PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On the Biology of Stereum hirsutum (Fr.)

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V. *On the Biology of* Stereum hirsutum (Fr.).

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[Plates 17–21.]

Several anatomical studies of this well-known and common Hymenomycete are to hand, of which the best are those of De Bary* on the general features of the young sporophore,† of Hartig‡ on the anatomy of the adult fungus and its behaviour in oak, the wood of which it destroys, of Massee,§ who treats of it in his account of the Thelephoreæ, and of Istvanffi,‖ who is concerned with the distribution of the tissue systems. The only attempts known to me at cultivating *Stereum hirsutum* from the spore are those of Brefeld,¶ and these were not successful beyond the early stages of germination and development of a mycelium.

During the past year I have been engaged in cultivating this fungus in various ways on different media, the primary object having been to see if any trace of the enzyme or enzymes it must contain could be extracted, and dealt with separately. The progress of events led the inquiry in other directions, since it was found possible to obtain excellent cultures in a pure condition and bring them after some months to the development of the fructification, with the basidia and basidiospores normally matured, and as this had not been done previously, it appeared worth while to pursue the results further.**

The cultures were obtained in the first place by allowing sporophores of the fungus to lie overnight on clean glass plates, on which they deposited the spores in quantity. Some of these spores were then washed into a tube of sterile water, and drop-

* 'Comp. Morph. and Biol. of Fungi,' English edition, p. 53.

† In the English edition, in the sixteenth line from top of p. 54, "upper surface" should be "lower surface" according to the German edition of 1866 (p. 62).

‡ 'Die Zersetzungserscheinungen des Holzes.'

§ 'Journ. Linn. Soc.,' Botany, vol. 25, pp. 107 ff.

‖ 'Pringsheim's Jahrbücher f. Wiss. Bot.,' 1896, vol. 29, p. 391, Plate 4, fig. 15.

¶ 'Unters. aus d. Gesammtgeb. d. Mykol.,' H. 8, p. 21.

** The fact of this successful culture was announced at the Toronto meeting of the British Association, September, 1897.

cultures and plates made with gelatine or agar containing 1 to 2 per cent. of sugar or a little raisin juice, in which they germinate readily.

The mycelia grew rapidly, and were easily isolated and grown further on various media, tubes of agar consisting of 1·5 per cent. of agar with 1 to 2 per cent. of brown sugar, and made with tap-water, yielded excellent results.

The early stages of germination of the spores were followed in detail under high powers; but, as they offer nothing new, I pass to the mycelium. A pure culture forms in a few days a thick white cushion exactly like cotton-wool, which, particularly on sugar-agar, turns yellow-brown as it ages, and appears dense and felt-like.

No further developments were obtained on these tube-, plate-, or drop-cultures; and in the course of two or three months the dried up tube-cultures showed no trace of spores or other organs of reproduction.

During the course of the experiments I transferred isolated mycelia to blocks of horse-chestnut prepared as follows:—

Pieces of the wood, about 2 inches long by $\frac{3}{4}$ inch broad and $\frac{1}{4}$ inch thick, were split from the alburnum of a stem some 10 inches or so in diameter. Each block was put into a sterile tube plugged below and above, covered with water—in some cases with 1 per cent. sugar solution—and steamed. The surplus liquid was then poured off, and the sodden block resting on the wet plug below then properly sterilised.*

A trace of the pure mycelium transferred to such a block grows rapidly, and in many cases three or four days suffice for the development of a white patch, which, in a week or so, spreads over the whole surface as a delicate felt like cotton-wool; and sections show that the hyphæ have begun to penetrate into the wood.

This white felt goes on increasing in thickness for weeks, and in from two to four months it stands up from the surface in cushion-like masses $\frac{1}{8}$ to $\frac{1}{4}$ inch high, so that the invested wood appears to be hidden in cotton-wool (Plate 17, fig. 3).

Excepting that the primary growth was more rapid on the wood soaked in 1 per cent. sugar solution, I could detect little difference between the various blocks at the same temperature. The growth is best at ordinary temperatures (15°–18° C.), though it progresses rapidly for a time at 20°–25° C. At 30° and upwards it is distinctly poor, though I have not made exact experiments to determine the limits.

I may add that my earlier work was directed to attempts to obtain direct evidence of the enzymes which must be assumed to exist in such a fungus, and the sugar solution was added to the wood because it seemed probable that, in the destruction of wood, the splitting of glucoside-like bodies occurs, and sugars become available for food; the addition of a little sugar, therefore, would possibly accelerate the growth and activity of the fungus.

For the present, however, the hope of obtaining such proof of the existence of

* The employment of wood for certain cultures has been practised by Costantin and Matruchot ('Compt. Rend.,' vol. 119, p. 752).

definite enzymes as I was seeking has had to be abandoned, though, as will be seen, plenty of indirect proof of them is forthcoming.

On some of the blocks, after about three months' culture, the mycelial cushions, hitherto pure white like cotton-wool, showed yellowish patches here and there. The area of discoloration spread, the colour deepened to buff, or almost orange, and, during the fourth month, the surfaces appeared raised as thickish bosses, in some cases accompanied by tears of clear watery liquid, apparently exuding from them. As the colour deepened the bosses seemed waxy or dry, sometimes with a bloom, as if powdered with mustard or yellowish chalk (fig. 2).

I thought at first that an intruder had made its way into the culture, though it was not easy to see how this should be, considering the precautions taken. However, the regularity and uniformity of appearance of just these yellow bosses, and no other change, in one tube after another during the fourth to sixth month were not consistent with the invasion of a foreign organism.

Sections through these yellow bosses showed a dense meshwork of radiating hyphæ and innumerable minute spore-like bodies, evidently arising at the powdered surface. At first I thought a sort of conidial stage had been disclosed,* but these fresh sections examined in water were not sufficiently clear to decide any such point.

Most interesting results were obtained on cutting properly hardened and stained sections of the more prominent and somewhat waxy-looking older humps on cultures four months old and upwards, for the surface was found studded with typical basidia and basidiospores, showing that these humps were really small hymenophores.

Fig. 2 shows a group of these hymenophores on a block of *Æsculus* wood, with sugar, infected four months previously. This culture had been kept at ordinary temperatures, in the dark, from March 7 (the date of infection) to May 25, thence onwards to July 2, in diffuse daylight at the same temperatures, *i.e.*, about 15°–16° C., except on occasional hot days.

Since the same buff-yellow, waxy-looking hymenial humps appeared on tubes of the same batch kept in the dark the whole time, I cannot attribute anything to the light.

A section across one of these humps is figured in fig. 4 under a low power.

It shows three principal regions:—(1) a base or core of loose hyphæ, crossing and anastomosing in all directions, and with large interspaces: this is indistinguishable from the vegetative mycelium, the cotton-wool appearance of which is due to the air entangled in the meshes; (2) an intermediate region of much more closely packed hyphæ, radiating from this core; and (3) the densely packed palisade-like ends of these radiating hyphæ, forming the superficial hymenium.

As I understand matters, the first region corresponds to the *subiculum* of

* Massee (*loc. cit.*, p. 120, and Plate 46, fig. 7) describes and figures conidiophores, but I have not found anything resembling his figure.

systematists,* the second to the *sub-hymenial layer*, and the third is certainly the *hymenium* of basidia and barren ends, and I shall refer to them by these names in the following analysis.

If a portion of a vertical section is more highly magnified, as in fig. 5 (Plate 18), the *subiculum* is seen to consist of loosely interwoven hyphæ, with dark spots in the meshes; the *sub-hymenial* region shows radiating, branched hyphæ of two kinds, one of which is stained more deeply than the other; and the *hymenium* itself forms a deeply stained palisade-like layer of basidia, each with four long sterigmata bearing the spores.

Under high powers the dark spots in the *subiculum* are found to be pyriform swellings densely filled with contents which stain deeply, and are evidently developed as the swollen ends of the hyphæ (fig. 6); the conspicuous radiating hyphæ of the sub-hymenial layer are shown to be thin-walled, multiseptate filaments, with highly vacuolated contents, and branching in a sort of fan-like or falsely dichotomous manner, reminding one of the growth of many filamentous algæ. The contents stain so much more deeply than do those of the thicker-walled hyphæ in which they grow and intrude as into a matrix (fig. 7), that this system of hyphæ is sharply marked as distinct.

High-power sections, taken vertically through the *hymenium* (fig. 8), present little that is not known, but attention may be drawn to the facts that the young spores are round, and that they become more and more elongated as they mature; that the large basidia are clearly the ends of hyphal branches coming up from the sub-hymenium; and that many of the ends of the branching hyphæ remain barren. As fig. 9 shows, some of these barren ends grow out like *paraphyses* or *cystidia* here and there, but I am not sure whether this is a mere accident or an expression of the fact that, after the present crop of basidiospores is matured, these intermediate hyphæ grow forward, and develop the new hymenial layer, thus accounting for the periodic zones of growth found in older hymenophores.†

The observation that the sub-hymenium of *Stereum hirsutum* contains two kinds of hyphæ was apparently new, though the presence of a sort of laticiferous system in other species of this genus is well known,‡ and on cutting sections of the mature sporophore of specimens kindly sent me by Mr. Massee I find them there also, thus confirming Istvanffi,§ who had already figured them. They are quite evident in thin sections parallel to the surface of the hymenium, and therefore transverse to the course of the hyphæ themselves.

