THE

JOURNAL OF BOTANY

BRITISH AND FOREIGN

EDITED BY

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VOL. LVI.

LONDON
TAYLOR AND FRANCIS
RED LION COURT, FLEET STREET
1918.

and long-ciliate ligule-tips. These points all agree with the description of var. *vestitum* (Journ. Bot. 1911, p. 353); and they are even more marked in character than Ley's specimens from High Street, Westmorland, in my herbarium, which belong to this. Not previously known from Scotland.

THE RELATION BETWEEN GONIDIA AND HYPHÆ IN LICHENS.

By A. N. Danilov.

[The paper of which (by permission of the Director of the Imperial Garden) a translation follows was published in the *Bulletin du Jardin Impérial Botanique de St. Pétersbourg*, tom. x. livr. 2 (1910). The translation has been made by Messrs. R. Paulson and Somerville Hastings, who, while feeling that the importance of the paper justifies its publication in a language more generally understood by botanists than the Russian in which it originally appeared, are not to be understood as accepting all the author's conclusions. The original is accompanied by plates and figures, which it has not been thought necessary to reproduce.

Since the publication of the paper, Elfving has printed (Acta Soc. Sci. Fenn. xliv. no. 2: 1913) a further account of his investigations in which he supports his view that the gonidium of a lichen is genetically derived from the hypha.—R. P.]

The present paper was undertaken with a wish to throw some light on the interesting subject of the association of the alga and fungus in a lichen, for there has been some uncertainty regarding this matter. The chief aim has been to verify and explain those observations which led Prof. Elfving of the University of Helsingfors to revive the views of Wallroth on lichens.

In 1905 at the meeting of naturalists and doctors at Helsingfors, Elfving presented a report in which he maintained that the fungus and the alga in a lichen are not two independent organisms, as Schwendener declared, but two different stages of development of one and the same fungus. According to Elfving, the so-called algal portion differs from the hyphal part of the fungus in that the hyphæ undergo more or less complex changes. The hyphæ grow rapidly and throw out spherical cells, which, as they develop, resemble the cells of algæ more and more, in size and shape: later on these cells assume a green colour and finally break off entirely from the mother hyphæ. These separated greenish cells become the gonidia, and multiply by division within the thallus of the lichen. The process of separation of the gonidia from the hyphæ only takes place, according to Elfving, in the spring. Elfving carried out investigations on *Peltigera canina*, *Evernia*, *Parmelia*, *Ramalina farinacea*, *Usnea barbata*, *Lecanora peralbella*, *Cladonia rangiferina*, and on other lichens gathered in early spring, from which he obtained preparations for microscopical examination, which led him to these very definite conclusions. He exhibited his preparations after he had presented his report, and,

according to the late Prof. M. S. Voronin, they certainly gave the impression that the hyphæ develop algal cells.

The question is one of extreme interest. Acting at the suggestion of A. A. Elenkin and with his closest assistance in the spring of 1909, I made observations with a view of explaining what Elfving had seen in his preparations, as he had apparently obtained a sufficient foundation for the publication of conclusions entirely opposed to the views on the nature of lichens strongly held since the time of Schwendener, viz. a fungus in symbiotic relationship with an alga.

Before we come to the explanation of the facts observed, it will not be superfluous to indicate the materials on which my investigations were made. From the 20th April to the end of May, material was being collected in the neighbourhood of St. Petersburg, and afterwards throughout the whole summer in the Vitebsk province—an interval of two weeks occurred between the former and the latter collections. The observations were made principally on *Evernia prunastri* and *E. furfuracea*. In addition, the following were also examined for purposes of comparison: *Parmelia sulcata*, *Ramalina farinacea*, *Usnea barbata*, *Cladonia rangiferina*, *Xanthoria parietina*, *Lecanora angulosa*, and also the separately growing *Chlorococcum*. Part of the material obtained was fixed, immediately after collection, in 75 % or in absolute alcohol.

