The Panama Disease.

BY

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With Plate XXIX.

II

THE research was continued in order to control the results obtained at first and to study the development of the fungus in detail. The results are such as to enable me to add more particulars concerning the at first puzzling pleomorphism, and to elucidate and slightly correct some statements made in my first paper.

Sclerotia. As such were described structures arising from single hyphae, or even parts of them, whereas a true sclerotium is a structure formed by the interlocking of a number of hyphae, which give rise to a pseudo-parenchyma, often with distinct cortical and medullary parts. Resting mycelia is a useful general term; but it appears to me preferable to use a special term for a definite structure: in this case I propose to use the term *pegmatium* to indicate structures arising from well-nourished hyphae, or even portions of them, which passing or not through a stage of slimy dissolution of their walls, harden into gristly or gummy bodies having the power to regenerate the fungus mediately by chlamydospores —into which they break up under favourable conditions—or immediately by mycelia arising from them without the interposition of a spore stage.

These pegmatia are found to consist of a sterile and a fertile part; the first one, hard and gummy, is derived from the hyphal walls; the latter, more or less gelatinous, from the protoplasmic contents of the hyphal cells. For the sterile part I wish to introduce the term *mycoporoma*, and for the fertile part the term *myclomyxa*.

Pegmatia. In hyphae from which some pegmatia arise, the protoplasmic contents are seen to break up into numerous globules, spore-initials apparent by their greenish opacity. In the one case the cell-walls then begin to thicken and gradually pass to a gelatinous substance, in which the spore-initials are hardly perceptible; they become quite indistinct as soon as the mass begins to harden and to assume a yellow to brown hue. In

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another case the spore-initials are seen to prepare themselves a way through the hyphal walls, so that at last they are found densely massed on the surface of the hyphae, more or less deeply embedded (see Pl. XXIX, Fig. 1). During the migration of the spores, the hyphae swell to twice or thrice their original thickness and then harden into pegmatia. In a third case the hyphae become somewhat toruloid, some parts being largely distended. Here and there spore-initials are seen lying within or embedded in the cell-walls. Before pegmatium-formation sets in, the hyphae assume a smoky hue, the lumina become very much narrowed, and the outlines quaintly altered. Then the dissolution of the cell-walls ensues, giving rise to a gelatinous mass, out of which the pegmatia arise as yellowish, greenish, or dark-brown bodies. In a fourth case, gelatinous opaque masses exude through invisible openings in the cell-walls (see Fig. 2, *a*) ; these masses consist of the entire or partial contents of large hyphal cells, which arose from different adjacent cells by the absorption of the septa ; they harden in the end into pegmatia, of which the mycoporomatic coat is formed out of the gelatinous substance, and not from the hyphal walls. This mycoporoma may be compared with the hypothallus of some Myxogasteres. In a fifth case, terminal or interstitial cells of special hyphae emit little knob-like outgrowths, which gradually assume a cup shape. They are at first transparent, but gradually a thick mycoporomatic coat is formed, enclosing a glairy gelatinous inner part; this is best seen when viewed through the upper surface, where the coat seems to be thinner and somewhat translucent. These bodies may arise in large numbers beside each other, and may not or do closely abut on each other ; complete fusion seems to be rare (see Fig. 2, *a*). They may be compared to the chlamydosporangia of *Sorosporium* and some other Ustilagineae, in which, however, the wall is formed out of infertile hyphae instead of the walls of fertile hyphae. In some cases, when originating on the apices of hyphae, they assume a globular shape ; the wall may then be a thin mycoporomatic coat, and the bodies may be fertile or not ; in the latter case large spore-like bodies, which are empty and therefore sterile, are seen to form within. Finally the bulk of the mycelium in the decaying plant may turn at the end of its vegetative development to the resting condition, enclosing the tissue-remnants within pegmatia. When plenty of moisture is present the pegmatia give rise to chlamydospores in huge numbers, which are at first polyhedric lumps, but gradually assume their definite shape. The exosporium appears to be formed out of the mycoporoma ; at any rate it always has the same colour, a reason why the chlamydospores arising from the pegmatia in the plant tissues show a great variety of colour, which may be some shade between yellow and dark brown. When a limited quantity of moisture is present the pegmatia at once germinate into new mycelia or they may give rise to fruit bodies, as was shown in my first paper.

