ANNALS OF BOTANY

VOL. XIII

Oxford
PRINTED AT THE CLARENDON PRESS
BY HORACE HART, M A.
PRINTER TO THE UNIVERSITY

Charles Darwin

ANNALS OF BOTANY

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VOLUME XIII

London

HENRY FROWDE, M.A., AMEN CORNER, E.C.

OXFORD: CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1899

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No. LII, December, 1899.

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THE BOTANICAL WORK OF DARWIN.

CHARLES DARWIN'S botanical work falls naturally into two periods, of which the first ends with the publication of the Origin of Species in 1859, while the second begins with the appearance of the Fertilization of Orchids in 1862.

The chronological grouping, however, only holds good to a limited extent, and the work can be more accurately classified according to its nature and aim. Thus the botanical part of the Variation of Animals and Plants is principally characteristic work of the first period, though the book appeared six years after the Fertilization of Orchids; but here, as in many other instances, the date of publication is no guide as to the date of research, and, as a fact, the work in question was largely the fruit of earlier years.

In the first or evolutionary period, plants, like animals, were the material on which he tested his 'species theory.' In the second period he worked in detail at varied problems in plant physiology.

I believe the classification of his work into evolutionary and physiological will be a convenient basis for discussing his results, although a somewhat vague line separates the classes. Thus, his original interest in the fertilization of flowers was purely evolutionary. 'I was led,' he says, 'to attend to the cross-fertilization of flowers by the aid of insects, from having come to the conclusion, in my speculations on the origin of species, that crossing played an important part in keeping specific forms constant[1].' But he continued to study the means of fertilization of Orchids, &c.,

[1] Life and Letters, Vol. i, p. 90.

principally because of his irresistible desire to understand the machinery of living things. It is true that in elucidating the machinery he supplied the most brilliant evidence in favour of the validity of natural selection as the great moulding force in Nature. But I do not think this was his object, it was rather a by-product of work carried on for the love of doing it. It is true that he felt the importance of the evidence in regard to evolution, for he says:—'It will perhaps serve to show how natural history may be worked under the belief of the modification of species[1].'

During the long years of the first period he was learning to know plants as he used them in the building of his theory; and then the tables were turned, and the theory served him as a powerful engine to break still further into the secrets of plants, and the engine seemed all the more marvellously effective because he employed it on problems already half conquered in his earlier work. Instances of the pervasion of the second group of works by evolutionary characteristics will be given later.

Evolutionary Period.

I shall not attempt to deal at any length with the Botany of the evolutionary period; the nature of the work may be fairly estimated by a study of the Origin, first edition, 1859; Animals and Plants, 1868; and the Life and Letters. Taking the Origin first, a striking feature is the use made of a mass of botanical facts in parts of the science in which he had no special first-hand knowledge. Mr. Huxley has pointed out that in Zoology, by the eight years given to the Cirripedes, he had made himself a master in the trade; he knew the raw material, and could judge of the theoretical strain which that material would bear. But in Botany he had no such training. He had to test his theories on difficult problems in vegetable morphology, classification, and distribution, with an outfit of knowledge based almost entirely on reading, and on what he had learned from Henslow at Cambridge.

[1] Life and Letters, Vol. iii, p. 254.

It must, however, be remembered that his Cirripede work had done much more than make him a zoologist; it made him, in a general sense, a systematist, and this must have been of incalculable value.

In the matter of geographical distribution he had at least enjoyed the great advantage of seeing the vegetation of the world, but he had seen it with ignorant eyes. In 1836, immediately after his return from his voyage, he wrote:—'I felt very foolish when Mr. Don remarked on the beautiful appearance of some plant with an astounding long name, and asked me about its habitation. Some one else seemed quite surprised that I knew nothing about a Carex from I do not know where. I was at last forced to plead most entire innocence, and that I knew no more about the plants which I had collected than the man in the moon [1].'

To the end of his life he never made any pretence to be a botanist, or at best 'one of those botanists who do not know one plant from another,' a saying, attributed to Nägeli, which he was fond of quoting. Thus, too, he wrote to Asa Gray on being elected to the Botanical Section of the French Institute:—'It is rather a good joke that I should be elected in the Botanical Section, as the extent of my knowledge is little more than that a daisy is a compositous plant, and a pea a leguminous one [2].' He was in fact guilty of evolution, but with extenuating botanical circumstances.

It is perhaps not out of place to call attention, as I have done, to the poverty of professional training with which he attacked evolution on its botanical side; it not merely brings out his power of using the work of others, but it also brings out the value of his point of view, that he should, equipped as he was, have revolutionized botanical geography. Again, it makes clear to us the supreme value to him and to science of his lifelong friendship with Sir J. D. Hooker. All readers of the Life and Letters must have been struck with the paramount importance of the Hooker correspondence as

[1] Life and Letters, Vol. i, p. 275.
[2] Ibid., Vol. iii, p. 224.

a record of Darwin's life. But to me the thought is more pressingly present, that without Hooker's constant help his great task would not have been carried out on the botanical side. Only part of these letters are given in the Life and Letters, when the remainder are published they will only heighten the impression of the value to science of this memorable friendship. It was not merely information, guidance, explanation, that he received, but an inspiriting companionship, a fresh and vigorous influence giving continuous refreshment to the solitary worker.

The following list of subjects, taken at random from my father's letters to Sir Joseph, give an idea of the subjects discussed during the evolutionary period:—The dispersal of seeds, continental extension, geographical barriers, the arctic Flora, alpine plants, large genera varying, coal plants, island Floras[1], aberrant genera, direct action of conditions, rarity and extinction, sterility, graft hybrids. Many of these were also the subject of correspondence with Asa Gray, H. C. Watson, and others, with results familiar to us in the Origin.

One of the few pieces of his published botanical researches made use of in the Origin was his series of experiments on the flotation and vitality of seeds in salt water. The chief mass of his work on crossing plants was later than 1859, and it will be found that in the Origin his material comes chiefly from Knight, Kölreuter, and Gärtner.

He used to sneer at himself as a compiler:—'The inaccuracy of the blessed gang (of which I am one) of compilers passes all bounds[1].' But 'compiling' in his sense was not an easy art, the first requisite being an instinctive power of knowing the trustworthy authorities from the untrustworthy. In writing to Professor Huxley this was italicized, *The difficulty is to know what to trust.* He adds too, 'I have picked up most by reading really numberless special treatises, and *all* agricultural and horticultural journals; but it is a work of long years. . . . I have found it very important associating with fanciers and breeders[2].'

[1] Life and Letters, Vol. ii, p. 281, note.

[2] Ibid., p. 281.

The mass of information gathered in the last-named way and by reading is given in his Variation of Animals and Plants under Domestication, which, like the Descent of Man and the Expression of the Emotions, were amplifications of parts of the Origin, in which he gave in full the evidence referred to in that so-called 'Abstract.' In the Variation of Animals and Plants he was also able to include more of his own researches, partly because, as already explained, it was published in 1868, six years after the Orchid book, and partly because it was a more appropriate place for minute observations. Here, for instance, are given the remarkable facts on the similarity, or rather identity, of the seeds of various kinds of *Brassica*, in which the leaves or other vegetative parts have by man's selective power come to differ widely [1].

Among the subjects especially interesting to the botanist as distinguished from the horticulturist may be mentioned the contents of the well-known chapter xi of Vol. I, 'on bud variation, and on certain anomalous modes of reproduction and variation.' In Vol. II, again, there are well-known chapters or paragraphs on crossing, on Knight's Law [2], on the sterility of cultivated varieties, on the good effect of crossing and on self-sterility, where his own work and his correspondence with Fritz Müller are prominent; on the stimulating effect of changed conditions, and on the complex problem of sterility due to changed conditions; on the difference in fertility between varieties and species when crossed, and on hybridism; on the direct action of conditions.

My father's correspondence with Fritz Müller was, in its bearing on his work, second in importance only to that with Hooker. He had for Müller a stronger personal regard than that which bound him to his other unseen friends. Müller's letters were vividly interesting, with their constant stream of new observation on many biological subjects. Moreover,

[1] Animals and Plants, ed. 1, Vol. i, p. 323. The collections of seeds made for this purpose are now in the Botanical Museum at Cambridge.

[2] I have elsewhere discussed Knight's Law in relation to Darwin's work; see *Nature*, 1898.

there was, by an unformulated arrangement, a certain community of research on many subjects. For instance, on orchid-fertilization, self-sterility, heterostylism, and climbing plants the facts supplied by Müller were important contributions to the building up and extending of Darwin's theories.

It is needless to say more on the Variation of Animals and Plants, the book is in the hands of every one, and is familiar to botanists. I regret that space forbids me to quote the true and forcible description of the effect of the book on botanists and horticulturists, given by Sir W. Thiselton-Dyer in his classical essay on the Botanical Work of Darwin[1].

Second or Physiological Period.

The work of this period, though distinguishable in a certain sense from the purely evolutionary work, is yet bound to it by many interwoven ties. Thus, the Orchid book (taken in conjunction with Cross and Self-Fertilization) is the amplification of a passage in the first edition of the Origin[2]. 'The flowers of two distinct individuals of the same species would thus get crossed; and the act of crossing, we have good reason to believe (as will hereafter be more fully alluded to), would produce very vigorous seedlings, which consequently would have the best chance of flourishing and surviving.'

Its connexion, from another point of view, with the evolutionary work has already been made clear, and I have indicated its value as a demonstration of the efficiency of his theory of evolution as a guide in natural history work. He acknowledged the value of his Orchid work in relation to natural selection:—'I can show the meaning of some of the apparently meaningless ridges [and] horns; who will now venture to say that this or that structure is useless[3]?'

It is not every man who can prove so convincingly the

[1] Charles Darwin, Nature Series, 1882, p. 38.
[2] p. 92.
[3] Life and Letters, Vol. iii, p. 254.

temper of the tool he has forged, or who can hope to see his single arm multiply a thousandfold, and a great guild of craftsmen working with his aims and his methods. Yet this is what has happened, for the great mass of biological or natural history work of the last thirty years is part of that harvest of which the Fertilization of Orchids was the first-fruits. What Mr. Huxley has said is true here :—'Even a cursory glance at the history of the biological sciences during the last quarter of a century is sufficient to justify the assertion, that the most potent instrument for the extension of the realm of natural knowledge which has come into men's hands since the publication of Newton's Principia is Darwin's Origin of Species[1].'

But it is not possible to judge the Orchid book until Darwin's researches on Cross and Self Fertilization, published in 1876, are taken into account.

In the Orchid book he showed that certain machinery exists for insuring cross-fertilization. In Cross and Self Fertilization he showed, for the first time, the definitely measurable effect of cross-fertilization, and thus expressed in figures the value of the selective force at work in modifying the floral mechanism. And in Cross and Self Fertilization he generalized the position one degree further, by showing that the value of crossing does not depend on the union of two individuals as representatives of different sexes, but as representatives of different conditions of life. The light in this way thrown on the meaning of sexual reproduction is, in my judgement, one of his greatest achievements in science.

A great deal of work, part only of which has been published, was done on the floral mechanism of the *Papilionaceae*, on *Leschenaultia*, on *Melastomaceae*, and on *Clarkia*. In the two last-named examples it was the possession of two different sets of anthers which interested him, a state of things which has been shown in some cases to be a case of division of labour, one set supplying pollen as an attraction, while the remainder serve as fertilizers. The *Melastomaceae* are a good

[1] Life and Letters, Vol. ii, p. 204.

example of his persistence; they began to interest him in about 1862, and he was still hoping to attack them in 1881, as illustrated in letters to Sir W. Thiselton-Dyer and others, which I hope soon to publish.

His next great work in the domain of fertilization was his papers on Heterostylism, the well-known researches on *Primula*, *Linum*, *Lythrum*, &c., which were afterwards worked up with other cognate matter into his book on Forms of Flowers, 1877. This work was extremely laborious, but with the labour went an especial delight in reading the riddle. Thus he wrote:— 'I do not think anything in my scientific life has given me so much satisfaction as making out the meaning of the structure of these plants[1].' While the delight of unravelling a biological puzzle was, as usual with him, the chief incentive to work, he did not overlook any fraction of the lessons to be learned from his results. In the case of heterostylism, he thought that the essential value of the problem lay in its bearing on hybridism. The parallelism between hybridization and certain heterostyled unions is wonderfully close, so that it is hardly an exaggeration to say that 'illegitimately' reared seedlings are hybrids between members of a single species. This fact gave a death-blow to the doctrine (which has been so long in dying) that differences in sexual constitution are 'the very touchstone of specific distinction[2].'

Another group of his researches was connected with the irritability of plants as exhibited in movement. The earliest of these was his work on Climbing Plants, 1865, in which he followed Mohl and Palm, but with the addition of so much new matter that he practically made the subject his own.

In his Insectivorous Plants, 1875, he also discovered and thoroughly investigated a number of remarkable instances of plant movement. His account of the sensitiveness of *Drosera* to excessively minute weights, though since shown by Pfeffer to bear a somewhat different interpretation, yet remains one of the most wonderful instances of vegetable irritability.

[1] Life and Letters, Vol. i, p. 91.
[2] Forms of Flowers, p. 276.

His views on aggregation have been shown to be incorrect, but remain as the starting-point of a curious subject. The work on insectivorous plants is, however, more remarkable for the boldness and originality of the central idea of the book than for anything else. As he has said, 'The fact that a plant should secrete, when properly excited, a fluid containing an acid and ferment, closely analogous to the digestive fluid of an animal, was certainly a remarkable discovery [1].'

In his book on Climbing Plants he wrote:—'The conclusion is forced on our minds that the capacity of revolving, on which most climbers depend, is inherent, though undeveloped, in almost every plant in the vegetable kingdom [2].' This, extended and modified, forms the central conception of his book on the Power of Movement in Plants, 1880. He showed that circumnutation is a widely spread phenomenon, that practically all plants carry on their growth in a rhythmic manner, which is identical on a small scale with the revolving nutation of climbing plants. But, as is well known, he went much farther than this, and attempted to prove that the movements of plants have been evolved by various modifications of circumnutation. It is a point of view which harmonizes admirably with his evolutionary views, namely, the conception of a plant attaining an adaptive movement (e. g. geotropism) by selection from a tentative series of movements—in a way that calls to mind the selection and summation in a given direction of morphological variation. This view has not been accepted by botanists, and personally I doubt whether it should be accepted in the form in which it is stated; for I doubt whether we know enough of the machinery of curvature, as distinct from the machinery of rectilinear growth, to understand the connexion between the two. But, as Sir William Thiselton-Dyer has said, 'No one can doubt the importance of what Mr. Darwin has done in showing that for the future the phenomena of plant movement can and indeed must be studied from a single point of view [3].'

[1] Life and Letters, Vol. i, p. 96. [2] Climbing Plants, p. 205.
[3] Charles Darwin, Nature Series, 1882, p. 41.

In my opinion the most striking feature of the book is its rehabilitation of Dutrochet's[1] theory, that vegetable movements are 'spontanés, exécutés à l'occasion de l'influence d'un agent extérieur et non des mouvements imprimés par cet agent.' This remains a signal service to plant physiology, whether or no we accept Darwin's views as to the rôle of circumnutation.

The localization of geotropic and heliotropic sensitiveness in roots and in certain seedlings is perhaps the most striking of the discoveries published in the Power of Movement in Plants. By some persons these statements and conclusions were received with incredulity or contempt. The proof of their substantial accuracy is due to Pfeffer[2] and his pupils, Rothert, and Czapek.

Towards the end of his life my father more than once spoke of his physiological researches as being undertaken in place of the more trying evolutionary work, for which he felt himself too old. This has always struck me as remarkable; I should have believed that an old man in bad health would have more easily returned to the work on which he had spent his best years; I should have supposed it more difficult to attack a comparatively new subject with new methods and new lines of thought. When I remember the amount of labour necessitated by the Power of Movement, I am astonished at his courage and unflagging energy. His manner of attack has been so truly described by Sir W. Thiselton-Dyer, that I cannot resist the pleasure of quoting it[3]. 'He turned his attention to plants doubtless because they were convenient objects for studying organic phenomena in their least complicated forms; and this point of view, which, if one may use the expression without disrespect, had something of the

[1] Pfeffer was the first to call attention to Dutrochet's remarkable utterance. In my address to Section D of the British Association, 1891, I have expressed my appreciation of the importance of Pfeffer's contributions to the study of the irritability of plants.

[2] The most brilliant demonstration of the 'brain-function' of the root-tip was published by Pfeffer in the Annals of Botany, 1894.

[3] Charles Darwin, Nature Series, p. 43.

amateur about it, was in itself of the greatest importance. For, from not being, till he took up any point, familiar with the literature bearing on it, his mind was absolutely free from any prepossession. He was never afraid of his facts or of framing any hypothesis, however startling, which seemed to explain them. . . . In any one else such an attitude would have produced much work that was crude and rash. But Mr. Darwin —if one may venture on language which will strike no one who had conversed with him as overstrained—seemed by gentle persuasion to have penetrated that reserve of nature which baffles smaller men.'

It may have been an unconscious perception of this quality which forced one, while recognizing the boldness of his course, to feel certain of his success.

The portrait of Charles Darwin is by Messrs. Maull and Fox, who have been good enough to permit its reproduction. The date of the photograph is probably 1854; it is, however, impossible to be certain on this point, the books of Messrs. Maull and Fox having been destroyed by fire. The reproduction is by Mr. Dew-Smith, who has been at some disadvantage, having only an old and faded print to work from.

FRANCIS DARWIN.

September, 1899.

Symbiotic Saprophytism[1].

BY

DANIEL TREMBLY MACDOUGAL.

University of Minnesota.

With Plates I and II, and one Figure in the Text.

THE extension of experimental evidence that plants of the most diverse physiological constitution may use organic compounds as food, seems to point to the conclusion that all plants may take up and use more or less material of high molecular complexity. The green plant is essentially an organism adapted to the manufacture of its food from simple compounds by the aid of radiant energy. Any variation from this type of nutrition, by which the energy stored up in complex food-substances may become available, entails a corresponding modification of the entire metabolism of the organism, and is accompanied or followed by anatomical changes. The extent of such alteration will roughly correspond with the proportionate amount of complex food used, and will be most apparent in the absorbing organs, chlorophyll-apparatus, and transpiratory surfaces. It is

[1] An abstract of this paper was read before the Botanical Society of America, Boston, August 20, 1898.

[Annals of Botany, Vol. XIII. No. XLIX. March, 1899.]

customary to designate all species that have lost the chlorophyll-apparatus as holosaprophytes; and all those which show only a slight degeneration of the structures mentioned above, as hemisaprophytes. As a matter of physiological fact, the hemisaprophytes are not sharply separated from the autophytes, since all of the latter class are in a sense hemisaprophytic.

The acquisition of complex compounds by the higher plants may be accomplished by three methods. In one the limiting membranes of the absorbing organs are modified to permit the passage of organic food-substances. Complete saprophytism by this method has been accomplished by one species only, *Wullschlaegelia aphylla*, a colourless Orchid of the West Indies, according to Johow's investigations. In my own work upon the subject it has been demonstrated that this capacity is shared by *Cephalanthera oregana*, a waxy white plant from western North America.

The second method of saprophytism is that by which the 'carnivorous' plants entrap and receive the bodies of plants and animals in differentiated portions of the shoot, and take up the products of their decomposition by means of specialized absorbing cells. About six hundred carnivorous species are known, and while the material obtained in this way is beneficial to the plant, it is not absolutely necessary to its existence or normal development. It follows quite naturally that no species has attained complete saprophytism by this method.

By a third method the higher plant gains complex substances through the agency of Fungi interposed between its own protoplasts and the nutritive substratum. The fungal hyphae may replace the external layers of the underground absorbing organs, may occupy these layers, or may penetrate into the cortex either between or through the cells. In any case the arrangement is such as to interpose the walls of the Fungus alone between the nutritive medium and the protoplasts of the higher plant. The walls of the Fungus have developed a capacity for the osmotic passage of organic

material, and hence the transmission of these substances to the higher plant is comparatively easy. The exact metabolic relations of the two organisms is not yet determined, although the mere contact of the protoplasts of the Fungus and those of the higher plant would imply some interchange of material. The higher plant surely affords some mechanical advantages to the Fungus, and to that extent at least the arrangement constitutes a symbiosis. With regard to the higher plant, therefore, I have temporarily termed this adaptation *symbiotic saprophytism.* The number of species that have attained complete saprophytism by this method is very large, while partial saprophytism in this manner is a widely spread feature of plant-life, though, as may be seen below, it is more common in tropical latitudes. Schlicht (36) examined 105 species growing in the north temperate zone in Europe, and found seventy to be furnished with mycorhizal adaptations of the roots. Among the number were such forms as *Ranunculus*, *Bellis*, and *Taraxacum*. Janse (16) examined forty-four Dicotyledons, fourteen Monocotyledons, five Conifers, and six Ferns and Liverworts growing in Java, and found mycorhiza on all except one Dicotyledon, three Monocotyledons, one Fern, and one *Equisetum*: that is to say, sixty-nine out of seventy-five species taken at random were more or less saprophytic by means of mycorhiza. Hoveler examined seventy-two species of native and cultivated plants growing in northern Germany, of which twenty-four were symbiotic saprophytes (14). Among the number were some not exhibiting mycorhiza elsewhere. Wahrlich examined 500 species of Orchids in cultivation at Moscow, all of which he reported as exhibiting mycorhiza (41). This number has been increased by subsequent observations by other investigators.

Identity of Fungal Symbionts.

Many attempts have been made to establish the identity of the Fungi entering into mycorhizal structures. Tulasne noted the fact that *Elaphomyces* forms coatings on the roots

of Pines (39), to which Boudier attributed a parasitic relation (1). Reissek determined the Fungus on *Platanthera bifolia* as *Fusisporium endorhizum* (34). Schacht found that the Fungus in the roots of *Limodorum* developed reproductive organs similar to *Eurotium* (35). Treub (38) and Bruchmann (2) conclude that that in the prothallia of *Lycopodium* is a *Pythium*. Bruns found that *Polysaccum* formed coatings on the roots of Pines, and that the relation is not a parasitic one (3). Woronin found mycelia on the roots of Conifers, *Salix*, *Populus*, *Corylus*, *Betula*, and of Grasses, which were undoubtedly formed by *Boleti* (42); and Rees identified the Fungus on the Conifers as an *Elaphomyces* (31). Frank made some unsuccessful attempts to grow the Fungi symbiotic with forest-trees, but failed (9). Janse had the same experience with the symbiont of *Coffea* (16); but determined the one associated with the roots of *Celtis* as *Celtidia duplicispora*, to be included with the Tuberaceae. Recently Rees and Fisch have examined the mycelium formed by *Elaphomyces granulatus* and *E. variegatus* on forest-trees, and conclude that the relation is not a parasitic one, but must be a symbiosis (32). Noack made some attempts to form mycorhiza by cultural methods in 1887–9 (27), and found that *Geaster fimbriatus* Fr. and *G. fornicatus* Fr. form mycorhiza with the roots of Conifers; *Agaricus terreus* Schaeff. with Beeches and Firs; *Lactarius piperatus* with *Fagus sylvatica* and *Quercus pedunculata*; *Cortinarius callisteus* Fr. and *C. coerulescens* Schaeff. with Beeches; *C. fulmineus* with Oaks; and he concluded that the Hymenomycetes and Gasteromycetes furnished the Fungal symbionts of all the forest-trees examined at that time. Wahrlich determined the Fungus of the mycorhiza of *Vanda tricolor* as *Nectria goroschankiana*; that of *Vanda suavis* as *N. vanda*; and all Fungi symbiotic with the Orchids were supposed to be Pyrenomycetous (41). Lendner made a re-examination of the Fungi of *Platanthera* and *Vanda* in 1895, and his observation of cultures made from spores confirmed Wahrlich's results (22). Chodat and Lendner (5) found that the mycorhizal

Fungus of *Listera cordata* resembles *Nectria* as originally described by Wahrlich on *Vanda*. It is to be seen that the greater number of mycorhizal Fungi remain to be identified. All known species may be included in the Oomycetes, Pyrenomycetes, Hymenomycetes, and Gasteromycetes. No attention has been paid to this phase of the subject in the work described in this paper, though reproductive bodies resembling *Penicillium* were observed on the roots of *Pterospora andromedea*.

It is apparent that the mere presence of the Fungus in or on the root of a higher plant by no means constitutes a mycorhiza, since in a great number of cases the relation is undeniable parasitism. Rees and Fisch call attention to the fact that the relation of a Fungus to the root of a specific plant is not always a fixed one (32). The chemical interchange may be so evenly balanced during a part of the season, or during a part of the lifetime of the Fungus or of the higher symbiont, as to constitute a symbiosis; but in other stages the presence of the lower form may result in positive damage or disadvantage to the higher plant. Thus Macfarlane concludes that the mycorhizal Fungus of *Philesia* is an ultimate disadvantage to the plant, since it hastens the death of the absorbing organs. The experimental tests of the identity and relationship of the Fungus and the higher plant have been the following methods: cultural examination of mycorhizal Fungi; formation of mycorhiza by the culture of Fungi on the roots of trees; and the growth of symbiotic plants in sterilized soils. As a general result of such experiments, it may be stated that any higher plant may form mycorhiza with only one or two species of Fungi; and that nearly all hemisaprophytes may develop normally, though with some decrease of stature, without the presence of the fungal symbiont. This is especially true of the forest-trees. On account of the great elasticity of the various factors to be considered in symbiotic saprophytism, it is to be seen that no definite conclusions may be drawn as to the relations of the attached plants without a general considera-

tion of their anatomy, with special attention to the chemical nature of the cells of the two organisms at the place of contact as well as to the substratum.

Scope and Methods of Investigation.

The work described in the following pages was undertaken for the purpose of extending knowledge of the occurrence of mycorhiza, the physiological relation of the two symbionts, the influence of mycorhizal arrangements upon the development of the higher plant with regard to the fate of a species, and the experimental formation and variation of such structures. Eight orchidaceous hemisaprophytes, two hemisaprophytic Dicotyledons, one holosaprophytic Dicotyledon, and four autophytes were examined. The principal facts concerning each species are presented in separate sections, and no attempt has been made to carry the anatomical examination of the plants beyond the point of interest in connexion with the saprophytic habit exhibited.

Aplectrum spicatum (*Aplectrum hyemale*, Nutt.).

Aplectrum spicatum is the sole representative of this North American genus of Orchidaceae, and it is found as far south as Georgia, as far north as the North-West Territory in Canada, extending across the continent. It finds most suitable conditions for growth in damp woods and swamps, in soils rich in humus.

The plant consists of a compressed globoid corm of a glistening white colour, composed of three internodes. From the extremity of the upper internode arises a single elliptic or ovate leaf, 10–15 cm. long and 1–3 cm. wide, in July and August, and persists until late in winter, or until the following spring in southern latitudes. The base of the leaf-stalk is sheathed by three scales, 3–7 cm. in height. The peduncle arises from the first internode in May or June, attaining a length of 25–50 cm., bearing three scale-leaves and a terminal raceme of dull yellowish flowers. One or two

offshoots, 2–4 cm. in length and composed of three or four internodes, are given off from the upper internode of the corm, and from their extremities arise new plants. The fourth or fifth internode becomes apogeotropic, turns upward and begins to swell, forming a new corm. About twelve to twenty unbranched roots are given off from the lower internode of the corm, attaining a length of 5–10 cm. The reserve material in the parent corm is drawn upon so slowly by the offspring that its contents are not exhausted until one or even three seasons have passed. In consequence of this fact plants may be found with two or three corms of different ages attached by the old offshoots.

The Roots. The roots are furnished with persistent root-hairs from the base to within 2 mm. of the apex, which bears a distinct root-cap. Thickly intermeshed with the hairs were great numbers of brown filaments derived from the mycorhizal strands of Oak, Maple, or other neighbouring trees. In several instances a mycorhiza of a tree was found applied to the side of roots from the base to a point near the apex so closely as to be incapable of separation, except by tearing the superficial layers of the root. Separate hyphae from the tree-mycorhiza were given off laterally and entered the epidermal layers as described below. It is thus to be seen that *Aplectrum*, the Fungus, and Oak or Maple trees form a nutritive union. It is difficult to estimate the symbiotic balance existing among the three members. The Fungus forms a complete coating on the roots of the tree, but covers only patches and strips on the *Aplectrum*. The presence of an endotropic mycorhiza in the roots of the *Aplectrum* would lessen the tendency to the formation of an ectotropic layer with a second Fungus. It seems reasonable to suppose therefore that the interchange between the tree and the Fungus is much greater than between the Fungus and *Aplectrum*. That the other two are parasitic upon the *Aplectrum* is rendered improbable by the fact that the largest and most robust specimens collected and received from elsewhere were abundantly covered with the ectotropic Fungus. The Fungus must therefore be

considered simply as a neutral bond between the other symbionts, and the symbiosis as dual and overlapping.

The epidermis is composed of elements elongated parallel to the axis of the root, with the radial walls variously folded and curved. The walls are slightly suberized, and the outer membrane of many of them is extended into root-hairs, also suberized. The root-hairs are seen to be traversed by pale, highly refractive hyphae, which extend from the cortical cells through the hypoderm into the epidermal cells, and out through the hairs into the soil. The epidermis is penetrated by the hyphae from the mycorhiza of trees. The hyphae traverse the epidermis in a longitudinal direction, sending off lateral branches which show a few convolutions inside some of the cells, or penetrate one or two walls tangentially, in one or two instances into the exoderm. In the instances in which the tree-mycorhiza was applied directly to the root, the outer walls had disappeared, and the layer was replaced by an entangled mass of the Fungus. In any given transverse section the Fungus-layer might include one-fifth of the circumference, while nearly all the epidermal cells were penetrated more or less through the lateral walls by the main hyphae or its branches. The exoderm consists of thin-walled rectangular cells in both cross and longitudinal section. The average length is equal to one and one-half or twice the short diameter. The protoplasmic content of both the outer layers is very spare. Numbers of the exodermal cells are almost cubical, and contain a large proportion of protoplasm and deeply-staining nuclei; such cells are penetrated by hyphae passing from the cortex to the epidermis. The cortex consists of four layers of oblong ovoid cells, increasing to six or eight layers with large intercellular spaces in older organs. The outer layers contain numbers of raphide-cells, and numbers of more or less anastomosed clumps of filaments. The internal layers contain great masses of closely interwoven hyphae, forming an irregularly ovoid or globose body lying against or nearly surrounding the nuclei. The general appearance of the

bundles of hyphae closely resembles those of *Corallorhiza* as figured by Thomas (37), except that the filaments are much more closely interwoven and curve more abruptly. It is noticeable that the hyphal masses may occupy all the cortical cells from the exoderm to the endodermis. The nuclei to which the myceliar masses are appressed are usually enlarged and hyperchromatic. The increase in size is accompanied by an enlargement of the nucleolus and the assumption of an irregular globoid or oblong ovoid form, although the actual outline is smooth and curving. The presence of the Fungus does not appear to affect the nucleus unfavourably. These endotropic hyphal clumps are found intact in old roots in which the ectotropic Fungi and the epidermal cells have decayed.

