIA UTRICULARIS & BREFELDIA MAXIMA.