I am quite unable to explain in detail the significance of the pyriform swellings in the *subiculum* and the two kinds of hyphæ in the *sub-hymenium*. Istvanffi speaks

* *E.g.*, Massee, *loc. cit.*, p. 119.

† See also De Bary and Hartig, *loc. cit.*

‡ Istvanffi and Olsen, 'Bot. Centr.,' vol. 29, 1887, p. 373; 'Pringsheim's Jahrb.,' 1896, vol. 29, p. 391; Massee, *loc. cit.*, p. 121.

§ *Loc. cit.*, p. 391, and Plate 4, fig. 15.

of a *Leitungssystem* in regard to one kind of hypha, but there seems no direct evidence of function. In some sections the pyriform swellings give out branches, and present a curious formal resemblance to ganglion-cells when stained, but whether these branches run up as the hyphæ which terminate in the basidia, or as those which end like paraphyses, I cannot as yet determine. It is also very difficult to determine which hyphæ are and which are not segmented. It would be an interesting point in relation to the phylogeny of this fungus if the hyphal arms connected with the pyriform bodies end in basidia, since the view which would suggest itself to the advocates of the BREFELD school of morphology would no doubt be that the pyriform bodies are the homologues of chlamydospores. At present I can throw no certain light on this. Up to the present, moreover, I have not been able to make the sporophores grow out into the bracket shape so commonly observed in such fungi in the open; the air and space at the disposal of the imprisoned fungus are, of course, limited, and no doubt affect this matter.

As already said, the only attempts at pure cultures of *Stereum* known to me are those of BREFELD,* who tried nine different species of this genus, including *S. hirsutum*, but failed to obtain anything beyond the barren mycelia. He says: "Die Mycelien endeten nach langer Cultur trotz riesiger Ausdehnungen auch im Luftmycel gänzlich steril an allen secundären Fruchtformen und auch an Basidienfruchten."

This is very definite, but since BREFELD does not allow us to know what medium he employs in the culture of these fungi, no suggestion can be offered in explanation of his failures.

BREFELD says the hyphæ of *Stereum* are devoid of clamp-connections in the young mycelia grown by him, but MASSEE, in his excellent "Monograph of the Thelephoreæ,"† says these are probably present in all species having septate hyphæ, and figures them in the closely allied *Corticium*. They certainly appear to occur occasionally, as seen in figs. 11 and 12, but they seem to be rare.

Another point in dispute is the size of the basidiospores of *Stereum hirsutum*. They are given by MASSEE‡ as globose, and 5 μ in diameter. WINTER also gives them as "Sporen kugelig, sehr klein."§ ZOPF‖ describes them as cylindrical, with rounded ends, 6–8 μ long and 2–3 μ broad. COOKE¶ makes no mention of their size or shape, nor does STEVENSON.** COSTANTIN and DUFOUR†† say the spores are cylindrical, but give no measurements.

* 'Unters. aus dem Gesammtgebiete d. Mykol.,' H. 8, 1889, p. 21.
† 'Journ. Linn. Soc.,' Botany, vol. 25, No. 170, 1889, p. 115.
‡ 'Brit. Fung. Flora,' vol. 1, p. 131.
§ RABENH., 'Die Pilze,' Abth. 1, p. 345
‖ 'Die Pilze,' p. 341.
¶ 'Handbook,' vol. 1, p. 316.
** 'Brit. Fungi,' vol. 2, p. 268.
†† 'Nouvelle Flore des Champignons,' 2nd ed., p. 182.

Brefeld* merely refers to "Kleine, farblose und Krumme sporen," and Hartig† says they are *birnförmigen*.

That some of the differences depend on the age of the spores measured may well be assumed. I find them long, ovoid, or pear-shaped to shortly cylindrical, and 5–6 μ long by 3–4 μ broad, and, in order to be sure that my earlier measurements, &c., were correct, I subsequently asked Mr. Massee to send me some fresh specimens of *S. hirsutum*, and confirmed them on the fresh spores (in water) obtained from the hymenia he kindly selected for me.

At an early stage in the investigation sections were made of the infected blocks of *Æsculus* wood put into absolute alcohol at various dates as the work proceeded. These gave very satisfactory preparations, and showed clearly that not only the medullary rays, but the vessels, tracheids, and all the elements of the wood, are invaded, the easiest passage for the pioneer hyphæ being the medullary rays exposed on tangential sections and the cut vessels on the transverse sections.

A week suffices for the entry of the hyphæ, some of which are found to have penetrated to from 6 to 10 elements deep from the tangential face; and in a month almost the whole of the wood is invaded, and the cavities of the vessels show the hyphæ, of various thicknesses, branching and growing into tufts in all directions.

In order that no question of a possible sweeping in of hyphæ from the outside, during the cutting, should arise, I wiped off the external mycelium from the block, and then scrubbed the whole surface with a hard brush before cutting.

Moreover, as the figures show (*e.g.*, figs. 10–12), it was quite easy in many cases to trace a hypha through several cells or tracheids, especially radially, and to see that it always passes through the pits. Not only so: thickish sections showed the hyphæ in the vessels which had not been opened by the razor. The lateral extension from the medullary rays or vessels into other elements is through the bordered or other pits (figs. 11 and 12); and it is very evident, in some transverse sections, that the spring wood is invaded much more rapidly than the autumn wood of the same annual ring (fig. 13).

Excellent preparations of these hyphæ in the wood can be made by overstaining the sections in very dilute Delafield's hæmatoxylin,‡ or by Gramm's method with gentian violet,§ and then washing out in water and successive concentrations of alcohol before clearing and mounting in xylol Canada balsam in the usual way.

The results are even better if such sections are prepared, not from alcohol material, but from blocks which have been boiled to fix the hyphæ *in situ*, and then passed through 50 per cent., 70 per cent., 90 per cent., and finally into absolute alcohol.

The action of the fungus on the walls of the elements, especially the fibres and

* Brefeld, *loc. cit.*, p. 21.
† Hartig, *loc. cit.*, p. 130.
‡ See Zimmermann's 'Botanical Microtechnique,' p. 180.
§ Zimmermann, p. 185.

tracheids, is particularly evident in transverse sections of *Æsculus* wood after three to four months.

Thin sections show that the thickening layers next the lumen are no longer homogeneous in appearance, but have swollen and separated from the layers next the middle lamella, and lie loose in the cavity, often contorted or wrinkled in curious shapes, evidently because their prison is too small for the swollen layers (see figs. 17–19). This is particularly clear in cases where the section has passed through a pit, for the swollen layers here form a ring, broken at the pit and folded or coiled according as the severed ends abut on one another or glide one over the other during the swelling (figs. 18 and 19).

It is a common event in such sections to obtain beautiful transverse sections of the hyphæ running vertically in the tracheids, &c., and these are evidently held in position by the gelatinised swollen layers referred to (figs. 18 and 19).

Sections such as the above placed in chlor-zinc-iodine at once undergo colour changes which betray what has happened, for while the middle lamella comes out deep yellow, and the lignified layers next it a paler, but still bright, yellow, the swollen layers turn blue, often deep blue-black, but varying in hue from that to pale violet. These swollen layers, then, have been delignified, and consist entirely, or almost entirely, of cellulose. These colour contrasts are very striking and beautiful, as shown in fig. 18.

Equally convincing and striking are similar sections treated with phloroglucin and hydrochloric acid (fig. 19). The middle lamella is deep rose-red, the layers next it paler, but still distinctly red, but the swollen layers remain colourless; they contain no lignin.

The results with aniline chloride, and with other reagents for differentiating lignified membranes from those devoid of lignin, are in harmony with the above, and it becomes certain that the action of the hyphæ is to slowly delignify the walls from the lumen outwards. That this is a progressive action is clear from testing the wood at various stages. During the first month I find no distinct reactions with the above reagents, and stains, such as DELAFIELD'S hæmatoxylin, do not colour the walls blue or purple, but merely brown or yellowish; but in some cases a thin lining layer is found to react in wood acted on by the fungus for six weeks to a couple of months, and the altered layer gets more and more decided as the action progresses, till the beautifully clear reactions above described are obtained after three to four months.

As the action proceeds—*e.g.*, after five or six months—the swollen layers gradually disappear from within outwards, and the layers next the middle lamella react less and less to phloroglucin and aniline chloride, and stain blue or violet in chlor-zinc-iodine or in hæmatoxylin, and may separate from the middle lamella.