The endodermis consists of brick-shaped cells with walls not thickened to any appreciable extent. The stele is tetrarch. Each bundle consists of two to four scalariform ducts, and a number of libriform vessels with transverse pores. The number of bundles increases to six or seven in old roots, and the conjunctive tissue becomes strongly sclerotized.

At the beginning of the growing-season some variations from the features described above were to be seen. At this time the cortex is heavily loaded with starch, giving the typical iodine-reaction. The hyphal clumps give a yellowish brown reaction with the same reagent. Great numbers of highly refractive and vacuolated hyphae are to be seen which stain yellowish with iodine, and these apparently break up into globose bacterioids staining reddish brown. The ultimate fate of these bodies is yet to be determined. Lateral branches of the hyphae develop into stalked globules of a diameter three or four times as great as the filament. The granular contents stain a deep reddish brown with iodine. These formations are to be seen occasionally in the mediocortex, frequently in the epidermis, and rarely in the root-hairs. In the last-named instance the entire cavity of the hair may be thus occupied. The structures in question may

be taken to correspond to the 'sporangioles' described by Janse, which he characterizes as occurring in non-orchidaceous species, and which are homologous with the 'vesicles' of the Fungi inhabiting the Orchids according to his conclusions (16).

The Corms. The compressed globose corms attain a volume of 2 to 5 cc., and have a glistening or pearly white appearance. The upper internode comprises about one-half of the bulk of the corm, and hence the nodal line marked by a black rudimentary scale occupies an equatorial position. The epidermal cells are outwardly convex, rich in protoplasm, with walls equally thickened. The hypoderm shows no special differentiation. The endodermis consists of irregular flattened cells separated from the hypoderm by large intercellular spaces, which give rise to the pearly white appearance of the corms. The walls of the three layers mentioned are copiously perforated, and contain numbers of starch-grains staining brownish red with iodine. The external layers of the conjunctive tissue contains numbers of raphide-cells, and the more slender cells near the scattered fibro-vascular bundles contain starch-grains giving similar reactions to those in the epidermal tissues. Small numbers of granules are scattered throughout the storage-tissue. The chief reserve substance, however, is a viscid mucilaginous carbo-hydrate soluble in water, and coagulated by 85 % alcohol into granular masses, which readily dissolve in water. Corms one year old are partially emptied of their reserve substances. The most noticeable fact in this connexion is the diminution of the bundles of raphides to about one-half their original size, showing that the crystals are not entirely excreta or waste-products. The Fungus of the root was not observed in any instance to penetrate into the corm.

The corm undergoes great variations in response to external conditions. Thus, if a portion of an offshoot giving rise to a corm is uncovered and exposed to the light, the resulting corm will consist of six or eight internodes, and will contain chlorophyll in the outer layers. A corm of this character,

after attaining full size, was detached from the offshoot and allowed to lie on its side in damp soil. Two offshoots were formed laterally, and a stem-like structure from the apex. The corm exhibited the usual reserve material. The offshoots contained quantities of starch in the cortical layers, and naturally in greatest quantity in the neighbourhood of the conducting cells. Trichomes were formed very sparingly on the offshoots, and on account of this fact, and also because the offshoots lay on the surface, the Fungus-guest had not gained entrance. This arrangement is of interest when the manner in which the Fungus is transmitted from the parent to the daughter-plant is considered (see p. 12). It was found, during a course of tests in the plant-house from 1896 to 1898, that the plant might be forced to form a succession of corms and stems without leaves or flowers, in much the same manner as *Solanum tuberosum*.

The Offshoot. The offshoot of a normal corm consists of a stem about 1·5 cm. long and 3 to 4 mm. thick, with two long or three short internodes. The nodes are sheathed by an encircling ragged scale composed of a single layer of cells and soon turning brown. The scales are from ·2 to 2 mm. in length, and bear in their axils the naked papillae of incipient branches. The branches may be detected in one or two nodes only, and, so far as specimens have been examined, do not develop in the normal plant. When the parent corm gives rise to two young plants, it does so by separate offshoots.

The epidermis consists of a layer of cells of externally convex elements, the radial diameter being generally less than the tangential or axial. The walls are slightly and evenly thickened. A few dark brown hyphae may be seen on the surface, and the sections show an occasional penetration of the outer walls. The epidermis is separated in places from the layer beneath by great intercellular spaces, while in other portions these are entirely absent. The layer underlying the epidermis is not clearly differentiated as a hypoderm. It is composed of rounded, flattened or irregular

cells with uniform walls which show oval perforations in the older stems. In places the large thin-walled cells of the cortex touch the epidermal cells directly. Many stomata are to be found opening into distinct air-chambers in the cortex. Both arrangements are apparently devices for the extrusion of superfluous water, though no direct experimental evidence of the fact was obtained.

The outer layers of the cortex exhibit many mucilaginous raphide-cells. In the median regions are many mycorhizal clumps of anastomosed filaments similar to those found in the root. No uniform distribution is to be made out, however, and in any given cross-section these formations may be confined entirely to one quadrant. Since the offshoot usually is not furnished with trichomes except at the base as noted below, there is no external development of the hyphae. The sheath consists of an ill-defined layer of prismatic elements with oblique ends, and walls evenly and sparingly thickened. In mature offshoots a double ring of bundles, with the conjunctive tissue sclerotized, is to be seen. The bundles consist of one or two spiral vessels, two or three scalariform ducts, and a number of sieve-tubes and companion-cells. The space separating the bundles is made up of libriform elements with numerous oblique perforations. It is to be seen that the offshoot is amply equipped for conduction, and that the cortex affords a ready means of exit for superfluous water.

A clump of trichomes is to be found on the offshoot near its base, and in the longitudinal section the fungal cysts are seen to occupy a mass of cells increasing in volume from the base of the clump of trichomes, and spreading out toward the apex of the root, until some appear on the upper side of the stele. The fungal hyphae are not to be found on the base of the offshoot or in the corm; and since the offshoot offers no other means of access, it is plain that the fungal hyphae from the root-hairs of the parent plant pass through the soil, a distance of a few millimeters, and enter the offshoot by the hairs near the base. The presence of the

Fungus in the offshoot is merely in transit. At the apex of the offshoot, the base of the corm, the roots spring from the offshoot and the Fungus passes into them directly, as could be seen in longitudinal sections. That this method of transmission of the Fungus from the parent to vegetative offspring is followed, is confirmed by the fact that the Fungus was not found in offshoots springing from corms on the surface of or above the soil.

The Leaves. The scales arising from the three nodes of the corm attain a length of 2 to 5 cm., and closely sheathe the base of the stalk of the terminal leaf. The scales are first whitish, like the corm, but they soon die and turn brown. This process is hastened by the presence of a parasitic fungus, probably *Phyllosticta Aplectri.*

The stalk upon which the leaf is borne is an obtusely four-angled structure with enclosed central cavity, and has a length of 3 to 5 cm. to a nodal point at the lower end of the lamina, denoted by a slight swelling immediately below a constriction. The constriction marks the outer edge of a layer of scission-tissue.

The epidermis consists chiefly of flattened cylindrical cells with the outer wall cuticularized. The hypoderm is separated from the cortex by large intercellular spaces giving rise to the whitish appearance of the stalk. Scattered stomata open through the epidermis into small air-cavities. Both outer layers are rich in protoplasm. The cortex is copiously developed, and the cylindrical elements exhibit very large intercellular spaces. The ectoplasmic layer is heavy and densely granular. The fibro-vascular bundles are about twenty-seven in number. The parenchyma-sheath is well defined. The xylem exhibits normal development, and its cross-section in mature stalks is equal to that of the phloem.

The lamina, which is ovate-lanceolate in outline, has the appearance of a hairy leaf. This is due to the development of cuticle in the form of knobs and ridges. Stomata are present on both surfaces. The epidermis is rich in protoplasm and remains so until disintegration begins. The palisade-

layer is but slightly differentiated. The external layers of chlorophyll-bearing cells of the lower side are not so regular in form as those of the upper side. The air-spaces are very large. The median cells are almost globular in form; numbers of them contain bundles of raphides. The xylem exhibits a strong development, and its cross-section is naturally greater than that of the phloem. Heavy strands of sclerenchyma lie externally above and below the bundles. The strand on the side toward an angle extends to the epidermis, greatly exceeding the bundle in cross-section and filling up the angles of the ribs appearing on both surfaces of the leaf.

The heavy cuticularization of the outer surface must be regarded as a device for the prevention of transpiration during the winter-season, when the absorptive capacity of the plant is diminished or nullified. The dense protoplasm is fitted for the endurance of low temperatures, and the enormous amount of mechanical tissue would prevent collapse during the absence of turgidity. The leaves live through several months in which the temperature of the air is 10° to 30° C., and the adaptations named are quite necessary for its existence.

Spontaneous variations. *Aplectrum* exhibits some variations which, if they become permanent, would change the entire complexion and habit of the species.

The chief variation from the normal consists in the development of the lateral buds of the offshoot into coralloid branches, similar in general appearance and structure to the underground stems of *Corallorhiza*, which has been recently described by the writer (24). So far as the information of the writer goes, this peculiarity was first reported by H. Gilman in 1876. Letters of inquiry to the principal herbaria of the country, to dealers in native plants, including a firm near the original collecting ground, failed to bring further information. Mr. Gilman (10) writes: 'I have lately discovered (April 9, 1876) in the woods north-east of Detroit, Mich., two adjoining plants of *Aplectrum hyemale*, Nutt., having branched and toothed coral-like roots, similar to those of the genus

Corallorhiza, immediately below the usual bulb or corm, which also had the ordinary rootlets. Each plant had the green leaf which the species sends up in autumn. The coral-like roots appear to be parasitic on the partly decayed bark of a tree-root. A large number of plants of the species (much more than one hundred), taken from the same locality at different times, presented no such peculiarity. This is an interesting and significant discovery, and, as Professor Gray (to whom I sent my specimens) adds, "indeed unexpected." I beg to call the attention of botanists to it, that we may learn whether the peculiarity exists elsewhere, and, if so, to what extent.' By the kindness of Professor B. L. Robinson I was able to examine the specimen collected by Mr. Gilman and sent to Professor Gray. It consists of a corm with the mature leaf attached, and the usual number of roots. Attached to an internode from which roots arise is a portion of a slightly branched coralloid offshoot. The offshoot is evidently one which has arisen from a parent corm which had been separated, leaving it attached to the young plant. The offshoot always arises from a median internode of the parent corm, and of course joins the young corm at its base, as the young corm is the enlarged apical internode of the offshoot. No anatomical investigation could be made of this specimen on account of its historical value, but the brown strands of mycorhizal filaments could be seen adhering to the roots and coralloid branches.

The writer collected a number of specimens near Lake Minnetonka, Minnesota, in September, 1897. Of these, four plants adhered in such manner as to form a clump. The clump was found to consist of a parent corm, the upper internode of which was plump and turgid, while the two lower ones were shrunken in such manner as to be quite obscure; from the latter arose three offshoots, bearing four plants of different sizes. The largest plant arose from an offshoot composed of three internodes with the surface roughened, and each giving off a short coralloid branch. A smaller plant was borne on an offshoot consisting of five

Fig. 1. *A*. An *Aplectrum* with coralloid offsets and three young plants: a fourth was originally present: *a*, old corm; the leaves of the coralloid specimens are smaller than in the normal. Photographed in October, 1897.

B. Normal specimen of an old corm with young plant connected by the cylindrical offset.

internodes, bearing longer and subdivided branches. The third offshoot branched near the point of origin, and the branches were subdivided in such manner as to form an almost solid mass of coralloid formations. Two plants arose from different branches of this offshoot. One of the plants from this offshoot was detached with a mass of the coralloid stem for anatomical examination, and the remainder were photographed and placed in a cold house for three months, where they perished by freezing. It was most noticeable that the formation of young plants had not greatly decreased the size of the parent corm. In this instance the growth of the offshoot had consumed the reserve material in the lower part of the corm; but if the upper part had undergone the usual shrinkage, it had subsequently regained its original plumpness. The formation of the great mass of coralloid offshoots not only did not involve the expenditure of the amount of food usually consumed by one plant, but was accompanied by an increase of the material in the parent corm, indicating a distinct change in the nutritive conditions.

The coralloid shoot differs chiefly from the type by its profuse branching and the enormous multiplication of the internodes, with a corresponding increase of surface. The extension of surface is carried still further by the formation of great numbers of trichomes, which replace the scale-leaves. The latter appear to be almost wholly lacking. The clumps of trichomes are so closely crowded as to give it the appearance of being covered with root-hairs to which the particles of humus cling, and are most numerous near the apices of the branches. The epidermis is composed of irregular flattened cells, with walls strongly thickened. The stomata are more numerous and larger than in normal offshoots. An ectotropic Fungus covers the surface in patches in much greater profusion than in the normal offshoot, and penetrates the epidermal cells in the same manner as in roots. The hypoderm consists of ovate cylindrical elements with thickened walls. The two layers are rich in protoplasm and are separated by intercellular spaces. The trichomes are large

and contain hyphae which may be traced to the cortex beneath. The outer layers of the cortex contain numbers of raphide-bundles. The cortical cells are globose or greatly irregular, and are arranged in about twelve to fifteen layers. The outer layers contain simple hyphae chiefly, but also some cysts of Fungi which are most numerous in the median portion. Numbers of these formations are to be found in the cells next to some of the inner cortical cells, and the sheath contains starch. It is impossible to distinguish clearly the exo-cortex, medio-cortex and endo-cortex, as made out by Groom (13) in other saprophytes, upon the basis of the relative distribution of the Fungus and of starch. The starch is found in greatest quantity in the tissues near the apex, and in that region granules were actually present in cells which harboured the Fungus even in the external layers. The sheath consists of flattened cells with directly transverse end-walls, and all of the members are slightly thickened. The sheath is interrupted in places by small passage-cells. The fibrovascular bundles occupy a single irregular circle with the phloem external, radial to the xylem, and consisting of sieve-tubes, companion-cells and parenchyma. The xylem consists of one or more spiral vessels, a few scalariform ducts, and some obliquely perforated libriform vessels. The inner circle of bundles is represented by a few irregularly placed spiral ducts which most probably represent atrophied bundles. It is to be seen that the cross-section of the conducting tissues is much less than in the normal offshoot. The apices of the branches so closely resemble that of *Corallorhiza*, as described by Reinke, that no detailed description is necessary (33). The apex of each branch is flattened and covered by arched scale-leaves which soon fall away leaving only the leaf-trace to mark the internode. The meristem-cells exhibit division in every direction, and only the dermatogen is clearly differentiated. The fibrovascular tissues extend to within ·5 mm. of the surface of the meristem. The method of branching is quite similar in detail to that figured by Irmisch (15) for *Corallorhiza*. The roots of coralloid plants are almost free

from the endotropic Fungus as well as reserve material, while the ectotropic Fungus is developed only sparingly.

A dozen specimens of *Aplectrum* received from Mr. F. H. Horsford, March 25, 1898, showed three in which coralloid structures were beginning to form on the offshoot. The coralloid formations have thus been found on specimens growing at three different points in a line fifteen hundred miles in length, extending through a region in which the character of the soil and climate is fairly identical. Lundström has described similar formations on *Calypso borealis* (23).

Experimental formation of coralloid structures or stem-mycorhiza. A theoretical consideration of the problem of the formation of the coralloid stems leads to the conclusion that these structures might result from the parasitism of the investing Fungus, from an effort on the part of the plant to increase its absorbing surface, or from the awakening of all of the lateral buds as a reaction to the destruction of the apical growing-points. The anatomical inspection described above failed to show any parasitism on the part of the mycorhizal Fungus, or the presence of a second organism having this effect. In order to determine to which of the remaining causes these formations were due, a series of tests were made as follows. In one series the apical buds of a number of young offsets were cut away and the plants placed under the most favourable cultural conditions. In another the roots were cut away from the bases of newly-formed corms at the extremities of offsets, leaving only the cylindrical offsets as absorbing organs. In another the old corms were taken away leaving the half-grown daughter-plant without its customary supply of reserve nutriment. In a fourth series, old corms which were nearly spent but which still showed living dormant buds, were separated from the daughter-plants. The first three sets of experiments gave only negative results. In no instance could the development of the lateral buds be induced by the decapitation of the offset. The excision of the roots or of the reserve food in the old corms tended only to induce the formation of new

roots, and failed to bring about any changes in form or function of fully formed offsets. In the fourth series of tests most beautiful and conclusive results were obtained. The awakened buds on old corms formed small delicate glistening white stems which branched repeatedly and which exhibited the long root-hair-like trichomes of the coralloid structures. From the results at hand, therefore, it seems conclusive that the coralloid structures are a direct adaptation whereby young plants may secure humous material in the event of the destruction of the reserve supply in the corm: or this adaptation may render possible the formation of young plants from an old corm when the previous offsets from it have been destroyed from any cause. This is, it is believed by the writer, the first successful attempt at the experimental formation of stem-mycorhiza. The tendency to form stem-mycorhiza by *Aplectrum* must be regarded as an individual variation, only called out by the deficiency of the reserve food-supply, since it has not been transmitted to any of the vegetative offspring of the plants under continuous observation.

Peramium repens (*Goodyera repens*, R. Br.).

Peramium repens consists of a creeping stem 4 to 7 mm. in diameter, with internodes 4 to 5 mm. long, the leaves at the terminal internodes being crowded into a tuft. The diageotropic rhizome attains a length of 8 to 12 cm., and is greenish in colour due to the presence of scattered chloroplasts in the cortex. The epidermal cells are rich in protoplasm: the outer walls are cuticularized. The cortex consists of twenty to thirty layers of loosely arranged ovoid cylindrical elements rich in starch. The fifteen to twenty fibrovascular bundles are arranged in an irregular circle. The thickish roots spring singly from the internodes and penetrate the substratum in both lateral and vertical directions. Root-hairs are abundant and persistent, and when the root is taken from the soil it brings away an extraordinary heavy coating of humus. The root-hairs are to be seen traversed

by as many as four or five colourless septate hyphae in each. The epidermis of the older parts of the roots show signs of disintegration, although no complete exfoliation occurs in the two or three years of the life of the organ. The exodermis consists of very large muriform elements with thin walls which are empty for the most part unless traversed by hyphae. The cortex consists of about ten layers of ovoid cylindrical cells of varying size. The outer layers contain more or less starch, and hyphae which form a small number of convolutions and branch near the nuclei. Passing inward, the convolutions and branching increases in density, until in the medio-cortex they form spindle-shaped masses with a roughened integument quite similar in all respects to the vesicles described elsewhere. The gradually increasing size and density of these structures from the loosely woven clumps of the outer cortex to the agglomerated masses in the medio-cortex, demonstrates beyond all question the absorptive character of these structures, and also the fact that these organs are not in any sense homologous with or analogous to the 'sporangioles' of non-orchidaceous species as indicated by Janse (16, p. 150). The clumps of neighbouring cells are connected by means of numerous hyphae: fifteen or even twenty may be seen in a single plane leading away from one clump. The clumps in this case are very large, occupying in some instances as much as a half of the volume of the cell, and slightly separated from the nucleus by a clear space. The influence of the presence of the clumps upon the nucleus is very marked: in all cells in which this formation occurs the nucleus is two to four times larger than the normal, and is densely granular. The form is modified in such manner that the nucleus resembles an oblong sac tapering to a point at one pole perhaps. In many instances the nucleus is curved: in others a constriction is to be seen which occasionally has been carried so far as cause an undeniable fragmentation of the nucleus into two or perhaps three portions, separated by a clear space: the fragments are equally hyperchromatic. The enlargement of the nucleus in the roots

of *Vanilla planifolia*, due to the presence of a mycorhizal Fungus, was noted by Cavara, but no division was found (4). Dangeard and Armand described variations of the structure of the nucleus in *Ophrys* in which a general similarity to the reactions of *Peramium* was noted (6), and with the above observation constitute the only known instances of the fragmentation of the nucleus due to the action of a symbiotic Fungus. In some instances the presence of the Fungus causes hypertrophy of the nucleus, accompanied by cell-multiplication. The inner cortex of the root is rich in starch. The endodermis could not be made out clearly. The irregularly arranged bundles show no marked reduction of the conducting-tissues. The hyphae of the infesting Fungus find their way a short distance into the tissues of the stem, and might pass quite easily to the bases of the roots at the nearest internodes, after the manner of *Aplectrum*, although no such behaviour came under observation. The beginning encroachment of the Fungus in the stem suggests the possibility that the species might easily pass into the category of plants furnished with stem-mycorhiza, losing the roots in the process. No experiments were made upon this point however. The upright oblong ovate leaves are plentifully provided with stomata on both the inferior and superior surfaces. The epidermal cells of the superior surface have strongly convex cuticularized outer walls. The chlorophyllous cells are globose or ovoid, and only in places is a palisade-layer evident. This species was examined by Wahrlich, but no details of structure are given beyond the fact that a mycorhizal Fungus was observed (41). Frank has figured the occurrence of the Fungus in the medio-cortex of the rhizome in a manner similar to that described above (9). The formation of the hyphal clumps, or absorbing organs, is similar to that described by the writer. Frank does not, however, figure the fragmentation of the nucleus of the higher plant. It is to be noted that, in the plants examined as above, the occurrence of the Fungus in the rhizome was very sparing. It seems probable therefore that this plant may in some

localities transmit the hyphae from internode to internode after the manner of *Aplectrum*, which would give opportunity for similar variation in the formation of the mycorhiza.

Cypripedium parviflorum (Salisb.).

The roots are long wavy cylindrical organs 1 to 2 mm. in diameter, arising from the lower nodes of the upright stems. The epidermal cells are flattened cylindrical, with the outer walls yellowish and thickened. So far as the material examined shows, there are no root-hairs or papillose extensions of the walls. The exoderm is composed of irregularly-angled cells with thin walls. These two outer layers of cells are rich in protoplasm, some of which contain starch-grains, and others convoluted masses of colourless hyphae.

The cortical cells are polygonal, with copiously perforate walls, and characteristically large nuclei. One or two of the outer layers contain a small number of cells with hyphal clumps, and the remainder of the tissue is very rich in starch. The central cylinder contains a relatively large cross-section of conducting tissue. The endodermis opposite the xylem-rays is composed of thin-walled passage-cells which do not become lignified even in old roots. The cell-walls opposite the phloem are evenly and heavily thickened to such an extent that the cavity is nearly obliterated. Slight traces of lignification are noticeable. The Fungus does not gain access to the underground stem. The hyphal clumps are comparatively few in number, and are small and irregular in shape. They are closely appressed to the nuclei, in which but little variation from the normal is shown. The relation here of the Fungus and the host-plant is quite an indifferent one. From the size of the chlorophyll-apparatus and the limited extent of the mycorhiza, it is to be inferred that the symbiotic saprophytism of the species is but little developed.

Cypripedium hirsutum (*Cypripedium pubescens*, Willd.).

The roots of *C. hirsutum* present the same general features as those of *C. parviflorum*, except that they are larger. The fungal filaments occupy the same layers, but are more copiously developed, and stain more deeply. In neither this nor the preceding species were any hyphae found outside the root. The hyphal convolutions in *C. parviflorum* fuse to form a 'vesicle-like' absorbing organ by the nucleus; but in this species the loosely-arranged mesh-character is preserved. The presence of the Fungus appears to be indifferent or perhaps in some instances parasitic, as certain included nuclei stained less deeply than the typical form.

Cypripedium acaule (Ait.).

The roots of *C. acaule* are large, 2 to 3 mm. in diameter, curved in an undulating manner, and attain a length of 8 to 15 cm. The epidermal layer, like that of *C. hirsutum* and *C. parviflorum*, is yellowish brown except in the very youngest portions, owing to the presence of colouring matters in the outer wall. The epidermal cells are irregular in the surface outline, and flattened oval in cross-section. The exoderm is composed of four- or five-sided, small, thin-walled cells. The cortex consists of nine to twelve layers of large cylindrical or globose elements rich in starch. Scattered at random through this tissue are clumps of hyphae almost filling the cells. A few vesicles are formed near the nuclei, and the hyphae may be traced through the exoderm and epidermis in a manner similar to that figured by Janse (Pl. IX, Fig. 14). The endodermis is ill-defined and consists of thin-walled elements. The central cylinder shows nine to twelve groups of xylem with great centripetal development, and a relatively large cross-section of conducting-tissue. The Fungus does not gain entrance into the stem-structures.

The nuclei of the cells inhabited by the Fungus are above the normal in size and stain deeply. No considerable

amount of complex substances can be acquired by mycorhizal arrangement in this species, however, because of the weak development of this adaptation.

Calypso bulbosa (*Calypso borealis*, Salisb.).

The underground portion of this plant consists of a small ovoid bulb bearing a small number of short simple roots, which are generally coated with humus though the habitat of the species shows the greatest variation. The vegetative reproduction of the plant is effected by the formation of a short offset, the apex of which becomes converted into the bulb of a young plant after the manner of *Aplectrum*. The specimens examined showed the presence of mycorhizal Fungi in the cortex of the roots, forming roughened vesicles closely appressed to the hyperchromatic nuclei. Hyphae were seen extending outward through the root-hairs. The conducting-tissues are but weakly developed, and the endodermis could not be clearly distinguished.

Spontaneous variations. Although this species is credited with 'coralloid roots' in many systematic descriptions, yet no structures of this character have been found on specimens examined by the writer. Their existence seems well-authenticated however. The following note in the Botanical Gazette records their presence in America (28): '*Calypso borealis* has not usually been credited with the possession of coralline roots. These were pointed out to Dr. Gray several years ago by Mr. Hitchings, of Boston, and the fact was called to mind lately by seeing such roots on fine specimens of this beautiful Orchid brought to the Botanic Garden at Cambridge from the White Mountains by Dr. Goodale.' A. N. Lundström observed the plant through a period of ten years beginning in 1862: 'bei Langviken unfern Pitèa' in Sweden (23). He found coralloid appendages on large numbers of old bulbs from which flower-stalks had sprung in previous years. These structures were found to be rhizomes densely branching in one plane, with rudimentary leaves of conical form arranged in a $\frac{1}{2}$ order. The general appearance was very similar to

that of *Corallorhiza* and *Epipogium*. Yellowish fungal clumps were found near the nuclei of the cells of the medio-cortex which he regarded as plasmodial: strands of protoplasm were seen extending between the nuclei of neighbouring cells, which he did not regard as hyphae. The examination given was preliminary, however, and it seems quite probable that the 'strands' were hyphal structures similar to others recently described in mycorhiza. No facts were brought to light to show that the coralloid structures were pathological formations, and they were regarded as similar in general nature to the coralloid underground structures of *Corallorhiza*. Since they were small and not of invariable occurrence, Lundström was disposed to regard them as vestigial structures of no present use to the plant, but as indicating a phylogenetic relationship between this genus and *Corallorhiza*. This last conclusion does not seem well-founded in the light of the facts concerning *Aplectrum* stated on p. 20. The development of the stem-mycorhiza on old bulbs more or less nearly spent, points to the conclusion that here, as in *Aplectrum*, it is an effort to supplement the scanty supply of food available to the young plant by absorption of humous products. From the observations of the writer on specimens in the field from Michigan westward to Idaho, and Washington, it appears that the individuals grouped under this species show great extremes of variation in leaf- and flower-characters, although unfortunately the subterranean organs were not examined on the same specimens. Some systematists are disposed to divide it into two species on account of these variations which as yet are fairly continuous. It is to be seen therefore that the genus *Calypso* is in a state of very unstable equilibrium, and it seems entirely justifiable to ascribe this in part to the fact that young plants may be formed from old bulbs by means of coralloid offsets, instead of by normal non-absorbing organs. The influence of such mycorhizal structures would of course be quickly apparent in leaves and flowers[1].

[1] Since this article was sent to press, the writer has re-discovered the coralloid formation.

Pogonia ophioglossoides (L.), Ker.

This plant grows in moist humus. The roots are few in number, arising from a short underground stem, and attaining a length of 3 to 8 centimetres. The epidermis consists of polygonal cells bearing very large root-hairs which are seen to be traversed by hyphae. The exodermis is mostly thick-walled, though here and there thinner-walled passage-cells are found. The four to six layers of cortex abound in hyphae. Numerous large vesicles are formed which are generally closely appressed to the nucleus. Invagination of the nucleus may progress so far as to give it a reniform appearance, yet in every instance it stains deeply, is greatly enlarged and apparently benefited by the presence of the Fungus.

The endodermis is not clearly defined, and the conducting-tissues are but moderately developed. The hyphal clumps are persistent even in members in which disintegration of the cortical layers has begun. The cells containing the hyphal clumps are distributed indiscriminately with others loaded with starch. The Fungus penetrates the cortical layers of the stem in rare instances, and the underground portions show conical or papillose extensions of the epidermal cells which would give a greater surface in contact with the humus.

Gyrostachys cernua (L.), Kuntze (*Spiranthes cernua*, L.).

The roots are cylindrical, nearly straight, 2–3 mm. in diameter, and a few centimetres in length, spreading out horizontally through the loose substratum. The apical portion is whitish, but the older sections are brownish with irregular patches of adhering humus. The shoot is furnished with a number of bract-like green leaves showing some variation in size.

The epidermal system of the roots is composed of three layers. The outer layer consists chiefly of shallow cup-shaped cells, with the outer walls concave and cuticularized.

The radial walls are marked with reticulate thickenings which are in general at right angles to the root, and the inner wall bears similar thickenings. The lateral and inner walls are plainly but sparingly perforate, and the outer wall, especially in old cells, appears to be pierced by minute openings. The layer is destitute of protoplasm, and the outer wall is seen to be coated with humus particles adhering directly to the wall. A few of the cells of this outer layer are prolonged outwardly into root-hairs which are traversed by fungal hyphae. The hyphae are to be found in some of the cup-shaped cells also. In many instances, the development of the cell toward the root-hairs proceeds only so far as to produce a capitate extension outside the bounding line of the organ, very rich in hyperchromatic protoplasm. The lateral and inner walls of the basal portions of the root-hairs are pitted and furnished with short cellulose ridges.