The first observations were made on sections cut with a razor, both from fresh material and from that fixed in spirit. The material was prepared for examination by being put through absolute alcohol and xylol and was embedded in paraffin; the sections varied from 1 μ to 15 μ in thickness; the 1 μ sections were however unsatisfactory, but fully satisfactory results were obtained from the 2 μ sections. Some of the sections, stained and unstained, were examined in a liquid medium, water or glycerine. In such cases the following method was employed in every instance: the section was first examined as a whole, then broken up under a cover glass, by careful tapping and pressure, in order that the separate hyphæ and gonidia might be observed more minutely. Various stains were used—carmine, fuchsin, methyl green, methyl blue, safranin, hæmatoxylin. Of these the most suited to my purpose were a mixture of fuchsin and methyl green, made acid with acetic acid. During both staining and clearing, the preparations were kept on slides in beakers filled with the same solutions as those used for staining in bulk. I at first washed with spirit of a different strength, but found it very difficult to ascertain the precise moment at which to cease washing, for decolouration takes place very rapidly when the spirit is either too strong or too weak. Spirit greatly diluted with water I found impossible to use for the work, as the preparations would absorb too much water and become impossible to fix. For this reason I began to use a solution of glycerine in strong spirit. In this solution decolouration proceeds less rapidly and thus gives a better opportunity to cease washing at the moment when the differentiation of the various parts of the preparation has attained its greatest distinctness. The fixed sections were mounted in glycerine jelly and the unfixed ones in glycerine. The most interesting preparations were examined with a Zeiss apochromatic objective magnifying 1000 diameters.

The problems presented at the commencement of the work had to be divided as follows: 1. What is the nature of the gonidia? 2. How close is the relation between the hyphæ and the gonidia, and what is its character? 3. Is the point of view of Elfving in any way justified? 4. Do the hyphæ penetrate the algal cells, *i. e.* do they form what is known as haustoria? 5. What is the further development of the hyphal extensions after entering the gonidia, and what is the effect upon the gonidia of their invasion by the haustoria? The following results obtained from these investigations more or less answer the questions.

In a thin section, made from the living thallus, certain areas can be clearly distinguished in which chloroplasts are enclosed in an algal cell wall. Side by side with the normal spherical cells with a well-developed cup-shaped chloroplast can be seen pale green cells obviously differing from the normal. The character of these abnormal cells is seen in the paleness of colour, the contracted and deformed chloroplast, and even in the entire absence of contents, so that one sees even empty cellulose envelopes of these gonidia. I cannot consider this a normal condition of the cells which will be described as "pale gonidia." They have a protoplasmic structure from which chlorophyll is entirely absent; these gonidia, in an altered condition, are often found in considerable numbers and present many successive changes from the normal green cell to the empty gonidial envelope. In this connection A. A. Elenkin investigated abundant material, and observed that the disorganisation of the gonidia increases in the deeper parts of the thallus, where the zonal character is found to be the result of the distribution of the dead remains of gonidial cells. These gonidial remains are scarcely noticed among the green gonidia and hyphæ, but it is only necessary for a reagent ClZnI to come into contact with the cellular tissue, to bring them distinctly into view in the form of violet-coloured spherical envelopes and shapeless little masses whose connection with the gonidia, without the use of a reagent, would be absolutely impossible to imagine. It is interesting to note that in the staining of the gonidia, different sections of the same thallus appear very dissimilar. In many preparations a whole series of changes in the gonidia could be observed, from deep staining to perfect absence of it. As far as it was possible to judge, the gonidia showing the greatest change from the normal were less deeply stained.

"Pale gonidia" at first sight appear to differ from normal ones only in the absence of colour, but a more careful examination proves beyond doubt that the difference goes considerably further. The contents of the white gonidial cell stains practically the same as that of the hyphæ and are seen as small masses or even scattered about in the form of a number of knots. "Pale gonidia" are also found in which the contents have become separated into tiny spheres united into a single mass. On the addition of ClZnI the "pale gonidia" undergo the same changes as the normal green ones, the envelopes becoming violet and the contents brown from the iodine, though perhaps not to so marked an extent. When a watery solution of methyl green is applied to a fresh section from the plant, the contents of the "pale gonidia," as also those of the hyphal threads, takes a

turquoise-green colour, and the pale gonidia then stand out very clearly in contrast with the normal green, their contents becoming sharply outlined. It is interesting to note that pale cells are met with in freely growing *Chlorococcum*. They appear to a very large extent to be invaded by fungoid hyphæ in some specimens. There is every indication that these pale cells of the *Chlorococcum* are identical with the "pale cells" of lichens, but, as far as I know, nothing has been said about them in the literature of the subject. It is evident that when Prof. Elfving examined these "pale gonidia," he inferred that gonidia were formed by the transformation of the extremities of hyphal cells into spherical masses.