The above results demonstrate in detail that the hyphæ attack the walls of the tracheids and other wood elements of *Æsculus* from within, gradually delignify them layer by layer, and then consume the swollen cellulose matrix. HARTIG had

already stated in general terms that *Stereum hirsutum* acts thus in the oak, but he also says that in some cases the elements of oak wood are isolated by the middle lamella being first dissolved. Whether this depends on differences in the action on oak, or on his having some other fungus present—he employed diseased wood from the forest for his examinations, and so ran the risk of contamination—I cannot yet say, and my cultures on oak are still too young to determine the point, nor have I yet succeeded in extracting from my cultures the enzyme, which must be assumed to exist, which effects the delignification.*

Experiments were made to test the growth on other woods than *Æsculus*. Blocks of *Pinus* and of Willow were used, prepared as before, with water only, and gave the same results so far as the sap-wood is concerned, but it happened that in blocks which contained heart-wood only the growth appeared to be arrested (see fig. 1). I therefore had series of blocks cut entirely from the heart and entirely from the sap-wood of both pine and willow. In all cases the growth on the sap-wood of both proceeded rapidly, whereas scarcely any mycelium at all was visible on the heart-wood of either pine or willow. Even after three months the cultures on the heart-wood show only a sparse cobweb of mycelium, not hiding the wood, and indeed often scarcely visible; whereas the mycelium on the sap-wood, same age and grown side by side, is a thick white, cotton-wool-like cushion, completely hiding the invested wood (fig. 1).

In order to test more completely the action of the fungus on heart-wood and sap-wood respectively, I made the following cultures:—

Three tubes each of sap-wood of oak, horse-chestnut, willow, and pine, and three of the heart-wood of each, were infected in July and placed side by side and kept under like conditions—15°–18° C. in the dark.

In October, after three months of undisturbed growth, all the cultures had succeeded, and showed marked contrasts between sap and heart-wood in each case, but especially in those of oak, willow, and pine, where the differences between sap-wood and heart are most pronounced.

The mycelia in the heart-wood cultures were almost entirely confined to thin, cobweb-like, superficial growths, which scarcely obscured the view of the wood beneath, even in the most luxuriant specimens, and in many cases were so inconspicuous that the wood appeared unaltered, and no mycelium could be seen at all until close examination in an oblique light betrayed it. In these cases I am speaking of the naked eye characters.

On the sap-wood, on the contrary, the mycelium had formed a dense white felted covering, like cotton-wool, and even in the feeblest specimens the wood was entirely

* I use this term for the sake of brevity, in spite of the fact that lignin and lignification can no longer be regarded as the simple matters they were once supposed to be. BOURQUELOT (see 'Zeitschr. f. Pflanzenkrankh,' 1895, p. 39, for reference) finds emulsin-like enzymes in these wood fungi, which split glucosides like æsculin, coniferin, &c., into sugars and other bodies.

covered. Evidently, therefore, the fungus grows more luxuriantly in sap-wood than in heart-wood—at any rate, of the species of tree examined.

The same truth comes out on examining the specimens with regard to the presence or absence of young hymenia, the waxy yellow humps showing up distinctly on several of the richer growths on sap-wood of oak especially; whereas no traces of incipient hymenia were visible on the poorer growths on the heart-wood.

That light is not necessary to the development of the fungus, or of the hymenia, comes out clearly from the above. But, side by side with the series referred to, I had prepared in July an exactly corresponding series, *i.e.*, three tubes each of heart- and sap-wood of oak, willow, *Æsculus*, and pine, infected at the same time and kept side by side at 15°–18° C. in the light.

The same facts came out on examining the three months' cultures in October. In all cases the growth was luxuriant on the sap-wood, and in some cases had proceeded so far that incipient hymenia were visible; whereas on the heart-wood the mycelium was much more feebly developed, and no hymenial beginnings were perceptible.

It is, perhaps, premature to speculate on the causes of these differences, since they may reside in difference of food-materials in the walls of heart- and sap-wood respectively, in the existence of antiseptic bodies, in differences of mechanical obstruction to the entry of the hyphæ, or in other factors; but taking all observations together, I suspect that aëration—ventilation—is at the bottom of the whole question, and I am now carrying on experiments which will, I hope, settle the matter.

In this connection it may be interesting to compare the normal structure of the woods of *Æsculus*, *Salix*, *Quercus*, and *Pinus*. The latter consists, as is well known, of tracheids only, apart from a few wood-parenchyma cells near the resin passages, and since the heart-wood is full of resin, and the tracheids closed, the fungus hyphæ will have peculiar difficulty in obtaining air. Apart from other causes, therefore, I attribute the poor growth on pine to this difficulty.

In oak we find large vessels in the spring wood, and smaller ones throughout the summer wood, together with tracheids, wood-parenchyma, and fibres; moreover, the medullary rays are in part very broad, whereas in *Pinus* they are all very narrow. But in the heart-wood the vessels are rapidly stopped by tyloses, and the sap-wood is very narrow. Hence the ventilation of the heart-wood is difficult.

Æsculus and *Salix* both have small numerous vessels and much fibre, but no tracheids and very little wood-parenchyma. Whereas *Salix* forms a distinct heart-wood, that of *Æsculus* is very little, if at all, different from the sap-wood, and no doubt the much freer growth of the hyphæ in the "heart-wood" of *Æsculus* is correlated with this fact. At the same time the matter deserves further examination.

Hartig in his work, 'Die Zersetzungserscheinungen des Holzes' (p. 129), gives an account of the destruction of oak-wood by *S. hirsutum*, but his description refers to that wood only, and to the rot as it occurs in the forest; no question of pure cultures is touched by him.

He regards the fungus as a saprophyte, though much damage accrues to living trees owing to its spreading from dead wood into the trunk.

It is significant that HARTIG found two modes of action of the fungus. In some cases, the middle lamella is first destroyed, and the elements isolated; in others, the walls of the elements are destroyed from within outwards. In the first case, the walls are delignified and converted into cellulose before final solution; in the second case, no such conversion to cellulose precedes solution.

His description does not refer to any mixture of bacteria or other fungi as being present, but it seems very likely that such would be the case in wood taken from such rotten branches, and if so, the question arises how far the changes depicted are due to *Stereum* alone, and how far to other organisms.

I have made a few preliminary experiments with promising results. Blocks of oak were prepared as usual, but before infection I poured into the tubes a few drops of water in which rotting wood, swarming with bacteria, had been crushed in a mortar, and which had been filtered through ordinary filter paper. Of course, the method of pure cultures is here abandoned, but since one of the blocks developed a crop of *Stereum* hymenophores, it will be worth while to go further into this question of possible symbiosis.

I have also tried a method of obtaining the wood blocks free from organisms without sterilisation by heat; but as yet certain difficulties have not been overcome.

EXPLANATION OF THE FIGURES.*

PLATE 17.

Fig. 1. Pure cultures of *Stereum* on blocks of willow wood, sterilised in water only, and grown for 91 days at 22° C. in the dark. The block to the left is of sap-wood, and is covered by an abundant mycelial felt, like cotton-wool; the block to the right (heart-wood) shows a much poorer development, though both were treated alike. Photographed from the original culture.

Fig. 2. A culture on *Æsculus* wood, to which 1 per cent. solution of sugar was added. The growth is 112 days (*i.e.*, nearly 4 months) old, and was kept at 20° in the dark; the small shaded patches are incipient yellow hymenial cushions. Photographed from the original culture.

Fig. 3. A culture on sterile *Æsculus* wood, to which 1 per cent. sugar solution was added, after 117 days (*i.e.*, about 4 months) at 15° C., during the first 79 days in the dark, the rest of the time in diffused light. An

* The photographs and figs. 4–11 were prepared by Mr. W. G. P. ELLIS; figs. 12, 16, and 17 by Mr. HILL; and figs. 13–15 by Miss PERTZ. I have great pleasure in recording my thanks for this help.

abundant crop of hymenial cushions has been developed on the surface of the cotton-wool-like mycelium. Photographed from the original culture.

Fig. 4. Vertical section through one of the yellow waxy hymenial cushions of fig. 3 under a low power. Three regions are visible, viz., (1) a central basal one of loosely felted hyphæ, with small swellings here and there showing as dark points; (2) a more peripheral region, with the hyphæ more densely packed and radially directed; and (3) the outer layer of basidia covering the surface.

PLATE 18.

Fig. 5. A portion of the cushion more highly magnified, and showing the three regions still more distinctly. The intermediate layer—No. 2 in fig. 4—is composed of two kinds of hyphæ. The dark spots in the basal region are pyriform swellings of certain branches.

Fig. 6. Portion of a section taken vertically through the basidial layer under a high power, and showing basidia and basidiospores, with intervening barren filaments, in various stages of development.

Fig. 7. Part of vertical section through the central basal region of fig. 4, highly magnified, and showing the pyriform swellings on certain of the hyphæ, which are the dots in the figure referred to.

Fig. 8. Part of the region of radiating hyphæ between the basidial and basal regions last figured, and comprising No. 2 in fig. 4. The hyphæ are seen to be of two kinds. One set, with vacuolated contents and distinct segmentation and branching, stain more deeply than the faintly marked hyphæ which serve as a matrix for them.

Fig. 9. Vertical section of hymenium, showing the prolonged cystid-like paraphyses.

PLATE 19.

Fig. 10. Transverse section of *Æsculus* wood from a block infected 2 months previously, showing hyphæ in the vessels and other elements, and passing radially from one to another.

Fig. 11. Tangential section of same block, showing hyphæ of two sizes in the large vessel to the left, and smaller ones passing from fibre to fibre through the simple pits.