The second layer consists of irregular thin-walled cells rich in protoplasm. The cells underneath the root-hairs are enlarged, and the protoplasm is hyperchromatic. The fungal hyphae make a few convolutions in these elements as they pass to the cortex. The inner epidermoidal layer is composed of smaller elements often with folded walls, and rich in protoplasm.

The cortex consists of ten to twelve layers of cells loosely arranged in radii. A small number contain starch. The Fungus is distributed in irregular sections of the outer and medio-cortex, vesicles being formed in the neighbourhood of the nuclei, connected by two or three filaments only with the vesicles in adjoining cells. The cortical cells in the region internal to portions inhabited by the Fungus show an increased radial diameter.

The vesicles are more or less closely applied to the nuclei, which do not differ greatly from the normal in form, size, or staining properties. This, and the fact that the chlorophyll-surfaces are comparatively limited, leads to the conclusion that the fungal symbiont must participate to a marked degree in the acquisition of food-substances.

The endodermis consists of evenly and slightly thickened cells, oblong-ovate in cross-section. The fibrovascular bundles are twelve to sixteen in number, and are arranged in the form of a thin ring. Each bundle shows one to three vessels with no centripetal development. The medulla is large, and composed of very loosely arranged elements.

The formation of a third epidermal layer resembling a velamen is not easy of interpretation, even in the light of Groom's experiments upon the formation of such tissues in terrestrial species. The presence of a velamen may be an adaptation for aeration, absorption, protection against loss of water, or a vestigial character of ancestral species which were formerly epiphytic. So far as this last contingency is concerned, the entire genus is terrestrial, and the nearest epiphytes are so distant that no relationship can be traced. An oriental species examined by Groom, *Spiranthes australis* (*G. australis*), exhibits similar features as follows (12, p. 205): 'Outside is a persistent *piliferous* layer of one layer of cells, the outer walls of which are feebly cuticularized. The walls are marked with reticulate cellulose thickenings which run in a direction at right angles to the axis of the root. Some of the cells grow out into very long slender root-hairs. Mycorhizal hyphae penetrate by means of these hairs, and by them alone. Within is a typical exodermis with suberized walls and passage-cells.' Groom also found that root-hairs were entirely absent from the two-layered epidermal system of *Lecanorchis malaccensis*, and that the outer layer was converted into velamen (12, p. 184).

The same author has pointed out that *Grammatophyllum speciosum*, which grows both as an epiphytic and terrestrial plant, develops a velamen more strongly in the latter form. It is evident that the only purpose that could be subserved by a velamen on terrestrial roots would be that of absorption; an adaptation that would be highly useful to a plant growing under conditions of temperature or soil which would destroy root-hairs. Furthermore the cells of the velamen would be constantly filled with water attracted by capillarity, which

would contain the humous products, and be available for the fungal symbiont. This arrangement would obviate the necessity for the external development of the hyphae. The presence of mycorhizal Fungi in epiphytic roots suggests a similar use of the velamen in them. It would be impossible for the hyphae in these organs to maintain an external form of sufficient size to be of any appreciable use in absorption. When the dripping water is drawn into the velamen, however, the organic compounds contained are readily taken up, and in turn transmitted to the higher symbiont. The velamen then is an absorbing device in some instances, of especial value in mycorhizal forms.

Sarracenia purpurea, L.

Sarracenia, as an example of the carnivorous plants, was examined to ascertain whether a second method of saprophytism might also occur. The simply branching-roots are yellowish brown in colour, due to the presence of globules of colouring-matter in the exoderm, which is modified in many instances by the network of hyphae ramifying over the surface. These hyphae penetrate the epidermis in many places, and pass through the exoderm by way of certain cells free from colouring-matter, forming clumps of coils near the nuclei of the cortical elements. The distribution is most unequal, and the Fungus must be considered as a parasite, though it is not present in sufficient abundance to work material harm to the host. Such semi-aquatic species as *Sarracenia* are generally without mycorhiza: moreover, mycorhiza has not been detected in any of the pitchered carnivorous plants. The same is true of the Droseraceae. The acquisition of a certain amount of organic food-material by the carnivorous habit would probably prevent any adaptation of the roots for a similar purpose.

Coptis trifolia (L.), Salisb.

The long slender creeping stems of *Coptis* give rise to a few thin unbranched roots which penetrate the loose substratum of decaying moss or humus in every direction. The

roots are covered with a network of septate branching hyphae which extend nearly to the apex, and become brownish with age. The epidermal cells in places are extended into papilla-like root-hairs, but in no instance does the Fungus gain entrance through these structures. Penetration of the epidermal cells is made directly through the outer wall, and clumps are formed in these and the exodermal cells. The arrangement is to be considered as an incipient ectotropic mycorhiza, since the state of the external layers appears to denote a heightened nutrition.

Various other species examined.

The roots of *Clintonia borealis*, *Sanguinaria canadensis*, *Helonias bullata*, and *Podophyllum peltatum* were examined and found to be free from Fungi, except an occasional adherent hypha on root-hairs and decaying epidermal cells.

Pterospora andromedea, Nutt.

Pterospora andromedea is the sole representative of this North American genus, and it ranges from Quebec, New Hampshire and Pennsylvania, westward through Michigan and Wisconsin to the Rocky Mountains, extending northward into British Columbia and southward into Arizona. Near the southern limit it is found only above altitudes of two thousand meters. Its habitat is in humus-soils under or near Oaks and Pines, or rarely other coniferous trees. The eight genera of Monotropeae are saprophytic, although some closely related genera are parasitic. *Monotropa* has been somewhat thoroughly investigated, and Oliver has recently published a paper in this journal descriptive of his morphological researches upon *Sarcodes sanguinea* (29). It is to be seen by reference to Oliver's work that many of the characteristics of *Sarcodes* are duplicated in *Pterospora*. The results of some observations upon herbarium-specimens of *Pterospora* are given by Oliver.

Nearly all the discussions of the dicotyledonous holosapro-

phytes have centered in the facts obtained from the study of *Monotropa*, *M. Hypopitys* in particular. It will be of interest to recall that three distinct theories have been advanced at different times in explanation of the nutritive system of this group. Unger, as a result of his investigations in 1840, concluded that *Monotropa* was a parasite upon the roots of trees (40). Various contradictory and indeterminate results were brought forward by a number of workers in the next forty years, until Kamienski demonstrated in 1881 (19) that it was not parasitic, and later gave an inclusive history of its development with careful attention to the relation of the higher plant to the mycelium sheathing the roots (20). After Kamienski had proved that *Monotropa* has no nutritive contact with other seed-forming plants, Frank advanced the opinion that it was actually parasitic upon the Fungus attached to the roots (9); a theory which is also supported by Kerner (21), who says, 'Since it is quite destitute of chlorophyll, and its aerial stems and leaves display no trace of stomata, the possibility of creating organic matter and of at all adding to its substance by means of aerial parts is excluded.' It may be said, in comment, that no actual proof is at hand to show that no advantage does accrue to the Fungus by its association with the higher plant. The possibility is by no means excluded that the shoot may absorb heat or light and use such energy in the promotion of metabolic processes, the products of which might be available for the Fungus. The union of the cells of the Fungus and the higher plant is of such an intimate nature that the assumption of no interchange of material is not justifiable. The mere mechanical lodgement of the Fungus on the roots of the other plant may be of benefit to it. The argument from the structure of the higher plant is invalidated from the fact that while the general relation of the Fungus and the higher plant is the same in *Monotropa* and *Pterospora*, the latter is furnished with stomata and other mechanisms for gaseous interchange. The symbiosis of the Monotropeae and the associated Fungi must be regarded as

established in the light of all known facts, though many points remain to be explained.

The work of the writer upon *Pterospora* was carried on in the field in the Rocky Mountains from Idaho to Arizona, and upon alcohol- and herbarium-material obtained from numerous points in its range.

Pterospora is furnished with an ovoid mass of dark brown club-shaped roots which ramify densely through a space of not more than 150 to 200 cc., in which the roots occupy a much greater proportion of the volume than the included humus. A heavy cylindrical shoot arising from this comparatively small mass of roots attains a height of 50 to 150 cm., and bears a profuse raceme of pendulous flowers. The stem is smaller at the base, swelling a few centimeters above to a thickness nearly double that of the base, then tapering to the apex. Glandular, fringed, oblong-lanceolate scales, 1 to 2 cm. long, are found on the lower part of the stem, decreasing as they ascend until they form but minute bracts subtending the pedicels. The stems bear elongated septate hairs and stalked glands, the latter affording a sticky secretion that renders the plant extremely viscid to the touch, and causes the adherence of spores, of its own seeds, and of organisms from the atmospheric plankton and even fragments of leaves and flowers. The entire plant is reddish or purplish-brown from the presence of an anthocyan or some tannin-derivative in the epidermal system. The colouring-substance is a pale eosin-red in alcoholic solution. The examination of such a solution which had been made in the field a month before, failed to show any distinct absorption-bands, a fact which does not preclude a possible relation to light useful to the plant. The sap has a decidedly astringent quality. Some of the clumps examined showed two or three stumps of old stems, and others having one or two living shoots exhibited two or three buds. From the general aspect of the clumps it seems reasonable to conclude that the plant has a somewhat protracted existence as a seedling, slowly developing the small compact mass of roots,

accumulating reserve food, and finally developing the enormous reproductive shoots. In no other manner does it seem possible to account for the great discrepancy between the root-system and the shoot. The formation of reproductive branches continues for two or possibly three seasons, and then the entire plant dies. During the winter season the old shoots die away, but in some instances remain attached to the clump. As a matter of fact the stems begin to dry and harden with the ripening of the seeds in August. The extensive degeneration of the root-system without a corresponding alteration in the shoot, is a phase of this species for which no satisfactory explanation is offered by the facts at hand. It certainly has the appearance of tending to the rapid extinction of the species.

The Roots. The absorbing organs of *Pterospora* consist of several primary roots, attaining a length of 9 to 20 mm., giving off a number of club-shaped rootlets 6 to 9 mm. long which in turn show short cylindrical or flattened branches of the third order. The roots and branches intertwine to form a compact globoid mass the boundaries of which are sharply defined, as no branches or tips project beyond the general outline. The apices of rootlets on emerging from the mass appear to curve back toward it, in a manner indicative of chemotropic reaction. A section of the mass shows the tissue of the roots separated by thin streaks of mycelia and humus. The slice will be quite firm and coherent both in living and alcohol-material, having the consistency of a potato-tuber. The sections of the different roots do not separate entirely even when made thin enough for microscopical examination. The amount of humus included in these layers is small and quite insufficient for the needs of the plant, but it is to be seen that the hyphae ramify thickly through the soil in all directions. The arrangement of the roots permits no clamping action on the substratum, and hence these organs may be said to have lost their primitive function of fixation. Kamienski found that the finer branches of the roots of *Monotropa Hypopitys* extend out into the soil a distance of

a foot and a half. Oliver's examination of *Sarcodes* was made upon alcohol-material, and hence no measurements could be made of the penetration of the soil. Since this plant has a strong development of the root-system, by which it has a bulk much in excess of that of the shoot, it seems that *Pterospora* has a much more reduced absorbing system than any saprophyte hitherto examined.

The epidermis is coated with a dense brownish mycelium of a thickness equal to two or three times the radial diameter of the epidermal cells. The hyphae are septate and the mycelium is firm throughout, the hyphae which pass into the substratum being given off directly from the mass with no loosely arranged transition-layer as in *Sarcodes*. Numerous reproductive branches, resembling those of *Penicillium*, are to be seen on the external surface. A great number of sections were cut for the purpose, but it was impossible to identify these structures with the mycelium beyond doubt. It seems probable that they are continuous however.

The epidermis is made up of ovoid elements with the narrower end between the walls of the layer beneath and the broader outer ends separated by a mycelial layer. The exfoliation of the epidermis and mycelium in the older roots results in leaving the outer convex and lateral walls of the subepidermal layer free nearly to the base or inner end. The hyphae penetrate the epidermal cells forming irregular vesicles and variously distorting the nuclei. Hyphae are seen to enter the subepidermal layer in some instances, though whether these were derived from the mycelium or not could not be determined.

The root-cap of *Pterospora* is more than two layers in thickness and resembles that of *Sarcodes*. In free tips the mycelium coats the cap and penetrates the older cells, but in the interior of the mass of roots, where the rootlets find themselves in a *cul-de-sac*, the mycelium penetrates the tissues beneath the cap. Backward along the root in all cases the old cells of the cap are to be seen held in the meshes of the mycelium.

The roots of *Pterospora* branch exogenously. The seven to ten layers of the cortex are composed of ovoid cylindrical elements with the tangential greater than the radial diameter, an important deviation from the general arrangement of this tissue in the roots of saprophytes. The subepidermal layer undergoes radial division in older cells during or after exfoliation of the epidermis. Storage-material in the form of starch is to be seen in the cortex of the apical portions. The central cylinder resembles that of *Monotropa* most closely. The tracheal elements are lattice-cells. Secondary thickening occurs, while sclerotization and lignification are entirely lacking in contrast with *Sarcodes*. The periblem and plerome are easily separable. The entire embryonic region is heavily loaded with starch, staining first a reddish brown then a bluish black with iodine solution.

The Shoot. The shoot of *Pterospora* consists of a short rhizome which sends up each year a cylindrical stem bearing a terminal raceme of pendulous flowers and a number of scale-leaves of reduced size. The stem-system of this plant exceeds that of the root many times in bulk, and it exhibits minor anatomical differences from *Monotropa* and *Sarcodes*, chiefly as a physiological result of its immense size. The medulla is from 3 to 6 mm. in diameter, making up about half of the cross-section of the stem, and is composed of cylindrical elements with ample intercellular spaces. The fibrovascular bundles contain one or two annular and two or three spiral vessels, with a few elongated elements of slight differentiation which are perhaps tracheides. A number of closed sieve-tubes are present. The xylem-bundles do not form a ring, but immediately external to the bast-region is a complete cylinder of heavy sclerenchyma composed of ten to fifteen layers of cells. Both the xylem and the sclerenchyma are distinctly lignified. Of other saprophytes examined, a species of *Burmannia* alone exhibits lignification of the shoot (Groom, 13). The cortex is composed of cylindrical elements arranged in circles with intercellular spaces which increase in size outwardly. The two or three

peripheral layers of the stem are furnished with numerous yellowish-brown bodies which give the characteristic colour to the plant. The epidermis is composed of elongated spindle-form elements of the greatest irregularity of size and arrangement. The epidermis and the two underlying layers are slightly lignified, thus giving the shoot two distinct cylinders of lignified tissue. The outline of the epidermis is exceedingly crooked, and shows deep invaginations directly over large air-chambers, while in other places distinct fixed stomata are to be found. The surface is thickly covered with two forms of trichomes. In some places short lozenge-shaped cells are extended outwardly into club-shaped hairs septate at the base only, or prolonged into a number of ovoid cylindrical cells reminiscent of the staminal hairs of *Tradescantia*. The trichomes of the second type consist of a globular many-celled gland, borne on a stalk consisting of a central column of cylindrical cells and an outer circle of flattened elements, the entire structure being derived from a small group of epidermal cells. The gland is seen to be covered with a viscous secretion, the chemical composition of which could not be determined, since it was apparently dissolved from the alcohol-specimens brought to the laboratory. Whether the secretion is for the purpose of preventing the visit of unwelcome guests to the flowers or whether it bears some relation to the transpiratory processes, cannot be determined without further observation. Spores find lodgement in the secretion and germinate, penetrating the gland and its stalk, but never effecting entrance into the basal cells or the epidermis.

The Scales. The scales near the base of the stem are smooth but with thin margins attenuated to form a fringe of stalked glands similar in structure to those found on the surface of the stem, except that the stalk is made up of a greater number of cells. The scales decrease in size upward along the stem. The basal scales are flecked with irregular patches of yellowish-brown areas, due to the penetration of the epidermal cells by brownish hyphae which completely fill them and extend over the surface of the

scales in a network. The scales are crescentic in cross-section with a row of simple fibrovascular bundles near the inner side. The parenchymatous tissue is provided with occasional large intercellular air-spaces. The scales on the lower part of the stem are furnished with a number of stomata on the outer or lower surface. Higher up these organs are replaced by trichomes of the simpler types found on the stem, which also extend to the pedicels and sepals.

Conclusions. The absorbing organs of *Pterospora* have undergone a very great degeneration, and reduction in size. The investing mycelium appears to be encroaching upon the tissues of the root by entering the epidermal cells, in which it differs from *Monotropa* and *Sarcodes*. The underground stem of *Pterospora* has undergone such changes that it would be impossible to replace the roots by stem-mycorhiza in case of further degeneration of the roots, a fact which may lead to the final extinction of the species because of the saprophytic habit. The alteration of the shoot has resulted in the loss of the chlorophyll-habit, and the reduction of the leaves. The retention of the probable original size of the axis and of the large inflorescence has been accompanied by an adaptation of the epidermal layers for the conduction of water, the use of the large medulla as a reservoir for water, and the retention of the transpiratory mechanism. It is to be noted that *Pterospora* is the only dicotyledonous plant without chlorophyll, beside *Cotylanthera*, that is furnished with stomata. The small size of the shoot of the latter shows that no correlation can be traced between the bulk of the shoot and the presence of the stomata. It is much more probable that the matter is due to the habitat.

General Conclusions.

The independent saprophytes comprise organisms that have undergone certain adaptations whereby complex food-substances may be used. The necessary adaptation may be principally of the absorptive mechanism, or it may entail

changes in the metabolic activity of the protoplasm as a body. The passage of 'organic' substance through typical ectoplasmic layers is admittedly accomplished with difficulty, and the modifications to facilitate absorption would consist in alterations of the diosmotic properties of the limiting membranes, rendering them permeable to such substances, or promoting the transmission of the ions of a substance separately; or in such changes in character as to enable the membranes to unite with compounds yielding them internally, in their original form; or in the formation of special canals for chemical transmission; or in the establishment of electrolytic action, or a degree of potential that would facilitate absorption (Pfeffer, 30). On the other hand, the essential feature of saprophytism may consist in the ability to use feebly oxidized bodies such as are utilized by Fungi. It is indeed probable that saprophytism implies modifications along several of the lines indicated. The saprophytic adaptation is highly characteristic of the Fungi, and physiologically must be but little removed from parasitism so far as the nature of the adaptation is concerned.

The Fungi are often united with other forms as parasites and in a manner in which the second plant also receives some benefit. The union of Fungi and Algae in this manner has resulted in the formation of certain definite types of morphological constitution of such constant character as to constitute a distinct taxonomic group, the Lichens. In symbiotic unions with Pteridophytes and Phanerogams, the higher plant, by reason of its superior organization and greater physiological inertia, has retained its individuality more nearly, and the association results only in modifications of certain organs. It is difficult to account for the origin of mycorhizal arrangements. Presumably Fungi are attracted to the bodies of other plants chemotropically, and in both parasitism and symbiosis it has formed coating mycelia, and has penetrated into various tissues in different plants. The critical fact, however, which resulted in the absorption of material from the higher plant by the parasite, or set up an interchange

between the two, is not yet above the horizon. Presumably the immersion of the free portion of the mycelium in a substratum rich in available food is a pre-requisite in symbiosis of this character. Given then a fungal body with one portion immersed in a nutritive substratum and another in or on the tissues of a higher plant, it seems reasonable to suppose that certain substances would be most easily obtainable from the soil and others from the body of the higher plant. The same reasoning may apply to the higher plant, and it may be supposed to withdraw from the fungal protoplasm substances which may not be so easily obtained elsewhere by the laws of absorption. The deprivation of the Fungus of a nutritive substratum would certainly result in parasitism. As may be seen by reference to the anatomical details given by previous investigators, the protoplasm of the two symbionts is generally very closely united, thus avoiding the necessity for the special adaptations mentioned above.

The chemical interchange between the symbionts must be that which would result from the capacity of the Fungus to assimilate compounds of nitrogen poor or lacking in oxygen, and the superior ability of the higher plant to carry on metabolism of the carbohydrates, in accordance with Groom's well-founded conclusions (13).

The Fungi entering into the formation of mycorhizal structures are comprised in the Oomycetes, Gasteromycetes, Hymenomycetes, and Pyrenomycetes, an accident of habitat rather than morphological constitution so far as present information goes. These Fungi are capable of an independent existence outside the body of the higher plant. The period of existence of the portion of the mycelium in contact with the other symbiont may be identical with that of the absorbing organ; may exceed it as the writer found in *Aplectrum* and *Pogonia*, and Groom in *Thismia* (13); or the mycelium may perish early, thus hastening the death of the absorbing organs, as Mangin concludes in reference to the ectotropic mycelia of forest-trees (26).

The hyphae of Fungi forming ectotropic mycelia show

no important deviation from normal structure; but those of endophytic habit exhibit such important modifications that it is sometimes difficult to establish their identity with hyphae outside the roots, a fact accounting for the small number of determinations of mycorhizal Fungi. These modifications consist in reductions in the size of the hyphae, the character and appearance of the wall, and in the formation of organs or branches for reproduction or nutritive interchange. Bruchmann observed the formation of oospores on *Pythium* in the prothallia of *Lycopodium* (2), a fact confirmed by Janse, who also saw the ascospores of *Celtidia* in the roots of *Celtis* (16). Apart from these purely reproductive organs are others of similar origin which have perhaps become modified to serve vegetative functions. Among these are the 'sporangioles,' 'vesicles,' and hyphal clumps found in or between the cells. The vesicles and sporangioles of various writers are short lateral branches of the hyphae which undergo a globular enlargement at the apex, with more or less variation as to content. These organs are notably large in the Orchidaceae; and since they are generally, though not always, formed in the vicinity of the nuclei in the region of greatest metabolic activity of the higher symbiont, are to be regarded as organs for absorption and interchange. The occurrence of these structures between the host-cells would indicate the retention of the original function of reproduction. The hyphal clumps which have been noticed by the author in many of the mycorhizas described in this article, seem to be due to the repeated branching and chemotropic attraction of the apices of the branches toward a centre near the nucleus, without the formation of sporangioles. Several writers have noted that in some mycorhizas the sporangioles gave off numerous branches which became converted into the hyphal clumps, but in the material examined the intervention of the sporangioles could not be detected.

The Fungus may be associated with prothallia, roots, or stems in the formation of mycorhiza. This union usually

results in the degeneration of the organ invested by the Fungus. The degeneration of roots may amount to a reduction to a rudimentary condition, in which instance the Fungus is next associated with the stem, a state of affairs to be found in some holosaprophytes. Again, the degeneration might result from the modification of some other organ.

It is evident that species of seed-forming plants receiving a portion of their food from fungal symbionts, are in a condition to undergo much more rapid alteration or development than typical autophytes, because in addition to the influence of the ordinary environmental factors, the constantly increasing amount of complex food available has a tendency to set other forces in action which result in the degeneration of the absorbing organs, the chlorophyll-apparatus, and the transpiratory surfaces. *Calypso* and *Aplectrum* are of especial interest in this connexion, since these species afford still further opportunity for variation in the presence of the Fungus in a state of transit into a non-absorbing organ. Any cause which injures or lessens the supply of food available from the absorbing organs, or from the storage-tissues, will result in the outward and external development of the mycelium in transit, and an accompanying adaptation of the non-absorbing organ or offset into a coralloid mycorhiza, a fact which has been demonstrated experimentally by the writer. *Aplectrum* exhibits such variations that the descendants of the individuals now in existence may be divisible into three groups: viz, a form with ectotropic mycorhiza connected with the roots of Oaks and Maples; a second form with coralloid absorbing organs resembling *Corallorhiza*; and a third approximately similar to the type now prevalent. The second mode of variation seems to be more firmly fixed in *Calypso*, so that great differences are found in the individuals now composing the species.

The variation of the root and shoot as saprophytism increases shows the greatest diversity, and categorical distinctions of degree based on anatomical characters are not to be relied upon. This is especially apparent when saprophytes

retain such comparatively enormous shoots as does *Pterospora*, with stomata, the latter organs being also found on another dicotyledonous saprophyte with relatively small aerial members.

Recapitulation.

1. Saprophytism is an adaptation of the absorbent mechanism, or of the character of the metabolic capacity of an organism. Symbiotic saprophytism is the natural result of the supplemental capacities of two organisms brought into nutritive contact chemotropically.

2. Fungi comprised in the Oomycetes, Gasteromycetes, Hymenomycetes, and Pyrenomycetes, may form mycorhiza. They are capable of independent existence, and undergo modifications of the hyphae and reproductive organs in the portion of the mycelium in contact with the protoplasm of the higher symbiont. Sporangioles, vesicles, and hyphal clumps are organs of nutritive interchange.

3. The changes of the member of the higher symbiont harbouring the Fungus are of two kinds. One by which the degeneration of normal structures results, a very common condition. A second by which special cells are formed for the accommodation of the Fungus.

4. The cells entered by the hyphae generally show a decrease of carbohydrate, and an increase of proteid content. The morphological changes consist in fragmentation, distortion, increase in size and staining properties, and sometimes position of the nuclei, and in certain instances in repeated cell-division.

5. The velamen of terrestrial and aerial roots, and the trichomes of coralloid structures, are devices which facilitate absorption by endotropic Fungi.

6. Generally mycorhiza is not formed on specialized storage-organs. The fleshy-rooted Orchids are an exception however.

7. Mycorhizal Fungi sometimes penetrate non-absorbing organs. This is found in *Peramium* (*Goodyera*), *Aplectrum*, and probably *Calypso* and other Orchids.

8. The Fungus symbiotic with *Aplectrum* forms endotropic mycorhiza with the roots, passing out through the root-hairs into the substratum. The Fungus is transmitted to the vegetative offspring by entering the offset through the special trichomes near the base, traversing its length in response to chemotropic stimuli, and descending into the young roots directly. No degeneration of the stele occurs, and the storage-tissues at either end of the offset are not penetrated.

9. The acquisition of the habit of symbiotic saprophytism renders a group of plants extremely unstable as to specific characters.

10. The penetration of non-absorbent organs by the fungal symbiont affords opportunity for the utilization of these organs for absorption in case of a diminished supply of food from the ordinary sources.

11. The experimental formation of mycorhizal structures from non-absorbent organs penetrated by the symbiotic Fungus has been accomplished by the writer.

12. There are but few constant anatomical characters indicative of partial or complete saprophytism. The presence of a symbiotic Fungus in prothallia, and in root- and stem-mycorhiza may be taken as an indication of the use of complex foods obtained from without. Lack of chlorophyll is the only invariable accompaniment of holosaprophytism, while degeneration or alteration of the stele is not always consequent. Stomata, usually thought to be lacking in Phanerogams destitute of chlorophyll, are present in *Epipogium*, *Aphyllorchis*, *Lecanorchis*, *Cotylanthera*, and *Pterospora*.

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EXPLANATION OF FIGURES IN PLATES I AND II.

Illustrating Prof. MacDougal's Paper on Symbiotic Saprophytism.

PLATE I.

Fig. 1. Typical specimen of *Pterospora andromedea*, actual height 80 cm. The apical flowers are unopened, while seeds have been matured in the basal ones. The entire system of roots is present. Drawn from a specimen collected by the author at Schultze's Pass, San Francisco mountains, Arizona, August 3, 1898.

Fig. 2. Young specimen of *Pterospora*, showing buds *b*, *b*, *b* at base of stem. The portion of the root-system cut away is indicated by the dotted outline.

Fig. 3. Cross-section of root-system showing relation of roots to enclosed humus. × 20.

PLATE II.

Fig. 1. Mature specimen of *Aplectrum* developed from coralloid offset. *m*, coralloid offset or stem-mycorhiza. *r*, separatory layer of leaf.

Fig. 2. Apex of coralloid formation (stem-mycorhiza) of *Calypso*, showing rudimentary leaves. After Lundström.

Fig. 3. Apex of stem-mycorhiza of *Calypso* without leaves. After Lundström.

Fig. 4. Diagram of the method of branching of the stem-mycorhiza of *Calypso*. After Lundström.

Fig. 5. Diagram of the method of branching of root-mycorhiza of *Pterospora*.

Fig. 6. Apical portion of rootlet of *Pterospora* showing the branches of the third order as conical projections.

Fig. 7. Outer layers of root of *Pterospora* and mycelium: *n*. nucleus of epidermal cells; *v*. vesicular formation of Fungus.

Fig. 8. Diagram of cross-section of *Aplectrum* with adhering tree-mycorhiza.

Fig. 9. *Aplectrum*. *m*. coralloid mycorhiza: *s*. scission layer.

Fig. 1.

Fig. 2.

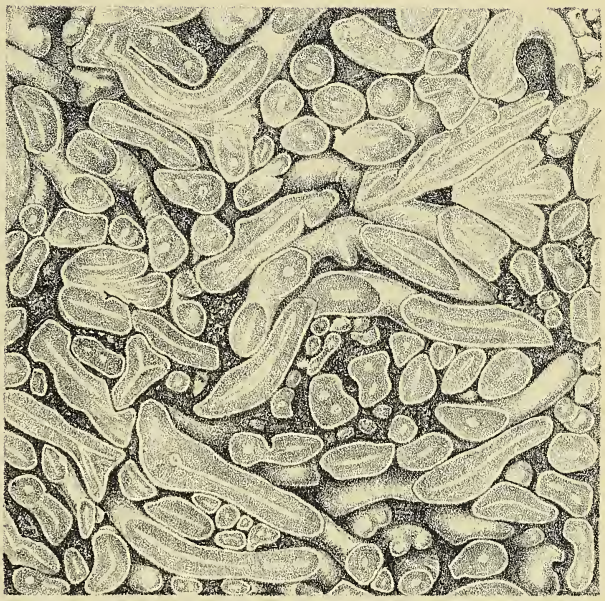

Fig. 3.

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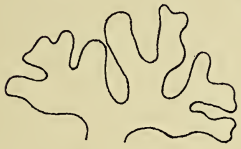
Fig. 5.