Regarding the connection between the hyphæ and the gonidia in the substance of the lichen, the following facts must be noted. Thin microscopical sections make it possible to observe with certainty that all the gonidia have not a close connection with the hyphæ. Some, generally a small number, are either not in contact at all with the hyphæ, as is particularly the case in the more or less strongly developed groups of gonidia; or the contact is so superficial that pressure on thin sections, by means of the cover glass, causes a certain number of the gonidia to become separate from the thallus: so that, even with the most careful observation, it is absolutely impossible to detect any adherent particles of hyphæ or of anything else. Such gonidia, in the great majority of cases, appear to be well-developed spherical cells with a regular cup-shaped chloroplast. The fixed sections of such gonidia, from which chlorophyll has been withdrawn, take a rather deeper tint on staining. But the correct interpretation may be that most gonidia are more or less closely connected with the hyphæ, varying from a simple contact to actual fusion with them.

Most often the gonidia are found in a network of hyphæ, as the figures in every textbook of botany will show. The surrounding hyphæ are stimulated to bud and many fine hyphal threads cover the gonidia all over, and in this way hold the algal cells in a hyphal envelope. This fact, in my opinion, speaks for itself.

The conditions appear to be quite natural; the hyphæ being brought into contact with the gonidia acquire an abnormal generative power which shows itself in an abundant budding and in the formation of short processes, generally filled with protoplasm, spread over the surface of the gonidial cells and extending from one algal cell to all the others in the neighbourhood. In this way whole groups of gonidia are commonly observed which are woven and tied into a single mass by short branches of the hyphæ. These offshoots of the hyphæ, first described circumstantially by Bornet, were named extracellular haustoria by Schwendener.

The above general description of the connection between hyphæ and gonidia must be supplemented by a more detailed examination of one particular example. Elfving made the following observation, which he misinterpreted. From the parts of the hyphæ spread over the gonidial layer, and also from the other hyphal masses, pear-shaped swellings extrude, which adhere so closely to the gonidia that they appear one with them. Such swellings generally leave the hyphal branches as lateral offshoots, but sometimes the hyphæ terminate as

club-shaped expansions with gonidia at the end. The club-shaped branches are often met with. They are sometimes in groups, but are always short. If this structure be examined without staining the gonidial cell-walls, one is led to imagine that the swollen part of the hypha has budded off an algal cell. Such an impression is confirmed by the observation that the hyphal swellings just described have a somewhat thin envelope and the protoplasmic contents show a feeble turquoise-green colour to the light. The suggestion that the gonidia are budded off from the hyphæ is increased where a few rounded hyphal swellings are next in order to the end of the club-shaped expansion adhering to the algal cell. In this case a superficial observation may unconsciously put the consecutive swellings not merely into connection with one another, but will include in this consecutive series the gonidium at the end of the chain, the more so, perhaps, because of the close connection with the hyphal branches, for even the most minute examination fails to show the line of union without the aid of reagents. This chain-like appearance can easily be observed both in those gonidia attached to a hypha and to those beaded in a row should they be close to a hypha. In such cases as I observed, the nearest gonidium was the least coloured with chlorophyll and the gonidium at the end of the row the most highly coloured. I shall not go so far as to assert that Elfving came to his conclusions on the basis of the above connection of the hyphæ and gonidia in the lichen thallus, as no drawings accompanied his paper; but in any case the microscopical drawing I have described might well serve to illustrate it.

The misinterpretation respecting the hyphal origin of the algal cells must finally be abandoned if reagents are applied to the specimen, for the staining reaction will be only apparent in the gonidial envelopes, leaving the hyphal cell-walls unchanged. Personally I always prefer ClZnI. On the application of this reagent the gonidial envelope became violet and the limits of the gonidial and hyphal junction were sharply defined.

There can be no doubt that the hyphal club-shaped swellings attach themselves to the envelope of the gonidia. In the first place the attachment takes place, apparently, only on a small part of the gonidial envelope in comparison with the later development. The club-shaped swelling, as it continues to grow, then increases the area of contact, spreading its broad base over the envelope of the gonidium. This may easily remain unnoticed, making it necessary after the use of ClZnI for the gonidia to be viewed in different planes. Besides this, the club-shaped swellings may give rise to buds as full of protoplasm as the swellings themselves. The secondary buds may be obscured by the neighbouring gonidia, thus forming, out of the gonidia and the thick hyphal distention filled with protoplasm, even more shapeless masses than the network of gonidia and short hyphal cells described above.