Fig. 12. Part of a tangential section of *Æsculus* wood exposed 4 months to the action of the fungus. Large irregular holes are to be seen in some of the walls, due to the solvent action of the hyphæ passing through them.

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PLATE 20.

Fig. 13. Transverse section through *Æsculus* wood after 4 months' exposure to action of the fungus. The autumn wood of the annual ring has the walls of the elements still intact; those of the spring wood have the inner layers swollen and separating, under the action of the contained hyphæ.

Fig. 14. Portion of a radial section through same block as fig. 13. The hyphæ have invaded all the medullary ray cells.

Fig. 15. Tangential section of same.

Fig. 16. Part of transverse section of *Æsculus* wood not attacked by *Stereum*—*i.e.*, normal.

PLATE 21.

Fig. 17. Similar section of wood invaded by the fungus, and exposed 5 months to its action. The inner walls of the wood elements are seen swollen and wrinkled.

Fig. 18. Portion of a similar section in chlor-zinc-iodine. The swollen inner cell-wall layers turn blue or violet, while the enclosed hyphæ and the still lignified parts of the walls are yellow.

Fig. 19. Similar section in phloroglucin and H.Cl. The lignified parts are coloured rose-red, while the delignified swollen inner layers are not coloured.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fig. 1.

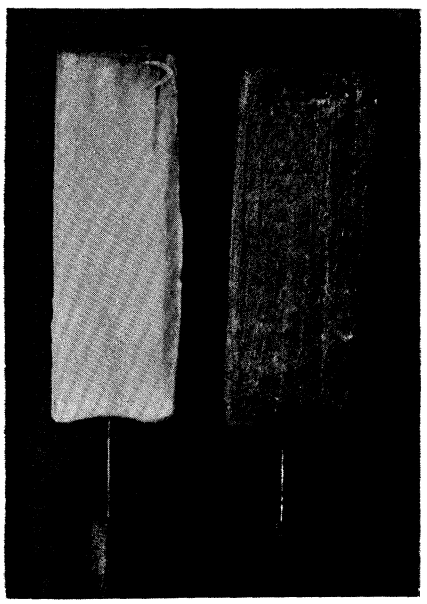
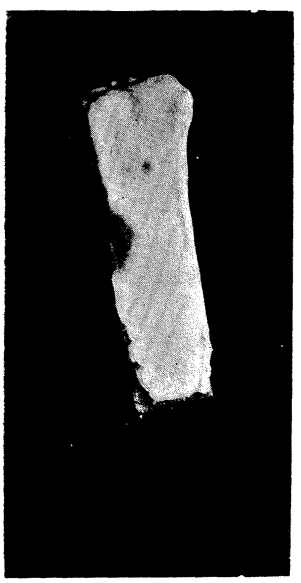


Fig. 2.

Fig. 3.



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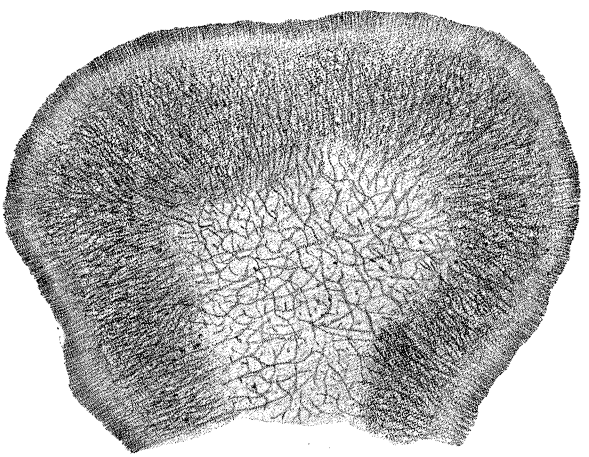

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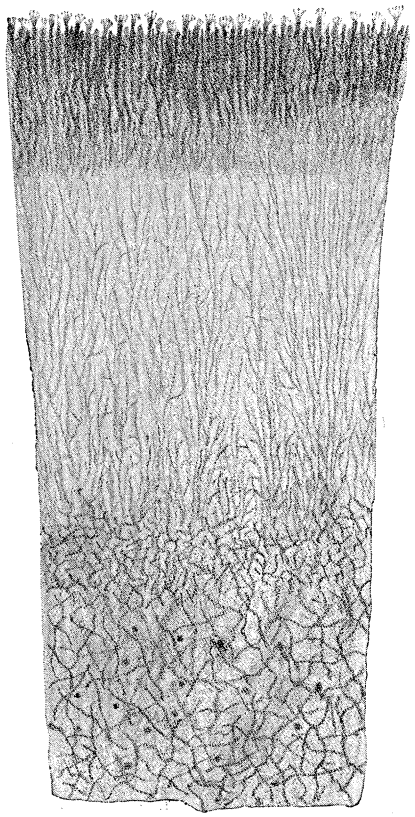
Fig. 5.



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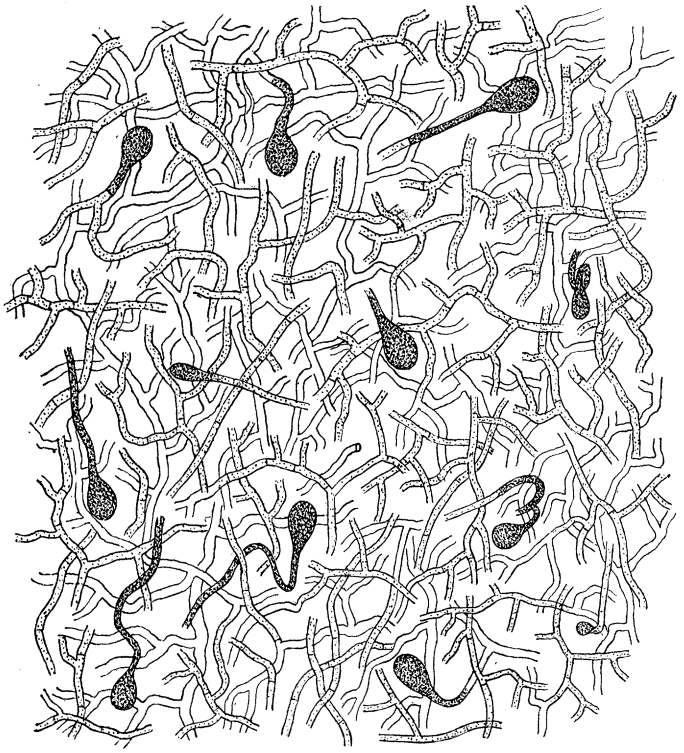

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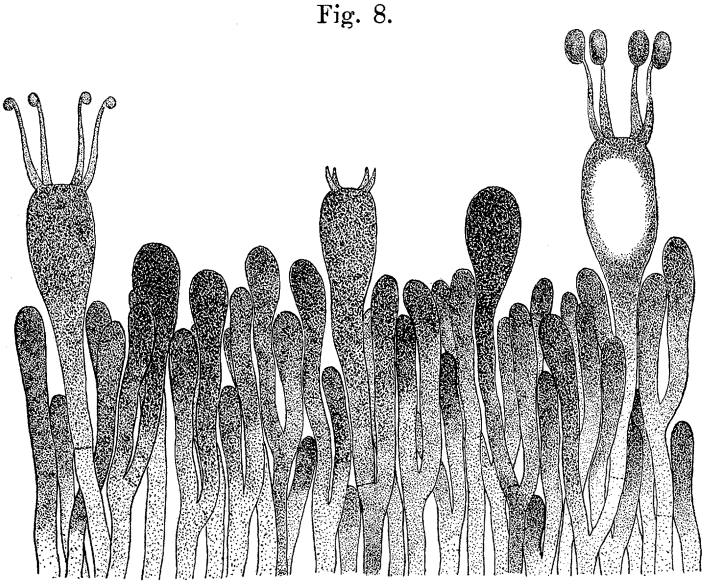
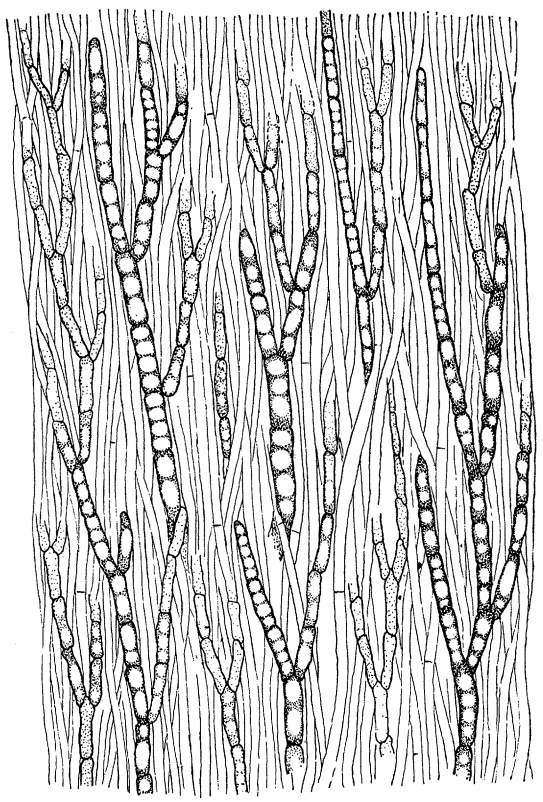