Spores. The reproductive organs may be divided into ascospores—of which nothing definite can be said as yet—conidia, and chlamydospores, including oidia. The conidia are all more or less sickle-shaped, and may be unicellular or compound (2–5 celled). The most frequently occurring are the bicellular and the 4-celled conidia, which may be considered typical for this fungus. In my first paper the conidia were described as being cut off at the apices of short conidiophores, but I found them later and most frequently borne on long conidiophores, either unbranched, or branched in different ways. The branching is of the racemose type, showing a tendency to become verticillate. The single conidiophores are tapering and pointed at the tips, often bearing a not fully abstricted conidium. The conidia are loosely kept together in heads, consisting only of bicellular or 4-cellular conidia, but sometimes also of a mixture of 1–5 celled conidia (see Fig. 2, *a*). As the most specialized type of conidium fructification may be considered the Stilboid bodies, which, as described in the first paper, anticipate the ascigerous fruit body. In fact, the hyphae remaining after the conidia are shed constitute the para-, or rather periphysial sheath, macroscopically appearing as orange-red, hair-like outgrowths surrounding the golden yellow fruit body with a pink-coloured stroma. All the conidia show a great tendency to turn to chlamydospores; the transformation seems to take place mainly under the influence of moisture. In assuming the character of chlamydospores the colour becomes dark—some shade of brown—and the exosporium very much thickened. The chlamydospores have, as is said before, different modes of origin. They may be formed intercalarily as in *Chlamydomucor* or *Entyloma*; at the apices of special hyphae as in *Hyponyces*; out of the myclomyxa resulting from the dissolution of the walls of fertile hyphae or out of pegmatia, the resting stage of fertile hyphae. The process of chlamydospore-formation in this fungus may be looked upon as a specialization of the conditions encountered in the group of Hemibasidii: the chlamydospore-fructification anticipated by the slimy dissolution of the hyphal walls as in *Ustilago*; the chlamydospores formed in the course of the hyphae as in *Entyloma*, the spores enclosed in sporangium-like bodies as in *Sorosporium*, *Doassansia*, *Uleiella*; moreover, the germination of the chlamydospores in *Ustilaginoidella* alone shows the same variations as is found in the Hemibasidii as a group: as in the latter we find them in the one case producing promycelia with numerous small conidia (sporidia) cut off apically and laterally; in the other, germinating vegetatively, which need not be wondered at in the case of Ascomycetes, the chlamydospores of which very often lose their most typical character. I may here mention another mode of chlamydospore-formation, which was found on liquid nutritive media. The hyphae become chain- or oidium-like, with round bodies in the chain or on the apices of side branches. These bodies attract a large quantity of protoplasm from adjacent cells, in

which one or more plasmic globules, described before as spore-initials, are seen to form. In maturing they assume a dark violet colour and are liberated as large chlamydospores, or rather chlamydosporangia, measuring 80–120 feet (see Pl. XXIX, Fig. 3). They do not differ at all from the bodies which were mentioned in my first paper under the name of giant chlamydospores. In germinating the spore coat was seen to burst, allowing the germ tube to emerge. This germ tube consisted of one or more club-shaped cells; from the peculiar way in which they adhere to each other I infer that each cell is a separate germ tube derived from a special spore-initial, and that the adherence is only due to compression caused by growth in a limited space (see Fig. 4). The colour of the germ tube is dark, producing a mycelium which at first is also dark, but gradually becomes hyaline (see under '*Pure cultures*' in first paper). Besides the mode of oidium-formation mentioned in my first paper, I found some hyphae of the mycelium treated of above forming chains of club-shaped bodies (i. e. oidia). Some of these oidia passed into chlamydospores. Sickle-shaped conidia on parts of the same mycelium also underwent the same transformation (see Fig. 3). The chlamydospore-fructification is undoubtedly the most prominent feature of the reproductive habit of this fungus.