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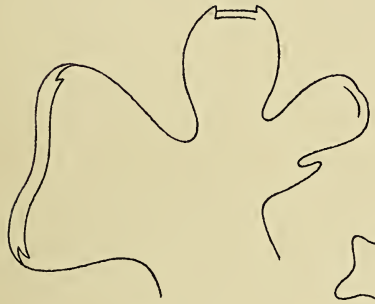

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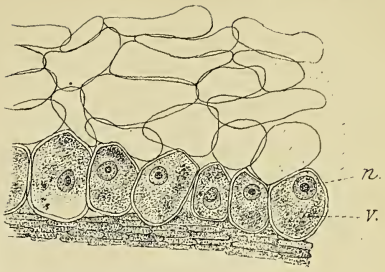

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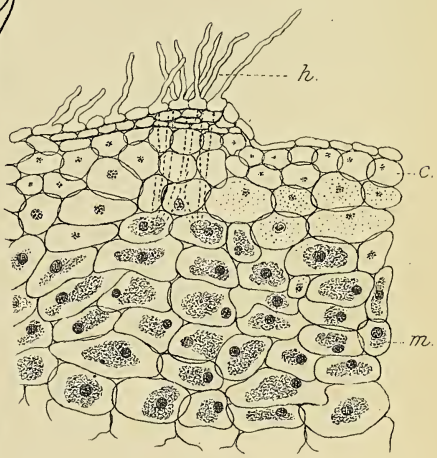

Fig. 9.

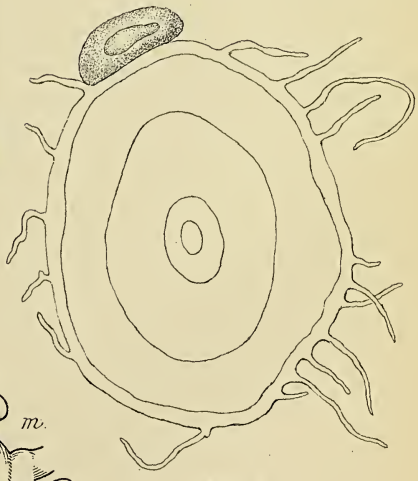

Fig. 8.

University Press, Oxford.

Cellulose-Enzymes.

BY

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I. Historical.

APART from a few Bacteria, there have been, to my knowledge, but four plants reported from which a cellulose-dissolving enzyme has been extracted. In 1886 de Bary[1] published an account of *Peziza Sclerotiorum*, whose mycelium was able to penetrate cell-membranes and to gelatinize them. The expressed juice of this Fungus, especially that of its sclerotia, dissolved the middle lamella and gelatinized the inner lamella of cell-walls of the roots of the Carrot and Turnip. The expressed juice of cultures of the Fungus on the Carrot and Turnip, when treated with an excess of alcohol, sent down a flocculent precipitate, which, when dried and redissolved in water and acidulated, had the same effect on cellulose membranes as did the untreated expressed juice. In 1888 Marshall Ward[2], by methods similar to those of de Bary, demonstrated a like ferment

[1] Ueber einige Sclerotinien und Sclerotienkrankheiten: Bot. Zeit., xliv. 377.
[2] On a Lily-disease: Ann. of Bot. ii. 319.

[Annals of Botany, Vol. XIII. No. XLIX. March, 1899.]

in a closely allied Fungus,—an undetermined *Botrytis* which is probably a *Peziza*. Brown and Morris[1], in 1890, in studying the germination of the Barley, observed, as others had before them, that the cell-membranes of the endosperm were thinned down before the starch-grains were corroded. Later experiments by these authors showed that when sections of a Barley-grain were laid in a simple watery extract of Barley-malt, or in a watery solution of the alcoholic precipitate of the extract, the cell-membranes underwent solution as in normal germination. Green[2], in 1887, supposed that he had obtained a weak cytohydrolytic ferment in a glycerin-extract of the cotyledons of Date-seedlings. Subsequently, however, Brown and Morris[3] could find no cellulose-dissolving enzyme in extract of Date-cotyledons; and more recently Green[4] himself has declared that 'the search for a cytohydrolyst in the Palms has not been successful.' In 1894 Grüss[5] reported that sections of the Date-endosperm, after lying for two months in a glycerin-extract of sixty cotyledons of Date-seedlings, appeared corroded at the edges, and portions of the walls had begun to 'melt.' From the fact, however, that Grüss in his subsequent publications nowhere refers to the result of this experiment, one may infer that he regards it somewhat dubiously. Certain it is that we need stronger evidence to prove that a cytohydrolyst can be extracted from the Date.

Not all kinds of cellulose are soluble in the Barley-ferment, but probably only hemicellulose or that which is loosely called *reserve cellulose*. Brown and Morris[6] give a number of plant-organs whose membranes are attacked by the Barley-malt-extract, and others which are not affected.

[1] The Germination of some of the Gramineae: Jour. Chem. Soc. Lond., 57, p. 497.

[2] On the Changes in the Proteids in the Seed which accompany Germination: Phil. Trans. Roy. Soc. Lond., 178 B, 37.

[3] The Germination of some Gramineae: Jour. Chem. Soc. Lond., 57, p. 497.

[4] On Vegetable Ferments: Ann. of Bot. vii. p. 94.

[5] Ueber die Einwirkung der Diastase-Fermente auf Reservecellulose; Ber. d. d. b. Gesellsch., xii, Generalver., Hft., p. 60.

[6] L. c.

Grüss [1] has corrected this list, and finds hemicellulose present in all walls in which the Barley-ferment effects a change. The presence of acid was found by de Bary [2] and by Brown and Morris [3] to promote the action of the ferment.

In their paper already cited, Brown and Morris express the view that the cellulose-dissolving power of the Barley is due to a special enzyme, and not to the diastase. They found that by heating the Barley-extract for thirty minutes at a temperature of 60°, its cytohydrolytic power was almost wholly paralyzed; while its amylohydrolytic power was not diminished by heating to 70°. Grüss [4], without more evidence than that furnished by the fact that Barley-malt-extract dissolves reserve cellulose, and the fact that extract from Date-cotyledons attacks starch, has assumed in all his writings that diastase is a cellulose-enzyme, and also that wherever cellulose-enzymes are known, they are diastase. He suggests that the failure of the Barley-extract to attack cellulose after the extract had been heated to 60° by Brown and Morris may have been due to a weakening of the diastase by heating. A strong diastase solution, he says, will hydrolyze reserve cellulose.

Reinitzer [5], in a recent paper, seeks to strengthen Grüss's position by demonstrating what has long been known, that the wall-substance of the Barley-endosperm is easily hydrolyzed, and thereby suggesting the probability of the starch-enzyme being also the cell-wall enzyme.

It is believed that the following pages, besides extending our knowledge of the distribution of enzymes, will show that the assumption of the identity of all wall-dissolving enzymes with diastase is at present far from justified.

[1] Ueber das Verhalten des diastatischen Enzyms in der Keimpflanze: Jahrb. f. wiss. Bot., xxvi. 379.

[2] L. c. [3] L. c.

[4] Ueber das Verhalten des diastatischen Enzyms in der Keimpflanze; Jahrb. f. wiss. Bot., xxvi, (1894), 379: Ueber die Einwirkung der Diastase-Fermente auf Reservecellulose; Ber. d. d. b. Gesellsch., xii, Generalver. Hft., p. 60: Ueber Lösung von Cellulose durch Enzyme (Cytase); Abstract in Chem. Centrlblt., 1896, Bd. i, 313.

[5] Ueber das Zellwandlösende Enzym der Gerste; Hoppe-Seyler's Zeitschr. f. physiol. Chemie, xxiii. 175.

II. Enzymes which dissolve Reserve Cellulose [1].

A. Extract of Barley-malt.

In order to have a means of comparison for the ferments whose action has hitherto not been studied, a series of orientation experiments was made with the extract of Barley-malt, that is with the so-called *diastase*. The enzyme was obtained in the usual way, by extraction with water and precipitation with alcohol. Some means was employed to remove partially the nitrogenous material and sugar from the extract, and a very active enzyme was obtained by the following method:—at first sufficient alcohol was added to the watery extract to make the whole volume 40 % alcoholic. The resulting precipitate must have contained, besides some amount of enzyme, a large quantity of sugar, dextrine, and proteid matter. This precipitate was rejected, and the filtrate raised to 80 % alcoholic mixture. This precipitate now collected was used in several of the following experiments. In other cases, the dried precipitate last mentioned was partially redissolved in water, and again precipitated by adding alcohol to the watery solution. Thus there was obtained an extract tolerably free from carbohydrates, as determined by Lintner [2].

As a test for the action of the ferment upon reserve cellulose, sections of the endosperm of *Hordeum vulgare* and of the cotyledons of *Lupinus albus* were employed. A dry Barley-grain was split longitudinally, and cross-sections taken only from the middle region of the half-grain. Thus the sections were all closely alike in composition. They were immediately killed and extracted in chloroform, washed for fifteen to thirty minutes in water, and then left in dilute saliva for twenty-four hours, at about 22° temperature. To prevent

[1] For many preliminary tests for this part of the work, the author is indebted to Miss Edna D. Day, a graduate student in botany. Miss Day performed her work with rare patience, skill, and understanding.

[2] Jour. f. prak. Chemie, 142, p. 386.

the growth of Bacteria and Moulds, a little chloroform was always added to such a preparation. At the end of twenty-four hours, sections wholly freed from starch, and all not more than two or three cells thick, were selected with the aid of the microscope, washed in water, and placed in the ferment.

Ferment-solutions of various strengths were employed, from 150 mg. of the dry powder in 10 cc. of water to 150 mg. in 3 cc. of water. In no case was the whole of the solid ferment dissolved, but the clear, supernatant liquid, after standing for twenty-four hours in the presence of the solid material, was removed for experimentation. During this process a little chloroform was employed to hold micro-organisms in check. All the ferment-solutions were acidulated with hydrochloric acid not to exceed ·05 per cent.

Various methods were used in testing the action of the ferment on the cellulose walls, but the one found most satisfactory was the following:—on a glass slide were placed three or four drops of the ferment-solution, and a Barley-section, prepared as before described, was introduced. Three bits of tinfoil were then properly disposed around the liquid on the slide, and on these a cover-glass was laid. The slides thus prepared rested on a support in a closed dish whose bottom was covered with water and chloroform. The dish was kept in an incubator whose temperature varied between 32° and 34°. By this method the evaporation of the ferment-solution was very slight and the growth of micro-organisms was prevented.

Examining these preparations at various intervals, it was found that the walls of the starch-bearing cells of the Barley-endosperm are dissolved down to the middle lamella within five to ten hours, according to the strength of the solution used. The middle lamella persists for a considerable period, the shortest period of solution being noted as twenty-seven hours, and the longest as two hundred and fifty-seven hours; during the latter period the ferment was renewed several times. The walls of the aleurone-layer, as noted by Brown

and Morris [1], are much more resistant to solution than those of the starch-bearing cells. One set of preparations showed, in a very strong ferment-solution, these walls completely hyaline in twenty-seven hours; but in most of the solutions only after several days. Reinitzer[2] states that the membranes of the aleurone-layer of the Barley-endosperm are composed mostly of hemicellulose plus a little cellulose, and these walls, he says, the Barley-malt-extract will not attack. In this he is wrong; for not only do these walls show by the great change in their optical properties that they lose the most of their substance, but, after becoming hyaline, they very gradually 'melt' away. I have, with camera-drawings, followed very carefully the gradual wasting away of these walls for a period of two weeks. By this means I have seen exposed walls shorten and shorten and finally disappear. There is not the slightest doubt, therefore, that the Barley-malt-extract is capable of dissolving wholly the walls of the aleurone-layer.

Brown and Morris [3] have described the solution of the thin walls of the endosperm of the Barley as showing the same phenomena in normal germination of the grain and in the extracted enzyme. They found first a swelling of the membranes, then a stratification, then a breaking up into spindles which finally disappeared, the middle lamella disappearing last of all. My own observations confirm these in a general way. But I have found that the shredded appearance of the membrane, which Brown and Morris evidently referred to as a spindle-structure, is followed always by the appearance of a middle, narrow, bright band which is the middle lamella, flanked on each side by a broad, hyaline band, and this last bounded against the cell-lumen by a very narrow, bright band. In other words, the first part of the wall to become hyaline is a strip, on each side, between the middle of the wall and the free edge. The resistant band

[1] Jour. Chem. Soc. Lond., 57, p. 497.

[2] Ueber das zellwandlösende Enzym der Gerste: Zeitschr. f. physiol. Chemie, xxiii. 190.

[3] L. c.

lying next the cell-lumen soon breaks up into small granules, the hyaline zones gradually fade away, the middle lamella becomes thinner and thinner till it vanishes. Just at the time of the disappearance of the middle lamella, the employment of Bismarck-brown will demonstrate a gelatinous residue; a later test on a similar older preparation will fail, however, to show any remaining trace of a membrane.

To test the action of Barley-malt-extract on the reserve cellulose of *Lupinus albus*, seeds of that plant were softened for twelve to twenty-four hours in water, and thin, partial cross-sections taken from the middle of a cotyledon. These sections were extracted in chloroform or ether, then in water, and then immersed for twenty-four to forty-eight hours in a solution of extract of pancreas, by which the cell-contents were for the most part removed. As far as a microscopical examination could detect, the cell-walls suffered no change even when immersion in extract of pancreas was continued for two weeks. Preparations were made on glass slides, just as described for the Barley-sections; the temperature was maintained at 32° to 34°; and chloroform was employed, which successfully kept down the growth of Bacteria and Fungi.

It was found that a ferment-solution sufficiently active to convert, in a section of Barley-endosperm, all the thin walls except a middle lamella to the hyaline condition in nine hours, required as many days to reduce the walls in the Lupin to the hyaline condition. The process must be described in greater detail:—within twenty-four hours of the immersion of the section in the malt-extract, the thick membranes can be seen to have lost their brilliant appearance in a narrow zone next the cell-lumen. This change to a hyaline condition progresses centrifugally from the cell-centre, requiring one to two weeks to reach the outer part of the inner lamella. In the first few days of enzyme-action, the wall becomes distinctly laminated. The outer zone of the inner lamella is apparently more resistant than the remainder of this layer, for it remains brilliant, even in very thin sections of the cotyledon, for a month after the action of the ferment begins. When a

preparation on a glass slide is continued for so long a period with such a small amount of ferment-solution, the ferment must necessarily be renewed from time to time. If much evaporation is allowed to take place, the section soon becomes encrusted with a crystalline deposit which does not subsequently dissolve, and which interferes with observation and possibly with ferment-action. I have practised, therefore, washing out the ferment with water, and renewing the active solution twice a week. By this means the section is always transparent, the increasingly hyaline condition of the wall can be followed, and finally the hyaline and almost invisible wall can be seen to become finely granular and wholly disappear. The application of Bismarck-brown shows no remnant of wall remaining. In strong Barley-malt-extract, therefore, the loss of substance from walls of the Lupin begins almost immediately after immersion; but, in the conditions here set forth, the complete dissolution is not effected, even in a thin section, for four or more weeks, and in this period only at the edges of the section. In this connexion it may be noted that Grüss[1] found sections of the endosperm of *Phoenix dactylifera* showing a hyaline condition and a solution at the exposed edges of walls first after two months' action of a strong solution of Barley-malt-extract.

The debated question of the thinning of the cotyledonary walls of the Lupin during germination will be considered later in the treatment of the enzyme of *Lupinus albus*.

Once for all a statement may be made regarding controls:—neither extract of pancreas nor saliva dissolved or made appreciable change in the membranes of the Barley-endosperm, though sections were kept in these enzymes for two weeks at 18° temperature. Similar sections of like material were kept for two weeks, at 32° to 34° temperature, in water acidulated to ·05 % with hydrochloric acid, without showing in their membranes more change than a slight fibrillar structure. Sections of the cotyledon of *Lupinus albus* after two weeks' immersion

[1] Ueber die Einwirkung der Diastase-Fermente auf Reservecellulose: Ber. d. d. b. Gesellsch., xii (1894), Generalver. Hft., 60.

in like acidulated water showed no other change than a slight swelling and lamination. Sections of the endosperm of *Phoenix dactylifera* were kept for three months at 32° to 34° temperature in water, both chloroformed and acidulated with hydrochloric acid, and then displayed no perceptible change under the microscope. These results should remove all doubt as to the solvent action of the extracts here used, and incidentally dispose of the caution suggested by Reinitzer [1] when he proposed that Grüss's [2] results with Barley-malt-extract on Date-endosperm might have been due to the conversion of chloroform to hydrochloric acid.

B. *Extract of Aspergillus Oryzae.*

It has been known for many years that the Fungus *Aspergillus Oryzae* has the power of converting starch into sugar. More recently an extract of this Fungus has been prepared as a proprietary article under the name *Taka-diastase*, which is sold as an aid to the digestion of starchy foods. As far as I know the extract has been tested merely with regard to its action on starch. We shall see that it actively hydrolyzes reserve cellulose also.

The commercial extract is a brownish-white powder almost wholly soluble in thirty times its weight of water. As with the Barley-malt-extract so with this, sections of the Barley-grain were employed to determine the cellulose-dissolving power. The sections were killed, freed from starch, and each mounted in three drops of the ferment-solution on a glass slide just as had been done in the Barley-extract. Here also the ferment-solution was acidulated with hydrochloric acid, and the preparations were placed in the presence of vapour of water and chloroform in an oven at a temperature of 32° to 34°. The endosperm-walls of the Barley became hyaline within a few hours, the process being different from what it was in the Barley-extract. In the latter, a middle band of the wall

[1] Ueber das Zellwandlösende Enzym der Gerste: Zeitschr. f. physiol. Chemie, xxiii. 180.

[2] L. c.

and the two contour bands lying next the cell-lumina remain brilliant for some hours after the intervening broader zones have become hyaline, while with the *Aspergillus*-ferment, the hyaline condition is first seen in the middle of the wall, and progresses toward the cell-lumina. It is somewhat remarkable though that when the wholly hyaline wall begins to disappear, it thins down from the borders toward the middle of the wall, so that a faint and thin middle lamella often persists for forty-eight hours after the beginning of the experiment. The walls of the aleurone-layer become hyaline in the same manner as with the Barley-ferment, that is, centrifugally from the cell-lumen to the middle of the wall. The action of this ferment has been followed far enough to demonstrate the gradual and complete solution of the whole wall-substance of the aleurone-layer, since the exposed ends of walls have been seen to 'melt' away after three to four days' immersion in several changes of ferment.

The sections of the cotyledons of the ungerminated seed of *Lupinus albus* were prepared for the extract of *Aspergillus* as they were prepared for the Barley-extract. Their cell-contents were removed with chloroform and extract of pancreas; they were placed in a few drops of ferment-solution on glass slides; the glass slides were enclosed in a moist chamber containing chloroform; and the moist chamber was kept in an oven at a temperature of 32° to 34°. The phenomena of membrane-solution are almost the same here as in the case of the Barley-ferment, except that the process requires less time. After one to two days the membrane appears slightly swollen and laminated. In three or four days it has become wholly hyaline except for a very narrow zone corresponding to the middle lamella. When the middle lamella is split, as occurs very often in this tissue in the formation of intercellular spaces, this brilliant, unaltered band lies, one half of it, upon each inner lamella. The inner lamellae become more and more transparent and gradually fade from view. The middle lamella also becomes hyaline, and I have seen it disintegrating without residue at the

exposed edges of the sections, an indication that with a long continued action of the ferment the whole wall would go into solution.

By two tests the *Aspergillus*-ferment shows itself more active than the Barley-ferment in attacking reserve cellulose. If the same weights of dry ferment of each kind are dissolved in equal volumes of water, the *Aspergillus*-ferment effects a solution of reserve cellulose the more quickly. This means of comparison has, however, little value, since it is unfair to compare the dry weights till more is known of the purity of the ferments in the extracts. A better method is the following:—if solutions of both ferments are made so that each will act upon starch with the same degree of intensity, then I have found that the Taka-ferment effects a solution of walls of Barley-endosperm and cotyledonary walls of the Lupin in shorter time than the Barley-ferment effects such solution.

The period required for the solution of cell-membranes is greatly prolonged when the ferment must penetrate to a considerable depth. For, though Grüss[1] has shown that cellulose-enzyme will penetrate a membrane and that diastase will migrate through a membrane, and my own observations recorded here show that the Taka-ferment causes the middle of the Barley-endosperm-wall to become hyaline before the outside of the wall, yet the cellulose-ferment works from the surface of a section comparatively slowly toward the interior, so that in a thin section of a cotyledon of the Lupin in a strong ferment often renewed, the walls of the most deeply lying cells may require many weeks to yield up all their substance to solution.

C. *Extract of Lupinus albus.*

Seedlings of *Lupinus albus* were grown in sawdust till they had attained a length of approximately 10 cm., when the cotyledons were gathered, minced, and dried in an oven

[1] Ueber das Verhalten des diastatischen Enzyms in der Keimpflanze: Jahrb. f. wiss. Bot., xxvi (1894), 379.

at a temperature of 35°. The material was then ground, extracted with water for an hour, filtered, and the filtrate mixed with alcohol so as to make the whole 20 % alcoholic. Following this treatment, there was a copious deposit of a medium slaty colour, which was rejected. To the supernatant, clear liquid there was added enough alcohol to make the whole 85 % alcoholic. The resulting precipitate was large in amount, and of a whitish slaty colour. This on drying over sulphuric acid in a vacuum became brown. Some of the following experiments were made with solutions of this dry material; while for the others a solution of this material was reprecipitated with alcohol and redissolved. Both ferment-solutions worked energetically on reserve cellulose, with an indication that the twice-dissolved precipitate was the more active. It is now known, therefore, that such a precipitate as the foregoing contains not only asparagin, proteids, and soluble carbohydrates, but also an enzyme-mixture with the properties of rennet, trypsin, diastase, and a cytohydrolyst.

It is probably because of the large content of albuminous material in the Lupin-extract that ferment-solutions from this extract are so difficult to keep free from Bacteria—much more difficult than the extract from any other plant I have used in these experiments. Bacteria grow in the Lupin-extract even when several drops of 2 % formalin are added to 5 cc. of the ferment-solution, and when a crystal of thymol is kept in the solution. They will not grow, however, in the presence of an abundant supply of chloroform, although I have found them active when enough chloroform was still present to enable a strong odour to be detected.

A solution of 100 mg. or less, of the dry Lupin-extract in 10 cc. of water acidulated with hydrochloric acid, shows itself very slow in its action on the cellulose of the endosperm of the Barley-grain. Sections of the Barley-grain for these tests were prepared as for the preceding ferments. Sections of similar dimensions were selected and their starch was dissolved out by saliva diluted with an equal volume of

water. When the ferment-solution was made with 150 mg. or more, of dried extract in 10 cc. of acidulated water, the action on the Barley-walls was rapid and convincing. All of the thin walls of the endosperm, except the middle lamellae, became hyaline in six to seven hours, at a temperature of 32° to 34°. In ten hours and thirty minutes the whole wall was hyaline. In several preparations no trace of a wall could be found twenty-four hours after the beginning of the enzyme-action; the hyaline wall had gradually 'melted' away.

As in the extract of Barley-malt and of *Aspergillus Oryzae*, so here the walls of the aleurone-layer of cells dissolve much more slowly than do those of the starch-bearing cells. They become hyaline in two to three days, and can be seen to waste away slowly at the exposed edges of the section, though after many weeks' action of the ferment not the whole row of aleurone-cells in the section will have disappeared. This gradual solution at the edges of the section has been demonstrated by repeated camera-drawings of a single section during a period of twelve days. The layer of wall lying next the cell-lumen is the last portion to become hyaline.

If in a strong solution of Lupin-extract is immersed a section of a cotyledon of an ungerminated seed of the same species, the section having been freed of cell-contents by the action of the extract of pancreas, and the preparation being maintained at a temperature of 32° to 34°, the following process can be observed:—within a few hours of the entrance into the solution, the section becomes flabby and tender, having lost its former rigidity and firmness: the walls swell somewhat, but not greatly, and become laminated. In twenty-four hours the laminated appearance has disappeared, the middle lamella shows partial or complete solution, and the thick inner lamella presents an inner zone against the cell-lumen, looking gelatinous and almost invisible, while the rest of the inner lamella still presents the firm and glistening appearance shown by the whole wall in the ungerminated seed. During the next twenty-four

hours the hyaline condition involves the whole wall. The section is now so tender that a little shaking will break it into fragments. The walls in becoming hyaline swell somewhat, but not greatly. Under the microscope they are almost if not entirely invisible, except for the boundary of the inner lamella lying farthest from the cell-cavity. Even after a week's action of the ferment, the application of Bismarck-brown will demonstrate the presence of the full 'ghost' of the inner lamella. A cover-glass pressed gently upon the section causes the whole to pass into a disorganized jelly-like, granular mass. A section kept in ferment-solution for twenty-three days showed under the microscope no remains of the inner lamellae except a thin zone corresponding to the border distal from the cell-centre. When Bismarck-brown was applied, it was shown, however, that the complete 'ghosts' of the interior cells of the section were still present; but in the outermost cells there had been a great loss in the original thickness of membrane, and there had been a gradual consumption of the entire wall at the edges of the section. The observation was not carried farther.

Comparing the results of the many preparations of the Lupin-ferment with those of the Barley- and *Aspergillus*-ferment, some solutions being weak and some the strongest possible with each ferment, it seems certain that the Lupin-enzyme attacks reserve cellulose more energetically than does the enzyme of either Barley or *Aspergillus*.

Regarding the behaviour of the thickened cell-walls of *Lupinus* seeds during germination, observers have differed. Schulze, Steiger, and Maxwell[1] working together upon *Lupinus luteus*, digested the seeds with dilute sulphuric acid, obtaining galactose and mucic acid. The authors submitted the solid matter left after this treatment to a microscopical examination by Cramer, who reported that there had been a *loss of substance* from the thick walls. The authors ascribed, therefore, the origin of the galactose and mucic acid to the

[1] Zur Chemie der Pflanzenmembranen, i: Zeitschr. f. physiol. Chemie, Bd. 14, p. 227.

substance of the thick cotyledonary walls, naming it *paragalactan*. Subsequently Schulze [1] tested the seeds of *Lupinus angustifolius* in the same way, showing by chemical analysis and by a microscopical examination by Dr. Pfister that almost all, if not all, the material dissolved out by the dilute sulphuric acid came from the thickenings of the cell-walls. It is to be noted here that neither Cramer nor Pfister maintained that the walls had become thinner by their treatment with acid. Tschirch [2], however, states positively that as germination progresses the walls become thinner and thinner. Nadelmann [3], examining the seedlings of *Lupinus albus* and *L. luteus* at various stages of growth, states that when the cotyledons had begun to shrivel, the seedlings being nineteen days old, nearly all the cotyledonary cells presented only thin walls. The thickened layers of wall first became laminated, then radial clefts appeared which widened later, corrosion ensued, and the membrane was finally reduced to the condition of thin-walled parenchyma. Elfert [4] a few years later observed the behaviour of the cotyledonary tissue in germinating *Lupinus albus*, *L. luteus*, *L. angustifolius*, and *L. maculatus*. He believed that the thickened walls contained no reserve cellulose, since he could find no thinning of membranes in the interior cells, and the evident thinning in the peripheral cells could be accounted for by the growth which these cells make during germination, the walls being thinned down while expanding in area.

My own observations made on many seedlings of *Lupinus albus* have convinced me that, for this species at least, Elfert is right as far as the non-reduction in thickness of walls is concerned; or I would prefer to say that if a reduction in thickness of interior cell-walls ever takes place in germinating

[1] Zur Chemie der pflanzlichen Zellmembranen, iii: Zeitschr. f. physiol. Chemie, Bd. 19, p. 38.

[2] Angewandte Pflanzenanatomie, p. 453.

[3] Ueber die Schleimendosperme der Leguminosen: Jahrb. f. wiss. Bot., xxi (1890), 670.

[4] Ueber die Auflösungsweise der secundären Zellmembranen der Samen bei ihrer Keimung: Bibliotheca Bot., 30 Heft, 1894.

Lupinus albus, it does not occur in all cases. That the four or five peripheral rows of cells of the cotyledons become thin-walled is true; but since these cells during germination increase considerably in size, one cannot be certain that all the material of the thick wall is not consumed in building the more extensive thin wall. As in the action of enzymes on the walls of cells in sections, so in normal germination, the membranes become hyaline, and the progress of this hyaline zone from the cell-cavity toward the middle lamella gives at first sight the impression that the wall is narrowing down, since the hyaline part is wellnigh invisible. This is probably the explanation of what I believe to be an error of fact in Tschirch's Angewandte Pflanzenanatomie. How then are Nadelmann's results to be regarded? The possibility has already been indicated of a difference in behaviour in different individuals of the same species. In a set of individuals of the same age, I have found in some withering cotyledons the thin-walled condition of the peripheral cells extending two or three rows deeper, and the thick walls of the interior cells looking much poorer in substance, than in other cotyledons of the same stage of development. I feel certain that when the cotyledons wither and die they are not all in the same stage of exhaustion. In this connexion it may be well to recall the various methods of solution of reserve cellulose in germination of seeds, as classified by Reiss[1].

Therefore, though I have not found corrosion and final disappearance of the cell-wall in germination, I am not disposed to assert that it never takes place in this plant, especially since I have seen the complete wall dissolve in sections immersed in an enzyme-solution. A word must be spoken, however, as to a possible source of error in Nadelmann's conclusions:—the pits in the walls of the cotyledonary cells of *Lupinus albus* look, when the walls are hyaline, quite like clefts and corrosion figures. Moreover, in cases of exhausted cotyledons ready to fall, I have several times

[1] Ueber die Natur der Reservecellulose, &c.: Ber. d. d. bot. Gesellsch., vii (1889), 322.

supposed that the cell-walls had lost all their thickenings, till I had made the very transparent zone visible by applying Bismarck-brown. It seems possible, therefore, that Nadelmann misinterpreted appearances, especially since he makes no mention of particular caution in observation.

Whether the wall finally becomes thin or not, is however a matter of small moment, and Elfert is certainly wrong in supposing that the walls do not contain reserve cellulose. Schulze[1] has very recently repeated his former analysis, finding that in three weeks' germination *Lupinus angustifolius* lost nearly three-fourths of its nitrogen-free material, and *L. luteus* in the same time lost more than one-half. The microscopic examination also shows that profound changes take place in the substance of the walls.