Fig. 7



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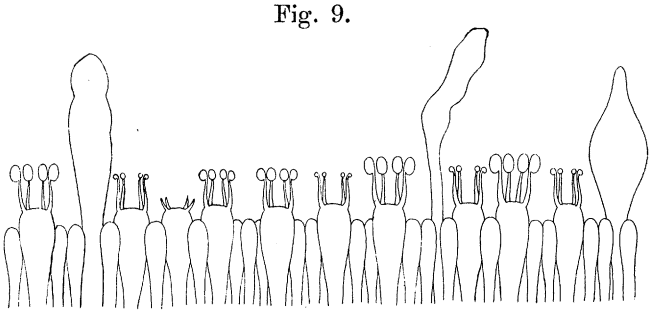

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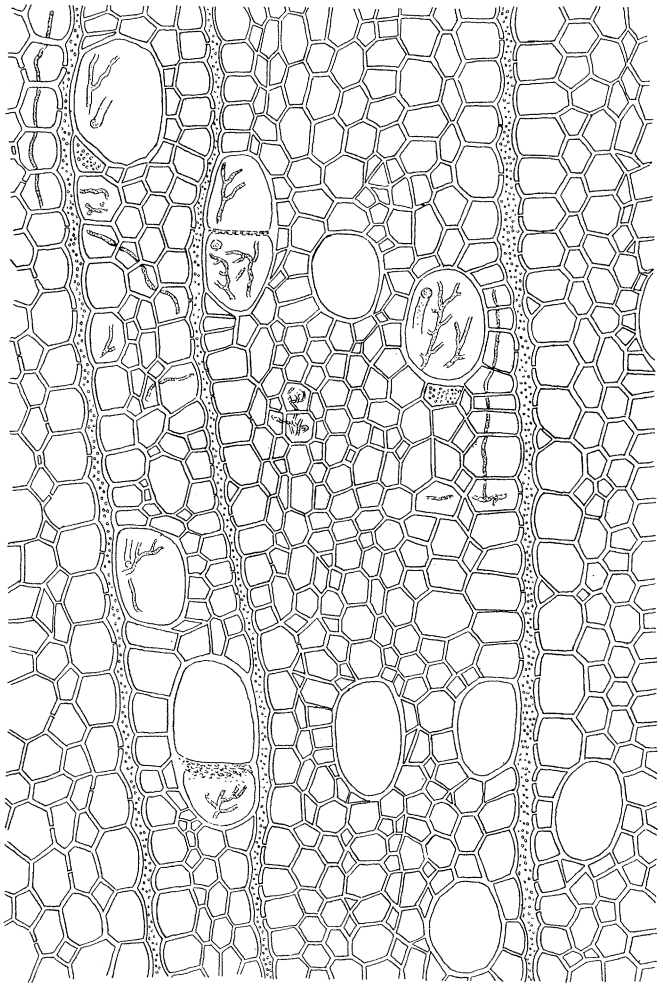
Fig. 10.



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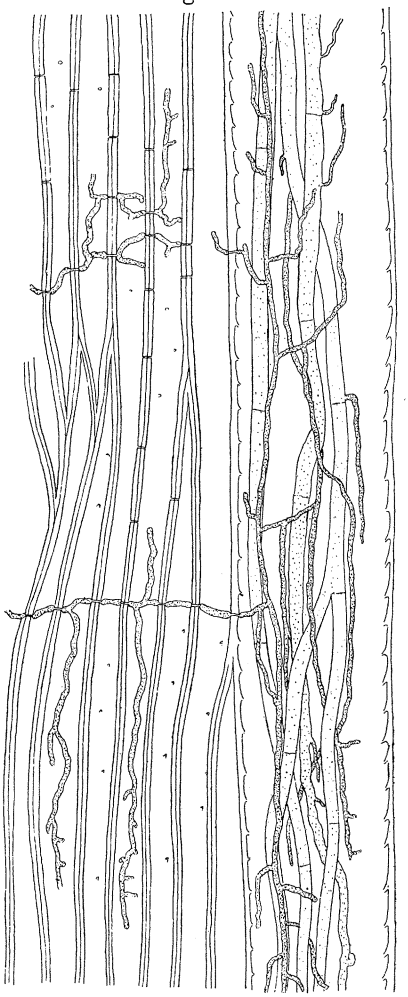

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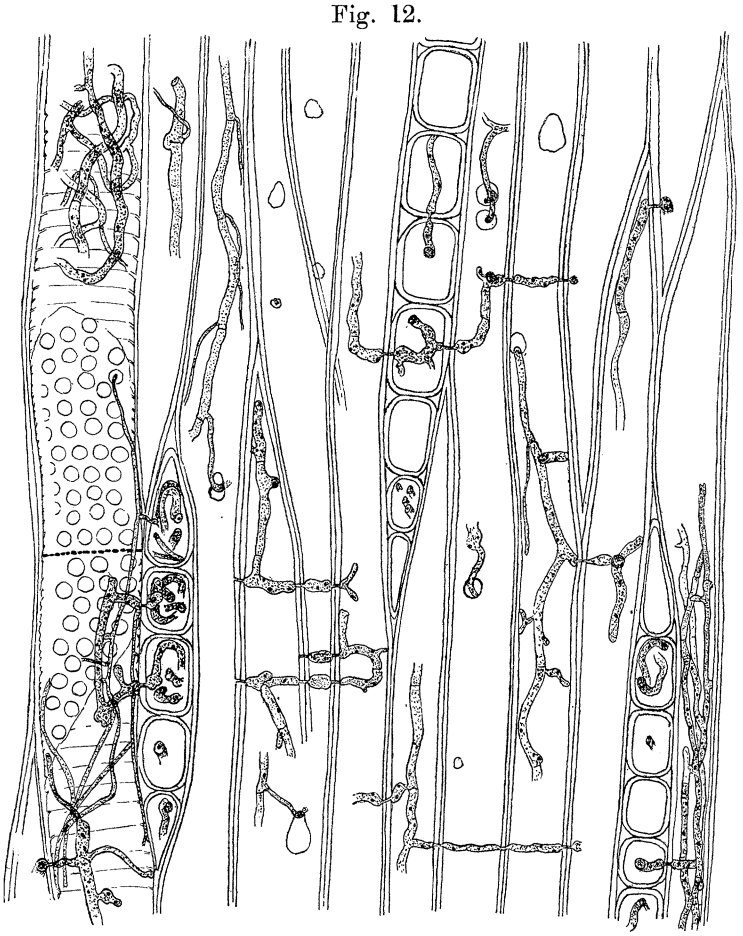
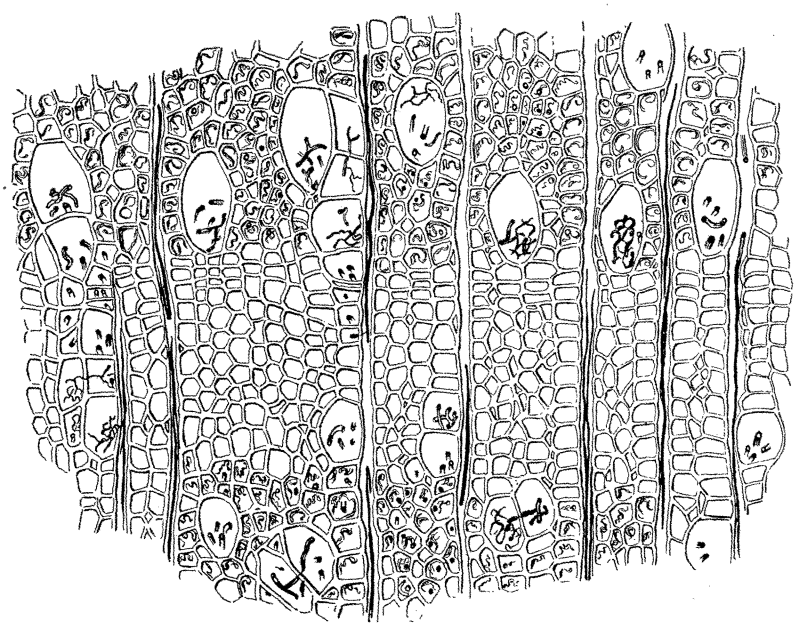


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fig. 13.