Haustoria. They arise as little knob-like excrescences, lateral or terminal, with opaque contents protected by a thin membrane. They grow out into flat saucer-like structures, or assume a funnel or a spoon shape, or become polypoid or tassel-like (see Fig. 5); they appear to assimilate food with the aid of their secretion, the turbidity of the protoplasm and the gummy degeneration of the walls of the cells, in which they arise, giving support to this assumption. The hyphae from which they arise become irregularly distended and turn in the end to pegmatia. The haustoria themselves may be wholly or partially transformed into mycoporoma. This takes place at the close of the parasitic mode of life of the fungus.

Pycnidia. Under this name were mentioned spore-masses found in the disintegrated tissues of the decaying rhizome and leaves. In fact they are better termed pseudo-pycnidia, since they arise in pre-existing cavities due to rupturing in consequence of contraction in the putrifying tissues. The shape is spheroidal or irregular. From all directions fertile hyphae enter the cavities and mainly crowd together in the lining cells. From this crowding together a felted mass ensues, out of which arise the mycoporomatic lining of the cavities and the huge number of chlamydospores filling them. Sometimes multicellular conidia are found among the chlamydospores; they are probably abstricted from hyphae, traversing the cavities before the chlamydospore-formation began (see Fig. 6). Considering the fact that some of these pseudo-pycnidia were mainly filled with conidia, and some conidia were found in a state of transformation into chlamydospores,

one may safely take for granted that the chlamydospores filling some pseudo-pycnidia are partly or even entirely derived from conidia.

Mycocecidia. Under this name mention was made of structures which on careful examination proved to be effluxes of pegmatia to the surface of the leaves, where the epidermis and subepidermal layers are absorbed. As will be seen in Fig. 7, remnants of absorbed tissue are enclosed in these mostly dome-shaped bodies, in which air-spaces are found, some of which contain chlamydospores in making or fully formed.

Enzymes. Judging from the histological preparations, there was good reason to think that the changes in the protoplasm and the cell-walls were caused by the action of an enzymic secretion of the fungus. To make sure, some cultures were raised on liquid sterilized banana extract in wide tubes. At the end of three weeks they were poured out on a filter and the liquid collected. To this absolute alcohol to four times the volume was added, when a yellowish precipitate was thrown down, which, collected on a filter and dried in the stove at a temperature of 37° C., had a weight of 110 mg. Dissolved in 22 grams of water (sterilized), and slices of a healthy banana sucker—cut off with the utmost precautions so as to secure sterility—being dropped in the solution contained in a large tube, which was then shut off with a lump of sterilized cotton-wool, it was noticed after three days that the solution became opaque and thickish, while the slices were very much swollen. After three more days the liquid became slimy, and the slices brought under the microscope (low power) showed that parts of the cell-walls were dissolved ; the spiral thickenings of the vessels were lying loose, and the hyaline transparency of the protoplasm was seriously disturbed. The aspect was the same as met with in the tissues of the diseased plant. The same experiment was repeated with the precipitate obtained from a watery extract of the mycelium ground down with sterilized sand. The results were identical. From the above-mentioned facts one might safely infer that the changes brought about by the secretion of the fungus are due most probably to at least two different enzymes, of which the one has properties approximating to cytase, if not actually cytase, and the other proteolytic qualities (vegetable trypsin). The first-mentioned enzyme, in fact, is one displaying the same qualities as the cytase of *Peziza sclerotiorum* described by de Bary—here also mention is made of ' curious organs of attachment in the shape of a kind of tassel ', which seem to be identical with the haustoria of the fungus described above. There was surely good reason to assume the presence of a second enzyme with proteolytic qualities ; for an enzyme with such a wide range of action, decomposing and gelatinizing the cellulose and pectose of the cell-walls and at the same time disintegrating the protoplasmic contents of the cells, was hardly conceivable. On the other hand it appeared to me very probable that the enzyme causing the disintegration of one or more of

the proteids in the protoplasm—the nucleus is not attacked, at any rate not primarily—was also the cause of the dissolution of the hyphal walls, which mainly consist of chitin, an albuminoid. In trying to prove this, I found that the action on the hyphal walls was very much enhanced when a few drops of ½ % HCl were added to the solution of the enzyme. I have satisfied myself as to the absence of any action of ½ % HCl alone. The change, however, was very slow in manifesting itself, and was not perceived sooner than on the eighth day, when the walls were found to gradually change into a highly refractive gelatinous mass. Along with this a large number of crystals of calcium oxalate were seen to be secreted, throwing light on the presence of the same in the tissues of the banana attacked by the fungus.