Compared with the protoplasm of the gonidia, the protoplasm of the hyphal distentions is less easily stained: it takes the stain only after a comparatively lengthy immersion in the staining fluid and loses it more readily when washed. Taking into consideration the

definite shape of the distentions above described when compared with the other hyphal threads, and their constant connection with the gonidia, no alternative remains but to consider these distentions definite organs of the plant, playing an important part in the relationship of the fungus to the green algal cell. Both the internal and external appearance of the gonidia to which these hyphal modifications are found to adhere is very varied: though perfectly normal as far as shape and external appearance are concerned, they are more or less changed internally—some are green, some pale, some devoid of protoplasmic contents.

In this connection three forms of external contact between gonidia and hyphæ may be described: (1) The absence of all superficial attachment or contact in the most literal sense—described by Schneider as "simple contact"; (2) Contact in the case of the surrounding of the gonidia with budded hyphal cells, a nearer form of contact, and, lastly; (3) Various stages of attachment, including in their number the union of the club-shaped hyphal distentions when the area of attachment and its strength are particularly significant. In Schneider's terminology, the second and third cases may be described as extracellular haustoria. The attachment of the hyphæ and gonidia is not confined to the external union of the hyphal and gonidial envelopes, the union is considerably closer. As far back as 1893 Hedlund showed, in relation to some drawings of *Lecanora*, *Lecidia*, and *Micarea*, that the hyphæ penetrate the gonidial envelopes in the form of club-shaped distended branches and stimulate the division of the algal cells into two daughter cells. Schneider gave very convincing facts concerning the penetration of the hyphæ into the algal cells, giving the penetrating hyphæ the name of intracellular haustoria. According to his description, this penetration has been observed, so far, in some specimens of *Cladonia*, *Parmelia*, and *Stereocaulon*, and consists in the haustorium piercing the envelope and entering the gonidial cell; but while this is taking place the hyphæ are branching within the cell, between the protoplasmic contents of the cell and the cellulose envelope, but not touching the contents. Peirce in 1899, as the result of his investigations, came to the conclusion that the hyphæ develop haustoria which penetrate the algal cells and absorb the protoplasmic contents of the gonidia, leaving only the empty cell-wall.

These intracellular haustoria were also investigated by Elenkin, and are described in a series of papers devoted to the question of the endosaprophytism of lichens, considering as of secondary importance, it is true, those occurring after the disappearance of the gonidial protoplasm, under the action of some enzeme-like substance acting for this purpose. The numerous microscopical drawings which I have made, to be described later, are, I think, sufficient evidence to show beyond all doubt that the hyphæ do actually penetrate the gonidia and in doing so take root in live algal cells and not only make their way within the cell-wall to the edge of the protoplasm, but actually penetrate the protoplasm itself, piercing it in all directions by slender processes. I believe this to be sufficiently proved both by the drawings of microscopical preparations appended to this paper [in its

original issue] and by the microphotographs. The degree to which the hyphal protoplasm absorbs the stain, compared with the gonidial, permits the possibility of differential staining, which results in the hyphal protoplasm standing out distinctly against the darker-coloured ground of gonidial substance.

In the hyphæ which are closely adherent to the gonidial cells, lateral offshoots extend from the area of contact and penetrate the cellulose envelope. The hyphæ having penetrated the algal cells change in appearance, and I believe that they do not fulfil the same functions. It seems to me necessary to distinguish two types of intra-gonidial hyphæ, standing in a close genetic relation to one another. The one to which the term haustorium is especially appropriate, having penetrated the gonidial envelope in the form of thin threads, encloses the protoplasm of the gonidia in a fine network. At first, following the perforation of the cell-wall, the network of tissue extends only over the surface of the protoplasm. During this process, it is sometimes possible to observe the formation of a very complete network of these hyphal filaments between the cell-wall and the protoplasm. In their further development the intragonidial hyphæ extend themselves into the protoplasm itself, piercing it in every direction. This first type of extremely delicate intragonidial hyphæ has the appearance of thin gelatinous branches—to use the accurate expression of Schneider,—which appear to me to be without hyphal envelopes: these thin branches, as may be supposed, are formed only of fungus protoplasm, the cell-wall being either entirely absent or so thin as to be beyond perception. It may be supposed that the hyphæ develop in this type of intracellular haustoria thin threads of protoplasm, or that the hyphal envelopes are destroyed through the activity of the living gonidial cells, so that only the hyphal filaments remain, unaltered in character, but minus the cell-wall.