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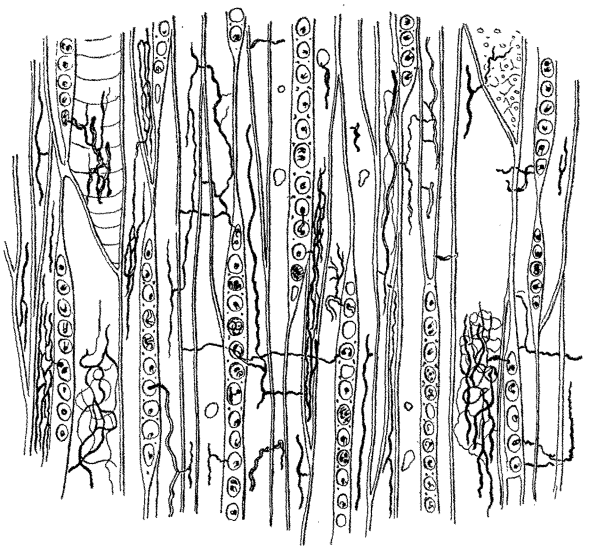

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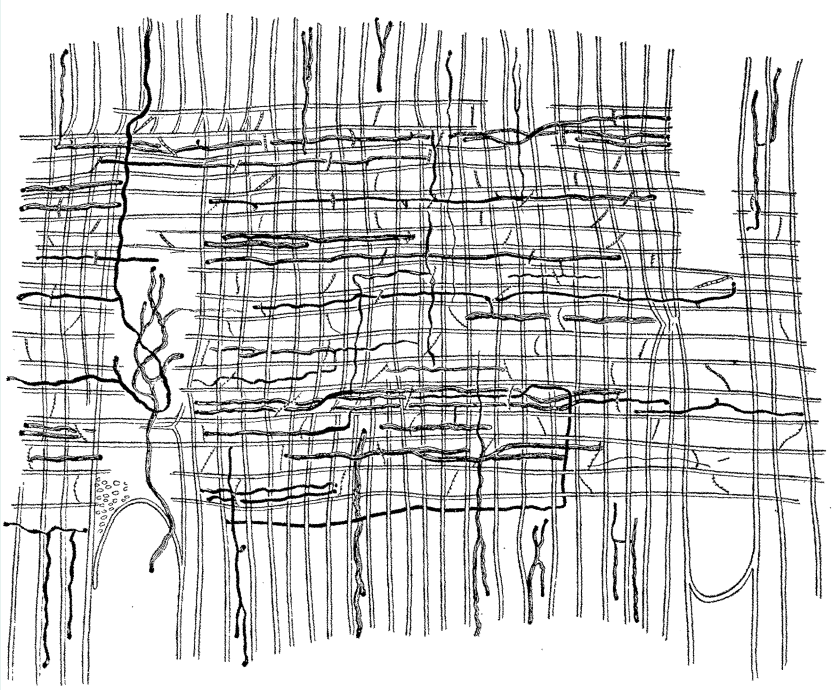

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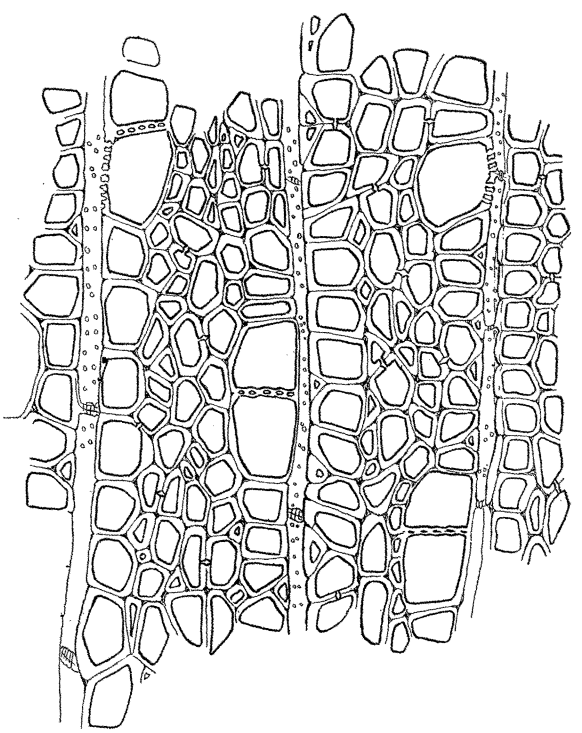
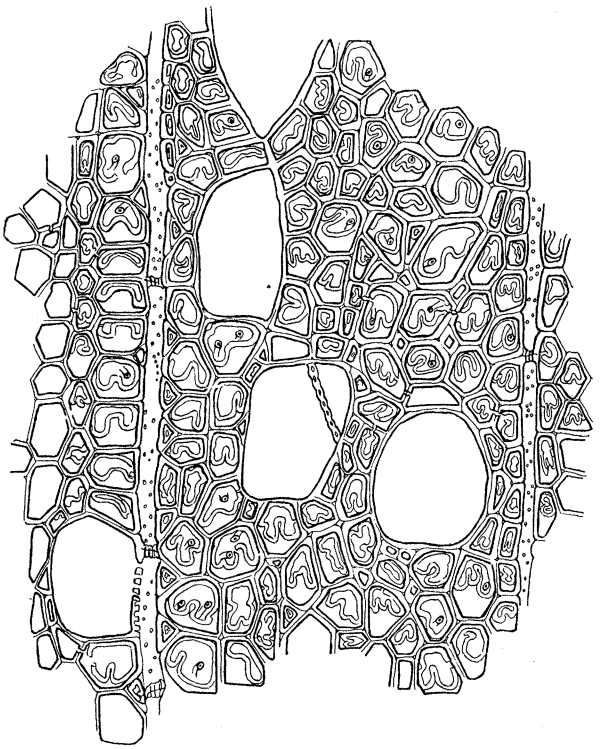


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fig. 17.



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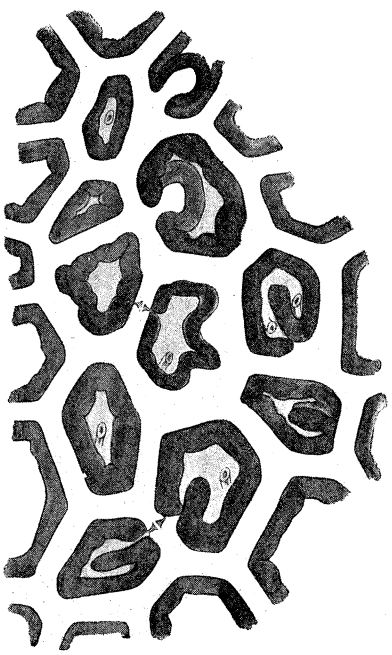

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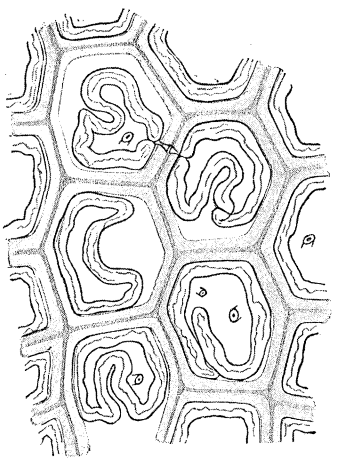
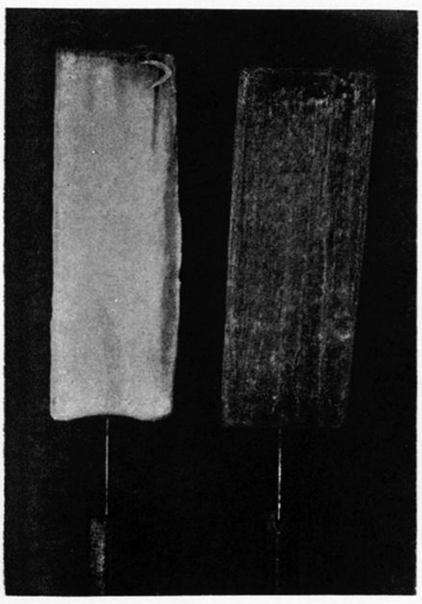


PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Fig. 1.



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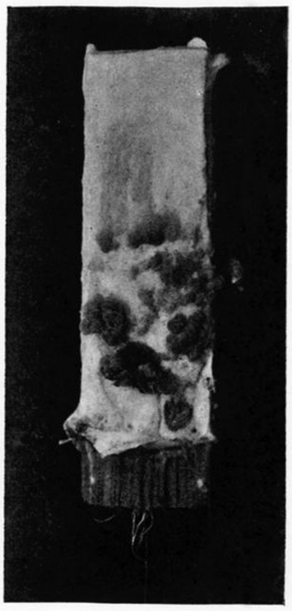

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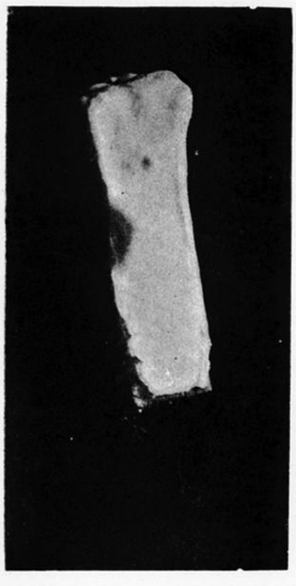

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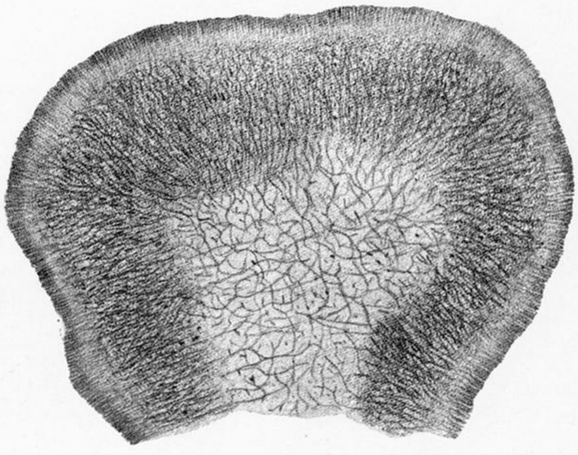

PLATE 17.