The coincidence of the softening of the hyphal walls, i. e. the secretion of enzyme, with the chlamydospore and pegmatium formation, gives a plausible explanation of the outbreak of the disease at the time of prominent seasonal changes, from drought to wet weather and *vice versa*, since chlamydospore-formation takes place when plenty of moisture is present and pegmatia arise when the water supply is limited, as was alluded to before.

The hyphal walls are composed nearly entirely of chitin, which was found in the following way: Pure cultures were raised on sterilized liquid banana extract in tubes of 3 cm. diameter. At the close of a fortnight a mycelium covering the surface of the liquid was removed to a dish of distilled water and well washed out so as to remove the adhering extract, and then transferred to a tube with Schweizer's reagent. It was left for four days, agitated from time to time. Removed from the solution, it was again thoroughly rinsed and left for four days in 2½ % NH_4OH, which was changed every day; washed out and dried in the stove at a temperature of 40° C. Brought under the microscope, not the least change could be detected, giving reason to infer the absence of cellulose. A fragment of 200 mg. was heated with KOH solution and subsequently treated with dilute H_2SO_4, 95 % alcohol and ether. A transparent horny substance was obtained of the same shape as the original material. When heated it was carbonized without preceding fusion. Other fragments proved to be only soluble in concentrated mineral acids and eau de Javelle, quite easily when heated; in this case the mycelium was carbonized before dissolution in H_2SO_4. The solution in hot HCl was evaporated till dry, when hygroscopic needles of glucosammonium chloride were seen to arise, which, dissolved in water and treated with KOH, gave rise to a precipitate of glucosamine. Another fragment was heated with alcoholic KOH solution and the mixture allowed to cool. The supernatant liquid was decanted and treated with dilute HCl in slight excess, when a comparatively small quantity of a gelatinous substance was thrown down, which, with the aid of staining methods recommended by Strasburger, proved to be pectine.

A very small percentage of carbohydrate was detected by heating 1 grm. of dried mycelium with 25 % H_2SO_4 for about two hours. The liquid was allowed to cool and filtered; the filtrate neutralized with KOH and the neutral solution treated with Fehling's solution, when a small amount of reducing carbohydrate was clearly demonstrated (the validity of the Fehling's solution was tested before). More elaborate investigation might throw more light on this matter. One thing may be now said: repeating this experiment several times, I was not always able to find carbohydrate on the one hand, or pectine on the other, from which I provisionally conclude that the amount of these constituents is rather varying.

Inoculation experiments. These experiments were carried out as follows:—

Two small beds in the kitchen garden of the Military Hospital were prepared by producing a fine tilth, digging the requisite number of plant-holes, and spraying the soil with a 20 % solution of formalin three times on three successive days. They were then left exposed to the sun for three days, when no vapours of formalin could be any longer detected. The suckers were all carefully examined as to their being healthy and the adhering soil removed from the spots to be inoculated. From each sucker a somewhat pyramidal fragment of the rhizome was cut out with the aid of a sharp knife, which was strongly heated every time before a stab was made; from this fragment the under part was cut off, so that the remaining upper part could be used as a lid on the opening produced. The inoculation liquid, previously prepared by shaking a portion of a pure culture in a small tube of sterilized water, was poured out on a bit of compressed cotton-wool, slightly dimpled in the middle to prevent the overflow of the fluid, and the cotton-wool pushed down the hollow with the inoculated surface downwards. At last the lid was tightly fitted in its place by the aid of another bit of cotton-wool spread over the opening. So, as was mentioned in my first paper, four suckers were inoculated with the fungus, four with fungus + Bacteria, four with Bacteria, whereas four were not inoculated and used as a check on the experiment. For convenience' sake I shall indicate the four groups by *f* = fungus, *fb* = fungus + Bacteria, *b* = Bacteria, and *c* = check. Two months after the inoculation I noticed on the leaves of one of *f*, tiny dark brown bodies, which, examined under the microscope, proved to be the mycocecidia described above. In the different sections made, hyphae were found, which at once disclosed their identity with the *Ustilaginoidella musaeperda.* All the outer leaf-sheaths of *f* and *fb* were ruptured longitudinally, but all the plants were still vigorous and healthy looking, and remained so until the middle of the fourth month, when the leaves of *f* and *fb* began to show signs of discoloration and marginal withering. A week after that, I found the outer leaves fallen back against the stems and the withering rapidly progressing and involving some of the