D. *Extract of Cotyledons of Phoenix dactylifera.*

Date-seedlings were grown in earth till the endosperm had been nearly half consumed, when the cotyledons, the petioles of the cotyledons, and the remaining endosperm were collected into separate groups. The cotyledons were removed from the enclosing endosperm by dividing the endosperm longitudinally by means of a knife and mallet, and then seizing the halves of the cotyledon with forceps. About one-third of the number of cotyledons were while still fresh rubbed in a mortar with infusorial earth and a few cc. of water, and the liquid was pressed through a cloth into a paper filter, giving a limpid filtrate. The other cotyledons, the petioles, and the pieces of endosperm were dried in an oven at 33°, and each kind of material ground separately in a mill. The ground material was in each case extracted with water, the liquid filtered off, and to each liquid five times the volume of 96 % alcohol added. After being dried the precipitate from the petioles appeared brown, and those from cotyledons and endosperm a greyish white. To each dried precipitate were added a few cc. of water acidulated to ·05 % with

[1] Ueber die Zellwandbestandtheile der Cotyledonen, &c.: Ber. d. d. bot. Gesellsch., xiv (1896), 66.

hydrochloric acid, and to each vial one or two drops of chloroform were given. Probably not one-half of any precipitate dissolved in the small amount of liquid.

The extract of petioles proved itself but slightly, if at all, active on the endosperm-walls of the Barley-grain, and was not used for farther experiment.

The unprecipitated extract of cotyledons was tested in its action on hemicellulose by making preparations of Barley-endosperm on slides as described before for other ferments. In some cases the sections were brushed under water till most of the starch was removed; in other cases the sections were immersed for twenty-four hours in dilute saliva, thus removing all their starch. The slide-preparations were all kept in an oven at 33°, in chloroform-vapour. The walls of the starch-bearing cells showed themselves in all cases reduced in twenty-four hours to one-third their first thickness. The middle lamella, however, dissolved slowly. I find in my notes the statement that an observation made sixty-seven hours after the beginning showed no remains of the middle lamella. How much sooner it disappeared I cannot say, certainly in not less than forty-eight hours after the beginning.

The precipitated and redissolved extract of cotyledons showed itself, in the solution used, more active upon the Barley-endosperm-walls than was the extract just described. In slide-preparations like those previously described, the walls of the starch-bearing cells of the Barley-endosperm, at 33° temperature, were reduced to a very thin middle lamella in twelve to twenty hours; the middle lamella in general disappeared within forty-eight hours from the beginning; and the aleurone-cell-walls became hyaline in a thin section within three to four days. We have therefore in this solution a Date-ferment whose action upon the hemicellulose of the Barley-endosperm shows the same energy as the extract of the Barley-malt and of *Aspergillus Oryzae*. Its method of solution of the Barley-walls is, however, that of the Barley-ferment, not that of the *Aspergillus*. It dissolves first a strip

of wall between the middle lamella and the layer bounding the cell-cavity; next these limiting layers disappear; and lastly the middle lamella. Control-preparations were made with sections in water acidulated with hydrochloric acid to the same degree as the ferment-solution. Here, however, in the same temperature as before, the walls remained intact for a week, when the preparations were discontinued.

Seeing that the attempts to extract by means of water a cytohydrolytic enzyme from the Date had, according to published reports, met with but poor success, and Green[1] having an indication of enzyme-action in a glycerin-extract, it was thought best to extract with glycerin the cotyledonary material that had already been extracted with water. The glycerin used was diluted with water to seven-tenths the full strength. Sections of Barley-endosperm freed from starch were put into this extract and kept at 33° for five days, when the walls showed only a thin middle lamella remaining. Sections of a cotyledon of a seed of *Lupinus albus*, killed and subsequently treated with pancreas-extract, showed, after five days' immersion in the glycerin-extract of the Date-cotyledons, a wholly hyaline condition except in the middle lamella. Similar sections from the Barley-endosperm and the *Lupinus*-cotyledon appeared unchanged when immersed for five days at the same temperature in glycerin of like strength. Several trials with sections from the Barley-endosperm showed, as might be expected, that the glycerin-extract of the cotyledonary residue was not as active upon the walls as was the watery extract previously taken from the same material.

E. *Extract of Endosperm of Phoenix dactylifera.*

No one has reported an enzyme in the extract of Date-endosperm. Green[2] expressed the belief that a cytohydrolytic

[1] On the Changes in the Proteids in the Seed which accompany Germination: Phil. Trans. Roy. Soc. Lond., 178 B, 37.

[2] Phil. Trans., l. c.

ferment does not exist there, but that the endosperm-walls are dissolved by contact with the cotyledon in whose cells the enzyme exists. While, as is well known, the endosperm-walls do not become softened in germination except within about 1 mm. of the cotyledon, yet that they do soften and begin solution, as Reiss[1] has shown, at this distance from the cotyledon, indicates that the enzyme travels from the cotyledon out into the endosperm; and therefore a cell-wall-ferment may be expected in an extract of the endosperm.

Since the method of removing the cotyledons from the endosperm, described in the preceding pages, may have allowed some of the enzyme from the cotyledons to be carried over to the endosperm, and thus vitiate the result possibly to be obtained with endosperm-extract, a more careful means was employed by which the endosperms received no juice from ruptured cells of the cotyledons when the latter were removed. In the first place the seedlings were allowed to grow till the remaining shell of endosperm was reduced to a thickness of 1 mm. to 2 mm., the leaf, as it appeared above ground, being cut back in order to compel the plant to draw all its food from the endosperm. The seedlings were then taken from the garden, the petiole of the cotyledon cut off close to the endosperm. Next, the endosperms were thoroughly cleaned with brush and water and the superficial water allowed to dry off. With a scalpel a slit was made along the endosperm just opposite the groove, when it was found that by a little effort with fore-fingers and thumbs the endosperm shell could be split into longitudinal halves, leaving the cotyledon intact. In not more than three or four out of seventy-two individuals, was the cotyledon cut into by the knife, or torn when breaking the endosperm apart. Thus it was certain that all the extract obtained from the endosperm, was normally present in the endosperm. It seems probable also that all the extract was contained in the softened layer of endosperm lying against

[1] Ueber die Natur der Reservecellulose und über ihre Auflösungsweise bei der Keimung der Samen: Ber. d. d. bot. Gesellsch., vii (1889), 322.

the cotyledon; since a granular mass containing considerable liquid could be scraped from the corroded surface of the endosperm, and beneath this was only hard substance.

The extract of this endosperm-material was prepared as before, the material being dried, ground, extracted with water, and the filtrate treated with alcohol. The precipitate was scanty and of an ashy white colour. Of this dried precipitate, 150 mg. were placed in a little vial with 5 cc. of water acidulated to ·05 % with hydrochloric acid. Probably about one-half the powder went into solution.

With this solution, preparations were made on glass slides as before, sections of Barley-endosperm being immersed in three drops of the liquid, and the slides kept at 33° in chloroform-vapour in a damp chamber. Sections were used from which the starch had been removed with saliva, and other sections in which starch remained. In both cases a marked thinning of the walls could be seen within six hours. In twenty-four hours only the thinnest middle lamella remained to represent the walls of the starch-bearing cells, and the entire wall showed solution at the edges of the sections. In most of the many tests made the entire wall in this thin-walled tissue disappeared within forty-eight hours of the beginning of the test.

This extract was able to dissolve the walls of the aleurone-layer of the Barley-endosperm also. The exposed walls of the aleurone-layer at the edges of the sections showed initial solution in twenty-four hours. In one case the whole aleurone-layer of one section was found hyaline in fifty-two hours after immersion in the ferment; while in a somewhat thick section the aleurone-layer showed, after nineteen days in ferment-solution, islands of hard wall in the hyaline.

Sections of a softened cotyledon from the seed of *Lupinus albus* were used to test the extract of Date-endosperm. The sections were first killed and extracted with chloroform, then extracted with water, and finally with extract of pancreas, before they were mounted on slides in a few drops of the Date-ferment solution. The slides were kept in a damp

chamber at a temperature of 33°, in chloroform-vapour. The walls of the cotyledonary cells became wholly hyaline in four to five days. One set of preparations was kept for forty-eight days, the Date-ferment being renewed twice each week. After a period of twenty-nine days, the middle lamella throughout the section seemed dissolved, except for a middle, indistinct, granular line. The inner lamellae were very little swollen, but were very transparent and apparently poor in substance. At the end of forty-eight days, when the observation was concluded, the section was allowed to dry. Thereupon the walls shrunk to about one-fifth their first thickness, showing certainly a great loss of substance. There is little doubt that the whole wall was dissolved at the edges of the sections, though this was not demonstrated as with other ferments. This conclusion is inferred from the similarity in behaviour of these Lupin-walls when treated with the Date-ferment, and when treated with those of *Hordeum*, *Aspergillus*, and *Lupinus* where entire solution is certain.

With these tests as elsewhere, controls were used, sections of Lupin-cotyledon being immersed in water acidulated to ·05 % with hydrochloric acid, and the preparations kept at 33° temperature. There was a slight swelling of the walls, but even after weeks no such hyaline condition appeared as in the Date-extract solution.

This same extract of Date-endosperm was also tested with sections of Date-endosperm. The endosperm was softened somewhat by soaking for two or three days in water, and rather large transverse sections were taken. These were suspended by means of a thread in a little vial of the extract solution, in the bottom of which was kept a drop of chloroform. The vial was corked and placed in an oven at 33° temperature. Microscopic examination was not made for thirty days. During this period the liquid remained free from Bacteria and Moulds, and transparent except for a very little cloud in the bottom of the vial. The sections had noticeably grown translucent toward the end of the period. Microscopic examination showed the same appearance

reported by Grüss [1] as taking place after two to three months' action of Barley-extract on Date-endosperm. The whole surface of the section was corroded, and the exposed walls frayed out. More deeply lying walls showed longitudinal, open channels. All of the walls had become hyaline. A subsequent examination two weeks afterward showed, by a comparison of camera-outlines, that the whole section had become considerably smaller. The dissolved walls had left no trace of a remnant, and therefore the whole of the wall is capable of solution by the ferment.

As noted elsewhere, proper controls showed that this solution of the endosperm was due neither to the hydrochloric acid nor to the chloroform.

F. *Extracts of Cotyledons of Pisum sativum and Fagopyrum esculentum.*

From the seedlings of the Pea and the Buckwheat, extracts were made as from the plants heretofore described, and strong solutions of these extracts were tested on Wheat-starch-grains, and on the membranes of the Barley-endosperm. Both extracts were found to exert a weak solvent power on the cell-walls as well as on the starch-grains. The strongest solution obtainable from either plant was not as active either on starch or on membranes as a moderately strong solution of the Barley-malt-extract.

III. Cytase or Diastase?

If in the present unsatisfactory condition of our knowledge of ferments, diastase be regarded as an enzyme whose chief characteristic is its ability to dissolve starch, then the following pages will show that not all of the extracts here treated as capable of dissolving reserve cellulose can be denominated *diastase*, though all of the cellulose-enzymes so far discovered show more or less solvent action on starch. If some of them cannot be regarded as diastase, they may be

[1] Studien über Reservecellulose: Bot. Centrlbt., lxx (1897), 242.

cytohydrolysts proper with weak action on starch; or the extracts may be mixtures, each of diastase and a cytohydrolyst. If the former of these two last hypotheses be true, then we must regard diastase as a cytohydrolyst also; since it is known to dissolve both starch and reserve cellulose. To my mind the evidence is fully as strong for the second hypothesis as for the first.

A. *Action of Ferments on Starch, when the Action on Membranes is nearly equally intense for all Extracts.*

In order to compare the solubility of starch with that of reserve cellulose by means of the cytohydrolysts here treated, powdery extracts of the malt of *Hordeum vulgare*, of the cotyledons of *Lupinus albus*, of the cotyledons of *Phoenix dactylifera*, and of the endosperm of *Phoenix dactylifera*, were prepared by the alcohol-method as described in the first section of this paper. From these solid extracts, and from the *Aspergillus*-extract, strong watery solutions were made which by repeated tests were brought to nearly like intensity of action on reserve cellulose. These solutions were acidulated to ·05 % with hydrochloric acid. In order to bring the ferment-solutions to nearly the same degree of strength relative to their action on reserve cellulose, very thin sections were made from dry Barley-grains; the sections were killed in alcohol, chloroform, or formalin, extracted for fifteen to thirty minutes in water, and the starch brushed out of their cells. Preparations with the various ferments were then made on glass slides as described on p. 53, chloroform being constantly present, and the temperature maintained at 32° to 34°.

To this method of measuring the activity of the enzymes the objection may justly be made that it cannot be precise. But want of great precision is not thought to vitiate the validity of the conclusions. In the first place, the intent of this paper does not require very close measurements; and

in the second place, much care has been taken to secure considerable accuracy. The number of tests made has been large, amounting to at least ten with each ferment-solution; the sections used were all taken from a small cube of endosperm, not more than 1 mm. in diameter, and so cut as to include the same kinds of cells in each section; the sections were selected by the aid of the microscope, and all over two cells in thickness were rejected.

The fact will be recalled that in the solution of Barley-endosperm-walls in an enzyme, the inner lamellae of the starch-bearing cells first dissolve, next the middle lamellae of the same cells, and last of all the walls of the aleurone-bearing cells. In the ferment-solutions now under consideration, the inner lamellae dissolved in fifteen to twenty-four hours, and the middle lamellae in twenty-four to forty-eight hours. In a few tests the middle lamella required more than forty-eight hours for solution. Of all the ferments, that of *Aspergillus* was perhaps most active, and that of *Hordeum* the least; the other three were very closely alike.

With the ferment-solutions thus brought nearly to the same strength respecting their action on reserve cellulose, tests were made of their behaviour toward starch. Sections from the Barley-endosperm were prepared just as for the last series of experiments, except that for this series the starch was only partially brushed out of the sections. The sections were then selected under the microscope so as to have all as closely as possible alike in size and in the amount of starch contained. The preparations were made on glass slides as before; chloroform was at all times present, and a temperature of 32° to 34° was maintained. Several series were carried through, each series containing preparations with all the kinds of ferments. In all cases the starch disappeared first of all in the Barley-extract, the time being twenty-four to thirty hours. With the *Aspergillus*-extract, the starch was dissolved in eight days; with *Phoenix*-cotyledonary-enzyme, in ten to fifteen days; with *Lupinus*-enzyme, in about thirty days; and with *Phoenix*-endosperm-

enzyme, in thirty-five days or more. In most of the series the ferment-solutions were renewed every two or three days; in one series no renewal was made for ten days after the beginning of the test. The quantity of ferment-solution given to the preparations was, as stated elsewhere, the same in every case.

It having been demonstrated that these ferment-solutions which act with almost like intensity on reserve cellulose show very unlike powers of dissolving starch-grains, it was decided to test the same solutions with starch-paste. A 1 % Wheat-starch-paste was made with distilled water alone, and the paste was then acidulated to ·001 % with hydrochloric acid. Such a preparation after standing two or three hours gives a clear liquid over a flocculent precipitate, and this clear liquid is very sensitive to the action of diastatic enzymes. When to equal volumes of this starch-paste in test-tubes, one-fourth volume of enzyme-solution was added, a little chloroform being placed in each tube, and the temperature kept at 18°, it was found by the iodine-test that with *Hordeum*-extract a brown colour free from purple appeared in two hours and ten minutes; with *Aspergillus*-extract, the same shade of brown appeared in five hours and twenty minutes; with *Phoenix*-cotyledonary-extract, in twenty hours; and with *Phoenix*-endosperm-extract, the same brown appeared a little later than one hundred and twenty hours. Unfortunately the *Lupinus*-extract was not included in this test. However, in another series of preparations, the *Lupinus*-extract was directly compared with that of the *Phoenix*-endosperm. The starch-paste was the same as in the preceding series, but the proportion of enzyme-solution to the volume of paste was as four to ten, and the temperature was kept at 30°. Here the brown colour appeared in the *Phoenix*-endosperm-extract between twenty and twenty-one hours after the beginning, while in the *Lupinus*-extract it appeared between nineteen and twenty hours from the beginning. Thus it is quite certain that had the *Lupinus*-extract been included in the former series of experiments, its period for bringing

in the brown colour would have been between one hundred and one hundred and fifteen hours.

Previous tests with the Date-endosperm-extract had shown such very slight action on starch that a control preparation was carried along with the first of the two series of experiments just described. This control consisted of a tube of the same starch-paste as used with the ferments, acidulated and chloroformed to the same degree as in the other tubes. After one hundred and fifteen hours at 18° temperature, the control paste showed still the blue colour with iodine. This result shows, therefore, that the Date-endosperm-extract is diastatic, though but very slightly so.

It is thus seen that with these enzyme-solutions, all dissolving reserve cellulose in nearly the same time, there is the greatest divergence in their behaviour toward starch, both in the form of grains and in solution.

B. *Action of Ferments on Reserve Cellulose when their Action on Starch is of like intensity for all Extracts.*

In order to study farther the relation of the power of these enzymes to dissolve starch to their power of dissolving reserve cellulose, it was decided to reduce all the ferment-solutions to the same degree of activity on starch, and then to test their action on cell-membranes. By this means among other conclusions it could be determined whether the inference drawn by Grüss[1] is correct—that only a strong solution of the Barley-enzyme would attack reserve cellulose.

The very strong solutions of *Lupinus*-extract and of *Phoenix*-endosperm-extract having been found to act the most feebly and almost equally on starch, the three other extracts employed were brought through dilution to the strength of these two, by many tests with starch-paste. The paste was prepared by Lintner's[2] method, and some

[1] Ueber das Verhalten des diastatischen Enzyms in der Keimpflanze: Jahrb. für wiss. Bot., xxvi (1894), 379.

[2] Jour. f. prak. Chemie, 34 (1886), 380.

of it by the method given on p. 74 of this paper. With the paste prepared by the latter method, the ferment-solutions in the proportion of four parts of ferment to ten parts of starch-paste were finally brought, by the iodine-test, to give the purely brown colour after twenty hours at 30° temperature, kept constantly in the presence of chloroform.

While the ferment-solutions were being brought to a common strength, sections from a dry Barley-grain were made ready. The sections were cut from the same part of the grain, killed, washed in water, those of like dimensions and appearance selected by the aid of the microscope, and put into solution of extract of pancreas till the starch was dissolved. That the pancreas-extract does not visibly affect the cell-walls is shown on p. 55 of this paper. With the five ferment-solutions and the prepared sections, two series of experiments were carried through. A section was put into three drops of each ferment on a glass slide, chloroform was added, the slides were enclosed in a damp chamber containing chloroform, and the chamber was kept in an oven whose temperature varied from 30° to 35°. The large variation of temperature should not have occurred, but it could not have affected the general results, since all preparations were alike subjected to it. Examining these preparations at frequent intervals, it was seen that the *Lupinus*-extract dissolved the inner lamellae of the starch-bearing cells in about nine hours; the *Phoenix*-endosperm-extract, in nine hours; the *Phoenix*-cotyledonary-extract, in twenty-one hours; the *Aspergillus*-extract, in one series in ninety-four hours, in the other series in one hundred and sixteen hours; and the *Hordeum*-extract, also in ninety-four and in one hundred and sixteen hours. The middle lamella was dissolved by the *Lupinus*-extract in the two series in twenty-one hours; by the *Phoenix*-endosperm-extract, in twenty-one hours[1]; by the *Phoenix*-cotyledonary-

[1] The lower limit of ten hours given for the Lupin-extract, and the upper limit of thirty-three hours given for the Date-endosperm-extract in my 'vorläufige Mittheilung' (Bot. Centrblt. lxxiii), are errors in transcription from my notes.

extract, in one hundred and eighteen hours; by the *Aspergillus*-extract and the *Hordeum*-extract, in some period longer than three hundred and twelve hours. That is to say, these last two preparations were examined for the last time three hundred and twelve hours after the beginning of the test, and then still contained traces of the middle lamella. In the preparations continuing for a long period, the ferment-solutions were renewed at intervals varying from two to four days, but these intervals were the same for all preparations.

Finally, in order to determine whether the almost complete inability of the Date-endosperm-extract and the Lupin-extract to dissolve starch might be due to some clogging component of the solutions, saturated solutions were made not only of these two extracts, but also of those of the Barley, *Aspergillus*, and Date-cotyledons. A portion of each of these solutions was then diluted with water to three-fourths full strength, and another portion to one-half full strength. Each ferment therefore was in solution in three strengths. Preparations were now made, as described before, by introducing into these ferment-solutions, on glass slides, starch-grains and sections of the Barley-endosperm. It was found for all kinds of ferments used that the strongest solution in each case acted most energetically on both starch and cell-membranes, and the most dilute solution acted in each case the least on both starch and cell-membranes.

IV. Review and Summary.

In reviewing and summarizing the results obtained in this work, it is to be noted first, that, in addition to the extract of Barley-malt, the extracts of *Aspergillus Oryzae*, seedlings of *Lupinus albus*, and seedlings of *Phoenix dactylifera* have been shown to be highly cytohydrolytic.

Testing these four extracts, each with three different kinds of walls, it is worth remembering that no extract has shown itself peculiarly active toward any one kind. All the extracts have dissolved the walls of the starch-bearing cells of the

Barley-endosperm in the shortest time; all have taken longer to render the walls of the aleurone-layer of the Barley-endosperm hyaline; and all have required the longest time to make the cotyledonary walls of *Lupinus albus* hyaline. This statement is not intended to indicate an exact similarity in the action of the ferments in question; for the preceding pages indicate a peculiarity in the action of *Aspergillus*-extract in the manner of solution of Barley-endosperm-walls, and a possibly selective action of the Lupin-ferment for the middle lamella of the walls of the Lupin-cotyledons. But these minor differences have not been studied fully, and hence cannot be extensively considered here.

All of the ferments with all the kinds of walls observed render the walls hyaline, and show full 'ghosts' before the walls begin to thin down or to 'melt' away. This condition indicates a progressive loss of substance from the walls, before the size decreases. That there is loss of substance from the walls in becoming hyaline has recently been practically demonstrated by Schulze[1].

Not less noteworthy than the last-named fact is the following one, that all the cell-walls used in these experiments do finally, though comparatively slowly, dissolve. Thin walls of the Barley-endosperm, walls of the aleurone-layer, walls of the Lupin-cotyledons, endosperm-walls of the Date, all finally wholly dissolve, and, so far as tested, all dissolve in each ferment used. If, as has been stated by some authors, some of these walls used as tests are composed partly of true cellulose, then these ferments can dissolve true cellulose. That true cellulose is not attacked at all by the extract of Barley-malt, as has been generally held, may be found to be erroneous, when a careful microscopic examination is made, and the test continued over a long period of time.

It will be noted also that the active or neutral behaviour of an enzyme toward reserve cellulose is not conditioned by the strength of the enzyme-solution. The enzymes tested,

[1] Ueber die Zellwandbestandtheile der Cotyledonen, &c.: Ber. d. d. bot. Gesellsch., xiv (1896), 66.

those of Barley-malt and *Aspergillus*, were still able to dissolve the endosperm-walls of the Barley when the ferment-solutions were so much diluted as to require ten times as long as required by strong solutions to convert the starch in a starch-paste.

Of the five strongly cytohydrolytic enzymes used in this work, all dissolve starch. But the extract of the Lupin and the two extracts from the Date-seedlings act so feebly on starch that they certainly cannot be denominated diastase. When the strongest solutions that could be made of the three ferments last mentioned required from twenty-four to fifty times as long as a strong Barley-malt-extract to dissolve starch-grains or hydrolyze the starch in starch-paste, and when such enzymes, so slightly acting upon starch, act very intensely on cell-membranes, obviously the enzymes of the Lupin and the Date are cytohydrolysts and not diastase.

The question proposes itself as to whether we have in each of the five enzyme-extracts under consideration a mixture of an amylohydrolytic and a cytohydrolytic ferment, or in each extract a single ferment with peculiar properties. So far as I can perceive there is no certain evidence for the one assumption or for the other. It would, however, to my mind, seem remarkable that two single ferments behaving so much alike toward reserve cellulose as do those of the Barley and the Lupin should attack starch with such unlike intensity, and yet should both attack it. With this hypothesis we should be compelled to assume that the extracts of the Barley and the *Aspergillus* are not diastase in the accepted meaning of the term, but ferments with the properties of both diastase and cytase possessed nearly equally; and we should be compelled to assume that the extracts of the Lupin and the Date are cytase which has the power of slightly dissolving starch.

On the other hand, there are various features favouring the view that these extracts with the properties of diastase and cytase are not simple, but are mixtures. In the first place, though a plant-diastase neutral toward reserve cellulose

has not as yet been found, one may yet be discovered; and on the animal side both saliva and extract of pancreas are active toward starch, neutral toward reserve cellulose. The discovery of such a plant-diastase would not of course prove the point, but it would lend some probability to the view. In the second place, relations are such in the cotyledons of the Lupin and of the Date that we should expect in the extract an admixture of diastase, even if cytase does exist there independently; for in the cotyledons of both plants more or less starch is found. In the third place, how is the remarkable difference in the behaviour of the extracts of the Date-cotyledons and the Date-endosperm to be explained?

As already shown, though the extract of the cotyledons compared with Barley-diastase is extremely feeble in its action toward starch, yet it dissolved starch-grains in less than one-half the time required by the Date-endosperm-extract, and it hydrolyzed starch-paste in one-sixth the time required by the endosperm-extract when both ferment-solutions acted with like intensity on cell-walls. Yet these two extracts are most likely both secreted by the Date-cotyledon, and though showing such a difference in properties are separated in the plant-tissue at most by a very few cells, probably by only a single cell-wall. We have here in the Date-seedling the possible conditions presented for securing a cytase free from diastase, if such separation ever really occurs. We can see no need of diastase in the dissolving endosperm, but it may be that a little is carried over by diffusion from the secreting cells of the cotyledon.

In brief the results of this work are the following:—

1. The enzyme extracted from *Aspergillus Oryzae* attacks reserve cellulose with greater intensity than it attacks starch.

2. The enzyme from the cotyledons of seedlings of *Lupinus albus* is very strongly cytohydrolytic, but very feebly amylo-hydrolytic.

3. The enzyme from the cotyledons of seedlings of *Phoenix dactylifera* is very strongly cytohydrolytic, and very feebly

amylohydrolytic, attacking starch a little more strongly than the extract of *Lupinus* does.

4. The enzyme from the endosperm of *Phoenix dactylifera* is very strongly cytohydrolytic, but amylohydrolytic to a little less degree than the extract of *Lupinus*, and to a considerably less degree than the enzyme from the cotyledons of *Phoenix*.

5. The very dilute enzyme of the malt of *Hordeum vulgare*, as well as that of the other plants here considered, attacks reserve cellulose; hence an enzyme-solution need not be strong to act on cell-walls.

6. None of the five enzyme-extracts used in this work shows itself, more than the other extracts, peculiarly suited to the solution of any one kind of reserve cellulose here employed.

7. With all the ferments the walls at first become hyaline appear gradually more and more transparent, and finally 'melt' wholly away in solution.

8. The enzymes from *Lupinus albus* and from *Phoenix dactylifera* act on starch so feebly, and on reserve cellulose so energetically, that they are to be regarded as cytase as distinguished from diastase.

Harveyella mirabilis (Schmitz and Reinke).

BY

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With Plates III and IV.

UNTIL recently only a single species (*H. mirabilis*) of the genus *Harveyella* (Schmitz and Reinke) was known. However, in December, 1896, I discovered a parasite on *Gracilaria confervoides* (Grev.) growing in Stokes Bay, Gosport, which Mr. E. M. Holmes considered to be identical with the *Choreocolax pachyderma* of Reinsch [1], but which, from the structure of its cystocarp, is undoubtedly a *Harveyella*. It has accordingly been placed in that genus as a second species under the provisional name of *Harveyella pachyderma* (Holmes and Batters). As *H. mirabilis* was also common in the same district, on the fronds of *Rhodomela subfusca*, I have thought it well to undertake a thorough examination of both species, and I give in this paper an account of my observations on *H. mirabilis*.

Harveyella mirabilis was first described by P. Reinsch [2] under the name of *Choreocolax mirabilis*. It was included with several other plants of apparently similar structure in

[1] P. Reinsch, Contrib. ad Algol. et Fungol., 1878.

[2] Loc. cit., p. 63, plates 53 and 54.

[Annals of Botany, Vol. XIII. No. XLIX. March, 1899.]

the genus *Choreocolax*, but the organs of reproduction of these plants were at this time unknown. In 1889 Reinke and Schmitz[1] founded a new genus, *Harveyella*, of which this plant was the typical and only species: they placed it in a new genus because the structure of its thallus differs from that of *Choreocolax Polysiphoniae*, the typical species of the genus *Choreocolax*. In their paper, after a short description of the development of *H. mirabilis*, it is stated that the cystocarp is formed in a similar way to the cystocarp of *Caulacanthus*, and that its structure shows that the Alga belongs to the Gelidiaceae. In 1891 Richards[2] discovered the cystocarp of *Choreocolax Polysiphoniae*, which differs essentially in structure from that of *H. mirabilis*, thus showing that the division of the original genus *Choreocolax* into two was fully justified. In 1889 the genus *Harveyella* was placed, by Schmitz[3], in a distinct family, the Harveyelleae, next after the Binderelleae—which includes *Binderella* and *Choreocolax*—at the beginning of the Gelidiaceae. In 1890–91 Holmes and Batters[4] included *H. mirabilis* in their list of British Marine Algae, and placed it in the same position as Schmitz had done. In 1896 the genus was described by Schmitz and Hauptfleisch[5] and maintained in the same position among the Gelidiaceae. After a short description of its morphological development, the sporangia are stated to be unknown. The antheridia are mentioned as covering the entire surface of small, flat 'Fruchtpolstern.' The carpogonial branches are formed in large numbers, are 3-celled, and affixed laterally to cells of the peripheral filaments. A short description of the mature cystocarp is given, but there is no mention of the actual development

[1] J. Reinke, Algenflora der westl. Ostsee Deutsch. Anth.: Berlin, 1889, p. 28.

[2] On the structure and development of *Choreocolax Polysiphoniae* (Reinsch); H. M. Richards, 1891, Proc. of the American Acad. of Arts and Sciences.

[3] Systemat. Uebersicht der bisher bekannten Gatt. der Florideen, 1889.

[4] Holmes and Batters, 'List of British Marine Algae,' Annals of Botany, 1890–91.

[5] Die Natürlichen Pflanzenfamilien, &c., Engler and Prantl; Gelidiaceae, Schmitz and Hauptfleisch, Lief. 142, p. 344. Leipzig, 1896.

of the carpogonium or of the gonimoblast having been observed.