Fig. 1. Pure cultures of *Stereum* on blocks of willow wood, sterilised in water only, and grown for 91 days at 22° C. in the dark. The block to the left is of sap-wood, and is covered by an abundant mycelial felt, like cotton-wool; the block to the right (heart-wood) shows a much poorer development, though both were treated alike. Photographed from the original culture.

Fig. 2. A culture on *Æsculus* wood, to which 1 per cent. solution of sugar was added. The growth is 112 days (*i.e.*, nearly 4 months) old, and was kept at 20° in the dark; the small shaded patches are incipient yellow hymenial cushions. Photographed from the original culture.

Fig. 3. A culture on sterile *Æsculus* wood, to which 1 per cent. sugar solution was added, after 117 days (*i.e.*, about 4 months) at 15° C., during the first 79 days in the dark, the rest of the time in diffused light. An abundant crop of hymenial cushions has been developed on the surface of the cotton-wool-like mycelium. Photographed from the original culture.

Fig. 4. Vertical section through one of the yellow waxy hymenial cushions of fig. 3 under a low power. Three regions are visible, viz., (1) a central basal one of loosely felted hyphæ, with small swellings here and there showing as dark points; (2) a more peripheral region, with the hyphæ more densely packed and radially directed; and (3) the outer layer of basidia covering the surface.

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Fig. 5.

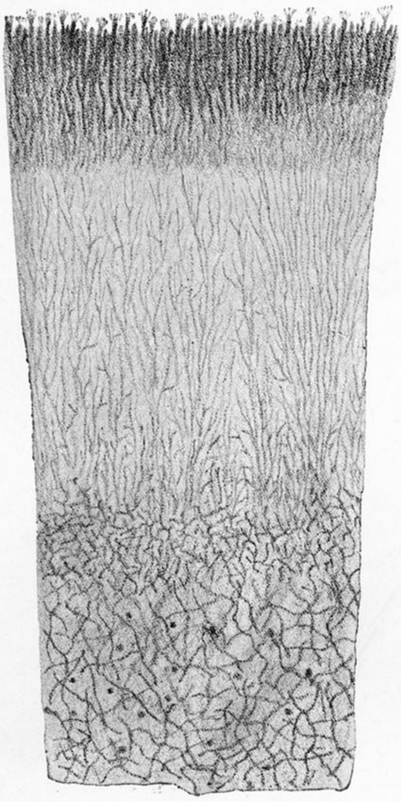
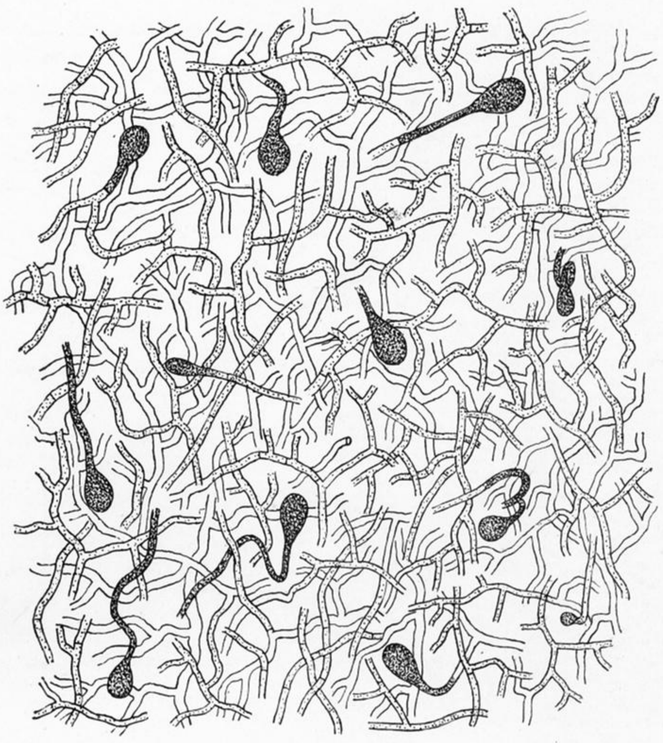


Fig 6.



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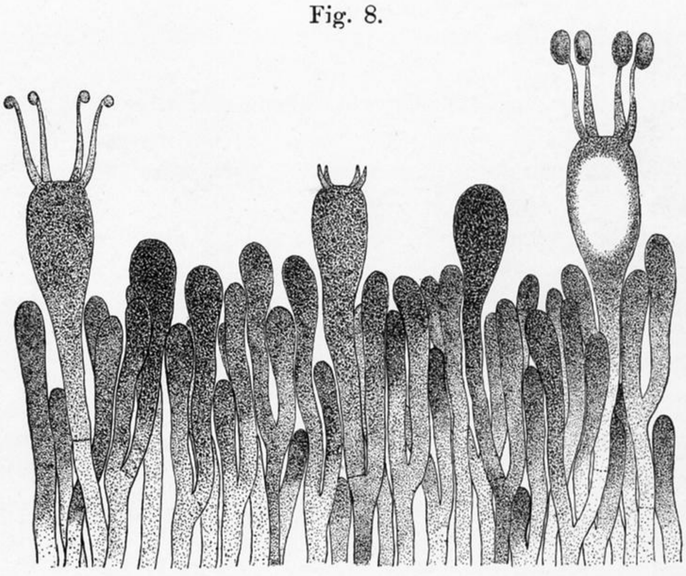
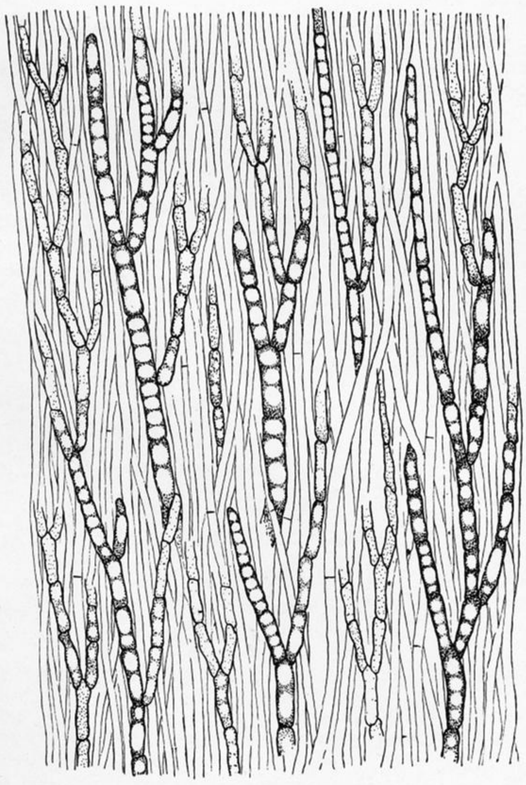


Fig. 7



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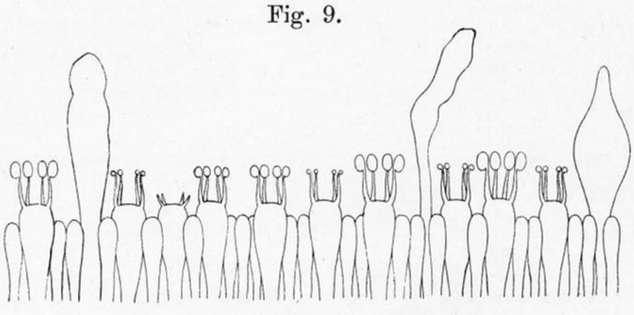

PLATE 18.

Fig. 5. A portion of the cushion more highly magnified, and showing the three regions still more distinctly. The intermediate layer—No. 2 in fig. 4—is composed of two kinds of hyphæ. The dark spots in the basal region are pyriform swellings of certain branches.

Fig. 6. Portion of a section taken vertically through the basidial layer under a high power, and showing basidia and basidiospores, with intervening barren filaments, in various stages of development.

Fig. 7. Part of vertical section through the central basal region of fig. 4, highly magnified, and showing the pyriform swellings on certain of the hyphæ, which are the dots in the figure referred to.

Fig. 8. Part of the region of radiating hyphæ between the basidial and basal regions last figured, and comprising No. 2 in fig. 4. The hyphæ are seen to be of two kinds. One set, with vacuolated contents and distinct segmentation and branching, stain more deeply than the faintly marked hyphæ which serve as a matrix for them.

Fig. 9. Vertical section of hymenium, showing the prolonged cystid-like paraphyses.