healthy-looking inner leaves, and at the close of the fifth month they were all dying ; *b* and *c* were still healthy-looking. All the plants were then dug out and examined; the rhizomes of *f* and *fb* showed the typical brown streaks and dots of the Panama disease, and moreover it was clear that the infection started from the inoculation spot ; the rhizomes of *b* and *c* were perfectly healthy. Microscopic scrutiny corroborated the macroscopic examination.

To meet objections as to the suitability of an open field for an experiment such as this, it was repeated with small but vigorous suckers carefully washed out under the tap, so as to remove all the adherent soil particles, facilitated by the removal of all rootlets. The suckers after inoculation were laid out in troughs, which were filled with soil sterilized in the oven at a temperature of 120° C., the first day during three hours, and the second day during two hours. The results were even more striking. At the end of four weeks it was found that the suckers were intensely infested by the fungus, showing the typical characteristics of the Panama disease. I believe I have convincingly proved that this disease, *as it occurs in Surinam*, is caused by the *Ustilaginoidella musaeperda*. I may here mention that the inoculation and the disclosure of the results took place in the presence of the majority of the staff of the Military Hospital, while the manager of the United Fruit Company, whom I wish to thank for much valuable information, had the opportunity of seeing the results of the last-mentioned experiment.

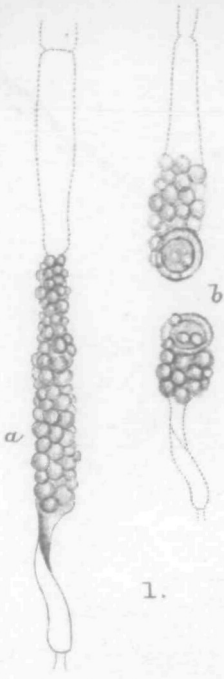
Final remarks. In the first paper I spoke of the Congo as a resistant variety, but since then it has proved not to be so resistant as was generally expected and believed at first. All efforts to discover a remedy against the plague were vain up till now; I myself tried steeping of the suckers in $CuSO_4$ solution and frequent spraying with $CuSO_4 + (NH_4)_2SO_4$ with no results. My experiment, however, was on a very small scale and was carried out under conditions which could not be looked upon as securing all chances of success. I do not mean to say, of course, that the application of the above-mentioned mixture should lead to success, but neither is the contrary proved, since no certainty could be obtained of the perfect sterility of the suckers used. At all events, I am not inclined to admit the incurability of the disease, as is generally thought here. It is very probable that no success can be secured unless with stringent, most expensive measures, but if they could lead to success, and the expenses could be divided over a certain number of years of a permanent crop, then I do not see why all attempts to fight even a most dangerous enemy should be abandoned so soon. One can hardly give any advice as to the way to follow in experimenting, when the opportunity for one's own experiments is so unfavourable, but I hope that experts of the different experimental stations interested in the West Indies and Guiana will be at one with me that some serious fighting

is indispensable, if scientific men do not wish to lose the confidence of the planters, who would so willingly put themselves under the protecting wings of science.

EXPLANATION OF FIGURES IN PLATE XXIX.

Illustrating Mr. Ed. Essed's paper on the Panama Disease. Part II.

Fig. 1. Migration of spores; (*a*) surface view of hypha; (*b*) transverse section of same. × 350.
Fig. 2. (*a*) Hypha with conidia and pegmatia. × 255. (*b*) Modes of branching of conidiophores.
Fig. 3. (*a*) Chlamydosporangia; (*b*) chlamydospores; (*c*) oidia. × 350.
Fig. 4. Germinating giant chlamydospores. × 255.
Fig. 5. Haustoria (see text). × 255.
Fig. 6. Pseudo-pycnidium in decaying rhizome. × 255.
Fig. 7. Mycocecidium; (*a*) tissue-remnants; (*b*) air-spaces; (*c*) chlamydospores. × 450.