In 1894 Dr. P. Kuckuck[1] published a description of the tetraspores of *Choreocolax albus*. He states that this plant is to be found in May, parasitic on *Rhodomela subfusca*, at Heligoland. From the figures of this plant it seems only to differ from *Harveyella mirabilis* in that the cells of the central mass of the parasite are not arranged in such obvious filaments as are the corresponding cells of *H. mirabilis*. But just at the time of the development of the tetraspores, the internal cells of *H. mirabilis* are occasionally so much gorged with contents, that the filamentous character of their arrangement is not very obvious, especially if the plane of section does not happen to coincide exactly with the long axis of the filaments of the parasite. I have frequently seen sections of *H. mirabilis* which corresponded exactly in appearance with the figures in Kuckuck's paper. As the tetraspores are exactly similar to those of *H. mirabilis*, and the host-plant is the same, and the external appearance of the parasite described by Kuckuck resembles exactly that of *H. mirabilis*, while I have never seen any other parasite of similar appearance on *Rhodomela subfusca*, I am of the opinion that *Choreocolax albus* (Kuckuck) is simply a tetraspore-bearing specimen of *Harveyella mirabilis* (Schmitz and Reinke). The tetraspores are stated to be sunk within the peripheral layer of the thallus, and to arise from wedge-shaped cells cut off from the growing peripheral cells. They are developed over the whole surface of the thallus, and are cruciately arranged.

Nowhere in the literature dealing with the genus *Harveyella* have I found any account of the development of the cystocarp from the fertilization of the carpogonium to the production of the carpospores. The mature cystocarps of *Harveyella mirabilis* are found in Stokes Bay from December to March; during this time the plant is common on the fronds of

[1] *Choreocolax albus*, von Dr. P. Kuckuck, Sitzungsberichte der Kön. Preuss. Akad. der Wiss., Berlin, 1894, xxxviii.

Rhodomela subfusca, but after March it becomes rarer, and I have never been able to find any specimens during July, August, or September. At the beginning of October certain stems of *Rhodomela subfusca* were noticed to be slightly swollen at various places, and on cutting sections of these swellings, it was found that a considerable development of *Harveyella*-filaments had taken place in the interior of the host; these filaments were growing between the cells of the *Rhodomela*, but none of them had yet reached its external cell-layer. The swollen appearance was caused by a great increase in number of the cortical cells of the *Rhodomela*. The probable course of the development of the parasite is as follows. After the spores are set free, from January to March, they in some manner make their way between the cortical cells of a *Rhodomela*, and then commence to send filaments between these cortical cells toward the large central cells of the host, as also among the cortical cells themselves: this is the earliest stage I have seen, and it is represented in Fig. 1. At this stage there is no external swelling of the *Rhodomela*-stems. Soon afterwards a considerable increase in the number of the cortical cells of the *Rhodomela* takes place, producing the swollen appearance (Fig. 2), and at the same time the filaments of the parasite grow rapidly between the large pericentral cells of the host (Fig. 2), where they are much branched and composed of well-developed cells. Fewer filaments, consisting of, as a rule, thinner cells, grow between the numerous small cortical cells which cause the external swellings of the *Rhodomela*-stems; these latter filaments eventually reach the surface (Fig. 2). Just beneath the external membrane of the *Rhodomela* these *Harveyella*-filaments become much branched, and give rise to a closely-packed layer of cells. At about this time the external membrane of the *Rhodomela* is broken and its place taken by the gelatinous external membrane of the *Harveyella*. A portion of one of the *Harveyella*-filaments which grow between the cells of the host is drawn in Fig. 18. The distal cell of the filament is very narrow,

tapering almost to a point, while the cells which follow it become larger and more rounded, in proportion as they are further removed from the distal cell. The walls between the *Rhodomela*-cells appear to become gradually swollen as the parasitic filaments force their way into them.

The parasite having reached the exterior of the host, rapidly develops branched filaments, usually consisting at this stage of rather elongated cells, which, however, soon become more full of contents and assume an oval or almost spherical shape. It is at this time that the first indications of a distinct peripheral layer are seen. The outermost cells of the frond are closely packed in a 1- or 2-celled layer; these cells are somewhat more regular in shape than the other cells of the parasite, being oblong with rounded ends. They stain more rapidly and deeply with Hoffmann's blue than the remaining cells. At this stage the swellings on the *Rhodomela*-stems have a greyish-white appearance.

In the meantime the *Harveyella*-filaments in the interior of the host have continued to force their way among the large central and pericentral cells until they occupy a space as large or larger than the mature external portion. Sometimes these internal filaments branch enormously, and form large fan-shaped masses of radiating filaments (Fig. 3). As these filaments grow they gradually absorb the contents of the host-cells which they surround. In Fig. 3 some of the host-cells have their contents partially absorbed, while others are quite empty, only their walls remaining. The external portion of the parasite now rapidly increases in size, owing to the growth and division of the distal cells of the peripheral chains. These growing cells divide in two ways. They are divided into two parts by a curved transverse wall, the lower half gradually increasing in size and passing over into the ordinary tissue of the frond, the upper half rapidly elongating and dividing again in a similar way. But frequently the distal cell is divided by a curved, oblique, longitudinal wall, producing in the ordinary way a sub-dichotomously branched filament. As the frond increases in size,

and the division and growth of the peripheral cells goes on, the three or four cells immediately beneath the growing distal cell of the chain are generally small, gradually increasing in size; thus there is a 3- or 4-celled peripheral layer, the cells of which pass over gradually into the larger cells of the central mass of the parasite.

This growth continues until the external part of the *Harveyella* is in the form of a more or less hemispherical cushion-like body, varying in size up to about 2 mm. in diameter. This cushion is, when fresh, often perfectly white, but sometimes it is of a brownish colour, owing to the external membrane and a few of the peripheral cells having become stained a dark brown. If a perfectly white specimen is kept in a bottle of sea-water for a day or two, it becomes stained in this way. When dried, all the specimens are of nearly the same colour as the dried *Rhodomela*-stems to which they are attached, very dark brown to black. This brown colour is, I believe, taken up by the external membrane and cells of the parasite, possibly from neighbouring dead cortical cells of the host.

When mature, *Harveyella mirabilis* appears to the naked eye as a small hemispherical cushion on the *Rhodomela*-stems (Fig. 4). Very frequently three or four of these bodies are situated close together, often encircling the stem; sometimes they are close together, but each one is distinct; at other times they are more or less joined. They are generally the product of one original spore, the external portion being developed wherever the internal filaments reach the surface of the host. If very many internal filaments reach the surface close together, they produce one large external thallus or frond: but if separated by some distance, they give rise to several smaller fronds, which may more or less coalesce as they increase in size. Thus the thallus, of which a section is shown in Fig. 3, was probably formed by the coalescence of at least three smaller fronds, all of which were originally derived from the same spore. Thus, when mature, *Harveyella mirabilis* consists of:—

1. The much-branched filaments growing in the interior of the host, which absorb nourishment from the contents of the host-cells. Glycerin-material stained with Hoffmann's blue, enables one to see distinct protoplasmic threads connecting the cell-contents of these filaments with the cell-contents of the *Rhodomela*. The distal cells grow and divide in a similar way to the distal cells of the peripheral chains, producing sub-dichotomously branched filaments, but these filaments are as a rule much more copiously branched than the peripheral chains.

2. A hemispherical external portion, consisting of—

(*a*) The small-celled peripheral layer, four or five cells deep, the cells being arranged in monosiphonous more or less branched filaments. This peripheral layer gradually passes over into

(*b*) The large central mass of larger cells. In the ordinary adult thallus these cells are oblong, slightly rounded, and obviously arranged in radiating branched filaments; but at the time of the development of trichogynes or tetraspores these cells become gorged with contents and are often almost circular in outline, and so crowded together that their filamentous arrangement is not so easily seen.

The whole external part of the plant is enclosed in a fairly thick gelatinous membrane, which is sometimes structureless, but can be occasionally seen to be made up, especially in its inner portion, of the fused gelatinous walls of the peripheral cells. The walls of all the cells are fairly thick. The contents of all the cells of *Harveyella mirabilis* are quite colourless, except when some of the outermost peripheral cells, as previously mentioned, become stained brown. Glycerin-material stained with Hoffmann's blue shows that the peripheral cells, and the cells of the filaments inside the host, stain much more deeply and rapidly than the cells of the central mass of tissue. The nuclei of the cells stain well with Hoffmann's blue.

During the growth of the parasite, a number of the cortical and some of the larger cells of the *Rhodomela* often become

surrounded by *Harveyella*-filaments, and are eventually isolated among the cells of the external portion of the parasite (Fig. 3). These cells are well shown in Kuckuck's paper on *Choreocolax albus*. They often remain for some time, but their contents are eventually absorbed, and their walls collapse. They are sometimes carried up by the growth of the parasite almost to its peripheral layer.

When the *Harveyella* is fully grown, it commences to develop antheridia and trichogynes. The earliest indications of the presence of *Harveyella*-filaments in the *Rhodomela*-stems were noticed on October 4, when the parasite was in the stage shown in Fig. 1. The first appearance of very young antheridial fronds was on October 23, and by the second week in November they had become very numerous, trichogynes appearing on other fronds at about the same time. The antheridia have been previously described as occurring on small, flat fronds[1]; but the antheridial fronds which I have found are of all sizes, being very frequently as large and spherical as the mature cystocarpic fronds. The antheridia are developed over the whole surface of the frond. The first indication of them is that the distal cells of the peripheral chains are elongated and divided by oblique curved longitudinal walls producing a small tuft of two or three very narrow cells (Fig. 5, *a*). These cells then cut off successively, at their distal ends, small more or less spherical cells (Fig. 5, *b*); so that, when mature, the whole surface of the antheridial frond is covered by a layer, three or four deep, of these small cells, embedded in the gelatinous external membrane. They are set free by the breaking away of this membrane.

The first trichogynes were noticed at the beginning of November, that is to say, one month after the parasite was first seen. The trichogyne is developed from a growing peripheral cell. As this cell commences to elongate at its upper end, three small cells are cut off successively at its base.

[1] Schmitz and Hauptfleisch, Die Natürlichen Pflanzenfamilien, Engler and Prantl.

Since, at the same time, the remaining peripheral cells have continued to grow, the young carpogonial branch is soon more or less buried in the frond. The carpogonial branch invariably consists of three small cells, upon which is seated the carpogonium with its trichogyne. The carpogonial branch is always attached to a cell of a peripheral chain, about one to four cells beneath the distal cell (Figs. 8 and 9). Soon after the appearance of the three small cells, the carpogonium becomes produced downwards at the side of this carpogonial branch (Figs. 9 and 10). At this time the trichogyne is also somewhat swollen at its upper extremity. While the trichogyne is developing, the frond still continues to grow at its periphery, and so the carpogonium slowly becomes more deeply buried in the frond, and when mature it is usually about four cells from the surface. The trichogyne continues to elongate until it reaches the surface of the external membrane of the frond, which it usually pierces without bending; but occasionally trichogynes are seen which, instead of breaking through this membrane, become bent and grow between the external peripheral cells and the outer surface of the membrane, and thus never reach the exterior. When the trichogyne has pierced the membrane, it grows for some distance beyond (Fig. 10), while the carpogonium continues to grow downwards at the side of the carpogonial branch. During the development of the trichogyne, the cell of a peripheral chain to which the carpogonial branch is attached increases in size and cuts off two small cells from its basal part, one on each side. These two cells rapidly develop into two short filaments, one of which consists of four cells—a 3-celled filament with a 1-celled branch from its proximal cell—and the other of two cells only (Fig. 10). Of these two filaments, the one consisting of only two cells is generally, if not always, attached nearer to the basal end of the peripheral cell from which they spring.

This peripheral cell, to which the carpogonial branch is attached, is the auxiliary cell, and it invariably gives rise to these two small sterile filaments before the trichogyne

is fertilized. If the trichogyne does not become fertilized, it eventually withers and disappears, while the auxiliary cell and its two small sterile filaments do not undergo any further development. They usually increase somewhat in size, and become pressed together by the growth of the ordinary thallus-cells surrounding them, until they form a small rosette of cells. They are often seen remaining in the mature cystocarpic plant in this state. When the trichogyne is ready for fertilization, it projects a considerable distance beyond the surface of the plant; it is covered by a thick gelatinous sheath, and is more or less swollen at its apex. The carpogonium is prolonged at the side of the 3-celled carpogonial branch until it nearly touches the upper part of the auxiliary cell, or even quite touches it (Fig. 11). But in all cases this part of the carpogonium and the upper part of the auxiliary cell are very near to each other. When mature, the trichogyne stains deeply and rapidly with Hoffmann's blue, as do also the three cells of the carpogonial branch, the auxiliary cell, its two small filaments, and frequently also the ordinary thallus-cell beneath the auxiliary cell, to which the latter is attached; this thallus-cell sometimes cuts off a small cell at its side (Fig. 11). When the trichogyne becomes aborted, the contents of all these cells become more granular, and only their nuclei stain deeply.

After fertilization, the trichogyne is cut off from the carpogonium by a transverse wall (Fig. 12); and then that part of the carpogonium which is almost touching the auxiliary cell fuses with it (Fig. 13). At this stage the auxiliary cell is unaltered: but immediately after fusion with the carpogonium, it becomes more or less fused with the cells of its two small sterile filaments. This fusion, which commences by an enlargement of the pit-connexions of the cells (Fig. 13), eventually produces a large cell of very irregular shape, which is soon divided by a curved transverse wall into an upper smaller hemispherical cell (Fig. 14, *gonbl.*) and a lower, larger, irregularly-shaped cell (Fig. 14). Both these cells are easily seen in the thallus, as they stain very rapidly and deeply

with Hoffmann's blue, the upper cell staining more deeply than the lower. This lower large cell, which includes also the two small filaments, undergoes no further change. The upper cell rapidly divides (Fig. 15) and gives rise to the young gonimoblast (Fig. 16). The gonimoblast branches copiously and ramifies horizontally in all directions among the cells of the thallus (Figs. 3 and 17). From the cells of these horizontal filaments arise numerous much-branched tufts of erect gonimoblast-filaments, which eventually bear the spores (Fig. 17). At about the time of the commencement of the development of the gonimoblast, the ordinary thallus-cells which are situated at the base of the peripheral layer, at about the same level as and near to the auxiliary cell, commence to elongate. It may be mentioned that just after fusion with the carpogonium, the auxiliary cell becomes connected with some of the thallus-cells near it (Fig. 15), and that these are the first ordinary thallus-cells to elongate. As the gonimoblast develops, these cells become more and more elongated, carrying up the peripheral layer with them. Those thallus-cells which are in immediate proximity to the fertilized auxiliary cell commence to elongate first; and as the gonimoblast develops further, so the thallus-cells, at about the same level, and among which it is forcing its way, also become elongated. Hence the elongation of ordinary vegetative cells, carrying up the peripheral layer, and thus providing space for the development of the erect gonimoblast-filaments which bear the spores, accompanies the development of the gonimoblast from its commencement. These lengthened cells are shown in the mature cystocarpic plant in Fig. 17. It may be noted that the peripheral cells in the mature cystocarpic plant are smaller and more circular in outline than in younger fronds.

The erect gonimoblast-filaments arise from the cells of the horizontal filaments of the gonimoblast. They form erect, much branched, often spreading tufts, which develop in the spaces between the elongated thallus-cells supporting the peripheral layer (Fig. 17). The cells of these erect filaments are usually

smaller than the other cells of the gonimoblast. The terminal cells of these filaments develop into spores, which are at first small and oval in shape; but when mature they are usually oval, though occasionally they are almost spherical. They are set free by the breaking away of a portion of the periphery, which in the mature cystocarpic plant is only attached to the central tissue by the very long and narrow cells.

The spores stain rapidly with Hoffmann's blue, as do also the cells of the erect gonimoblast-filaments, those nearest the spores staining most deeply. The nuclei of the spores stain well. The cells of the horizontal filaments of the gonimoblast stain deeply when young, but as they become older they only stain very slightly, resembling the ordinary thallus-cells.

Thus the cystocarp, when mature, consists of a much-branched gonimoblast derived from the product of the fusions of the auxiliary cell. This gonimoblast consists of filaments growing in all directions horizontally among the thallus-tissue, until the ramifications spread throughout almost the whole of the hemispherical cushion-like part of the plant which is outside the host, just beneath the peripheral layer, which is borne on very long and narrow vegetative cells. From these horizontal filaments arise erect much-branched tufts of gonimoblast-filaments, the terminal cells of which are developed into spores.

When fully mature, the whole plant—except the part inside the host—is cystocarpic; that is to say, there is a layer of erect gonimoblast-filaments bearing spores extending round the whole hemispherical external frond, just beneath the peripheral layer. This cystocarp is not contained in any special coat or wall, but is bounded on the exterior only by the slightly-modified ordinary peripheral cells, and on the inner side by the central tissue of the thallus.

Taking the view of an antithetic alternation in the life-history of the Florideae, it is worthy of note that in *Harveyella*

mirabilis the sporophyte consists of a much-branched filamentous thallus extending throughout the external portion of the gametophyte; and further, that, leaving out of consideration that portion of the thallus of the gametophyte which is inside the host, the sporophyte approaches very nearly in dimensions to the gametophyte. Both generations consist of a sub-dichotomously branched filamentous thallus. In the gametophyte a large portion ramifies among, and is parasitic on, the cells of the host-plant; while the sporophyte ramifies among the cells of the gametophyte, and may be said to be parasitic upon it. Thus in *Harveyella mirabilis* the two generations approach each other more nearly in vegetative characters than they do in possibly any other Floridean type.

The tetrasporangium is formed from one of the ordinary distal cells of a peripheral filament. This cell is divided in the usual way by an oblique wall. The cell (Fig. 6, *t*) cut off is the tetrasporangium, while the remaining cell (Fig. 6, *s*) becomes very attenuated, and its only further development is that it often divides once more transversely. The tetrasporangium rapidly increases in size, and its contents become more granular. These contents, like those of the carpospores, never show any trace of red or brown colour, and take up Hoffmann's blue very readily. After the tetrasporangium has reached its full size, its contents are divided into two by a transverse wall (Fig. 6, *a*). As a rule the lower half is divided by a longitudinal wall first, and then the upper half; or the longitudinal division in both upper and lower halves may take place at the same time. Thus a typically cruciate tetraspore is produced. Occasionally (as at Fig. 6, *c*) the longitudinal division in the upper part takes place in a plane at right-angles to the plane of division in the lower part: thus in Fig. 6, *c*, the other half of the upper part of the tetraspore is behind the half shown. This formation of tetraspores takes place around the whole periphery of the plant, and tetraspores in all stages of development are found at the same time; so that there is a layer, almost a nemathecium, made up of tetraspores, with a few ordinary peripheral

cells interspersed, and also the attenuated cells derived from the same original peripheral cell as the tetrasporangium, this layer being continuous over the whole surface of the external frond (Fig. 7). Tetraspore-bearing fronds, which are rare, are found in the spring.

The method of preparation adopted was to fix fresh material in a saturated solution of corrosive sublimate in sea-water, and then to cut fairly thick sections in mucilage on a freezing-microtome. The sections were afterwards stained with Hoffmann's blue in equal parts of glycerin and water. If the cell-contents become much contracted, they can be swollen out to their original size by gently warming the section.

The two sterile filaments, which are derived from the auxiliary cell in *Harveyella mirabilis*, and whose function is probably nutritive, are similar in character to those described for various species of the Rhodymeniales by Professor Phillips. For comparison I have added a short summary of the characters of these sterile filaments, taken from Professor Phillips' paper on the Rhodomelaceae [1]:—

'Sterile branches—two such invariably present.

'In *Rhodomela* and *Polysiphonia*—inferior branch 1-celled, lateral branch 2-celled at fertilization of the trichogyne, becoming 2-celled and 4-celled later.

'In *Chondria* and *Laurencia* both branches are luxuriantly branched at the time of fertilization, developing but little afterwards, and becoming partially absorbed on spore-formation.

'In *Dasya* the two branches are but slightly represented, if at all, at the time of fertilization, but become richly branched afterwards, still before spore-formation sets in.'

In *Harveyella mirabilis* the branches are respectively 2- and 4-celled, are developed before fertilization of the trichogyne, and are again absorbed by the auxiliary cell after fusion with the carpogonium and before the development of the gonimoblast.

[1] Development of the Cystocarp in Rhodomelaceae (II), Phillips, Annals of Botany, Vol. X, 1896, p. 201.

Up to the present time the genus *Harveyella* has been placed in the Gelidiaceae, the fourth division of the Nemalionales, a position first assigned to it by Schmitz. In the classification of the Florideae adopted in Engler and Prantl's 'Pflanzenfamilien[1],' the Nemalionales are characterized by the gonimoblast being developed directly from the fertilized egg-cell. Both the Gigartinales and the Rhodymeniales possess an auxiliary cell placed near to the carpogonium, this auxiliary cell, after copulation with the egg-cell, giving rise to the gonimoblast: but in the Gigartinales the auxiliary cell is developed before the fertilization of the carpogonium, while in the Rhodymeniales the auxiliary cells are for the most part differentiated only after this fertilization. The genus *Harveyella*, which possesses an auxiliary cell developed before fertilization, must therefore be removed from the Gelidiaceae, and may be placed in the Gigartinales.

It is worthy of notice that in Richards' paper on *Choreocolax Polysiphoniae*, the typical species of the parasitic genus *Choreocolax*[2], it is stated that beneath the trichogyne are three trichophoric cells, and that the cell on which the lowest of the trichophoric cells rests is the 'carpogenic cell' of the procarp. This 'carpogenic cell,' after the fertilization of the trichogyne, divides into a number of cells, which eventually give rise to the cystocarp. In the figures the carpogonial branch is so curved that the carpogonium, before fertilization, is brought near to this 'carpogenic cell.' This 'carpogenic cell' is evidently, therefore, an auxiliary cell which gives rise to the gonimoblast. This auxiliary cell in *Choreocolax* occupies a similar position to the auxiliary cell in *Harveyella*, that is, it is a cell of a peripheral chain, which, when the trichogyne is mature, is buried three or four cells deep in the frond. As the genus *Choreocolax* consists of small parasitic Algae of very similar appearance, and somewhat similar structure, to

[1] Die Natürlichen Pflanzenfamilien, A. Engler and K. Prantl, Leipzig, 1896, 141 Lief.; Rhodophyceae, Fr. Schmitz and P. Hauptfleisch.

[2] On the Structure and Development of *Choreocolax Polysiphoniae*, Reinsch; H. M. Richards, Proceedings of the American Academy of Arts and Sciences, 1891.

Harveyella, and as, so far as is at present known, its gonimoblast is developed from an auxiliary cell formed before fertilization of the carpogonium, I think that the genus should be provisionally transferred to the Gigartinales, and placed near *Harveyella*.

In both these genera, *Choreocolax* and *Harveyella*, the central portion of the thallus consists of a mass of cells more or less obviously arranged in filaments. Their thallus may be considered to be of the same type as those which possess a central strand of more or less interwoven branched filaments, but very much reduced owing to their parasitic habit. The following is suggested as a provisional classification of these two genera, the classification of the remainder of the Gigartinaceae being taken from the 'Pflanzenfamilien.'

GIGARTINACEAE.

A. Thallus with a single articulated central axis.	1. Endocladieae. 4. Mychodeeae.
B. Thallus with a median longitudinal strand of branched filaments.	
a. Strand obvious.	2. Gigartineae. 3. Tylocarpeae. 4. Mychodeeae. 5. Dicranemeae. 6. Callymenieae.
b. Strand much reduced.	
1. Cystocarp forming a fruit-cavity, in which are developed the spores. Cavity opens by an apical pore.	7. Choreocolaceae.
Parasitic thallus, with cells arranged in fairly distinct rows.	19. *Choreocolax*.
2. Gonimoblast branching freely among the thallus-cells beneath the peripheral layer.	8. Harveyelleae.
Parasitic thallus composed of subdichotomously branched filaments.	20. *Harveyella*.

With regard to the relation of this plant to the average temperature, as indicated by its life-history, I have unfortunately only been able to obtain the temperatures of the sea in Stokes Bay for a few months. As *H. mirabilis* is usually found just below low-water-mark, I have taken the surface-temperatures close to the shore. They differ very little from the shore-temperatures taken at Plymouth, which I have copied from Mr. Church's 'Table of Surface-temperatures[1].'

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	
Plymouth (Church).	7 to 8 to 11	6 to 8	7 to 9	8 to 10	10 to 13	12 to 16	13 to 17	14 to 18	16 to 14	14 to 12	13 to 11	12 to 9	Shore-waters in summer approximate higher numbers, the lesser being for open sea.
Stokes Bay, Gosport.							17	17·5	17·4	14·6			Average temp. for each month.
Vegetative Thallus	▬	▬	▬	▬	▬				—	▬	▬	▬	
Tetraspores		—	—										
Antheridia	—									—	▬	▬	
Trichogynes	—										▬	▬	
Cystocarps (ripe)	▬	▬										—	

The host-plant *Rhodomela subfusca* is a perennial, producing a large number of new branches in the early spring.

The spores of *Harveyella mirabilis* are set free in February, March, or possibly as late as April. In October the parasite was very slightly developed, consisting of only a few filaments, none of which had reached the exterior of the host. It seems probable that the spore, after being set free, finds its way to a position between the cortical cells of the *Rhodomela*. It

[1] A. H. Church, The Polymorphy of *Cutleria multifida*, Annals of Botany, XII, 1898.

either rests until the end of September, and then commences to develop, or the filaments are developed very slowly during the summer months. In either case the *Harveyella* reaches the earliest stage I have yet seen (Fig. 1) at the beginning of October. Thus it may be said that, as regards its temperature-conditions, *H. mirabilis* commences to develop as the temperature falls in the early autumn (average temperature at the end of September and the beginning of October was 15·5°), reaches its maximum during the winter months (6°–12°), and gradually dies away as the temperature rises in the spring. Specimens found in May are usually partially decomposed. Kuckuck's *Choreocolax albus*, as previously mentioned, bore tetraspores in Heligoland in May. The average monthly temperature for May in Heligoland is 8·5° (Church's table), corresponding to the temperature on the English south coast for February and March, during which months the tetraspores of *H. mirabilis* are developed. During the time when fertilization and copulation of the carpogonium with the auxiliary cell appeared to be most frequent —at the end of November—the average temperature was 9·5°.

Finally, I have to thank Mr. E. M. Holmes for his kind help in the identification of the two parasites, and Mr. A. H. Church for his valuable advice and assistance. My thanks are also due to Professor Vines for his invitation, of which I gladly availed myself, to carry out a portion of the work in the Laboratory of the Botanic Garden, Oxford. I hope to deal with the structure and development of *Harveyella pachyderma*, parasitic on *Gracilaria confervoides* (Grev.), in a future paper.

EXPLANATION OF FIGURES IN PLATES III AND IV.

Illustrating Mr. Sturch's paper on *Harveyella*.

The figures are to be regarded as of a semi-diagrammatic character. They were sketched by means of the camera lucida, but cells lying at different levels have often been figured together. All the figures have been slightly reduced in reproduction.

Abbreviations: *aux. c.*, auxiliary cell; *carp.*, carpogonium; *carp. br.*, carpogonial branch; *gonbl.*, gonimoblast-filament; *per. c.*, peripheral cell; *st. br.*, sterile branch.

Fig. 1. Longitudinal section of a *Rhodomela*-stem, with filaments of a young *Harveyella mirabilis* growing among the *Rhodomela*-cells. In Figs. 1 and 2 the cells of the *Harveyella* are dotted. Zeiss apochromat 16 mm., Oc. 8.

Fig. 2. Longitudinal section of a swollen *Rhodomela*-stem, with somewhat older *Harveyella mirabilis*. Zeiss apoc. 16 mm., Oc. 8.

Fig. 3. Longitudinal section of a *Rhodomela*-stem, with a mature cystocarpic *H. mirabilis*. The cells of the host-plant which contain food-material are dotted; the food-material has been exhausted in those cells of the host which are left blank. The cells of the gonimoblast-filaments of the *Harveyella* are black. Zeiss apoc. 16 mm., Oc. 8.

Fig. 4. A portion of a branch of *Rhodomela subfusca* with *Harveyella mirabilis* growing on it. ×3.

Fig. 5. Peripheral filaments bearing antheridia. Zeiss apoc. 8 mm., Oc. 18.

Fig. 6. Peripheral filaments bearing tetraspores. Zeiss apoc. 8 mm., Oc. 18.

Fig. 7. Portion of a section of a tetraspore-bearing frond. Zeiss apoc. 16 mm., Oc. 8.

Figs. 8 and 9. Stages in the development of the procarp. Zeiss apoc. 8 mm., Oc. 18: reduced $\frac{1}{2}$.

Fig. 10. Procarp and auxiliary cell after the development of the two sterile branches. Zeiss apoc. 8 mm., Oc. 18: reduced $\frac{1}{2}$.

The cells of the sterile branches have thickened outlines in Figs. 10, 11, and 12.

Fig. 11. Mature procarp ready for fertilization. The thallus-cell beneath the auxiliary cell has cut off a small cell from its basal part. Zeiss apoc., 8 mm., Oc. 18: reduced $\frac{1}{2}$.

Fig. 12. Procarp with fertilized carpogonium, which has not yet copulated with the auxiliary cell. Zeiss apoc. 8 mm., Oc. 18: reduced $\frac{1}{2}$.

Fig. 13. Fusion of carpogonium and auxiliary cell. The upper part of the carpogonium and the trichogyne were cut off in sectioning. Zeiss apoc. 8 mm., Oc. 18: reduced $\frac{1}{2}$.

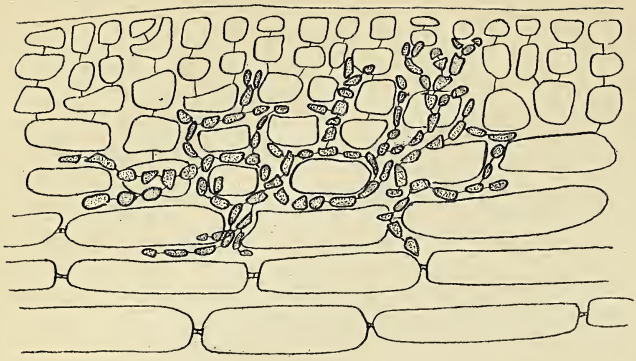
Fig. 14. Auxiliary cell after fusion, which has cut off an upper hemispherical cell. Zeiss apoc. 8 mm., Oc. 18 : reduced ½.