Before passing on to the second type of intragonidial hyphæ it is necessary to observe another kind of intragonidial formation, for this, as far as can be determined, appears to have no connection with the intracellular haustoria. At first sight this formation resembles drops of oil adhering to the periphery of the gonidial protoplasm in the shape of tiny ridges. The approach of a light shows them to be of a feeble turquoise-green colour, *i. e.* their refraction is the same as that of hyphal protoplasm. As far as is known, the literature of lichenology contains no description of these. At first I took them for drops of oil, but their persistence in the fixed microscopical preparations after the application of several changes of absolute alcohol to the specimen, followed by xylol, suggested the idea of more closely examining these problematical drops. In the first place, I had to assure myself that they were not oil drops. With this object in view, portions of fresh thallus, and also sections cut with a razor, were immersed in absolute alcohol for four days, the spirit being changed daily. Some were then transferred from the alcohol to chloroform, others to ether. The drops suspected of being oil were not dissolved: they did not give an alkaline reaction to alcohol in which they had been kept. A one per cent. solution of osmic acid, in spite of prolonged application, only gave them a brownish tint. This stain

brought out something of even greater interest than that which was sought: viz. extremely thin filaments embedded in the protoplasm, and, as it were, attaching to it the beaded prominence already described. These last stain less deeply than gonidial protoplasm, but nevertheless take a deeper colour that the hyphal protoplasm. In preparations kept long in a colouring agent (*e. g.*, during some days in weak solution) the beaded prominences retained their colour, while that of the hyphæ was lost on being washed.

It might naturally be supposed that these beaded prominences were some form of the gonidial protoplasm, but the detailed investigations which were undertaken must convince one that they are foreign organisms. As a rule, they are found, as described above, at the periphery of the protoplasm between the gonidial envelopes and contents, while in some cases they are somewhat separated from the protoplasm. In the latter case it may often be observed that thin filaments extending from them become embedded in the protoplasm, and also that the beaded distention is fixed on a thin stalk. Sometimes these are embedded in the protoplasm itself. A circumstance deserving attention is that, in the pale gonidia described above, the contents consist of a knotted and confused mass which, as I believe, is identical with the beaded distentions on the protoplasm of the green gonidia. On thin microscopical sections showing beaded distentions on their protoplasm the following picture is presented with the utmost distinctness: the gonidial protoplasm is intersected by fine threads forming in places knots of every size and shape; the beaded distentions are, apparently, derived from similar knots of filaments piercing the gonidial protoplasm, but differ in their much larger size. They are distributed especially on the periphery of the protoplasm. Their generally rounded shape becomes irregular, owing to the filaments which attach them to the protoplasm.

The morphology of the beaded distentions described above and their connection with the network of intracellular haustoria, their existence in gonidia, whose contents are obviously wanting, are sufficient in my opinion to prove that these distentions are not a formation of the gonidial cells themselves. The signs indicated, and the similar staining reactions with that of the hyphal protoplasm, must fix them as products of intracellular life. Something in the nature of the beaded distentions appears in the germination of spores in certain genera of lichens having large spores, such as *Thelotrema*, *Megalospora*, *Ochrolechia*, and *Pertusaria*, although so far it is impossible to draw a complete analogy between the formation I have described and the globular shoots on the germination of spores of the above-mentioned plants, but the analogy demands recognition. According to the investigations of De Bary the spores of the above-named lichens on leaving the endospore develop shoots which take a globular form. These shoots as they further develop burst through the exospore as promycelia, the threads of which, if they do not come in contact with algal cells become part of the substratum. According to the observations of A. Moller, two types of hyphal threads arise from the promycelium which are quite distinct in physiological function. Some typical threads become embedded in the substratum,

the others give off branches where they come in contact with algal cells and thus become closely connected with them. If the hyphæ on leaving the promycelium are prevented from fulfilling their function, they die. Generally speaking, the expansion of the hyphal filament into separate spherical distentions does not appear as anything exceptional.