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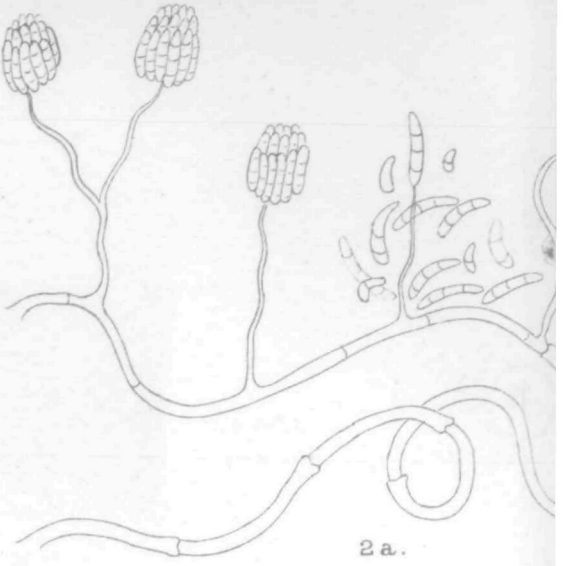

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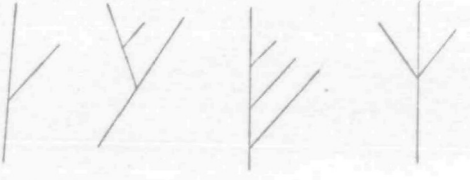

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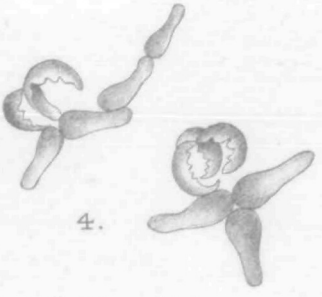

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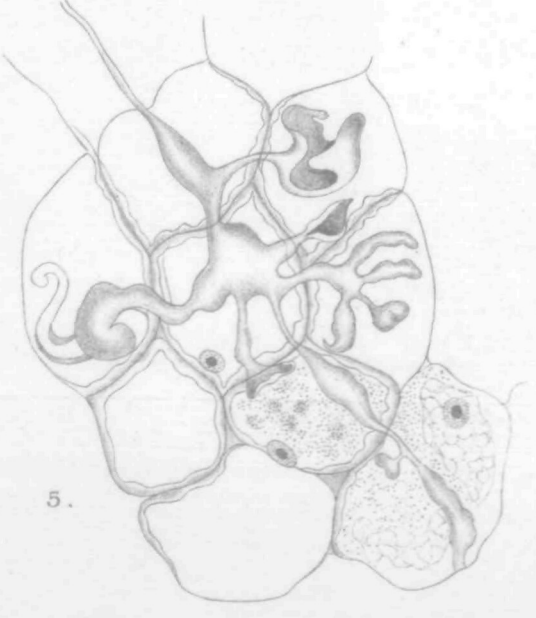

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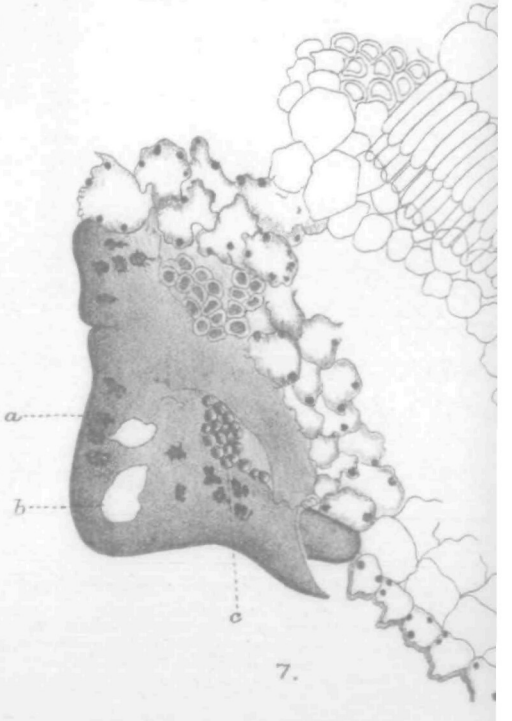