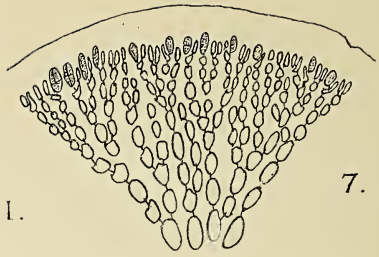
Figs. 15 and 16. Further stages in the development of the young gonimoblast. Zeiss apoc. 8 mm., Oc. 18 : reduced ½.

In Figs. 13, 14, 15 and 16 the carpogonium, the three small cells of the carpogonial branch, the auxiliary cell and its more or less fused sterile filaments, and the cells of the young gonimoblast are dotted.

Fig. 17. Part of a section of a mature cystocarpic frond of *H. mirabilis*. The remains of the auxiliary cell, the gonimoblast, and the spores in various stages of development are dotted. Zeiss apoc. 8 mm., Oc. 18 : reduced ½.

Fig. 18. Portions of filaments of *H. mirabilis* growing between the cells of *Rhodomela subfusca*. Cells of *Harveyella*-filaments are dotted. Zeiss apoc. 8 mm., Oc. 18 : reduced ½.





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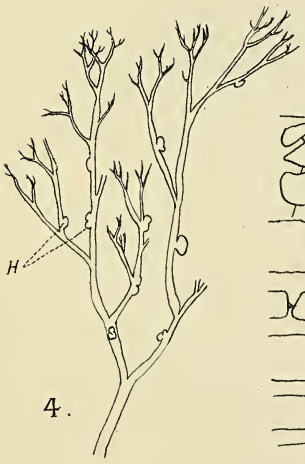

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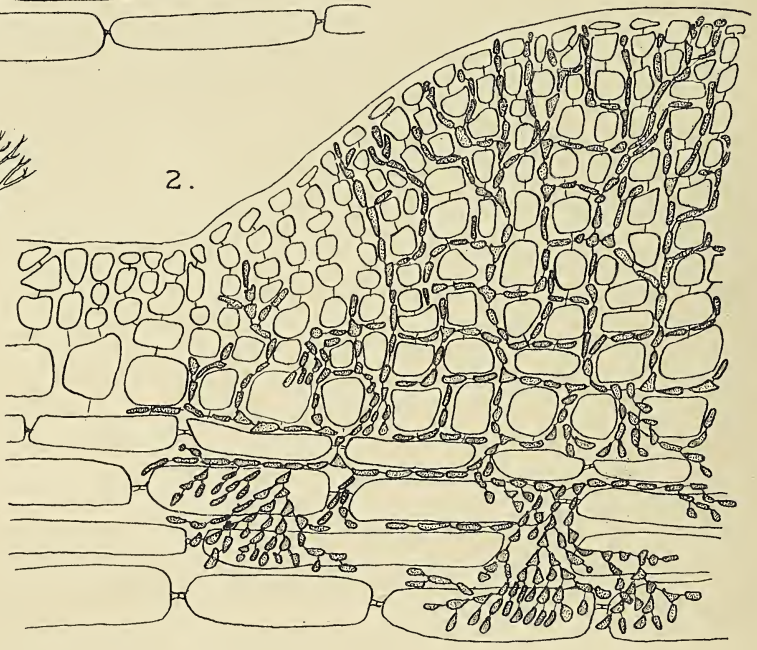

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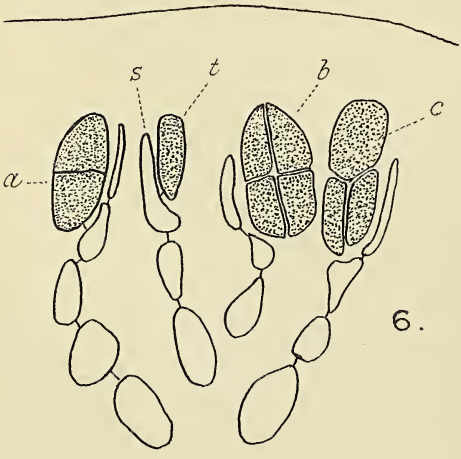

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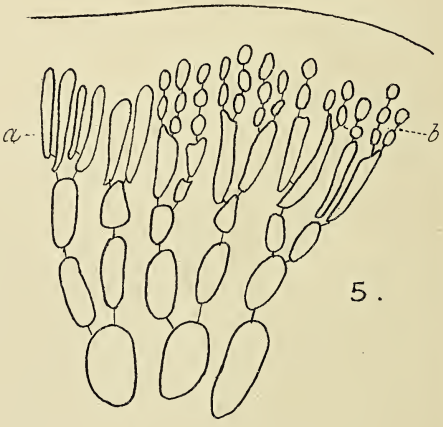

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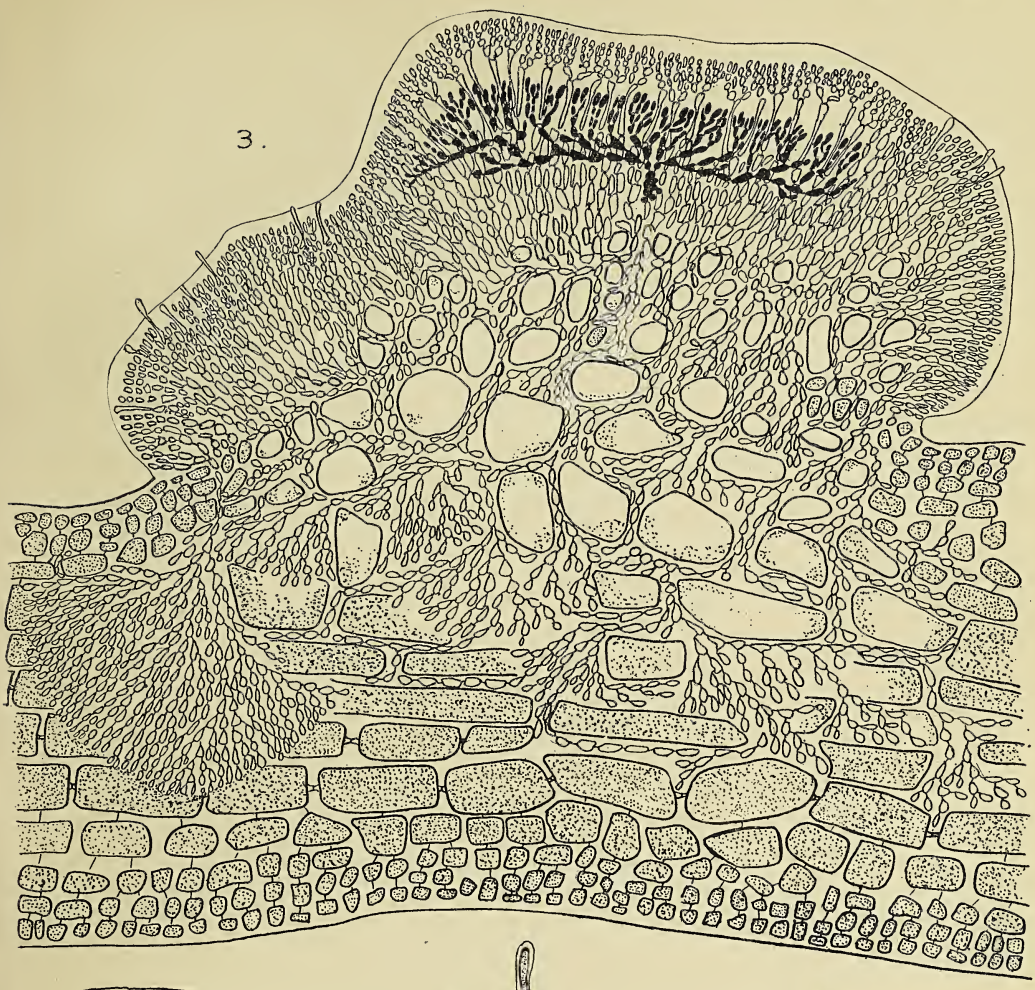
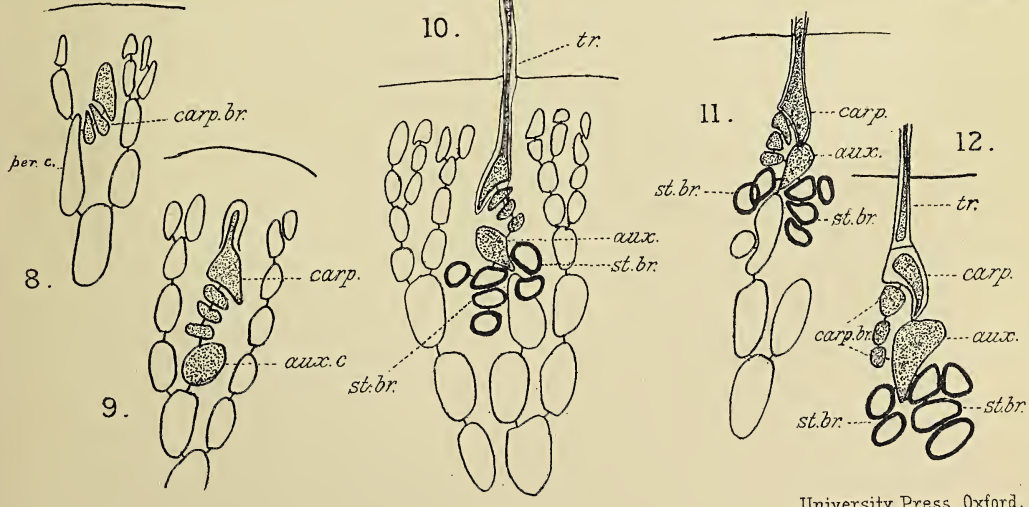
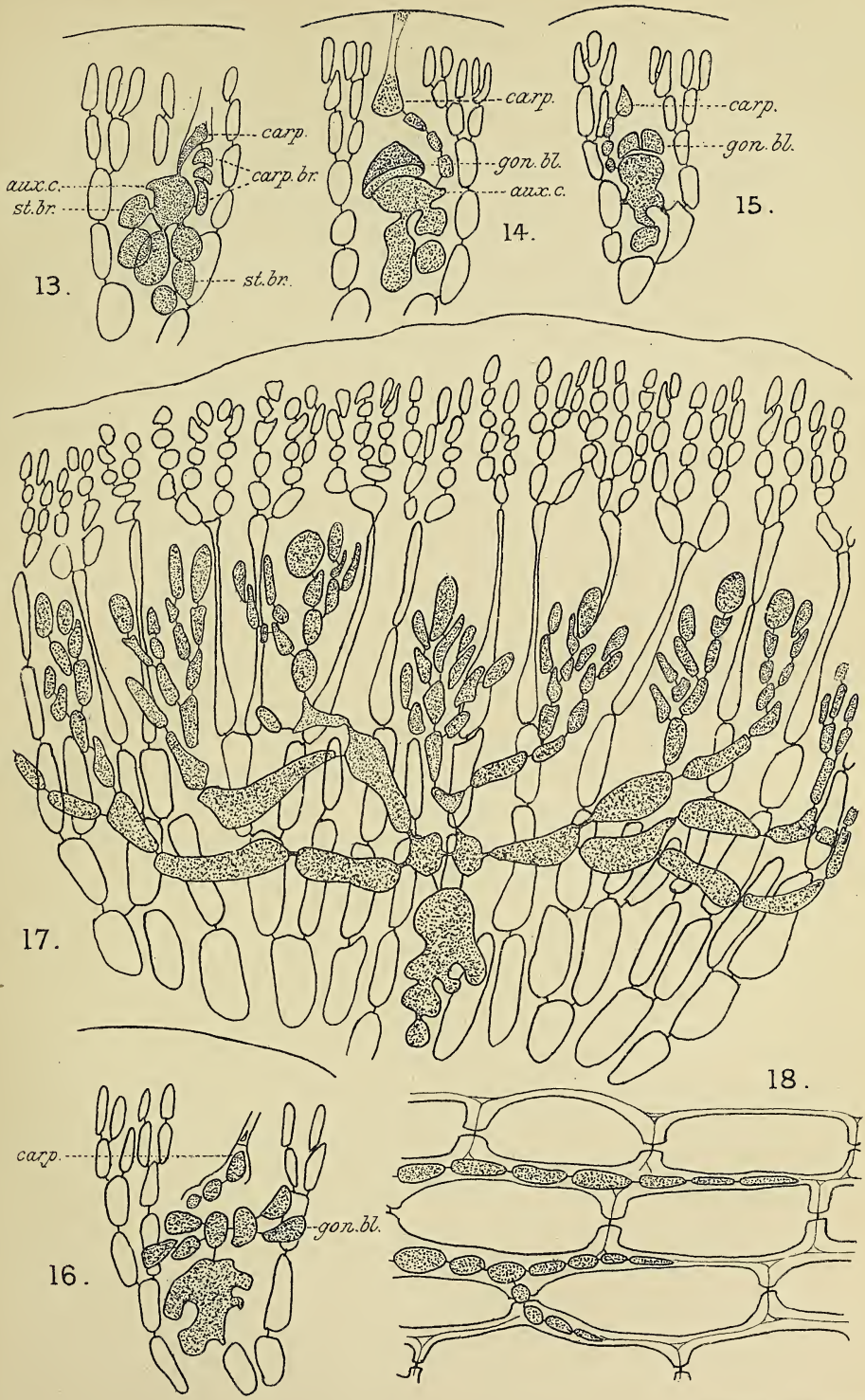
STURCH. — HARVEYELLA.





University Press, Oxford.



University Press, Oxford.

STURCH. — HARVEYELLA.

On the Genus Fissidens.

BY

ERNEST STANLEY SALMON.

With Plates V–VII.

Part I. Morphology.

THE structure of the leaf in the genus *Fissidens* is generally described as quite anomalous amongst Mosses. The leaf may be divided into three distinct parts: (1) the sheathing part, consisting of the two wings, or *vaginant laminae* (Fig. 1, *a*, *a*), inserted more or less horizontally on the stem; (2) the part above the vaginant laminae, constituting the *superior lamina* (*b*); (3) the whole length of the leaf below the nerve, the *inferior lamina* (*c*). Figs. 2, 3, and 11 show the position of the parts as seen in transverse section. This peculiar leaf-structure occurs, without exception, throughout the large genus of *Fissidens*, which numbers [including the subgenus *Conomitrium* (*Octodiceras*)] nearly 500 species.

The only variations from the type of leaf shown at Fig 1 are in the following points: (1) the relative size of the sheathing part—this is sometimes broad, and extends more than half the length of the leaf, or it may be small, and comparatively insignificant (Fig. 13); (2) the inferior lamina may be well developed to the base of the leaf, and inserted

[Annals of Botany, Vol. XIII. No. XLIX. March, 1899.]

on the stem with a prominent vertical insertion (Fig. 64), or it may fail to reach the stem; (3) the vaginant laminae may be equal at the apex, or unequal, with the smaller lamina either (in a few cases) ending in the nerve (Fig. 4), or, as is usually the case, between the nerve and the margin of the leaf; (4) the nerve may cease below the apex, or be strongly excurrent—in very rare cases it is quite rudimentary, if not absent.

Three principal theories have been advanced to explain the morphology of the leaf of *Fissidens*. The first theory—the one accepted by bryologists before 1819—considered that the whole structure corresponds to the leaf of other Mosses, and that the sheathing part results from a split in the thickness of the leaf. De la Pylaie (1), who monographed the genus in 1814, gives this theory in the following words:—the leaves present 'une fente ou dédoublement dans leur épaisseur, qui descend jusqu'à la nervure. Ce dédoublement . . . résulte de l'écartement et de la séparation de leurs deux surfaces, qui forment alors deux lames distinctes.'

The second theory was advanced by Robert Brown (2) in 1819, in the following words:—'In *Fissidens* . . . the leaves are universally described as presenting their margin instead of their disk to the stem, and as having a doubling of the lower half of their inner or upper margin, extending as far as the nerve. . . . It seems to me a much simpler explanation of the apparent anomaly to consider the supposed doubling or division of the leaf as its true disk, and the deviation from the usual structure as consequently consisting in the greater compression of the leaf, and in the addition of a dorsal and terminal wing. In support of this view, it may be observed that in the lower leaves of the stem both the additional wings are greatly reduced in size, and in some cases are entirely wanting, as they universally are in the perigonial leaves which have likewise the more ordinary form, being moderately concave and not even navicular.'

The view here expressed, that the sheathing part alone is the true leaf, and that all the rest is an outgrowth from

it, became for a time generally accepted. This was chiefly due to Bruch and Schimper (3), who in 1843 gave a detailed account of the leaf-structure; and as this account, which is an amplification of Robert Brown's theory, gives all the evidence up to the present known in favour of this theory, it is worth while to give their remarks:—'Les feuilles . . . dans les jeunes plantes, et à la partie inférieure de la tige . . . ne diffèrent en rien, par rapport à leur configuration et à leur insertion, des feuilles de Mousses ordinaires, et ce n'est qu'à mesure que les plantes se développent qu'elles prennent une forme tout anomale en produisant sur le revers de la côte médiane une aile verticale qui, par suite d'une dilatation extraordinaire et d'une prolongation considérable conjointement avec une des ailes normales de la feuille, finit par constituer la partie la plus considérable de l'expansion foliaire, de sorte que tous les auteurs ont pris cet appendice—car c'est ainsi qu'il faut qualifier cette production—pour la feuille elle-même. . . . Un examen détaillé et une appréciation exacte des différentes parties d'une feuille de Fissident ne tardent pas à montrer que l'aile dorsale n'est autre chose qu'une dilatation monstrueuse de la côte médiane, et que, considérée sous le point de vue morphologique, elle est tout à fait analogue aux lamelles qui se rencontrent sur la face interne de la côte médiane des feuilles de Polytric, de *Pottia*, et d'autres Mousses.'

Lindberg (4) advanced the third theory, according to which the whole expansion is the true leaf, with the exception of one of the wings of the sheathing part (that is, one of the vaginant laminae), which is considered to be a 'stipule'—a member, Lindberg says, not especially rare amongst Acro- and Pleurocarpous Mosses, e.g. *Epipterygium*. The stem is considered to have four straight rows of leaves—two rows of larger-nerved leaves, inserted at the side, and the remaining two of smaller nerveless leaves ('stipules'), coming out more from the front of the stem, and grown together by their lower margin with the middle of every one of the lateral leaves—so forming the sheathing part. Lindberg says that Robert

Brown's explanation seems sufficiently good when treating of the species of *Fissidens* that possess a nerve, but that it cannot be applied to those without a nerve.

Lindberg's theory has been supported by Braithwaite (5), who makes the following remarks on the different theories:— 'One of these [theories] was that the leaf to a certain extent was split vertically to embrace the stem; but this is not tenable, as each half of the split portion is of equal thickness to the rest of the leaf. Another view which has met with general acceptance is that the double portion alone is the true leaf, . . . and all the rest is an outgrowth from it. . . . However plausible this view may appear, it does not satisfy us, for there are species in which the duplicate part is nearly or altogether wanting, e.g. *F. dealbatus*. The most rational explanation seems to be this, that the additional lobule is of the nature of a stipule, arising on the opposite side of the stem, which has become adnate to the nerve by the whole lower margin, the upper margin being free and parallel to the corresponding margin of the leaf.'

Passing these three theories in review, we notice that according to Robert Brown's theory alone the true leaf (formed of the vaginant laminae) is considered to be horizontally inserted; in the two other theories the whole expansion is considered to be the leaf (either split on one side, or united to a stipule) with a vertical insertion. If, therefore, the internal structure of the leaf can decide the question of insertion, it will give important evidence in deciding between the theories. Such proof is, I think, furnished by the nerve-structure of certain species.

The usual type of nerve-structure in *Fissidens*, as shown in a transverse section near the base of the leaf, is represented at Figs. 2 and 3. We find two well-developed 'stereid' bands (*b*, *b'*), separated by a single or double row of large thin-walled cells. If we refer to the works of Lorentz (6, &c.) on the structure of the nerve in Mosses, we find that where, in the many hundred drawings of nerve-sections which he gives, two 'stereid' bands exist, they invariably mark the

dorsal and ventral surfaces of the nerve, that is to say, they never occur laterally. Therefore the nerve-structure, as shown in Figs. 2 and 3, would indicate by the two 'stereid' bands (*b*, *b'*) the upper and under surfaces of the leaf. But these sections are taken at a point where, according to Robert Brown's theory, the leaf bears an appendage at the back of the nerve; and to see the normal nerve-structure, we must select a species with leaves in which the appendage—the inferior lamina—dies out before reaching the stem. Such a species is *F. fasciculatus*, Hornsch., and Fig. 5 shows a section of the leaf just above its insertion. Here we see very plainly that the nerve-structure is that of an ordinary sheathing leaf, with the 'stereid' bands (*a*) (*b*) marking respectively the upper and under surfaces of the leaf. If we follow, by means of serial sections, the nerve-structure as we ascend the leaf, we shall see exactly how the arrangement shown at Figs. 2 and 3 has arisen. At Fig. 6 we find the first appearance of cells belonging to the inferior lamina, and at once the nerve-structure becomes altered. The stereid band marking the back of the leaf becomes divided by unthickened cells (the 'conjunctivae' of Lorentz) connecting the inferior lamina with the large cells ('Deuter') of the nerve proper. Fig. 7 shows the inferior lamina more developed, the two halves of the dorsal stereid band pressed more to the side, and the ventral stereid band beginning to die out. Fig. 8 corresponds to Figs. 2 and 3, and we can compare them. In Fig. 8 we see that the groups (*b*, *b'*) belong to a single dorsal band and that the cells (*a*) are the remains of a ventral stereid band, and mark the inner surface of the leaf: the rows of large cells connecting the laminae consist partly of the 'Deuter' of the original nerve-structure, and partly of cells placing the inferior lamina in connexion with these. In Figs. 2 and 3 the groups (*b*, *b'*) are similarly the divided halves of a dorsal band, and do not indicate the upper and under surfaces of the leaf; there is here no trace of a ventral stereid band, but we can see clearly that the upper surface of the leaf lies towards (*a*).

Continuing with *F. fasciculatus*, Fig. 9 needs some explanation. The bistratose portion between the vaginant laminae and the nerve is not to be considered as formed by the union of the two laminae, but is the beginning of the superior lamina. Fig. 10 shows the apex of the vaginant laminae, which are here, as is usually the case, unequal above. Section 11 is taken above the vaginant laminae, through the inferior and superior laminae, which are bistratose in this species.

Those species of *Fissidens* which, like *F. fasciculatus*, Hsch., *F. linealis*, Bryol. eur., &c., possess dorsal and ventral stereid bands, afford a clear proof of the horizontal insertion of the leaves, and the nerve-structure at the base of their leaves is quite irreconcilable with the theories that require a vertical insertion. These species are valuable in distinguishing for us so clearly, by means of the dorsal and ventral stereid bands, the under and upper surfaces of the true leaf: but we find, generally, among the rest of the species of *Fissidens*, a nerve-structure giving the same evidence. We often see, as in Fig. 12, a clear indication of a dorsal band, in the form of two principal groups, almost connected by small scattered groups of stereid cells. Moreover, in the large number of species examined, I have always found that, at the base of the leaf, even in those cases where the inferior lamina still existed, the two stereid bands approached each other towards the back of the leaf (vaginant laminae), indicating clearly a concavo-convex nerve.

The symmetry of the leaf, too, as seen in transverse section across the lower part, is all against Lindberg's theory of a 'stipule' having become adnate to the nerve—it can at once be seen, e.g. in Figs. 2 and 3, that it is not the presence of one of the vaginant laminae, but that of the inferior lamina, which makes the leaf irregular. Neither Lindberg's 'stipule' theory, nor the earlier one of the 'split leaf,' has any special evidence to support it; while Robert Brown's theory so admirably accounts for the internal structure of the leaf, that it may, I think, be safely accepted, provided that the objections that have been raised against it can be shown to be groundless.

Lindberg and Müller (11) have rejected Robert Brown's theory for the same reason. According to their interpretation of this theory, the inferior and superior laminae are a 'product' of the nerve; therefore, as nerveless species of *Fissidens* have been described, the theory fails to hold good. But even if we admitted that the outgrowth of these laminae was dependent on the presence of a nerve, we should hardly have reason to reject the theory.

Four species have been described as nerveless—*F. dealbatus*, Hook. f. & Wils.; *F. hyalinus*, Hook. f. & Wils.; *F. Metzgeria* (C. Müll.), Par., and *F. usambaricus*, Broth. *F. dealbatus* (Fig. 14) shows, in transverse section, cells which undoubtedly represent a nerve. The cells are unthickened [as in species of *Fissidens*, belonging to the subgenus *Conomitrium* (see Fig. 17)], and are more numerous near the base of the leaf, becoming less evident higher up (Fig 15), and ceasing just before the end of the vaginant laminae. *F. hyalinus* also shows the same trace of a nerve at the base of the leaves, but in this species the nerve dies out about halfway up the vaginant laminae. *F. usambaricus* has a group of about seven cells, which represent the nerve (Fig. 16). *F. Metzgeria* I have not been able to examine.

There seems, however, no reason for considering the laminar outgrowth of the superior and inferior laminae as in any way connected with the nerve. If we examine the leaves of the other Mosses in which similar outgrowths occur, e.g. *Polytrichum* (Fig. 18), *Catharinea* (Fig. 19), *Pottia* (Fig. 20), *Barbula* (Fig. 21), and *Tortula* (Fig. 22), we find that in all cases the outgrowths spring from unthickened cells, similar to those of the lamina, and that the nerve can only be considered as generally localizing the position of the outgrowths.

In the nerveless leaves of some Hepaticae, too, we see very clearly the origin of laminar outgrowths from the ordinary cells of the leaf. Mitten (12) has pointed out that certain species of *Schistochila* (*Gottschea*) and *Micropterygium* approach *Fissidens* in the shape of the leaf. Fig. 23 represents the leaf of a species of *Schistochila*. These leaves

are usually described as 2-lobed, with the smaller dorsal lobe (*a*) attached intramarginally on the larger ventral lobe (*b*, *b'*). It seems to me more probable, however, that we have here a keeled leaf bearing a laminar appendage (*b*), and that consequently a close approach is made to the structure of a *Fissidens* leaf. A transverse section in the lower part of these *Schistochila* leaves is shown at Fig. 24. It is not unusual to find in some species of *Schistochila* with this 'intramarginally' placed lobe, e.g. *S. alata* (Lehm.), Schffn., that instead of the single outgrowth (*b* in Fig. 24) at the back of the leaf, there are two of these (Fig. 25, *b*, *b*), when it is clearly impossible to consider both as anything but laminar outgrowths. This view of the morphology of the leaf is supported by the occurrence of species in the same genus, e.g. *S. lamellata* (N. ab E.), Dum., (Fig. 26), in which the whole back of the leaf is covered with numerous parallel laminae.

Goebel (13, 14) gives quite a different explanation of the leaf-structure of these Hepaticae. The part (*a*) of Fig. 23 is considered a 'lamella' borne on the upper surface of the leaf, which consists of the rest of the expansion (*b*, *b'*). According to this view there would be no real resemblance between the leaves of these species of *Schistochila* and those of *Fissidens*, for the 'lamella' of the *Schistochila* leaf (Fig. 24, *a*) would correspond to the inferior lamina of the *Fissidens* leaf (Fig. 3, *i. l.*). But in the face of the general occurrence of concave or keeled leaves in the genus *Schistochila*, as well as in such genera as *Diplophyllum* (Fig. 27) (which perhaps shows the primitive form of these leaves), it is difficult to accept Goebel's explanation. Such cases, too, as that mentioned above (Fig. 25) cannot possibly be explained on this theory.

The other objection to Robert Brown's theory is raised by Braithwaite, who says, 'there are species in which the duplicate part is nearly or altogether wanting, e.g. *F. dealbatus*.' I have not been able to find any leaves of a *Fissidens* with the vaginant laminae absent; in *F. dealbatus* (Fig. 13) these

laminae, although forming an insignificant part of the 'leaf,' seem to be always present.

The very few species (*F. hyalinus*, *F. usambaricus*, &c.) which, like *F. dealbatus*, possess rudimentary vaginant laminae, are extremely interesting in showing us the limit (as at present known) reached in the process of dwarfing the true leaf; at the other end of the series we have species like *F. bifrons* (Schpr.), C. Müll., (Figs. 28, 29) and *F. elamellosus*, C. Müll. and Hpe., in which the appendage of the leaf is the insignificant part—and between these extremes the other species arrange themselves in a perfectly continuous sequence.

A point must be mentioned here, of which, I think, Bruch and Schimper have given an incorrect explanation. These authors, accepting Robert Brown's theory, divide the leaf into four parts, viz. the two wings of the true leaf (i.e. the two vaginant laminae), the dorsal wing (ala dorsalis), borne at the back of the nerve of the vaginant laminae, and the vertical lamina (lamina verticalis), formed 'par la continuation d'une des ailes foliares [vag. lam.] et par l'aile dorsale.'

I do not think the last-named part has arisen in this way, for the following reasons. In many species the areolation is not the same in the different parts of the 'leaf,' and the signification of this does not seem to have been noticed.

F. floridanus, Lesq and James (Fig. 30) illustrates this feature well. Here the areolation of the vaginant laminae (*b*) is different to that of the superior and inferior laminae (*a*). The change in areolation occurs abruptly, and is seen most clearly in those species in which, as in *F. floridanus*, the vaginant laminae are unequal at their apex: there is then a distinct line from the apex of the inner vaginant lamina to the margin of the outer one (Fig. 30, *x*).

This difference of areolation is not uncommon among the species of *Fissidens* (e.g. *F. decipiens*, De Not., *F. lanceolatus*, Hampe, &c.), and in all cases it is the superior and inferior laminae which show one kind of areolation, and the vaginant laminae the other, with an abrupt separating line, as shown at Fig. 31. The different structure of the cells on each side

of this line indicates, I believe, a different origin, and would not occur on the hypothesis that one of the vaginant laminae has prolonged itself to form the superior lamina. In dealing below with an allied genus, this question of the origin of the superior lamina will be mentioned again, and I will only state here that I regard the superior and inferior laminae as one laminar outgrowth, arising primarily from the back of the leaf, quite independently of the nerve.