Of especial interest in this connection are the descriptions of Zukal, and then Fünfstück and Bachman's so-called spheroidal cells, most often met with in lichens on a substratum of limestone (calcivores). These were considered first as reservoirs of stored material and secondly as secretions. But if the spheroidal cells appear merely as outgrowths, consisting of substances not required for the fungoid organism, then perhaps the analogy between the distensions of intragonidial hyphæ I have described and the spherical cells is entirely superficial: similarly, the analogy between the spherical shoots of the germinating spores of *Pertusaria communis* etc. and between the same spheroidal cells must also be superficial. This interesting question demands further investigation.

Let us now pass on to the second type of intragonidial hyphæ, which differ from those described above by the obvious presence of a cell-wall. This type of intragonidial hypha is, as a matter of fact, in no way distinguishable from the ordinary hyphal thread. This class of intragonidial hypha was studied by Elenkin in *Lecidea atrobrunnea*. A very large number of gonidia, especially the largest, appeared as if crammed with thick-walled hyphæ containing drops of oil highly refractive in appearance. I have observed this type of intragonidial hypha only in those gonidia which have lost all or nearly all their protoplasm. Instead of the usual contents, in this case the gonidia were filled with hyphæ, which lay as a closely packed mass inside the gonidial envelope.

Having stated the facts observed, let us now endeavour to explain their true significance, and to deduce some conclusions as to the reciprocal relations of the different parts of a lichen, taking care not to lose sight of the fact that the following, like all deductions from observations made, needs experimental proof for its complete justification.

The direct contact of the hyphæ with the cell-walls of living gonidia makes possible an exchange by osmosis or food material between the fungus and the alga. The extended network of the hyphal cells around the gonidia and the enlargement of the surface of contact by means of the formation of a club-shaped hyphal distention, which I have noted, would, without doubt, to a large extent assist such an exchange; but the entire absence in this case of experimental proof of the possibility of osmosis through the hyphal and gonidial envelopes forbids a definite conclusion on this point. It will readily be granted that when two cells of different character come into contact, they may be equally pervious, or one of them may be semipervious, or, again, both may be semipervious in regard to the same substances, or both semipervious, but in regard to different substances, thereby creating a possibility of osmotic filtration of some substances into the fungus and others into the alga. In short, the

direction of osmosis in the case under consideration has yet to be confirmed, but the structure of the hyphæ as a network of long capillary tubes may easily allow, by a very similar physical process, a transference of food-substances in the hyphal filaments, on account of the uninterrupted capillary attraction of the hyphæ.

If it is impossible to speak with certainty of the importance or otherwise of the contact of the gonidia and the hyphæ, it is at least certain that the penetration of the gonidial cells at a later stage is attended with obviously fatal consequences to them. Their contents become deformed, contract, and finally the algal cell entirely perishes. As a consequence of this intracellular action, the algal cells sometimes cut off daughter cells, which appear free from haustoria. Perhaps by this method some of the algal cells, which are found in comparatively good condition, having freed themselves from the haustoria, continue their growth; or, possibly, by some other method the same result is attained. In spite of the assertions of Hedlund, Schneider, and Peirce, the algal cells do not always free themselves from the invading haustoria by fission into daughter cells, for the hyphæ, rapidly spreading out inside the mother cells, frequently destroy many of the gonidial daughter cells. The haustoria having penetrated into the gonidial cells appear devoid of cell-wall, perhaps owing to the action of the living protoplasm of the invaded gonidia. The protoplasmic hyphal filaments, feeding on the gonidial protoplasm form a network at its periphery, and gradually forcing their way form local swellings, or beaded distentions which gradually collect together and perhaps mark a definite stage of the development of the fungoid element in its intragonidial life. As the gonidial protoplasm disappears, the hyphal formations continue their development further and further, and when the contents of the gonidia have been entirely absorbed, the plasmatic branches from the hyphæ are extended until they fill the whole gonidial envelope. This phase of the development of intragonidial hyphæ seems to me to correspond with the "pale gonidia" described above. It cannot easily be stated exactly what further happens to these hyphal formations enclosed in the cell-wall of the destroyed gonidia.