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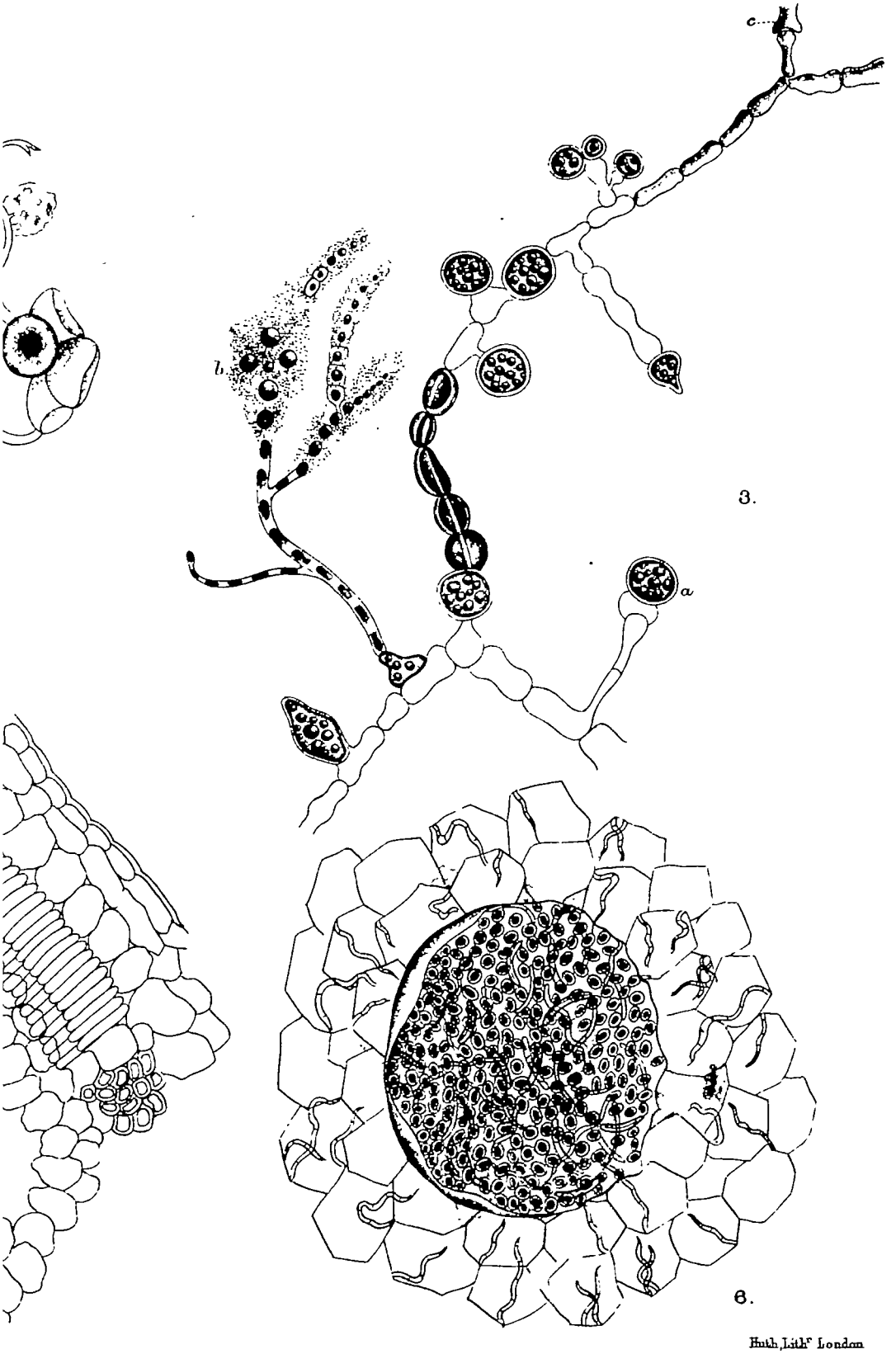

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