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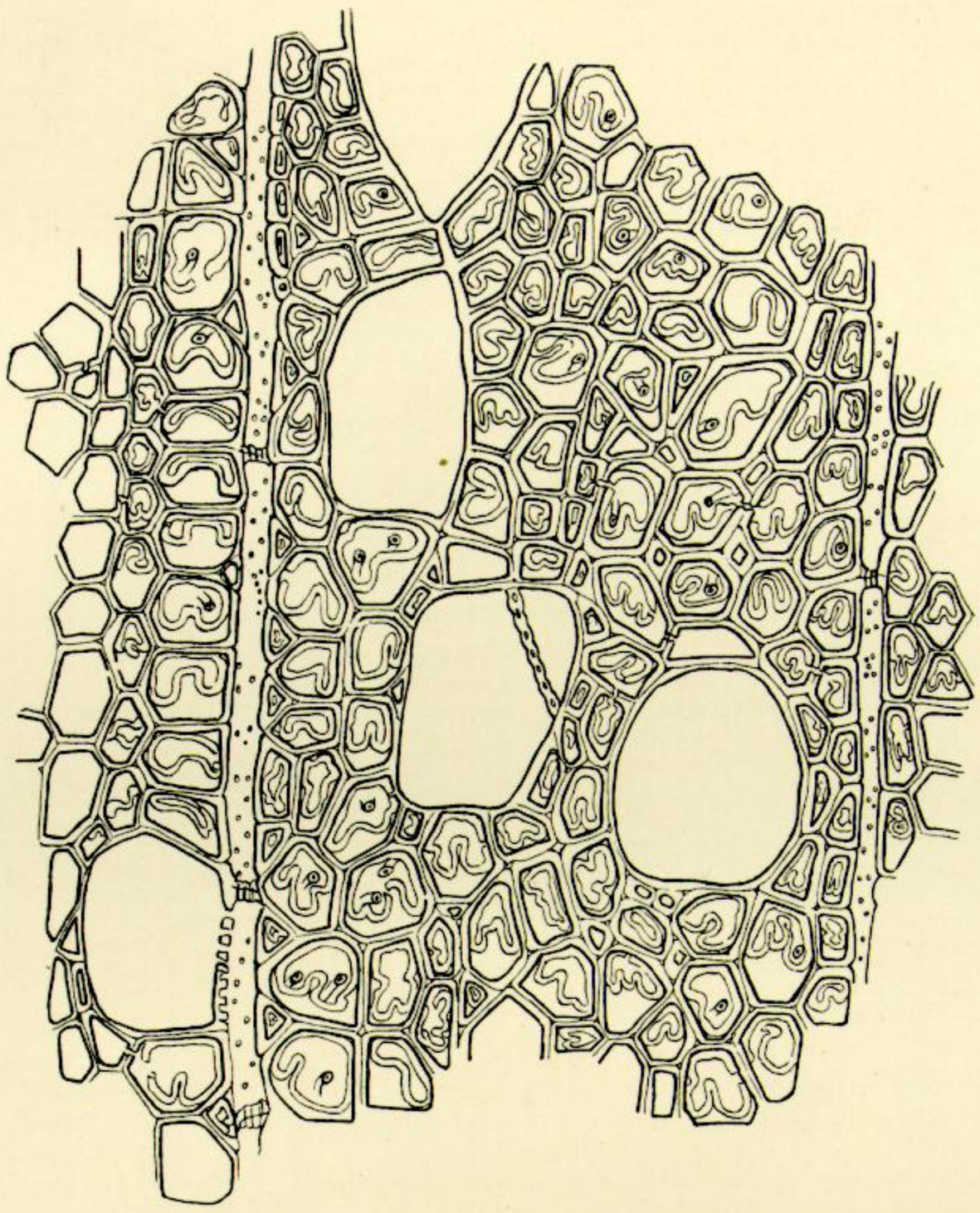
Fig. 17.



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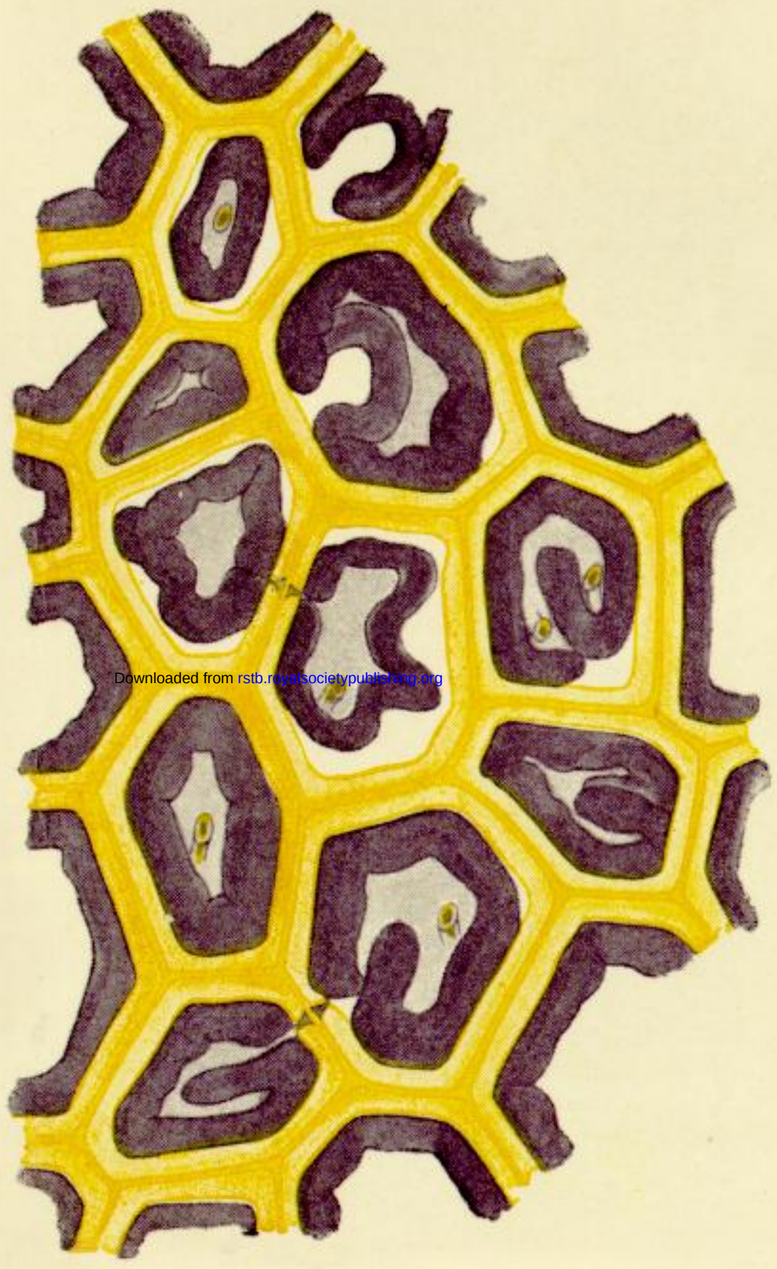

Fig. 19.

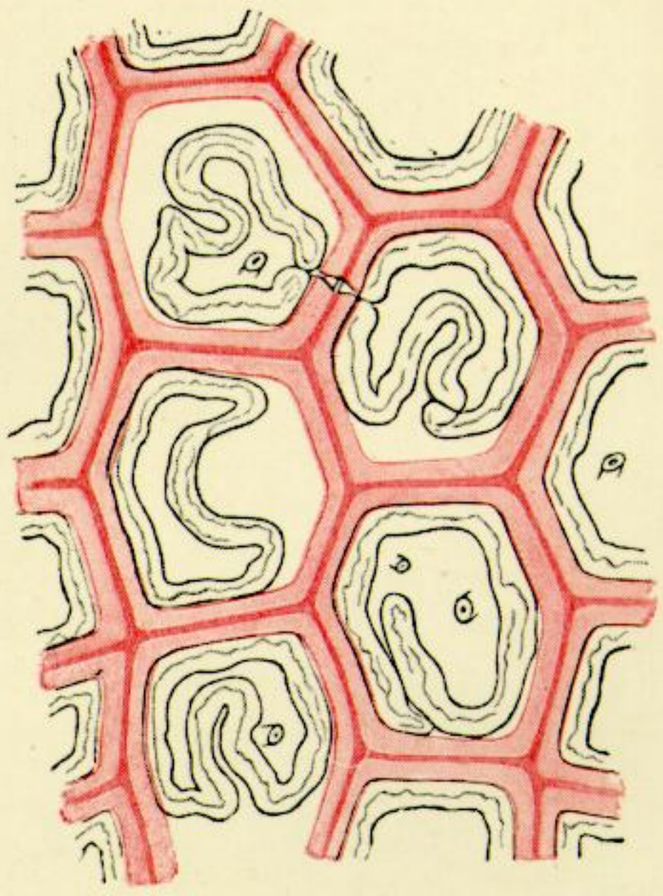

PLATE 21.

Fig. 17. Similar section of wood invaded by the fungus, and exposed 5 months to its action. The inner walls of the wood elements are seen swollen and wrinkled.

Fig. 18. Portion of a similar section in chlor-zinc-iodine. The swollen inner cell-wall layers turn blue or violet, while the enclosed hyphæ and the still lignified parts of the walls are yellow.

Fig. 19. Similar section in phloroglucin and H.Cl. The lignified parts are coloured rose-red, while the delignified swollen inner layers are not coloured.