I examined these gonidial envelopes, the contents of which consisted of numerous separate and disconnected masses similar to those contained in the interiors of the "pale gonidia." Some of these—generally speaking, those which were not deformed—were found lying in the perforated parts of the gonidial envelope, possibly as the result of pressure from the cover-glass. It is possible that such formations present us with some hitherto unexplained stage of fungoid development, or perhaps they, having no other function to fulfil save that of haustoria, become themselves covered with a thick envelope on reaching the inside of the gonidial cell-wall. Just as, in my opinion, occurs to the haustoria in other cases, when the gonidial protoplasm has been disorganised or destroyed. As the outcome of the above-described stage in the development of the haustoria we are in a position to observe such appearances as that of a gonidial envelope absolutely packed with closely interwoven hyphæ. The superficial fusion of the intragonidial hyphal growths with the external hyphæ

observed in the form of superstructure has evidently taken place as the result of the concrescence of the new thick envelopes formed on the unprotected surfaces of the internal haustoria with the old envelopes of the internal hyphæ. Developing further, the intra-gonidial hyphæ leave the gonidial envelopes and invade the neighbouring gonidia.

The processes explained above lead to a general conclusion which cannot easily be reconciled with the prevailing opinion on the question of the internal relations of the different components of a lichen. The distinct and definite individuality of a lichen as a plant organism, in view of the inexpressibility of the physiological relations between fungus and alga, may readily cause the lichen to be regarded as a plant not substantially different from any other assimilating plant. Wallroth held such a view of lichens, and Minks explained the facts he had observed from the standpoint of the same theory, pushing it to extremes. Elfving belongs to this group, owing to his opinions on the destruction of the gonidia by the club-shaped hyphal distentions. But, since the time of Schwendener the position has not only been made clear, but many times confirmed by experiment, that the lichen is a two-fold organism, consisting of two components, belonging to different classes of plant-systems, and all theories contradicting this basic proposition must be regarded to-day as errors.

Considering the lichen as an organism made up of two constituents, and finding it impossible to reconcile the well-being of the whole with antagonism of the constituent parts, De Bary in 1879 formulated the theory, prevalent to this day, of mutual symbiosis. By this the co-existence of fungus and alga in lichens was explained by their mutual needs and services resulting from their differences in organisation giving rise to joint existence to the mutual benefit of each, and as the final consequences the production of an organism with the power of resistance of a lichen.

The facts described above of the undoubted invasion of the gonidial protoplasm by the haustoria, and the destructive effect produced by the latter are entirely in accord with the theory of the mutual assistance of the fungus and alga. As far back as 1897, Schneider described and classified haustoria by means of which, in his opinion, the fungus in the end absorbs the contents of the gonidial cells. Somewhat later Peirce expressed an even more categorical conviction that the haustoria make use of the gonidial protoplasm, leaving the empty cell-wall. In spite of such views both the above lichenologists hold the mutualistic theory. Peirce acknowledges that he is unable to prove that the alga derives any benefit whatever from its co-existence with the fungus, but holds that the fungus portion of every lichen is absolutely dependent upon the gonidia for all its non-nitrogenous food. It is difficult to understand how the gonidia obtain nitrogen from the fungus, for Peirce himself declares that the hyphæ make use of the entire contents of the gonidial cells, as we have seen from the facts described above.

The numerous investigations of Elenkin also undoubtedly support the fact of the invasion of the gonidia by the fungus hyphæ. If the fungus attacks its partner and feeds on its body, it appears decidedly

difficult to speak of mutual service. Even in 1868 Schwendener in his investigations on the colour-changes of the blue-green algæ of lichens came to the conclusion that the fungus was a parasite upon the alga. Bornet also regarded the relationship between the fungus and alga as one of parasitism of the former, owing to the results of his convincing investigations on the destruction of the blue-green algæ by fungus hyphæ. Experiments with prepared cultures did not really give sufficient reason for the assertion of mutualism or antagonism between alga and fungus, but, nevertheless, gave ample evidence of their difference in composition, owing to their actions on independent substances. The experiments of Moller, Bonnier, and Haysen, pupils of Elfving, show that the fungus in the absence of algæ either does not develop at all or develops badly, and that spores do not develop a mycelium in the absence of gonidia. If, however, gonidia are introduced to such cultures, the mycelium begins to develop much better. Is not this direct evidence of the fact that the vitality of the fungus has been reduced to such an extent on account of its parasitic mode of life and that it has lost the power of living independently? Pure gonidial cultures separated from the thallus of the lichen lead to an exactly similar conclusion. In 1867 the experiments of Prof. Faminsen, together with those of Prof. Baranetsky of the University of Kiev, showed that if a lichen is left in water the fungus web becomes rotten, while the alga continues to live, grow, and multiply both by fission and by zoospores. The splendidly arranged experiments of Artari with pure cultures of gonidia from the thallus of *Xanthoria parietina* and *Gasparrinia murorum* show clearly that the gonidia are capable of existing independently, growing on a substratum containing the necessary mineral salts, but not developing so well as on media containing pepton and sugar. In this manner the two components closely united in the symbiosis of a lichen display distinctly varied characters when living independently, and consequently their relations to each other cannot be such as one would expect in a case of simple mutual symbiosis. The persistence of the gonidia is present to such an extent in a lichen thallus that it was sufficient to serve as the foundation of Elenkin's theory of the endosaprophytism of the fungus on the alga, while the facts described in the present work impress one even more strongly with the passive part played by the gonidia and clearly show the parasitism of the fungus. There is this difference between the gonidia and free chlorococci, that the former develop better in nourishing media containing substances with complex molecules (peptones), as is shown by the experiments of Artari. These circumstances can in no way be used as an argument in support of the mutualistic theory. The conditions of life of the gonidia competing with the fungus inside its mycelium might easily react on the algæ in some physiological manner, creating amongst other things that power of absorbing organic food more readily than of assimilating it from inorganic substances. It is possible that in the thallus of the lichen the gonidia make use both of the prepared peptones and of some of the organic material of the fungus, and that these circumstances, relieving the gonidial cells of some of their vital processes, assist them in their struggle against the

parasite, but in consequence of the changed conditions of life of the parasite the gonidia cannot in any way be compensated by this service on the part of the fungus. Such a correspondence in the physiological relations of fungus and alga as the reciprocal supply of each other's deficiencies in the numerous necessaries of the vital activities of the component parts, as is implied by the mutualistic theory, cannot be imagined.

In conclusion, we should note that the fact of the more or less certain absence of the cell-wall of the haustoria inside the living gonidia, while the protoplasm of the fungus carries on its physiological function in immediate contact with the gonidial protoplasm, recalls the teaching of the famous mycologist Eriksson. Perhaps the gonidia on becoming separated from the mother cells already carry in their protoplasm some beginning of the fungus, in this way nursing its own parasite. May not Eriksson perhaps be right in his supposition? It is unfortunate that this important theoretical and practical question has yet to be solved, and has not received its fair critical investigation in spite of the fact that its importance makes it deserve the most searching examination.

EAST WILTSHIRE MOSSES AND HEPATICS.

By C. P. Hurst.

Mosses.

The following mosses were gathered in the winter and spring 1917–1918 around Great Bedwyn, which lies about seven miles to the south-east of Marlborough in East Wiltshire, and is near Savernake Forest and not very far from the county boundary between Wiltshire and Berkshire. This border village is situated on the soft white *Marsupites testudinarius* zone of the Upper Chalk near the apex of the London Basin, but the calcareous *facies* of the moss flora is very much masked by the occurrence of Eocene outliers and Pleistocene layers of sand, gravel, and clay in the neighbourhood. The Kennet and Avon Canal passing through Great Bedwyn divides Wiltshire into the two vice-counties North Wilts (v.c. 7) and South Wilts (v.c. 8). All the localities and a number of the mosses are additional to those in my papers "East Wiltshire Mosses" and "County Lists of Mosses" in this Journal for 1916, pp. 17–24, 262–274. The arrangement and nomenclature of the *Census Catalogue of British Mosses* (1907) have been followed, and I am much indebted for kind assistance and notes to Messrs. H. N. Dixon, H. H. Knight, and W. Ingham. The list contains sixteen new vice-comital records for Wiltshire, the greater number of which were made close to Great Bedwyn, and forcibly illustrates the maxim that the more a district is examined the more it produces. 7 = North Wilts; 8 = South Wilts; c.fr. = with fruit; * = new vice-comital record.

Polytrichum nanum Neck. 7*. C.fr., rather plentiful in an old excavation for gravel near London Ride, Savernake Forest; abundant