There are still two more theories as to the morphology of the *Fissidens* leaf that must be mentioned. The first of these was put forward by Spruce (15) in these words:— 'The leaves of *Fissidens* were originally 3-lobed, with the medial and longer lobe, by a half turn on its axis, placed vertically, i.e. at right-angles to the other two lobes and to the base of the leaf, which is inserted transversely on the stem. As we now usually see them, the lobes have become connate; the two lateral lobes complicate into an equitant sheath, and at the keel, but especially at the apex, winged with the vertical medial lobe; but are still occasionally found more or less dissevered, as in the ancestral type.'

In support of this theory, Spruce mentions the occurrence of perigonial bracts 'not complicate, but concave, cloven at the apex into three subequal short obtuse lobes; the middle lobe twisted half round so as to set it at right-angles to the rest of the leaf,' and cites Bryol. Europ., fasc. 17, *F. bryoides*, Hedw. t. 2, f. 10, and Sullivant's Icones Muscorum, *F. obtusifolius*, Wils., t. 22, f. 20, as figuring similar male bracts, more or less distinctly 3-lobed.

An examination of the floral leaves of *Fissidens* shows, among not only the perigonial, but also among the perichaetial bracts, certain leaves of the 'trilobate' form mentioned by Spruce (Figs. 36–39). But taking the whole series of the leaves of either inflorescence, we can see that this 'third lobe' is simply the reduced or rudimentary 'lamina verticalis;' and if we are to take the most simple form of these leaves as representing the 'ancestral type,' we must choose the outermost simple concave leaves, which are quite

similar to those found on the base of the stem of most species. It is more probable, however, that these outermost floral leaves show a modified rather than a primitive form, and have arisen through the bud-like shape of the inflorescence.

The second theory has no evidence to support it, and hardly requires serious consideration. Debat (16), its author, examined the vegetative buds of *F. adiantoides*, and came to the conclusion that in the young leaves, up to a certain stage, the vaginant laminae are absent ('à 6 centièmes de millimètre on a une représentation exacte de ce que sera plus tard l'organe appendiculaire, moins la feuille normale'). For this reason Debat rejects Robert Brown's theory, and gives this explanation:—'Il nous semble que l'on pourrait assimiler très exactement l'évolution des lames foliacées de générations successives à celle de rameaux naissant les uns des autres par dichotomie. Ces rameaux seraient, à la vérité, modifiés par la production de deux ailes latérales; ils ont pris l'apparence de feuilles, et la feuille véritable serait adnée à la base.' Further, these 'branches' are supposed to be homologous with bracts—members, which (Debat says) 'suivant une théorie généralement admise . . . sont des pédoncules floraux modifiés par l'épanouissement d'un limbe.' To the bract the true leaf (i. e. one of the vaginant laminae) has become adnate by one margin.

It will be sufficient here to point out that Debat's statement as to the structure of the young leaf is not correct, as the very youngest leaves that can be dissected from the buds of *F. adiantoides* do possess at this stage—in common with other species of *Fissidens*—vaginant laminae, although this part is at the time very minute and relatively quite disproportionate in size. This fact is well seen in *F. serrulatus*, Brid. In the fully grown leaf of this species the vaginant laminae reach halfway the length of the leaf; in the young leaf, however, at about the time of cessation of apical growth, the relative size of the different parts is as shown in Fig. 40. To understand how this occurs, it is

necessary to follow the growth of the leaf-segment from its origin.

The embryology of the leaf of *Fissidens* has been thoroughly investigated by Lorentz (9) and Leitgeb (10), and shows some extremely interesting developmental details, for the full account of which the works of these authors must be consulted. An important point to be noticed is that the first two divisions in the leaf-segment cut off cells which later form the vaginant laminae. The true leaf is thus indicated from the first—a fact which gives the strongest support to Robert Brown's theory.

The subsequent growth of the leaf-rudiment is very curious. The 2-sided apical cell, after giving rise to the two cells mentioned above, becomes altered in shape in such a way that the segments now cut off lie in a plane at right angles to that of the first segments, and so form the vertical part of the *Fissidens* leaf. No growth of the cells of the vaginant laminae takes place for some time, while the vertical outgrowth continues to grow by the 2-sided apical cell in exactly the same manner as though it were a true leaf.

This process of development is true only for those leaves of *Fissidens* which show a well-developed superior and inferior lamina; in the case of the lower leaves, where the vertical outgrowth is small and chiefly terminal, the 'Drehung der Theilungsrichtung' of the apical cell takes place, as would be expected, only just before the conclusion of apical growth.

Taking the embryology of the typical *Fissidens* leaf, however, we have an instance of an interesting detail of development. The laminar outgrowth, which in the ancestral form of the leaf must have been merely a small wing at the back of the leaf, has here so increased in size and importance that it has assumed by its direct growth from the apical cell the character of a true leaf, while the rudiment of the latter, formed of the first two cells cut off, grows subsequently wholly by intercalary growth. The great effect which the abnormal structure of the leaf has on its embryology

tends to show that the abnormality must be of a remote origin.

Reverting to the different theories put forward as to the morphology of the leaf of *Fissidens*, it is to be noticed that all authors have considered the structure as quite anomalous amongst Mosses, and no attempt has been made to seek for any connecting link between this and the normal form of leaf of other Mosses.

It seems to me, however, that there are two genera which clearly supply this link, and which, moreover, give additional reasons for the acceptance of Robert Brown's theory. One of these genera is *Bryoxiphium*, Mitt. Here the leaves are of two shapes; the barren stems have simple sheathing leaves (Fig. 41), with or without a narrow wing at the back of the nerve; in the fertile stems the lower leaves are similar, but as they approach the apex the wing at the back of the nerve becomes broader, and the leaf becomes much longer, running out into a somewhat flexuose hair-like prolongation. Fig. 42 shows a leaf from the region a little below the apex; at the apex the prolongation often exceeds in length the rest of the leaf.

The structure of these upper leaves is very curious. Bescherelle (17), who monographed the genus, thus describes them:—'La nervure est pourvue d'une aile dorsale très étroite, presque nulle de la base au milieu et n'apparaissant souvent que vers le sommet de la feuille, où elle se confond avec le prolongement d'un des côtés du limbe foliaire pour former une lame spéciale très alongée, comme cela se voit dans les *Octodiceras*.'

If the sheathing part of the leaf really ended at the commencement of the prolongation, as Bescherelle states above, we should have practically the structure of a *Fissidens* leaf, but this is not the case. When the leaf is examined in surface view under the microscope, it can be seen by careful focussing that the wing on one side of the nerve in the prolongation is split to the apex. The side which is split is the one above the sheathing base, and is, in fact,

a direct upward growth of the whole of the true leaf. This is seen at once in a transverse section of the leaf near the apex (Fig. 43).

The structure of the leaf is not therefore so closely similar to that of *Fissidens* as Bescherelle asserts, but the genus *Bryoxiphium* is, nevertheless, very interesting in showing us the first start, so to speak, of a Moss with sheathing, horizontally inserted leaves, arranged distichously, producing a vertical expansion, in the shape of a wing at the back of the nerve. This wing, in the upper leaves, is prolonged upwards for a considerable distance, occupying one side of the nerve, while on the other side the true leaf has grown upwards from the sheathing base in the form of two narrow, vertically compressed wings.

The nerve-structure of *Bryoxiphium* is of interest in comparison with that of *Fissidens*. Fig. 44 is a section at the base of the leaf, where there is no dorsal wing; Fig. 45, where the wing is well developed. In the latter section we see that the presence of the wing has scarcely interfered with what may be considered the original structure of the nerve. There are, however, often a few unthickened cells beginning to grow through the stereid band in the direction of the dorsal wing; sometimes we find a row of these cells running obliquely right through, and in such cases we have a clear indication of the 'conjunctivae' which form so distinctive a feature of the *Fissidens* nerve.

The other genus is *Sorapilla*, Spruce and Mitt.: Fig. 46 shows the leaf of the single species *S. Sprucei*, Mitt. We see here at once a close resemblance to the leaf of *Fissidens*; also, that as compared with the typical form of the latter (Fig. 1), the relative size of the different parts of the leaf is reversed. The sheathing part of the *Sorapilla* leaf is seen obviously to be the true leaf, and forms by far the greater part of the expansion, and the vertical outgrowth from it, corresponding to the inferior and superior laminae, is insignificant in comparison. Figs. 48–52 show the nerve-structure. The nerve throughout is ill-defined, and consists of only

a few cells, variable in number, at the keel of the leaf, which are thickened in such a way as to leave a more or less rounded lumen; frequently the nerve-cells shade off gradually into those of the laminae. The gradual appearance of the outgrowth is shown in Figs. 49–52. A wide, but weak and ill-defined, nerve appears in the outgrowth; this is shown in section in Fig. 52, taken along the line *a*, *b*, in Fig. 46. The perichaetial leaves (Fig. 47) are nerveless, but have still a prominent appendage. *Bryoxiphium* and *Sorapilla* are, therefore, related to *Fissidens* in the structure of their leaves; the former also shows other signs of relationship.

The stem of *Fissidens*, as is well known, possesses the peculiarity of growing by means of a 2-sided apical cell. From this cell, segments are cut off alternately to the right and left, so that the leaves from the first have a distichous arrangement (Fig. 53). It is often stated that in this respect *Fissidens* stands alone amongst Mosses. Lorch (18) has, however, stated, although without giving any details, that a 2-sided apical cell is found in species of *Phyllogonium*, and Goebel (21) has recently said that the same is present 'in *Fissidens*, *Phyllogonium*, and perhaps still other Mosses with distichously arranged leaves.' In all other Mosses the stem has a 3-sided apical cell, from the three sides of which segments are cut off regularly (e.g. as in *Mnium*, Fig. 54), and the later distichous arrangement of the leaves, where it exists, is due to subsequent displacement.

Unfortunately from the herbarium material at my disposal, I have been unable to study in detail the apical growth of *Bryoxiphium* and *Sorapilla*. Campbell (19), however, has investigated the former, and states that the apical cell is 3-sided, and that therefore the distichous arrangement of the leaves is secondary. The arrangement of the young leaves in the bud is remarkably similar, however, in *Bryoxiphium* and *Fissidens* (cf. Figs. 55 and 56); in both cases the hairs, so prominent in the bud, persist in the axils of the mature leaves. The distichous arrangement of the leaves in *Bryoxiphium* is maintained up to the very apex of the stem, and I was

not able to find any signs of a 3-rowed arrangement of even the young leaves. This points to a very rapid displacement of the segments as they are cut off.

The stems of *Bryoxiphium* and *Fissidens* show a very similar structure (Figs. 57 and 58). There is a central strand of small, very thin-walled cells, surrounded by larger cells, the outer layers of which in the old stem become strongly cuticularized.

The fact that the apical cell is 3-sided in *Bryoxiphium* is not unfavourable to the view that this genus may represent the ancestral form of *Fissidens*, as the latter genus clearly shows signs of an origin from a Moss with this form of apical cell. We see this in the fact, discovered by Hofmeister (20), that the underground shoots of *Fissidens* still grow by means of a 3-sided apical cell, and also, that the branches of the stems above ground, possess at first a similarly shaped apical cell (Leitgeb, 10).

Leitgeb (loc. cit.) states as his opinion that it is 'im höchsten Grade wahrscheinlich, dass die Vorfahren unserer Fissidenten mit dreiseitiger Scheitelzelle wuchsen und eine dreizeilige Blattstellung zeigten, dass also die zweizeilige Stellung und ebenso die abnorme Ausbildung der Blätter eine erst später erworbene Eigenschaft sei.' But with the example of *Bryoxiphium* before us, we must conclude that the ancestors of *Fissidens*, although still possessing a 3-sided apical cell, had already acquired the distichous arrangement of the leaves.

Summary.

Considering the amount of evidence in favour of Robert Brown's theory, and the absence of any valid objections, I think this may be safely accepted as satisfactorily explaining the morphology of the leaf of *Fissidens*; and, further, that this 'leaf' must be considered as composed of two distinct parts, viz. (1) the vaginant laminae, the true leaf; (2) the superior and inferior laminae, formed of one laminar out-

growth from the back of the leaf, in which a nerve has been developed.

We can even, perhaps, get a glimpse at the evolution of the 'leaf.' Looking at the often twisted and contorted prolongations of the upper leaves of *Bryoxiphium* (a genus of three rare species), we may say that the attempt to produce a vertical expansion from the leaf was here frustrated by following the plan of elongating the whole leaf on one side, so that the prolongation remained double on that side to the apex; in *Sorapilla* (with one species known only from a single station in the Andes) a step in the right direction was taken in producing only a vertical outgrowth from the back of the leaf, but the nerve here is very poorly developed, and unable, perhaps, to keep in position any considerable vertical outgrowth; finally, we see that *Fissidens*, by enormously enlarging the outgrowth, and dwarfing and vertically compressing the true leaf, has made the whole assume the symmetrical form of a normal leaf, furnished with a strong nerve, and has attained the reward of spreading over the tropical and temperate regions of the world with its 500 species.

Part II. Classification.

The systematic position of *Fissidens* has been treated very differently by different authorities. The abnormal structure of the leaf has usually led to its being placed in a separate Order Fissidentaceae. Having regard only to the dicranoid peristome, many authors place this Order close to Dicranaceae. Others, on account of some of the species being cladocarpous, consider that the Order is intermediate between the Acro- and Pleurocarpi.

Schimper places the Order between Leucobryaceae and Seligeriaceae. Müller includes Fissidenteae in the first section *Distichophylla* of the Stegocarpous Acrocarpi, with the tribes Schistostegeae, Drepanophylleae, and Distichiaceae. Mitten places *Fissidens*, *Bryoxiphium*, *Sorapilla*, and *Eustichia* in

his tribe Skitophylleae, and this arrangement seems on the whole the most satisfactory, as the genera here associated are certainly, in their vegetative characters, closely related.

Geographical Distribution. The number of species of *Fissidens*, as mentioned above, is nearly 500, and these are distributed throughout the tropical and temperate regions of the world.

Taking the main divisions of land, and finding the total number of species, and the number of endemic species, for each, we have the following table :—

	Total No. of species.	Endemic species.
Europe	32	13
Asia	92	84
Africa	159	140
North America	74	49
South America	118	106
Pacific	60	50

There is thus, apparently, a large proportion of endemic species; on the other hand some species have a very wide distribution, e.g. the three commonest British species *F. taxifolius*, *F. adiantoides*, and *F. bryoides*.

F. taxifolius is common throughout Europe and North America; occurs in Asia in India and the Caucasus; and in Africa in the Canary, Madeira, and Mascarene Isles. *F. adiantoides* is found throughout Europe; in Japan and Hong Kong, Asia; Algeria, Africa; in the East and West of North America, and Canada; and in New Zealand and Tasmania. *F. bryoides* occurs in Europe, Asia, Africa, North America, Australia, and New Zealand.

During my studies, I examined the species of *Fissidens* in the Kew Herbarium, and the following notes refer (with one exception) to specimens found there.

Fissidens aequalis, sp. nov. (Figs. 59–68).

(*F. crispans*, Sch. ms. in Herb. Kew, pro parte.)

Terricola, dioicus, laxe caespitosus, flavescenti-viridis. Caulis

simplex, erectus, circiter 1 cm. altus, ad basin foliosus. Folia 10–14-iuga, remota, brevia, 1–1·5 mm. longa, oblongo-lanceolata, acuta, falcato-secunda, siccitate crispata, integerrima, limbata, limbo flavo angusto (in laminis veris latiore) ad apicem ipsum laminae apicalis dissoluto, nervo flavo crassiusculo infra summum apicem evanescente; laminae verae apice aequales, vel subaequales, ultra medium folii productae; lamina dorsalis basi valde decurrens; cellulae parvulae, circiter 5 μ diam., hexagonae, vel quadrato-hexagonae, laeves, parum incrassatae. Folia perichaetialia caulinis conformia, lamina dorsali breviore. Seta terminalis, erecta, vel obliqua, 4–5 mm. alta. Theca parvula, ovalis, subaequalis, inclinata, collo distinctiore instructa, peristomii dentibus infra medium bifidis, sporis laevibus 15–20 μ diam.

Planta mascula femineae similis, sed brevior, flore masculo terminali, foliis perigonialibus tribus, exterioribus foliis caulinis conformibus, sed lamina dorsali breviore, intimo lamina apicali scalpelliforme, lamina dorsali nulla, et laminis veris haud limbatis, paraphysibus nullis.

Caetera absunt.

Patria. Guatemala (Herb. Schimper).

F. rufescenti, Hornsch., proximus, sed habitu robustiore, laminis veris aequalibus, nervo haud excurrente differt.

There are two distinct Mosses named *F. crispans* in Schimper's Herbarium. One is described above as *F. aequalis*; the other, from the Cape of Good Hope, is barren, and I have not been able to identify it. It is evidently closely allied to *F. aequalis*, but has a narrower limb. The Guatemala plant (*F. aequalis*) has 'Hooker 174' on Schimper's label; this is probably a memorandum note, and is not the name of the collector, as Sir Joseph Hooker never visited Guatemala.

F. nitens, Rehmann, Musci austro-africani (1875–77) No. 289 (Figs. 69–74).

Dioicus ?, caespitosus, caespitibus olivaceo-viridibus, nitidis. Caulis simplex, interdum innovans, 4–6 mm. longus, rigidiusculus, curvato-prostratus, ad basin foliosus (rarissime subnudus). Folia 7–12-iuga, erecto-patentia, superne conferta, lingulato-lanceolata, acutiuscula, 1 mm. longa, inferna minora, remotiora, marginibus integris vel minutissime et irregulariter crenulatis hic illic incrassato-limbatis,

limbo bistratoso e cellulis parenchymaticis incrassatis composito, nervo crasso flavo-rufescente subflexuoso infra summum apicem evanescente; laminae verae apice inaequales ad medium folii productae, insertionem versus raptim angustatae; lamina dorsalis ad basin nervi rotundate enata; cellulae parvae, circiter 10 μ diam., rotundato-4–6-angulatae, pellucidae, laeves.

Planta feminea sterili similis, sed foliis 4–6-iugis remotioribus parum maioribus, foliis perichaetialibus caulinis similibus, paraphysibus nullis.

Caetera ignota.

Patria. Natal, Inanda (Rehmann, Musc. austr.-afric. 1875–77, No. 289).

F. Holstii, Broth., Engl. Bot. Jahrb., xx, p. 181 (1895), habitu simillimus, sed foliis nitidis minus acutis, marginibus subintegris plus incrassato-limbatis, nervo flavo-rufescente differt.

F. nitens, although published in Rehmann's Exsicc. (1875–77), does not appear to have been hitherto described. The shining appearance of *F. nitens* is apparently due to the presence of prominent oil guttulae in the leaf-cells. These guttulae, at first sight, look very like papillae or warts, but the cells are quite smooth.

F. nitens, Rehm. var., *neglectus* var. nov. (Figs. 75, 76).

Flavo-virens, flaccidior, haud nitidus. Folia latiora, marginibus ubique incrassato-limbatis; limbus bistratosus, in laminis veris latus, e cellulis prosenchymaticis incrassatis, in laminis aliis e cellulis parenchymaticis incrassatis, compositus; nervo flavo-virente.

Patria. Africa; Lutindi, Usambara (C. Holst, Fl. von Usamb., No. 3472) [inter *F. Holstii*, Broth., et *F. Usambarico*, Broth.].

F. Holstii and *F. nitens* and its variety are closely allied plants. The leaves of *F. Holstii* are described by Brotherus as 'elimbata,' but authentic specimens in the Kew Herbarium (C. Holst, Flora von Usamb., No. 3472) have the apical lamina here and there incrassate-limbate, just as in *F. nitens*, only more sporadically.

F. nitens, var. *neglectus*, is known at once by the broad limb of the vaginant laminae, but in other respects is somewhat intermediate, agreeing with *F. Holstii* in colour and habit, and with *F. nitens* in the subentire leaves with thickened margins. *F. Holstii* is distinct

in the margins of the leaves, being regularly crenate, especially those of the vaginant laminae, and it is only where the sporadic incrassation takes place in the apical lamina that the margin shows any signs of becoming entire; in *F. nitens* the margin is subentire, and the vaginant laminae, as well as the apical, show the sporadic incrassation, the limb of the vaginant laminae being sometimes composed of more or less prosenchymaticous cells; in *F. nitens*, var. *neglectus*, the incrassation is the most pronounced, and forms in the vaginant laminae a broad bistratose limb of prosenchymatous cells.

F. nigro-viridis, sp. nov. (Figs. 77–80).

(*F. geminiflorus*, Dzy. e. Mlkb., var. *nigro-viridis*, Schpr. ms. in Herb. Kew.)

Laxe caespitosus, subrigidus, olivaceo-viridis, infra nigrescens. Caulis erectus, 1½–2½ cm. altus, simplex vel parce ramosus, ad basin foliosus. Folia multiiuga, conferta, superiora plus minusve imbricata, inferiora remotiora, erecto-patentia, rigida, lanceolata, acuta, 1½ mm. longa, saepe paulum curvata, marginibus minutissime crenulatis vel subintegris, nervo validiore in apice dissoluto; laminae verae supra medium productae; lamina dorsalis ad basin folii descendens; cellulae 6–8 μ, rotundato-4–6-angulatae laeves. Caetera ignota.

Patria. Sarawak (O. Beccari, Crittog. di Borneo, No. 52).

F. multifloro, Thw. et Mitt., habitu simillimus, sed foliis longioribus angustioribus, laminis veris haud limbatis differt.

In Schimper's Herbarium this Moss is placed as a variety under *F. geminiflorus*, Dzy. e. Mlkb., but that species differs entirely in habit, and in the broader, distant and more patent leaves, with short laminae verae, usually percurrent nerve, and crenate serrate margins.

F. Nicholsonii, sp. nov. (Figs. 81–91).

Autoicus (rhiz- vel gonioautoicus), corticola, gregarius, laete viridis. Caulis erectus, vel ascendens, simplex, vel innovans 4–8 mm. altus, ad basin foliosus. Folia 4–12-iuga, ·75–1 mm. longa, oblongo-lanceolata, acuta, remota vel apicem versus approximata, subopaca, nervo hyalino flavido percurrente; laminae verae supra medium productae, apice subaequales, limbatae, limbo angusto sinuato-dentato flavido unistratoso e cellulis prosenchymaticis incrassatis composito; lamina

apicalis, et lamina dorsalis, haud limbatae, ob cellulis marginalibus prominentibus plus minus acutis minutissime serrulatae; lamina dorsalis ad caulem attingens; cellulae minutae, circiter 5 μ diam., hexagonae, verrucosae (cellulis laevibus ad faciem laminarum verarum interiorem). Folia perichaetialia caulinis conformia, sed angustiora, longiora. Seta terminalis, vel innovando pseudo-lateralis, tenuis, 6–8 mm. alta. Theca inclinata, plus minus inaequalis, parvula, cellulis externis magnis 25 μ diam., peristomii dentibus intus valde lamellosis, operculo rostellato, calyptra . . ., sporis laevibus 8–12 μ diam.

Flos masculus aut in foliorum caulinorum axillis, aut e radicibus ad caulis basin nascens.

Patria. Greenhouse, Kew Gardens, England; on a Tree Fern stem, brought from Jamaica in 1895 with *Trichomanes scandens* (leg. G. Nicholson, March, 1898).

F. Ravenelii, Sulliv., proximus, sed habitatione arborea, nec terrestri, foliis latioribus, laminae apicalis cellulis marginalibus acutis, nec truncatis, theca inclinata altius pedicellata distinctus.

It is probable that *F. Nicholsonii* is a native of Jamaica, and was brought from there with the *Trichomanes scandens*. *F. Ravenelii* occurs in Carolina and Florida, and is closely allied, but differs in the narrower leaves, the vaginant laminae less distinctly limbate, the more or less truncate marginal cells of the apical and dorsal laminae, and the erect capsule.

F. Ravenelii is described as being dioicous, but authentic specimens in the Kew Herbarium (Society Hill, S. Carolina, leg. Ravenel) are both autoicous and dioicous.

Occasionally small, separate male plants, 4–6 leaved, as described by Sullivant, occur, but more frequently the plants are rhizautoicous, the male flowers being attached to the rhizoids at the base of the female stems. I have also found gonioautoicous plants, with the male flowers in the axils of the lower stem-leaves, just as sometimes occurs in *F. Nicholsonii*.

(Since writing the above, I find that Wilson, according to MS. notes in his herbarium at South Kensington, had noticed as early as 1857 this variability of position of the male flower of *F. Ravenelii*. On one of his specimens is the note, 'decidedly monoicous! ♂ fl. even axillary (W. 3·57);' on another, '♂ fl. terminal on a stem,

or sometimes gemmiform and radical, and at times axillary (W. 3·57).')

Through innovation, the fertile stems of *F. Nicholsonii* often bear from two to four short branches, each of which terminates in a female flower, so that stems bearing a cluster of two to four capsules are found.

F. flavicans, Sch. ms. in Herb. Kew (Guadeloupe (L'Herminier)), is *F. radicans*, Mont. (Ann. Sci. Nat., Sér. 2, xiv, p. 345). Montagne (loc. cit.) and Mitten (Musc. Austr. Amer., p. 581) describe the fruit as terminal. It is, however, both terminal and truly lateral (cladocarpous). In the latter case a very short special branch is terminated by the perichaetium, just beneath which innovations often occur, in the same manner as when the perichaetium is terminal on the main stem. The species is autoicous, and the position of the male flower is variable. It may occur in the axils of the stem-leaves, just below the female flower, or it may terminate a short lateral branch, similar to that of the lateral female flowers; it sometimes occurs, at the base, on the lateral female branches. *F. radicans* has been recorded hitherto only from French Guiana and Brazil. Schimper's specimens have a slightly different facies from those from the above places, due to the plants being old, and the innovations consequently very numerous.

F. involvens, Sch. ms. in Herb. Kew (N. Hollandia), agrees well with small crisped forms of *F. glaucescens*, Hornsch. (Linnaea, 1841, p. 154), from Africa.

F. circinnans, Sch. ms. in Herb. Kew (non *F. circinans* (Sch.), C. Müll., Bot. Zeit., 1864, p. 340), from Java, is *F. Zippelianus*, Dzy. e. Mlkb., Bryol. Javan., 1, p. 2, t. 2.

F. macrostachys, Hpe. ined. (ad rup. hum. Austral., leg. Dr. F. Müller), in Schimper's Herbarium is *F. rigidulus*, Hook. f. and Wils.

F. angustifolius, Sch. ms. in Herb. Kew (non Sulliv., Proceed. Am. Acad., p. 275, 1861) (abundant on moist rocks in ravines on the Pouce, Mauritius (Ayres, July, 1862)), is *F. ovatus*, Brid., var. *planifolius*, Besch., hitherto recorded only from the island of Nossi Bé.

F. stolonifer, Rehm. Musc. austr.-afric. 1875–77, Nos. 294, 585; Kindb. Enum. (nomen nudum); from Cape Town is *F. bifrons* (Sch.), C. Müll., Bot. Zeit., 1859, p. 198.

F. gracilis, Sch. ms. in Herb. Kew (Trinidad (Krüger)), is *F. Kegelianus*, C. Müll., Syn. 1, p. 49.

F. Eckloni, Sch. ms. in Herb. Kew (Cape (Ecklon)), is *F. Lindigii* (Hpe.), Paris, recorded hitherto only from Nova Granata.

F. subcrispus, Besch., Not. Mouss. Parag., p. 260 (Mém. Soc. Sci. Nat. Cherb., t. xxi, 1877).

According to a note on a specimen of the above (Balansa, Pl. Parag., 1874–77, No. 1202) in the Kew Herbarium, Mitten considers this species to be the same as his *F. anguste-limbatus*. The leaves of these two Mosses, if examined before completely revived, look very similar in the opaque areolation and very narrow limb, but when the cells become fully expanded it is seen that those of *F. anguste-limbatus* are about twice the size of those of *F. subcrispus* (Figs. 93, 94), and there can be no doubt that the two species are distinct. The leaves of the former are also somewhat rounded at apex, and so blunter than in the latter. There is also a difference in inflorescence.

F. Balansaeanus, Besch., var. *limosus*, Besch., Not. Mouss. Parag., p. 261 (Mém. Soc. Sci. Nat. Cherb., t. xxi, 1877), was founded on No. 1245, Balansa, Pl. Parag., 1874–77. Mitten, on the specimen of this in the Kew Herbarium, refers the plant to *F. Kegelianus*, C. Müll., and it agrees well with that species, or rather the 'var. β' of it, described by Müller, Syn. 1, p. 49, which differs from the type in the less cuspidate leaves, and setae often in pairs. *F. Balansaeanus* appears distinct in the synoicous inflorescence and denticulate leaf-apex.

F. mollis, Mitt. (Musc. Austr.-Amer., p. 600), is not distinct from *F. macrophyllus*, Mitt. (loc. cit.). The specific descriptions of the two plants contain no separating characters, but in the key (loc. cit., p. 583) the species are thus arranged: *F. macrophyllus* 'folia elongata subspathulato-lanceolata'; *F. mollis*, 'folia lineari-elliptica.' In the authentic specimens at Kew, however, the leaves are identical in shape. It seems, moreover, that Mitten now unites these species, as on the type of *F. mollis* at Kew that author has written '*F. macrophyllus*.'

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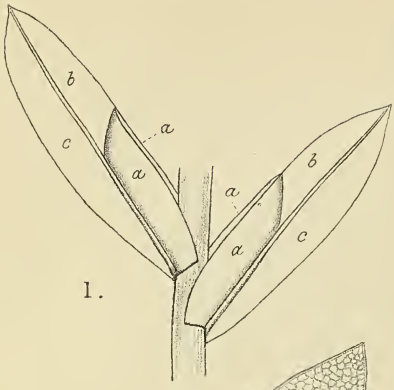
EXPLANATION OF FIGURES IN PLATES V–VII.

Illustrating Mr. Salmon's paper on *Fissidens*.

Fig. 1. *Fissidens bryoides*, part of stem showing insertion of two leaves; *a*, vaginant lamina; *b*, superior lamina; *c*, inferior lamina. × 25.
2. *F. taxifolius*, transverse section in lower half of leaf; *v. l.* vaginant laminae; *i. l.* inferior lamina. × 400.
3. *F. incurvus*, transverse section in lower half of leaf; *v. l.* vaginant laminae; *i. l.* inferior lamina. × 400.
4. *F. incurvus*, a leaf with one vaginant lamina ending in the nerve. × 25.
5–11. *F. fasciculatus*, transverse sections of leaf; *v. l.* vaginant laminae; *i. l.* inferior lamina; *s. l.* superior lamina. × 255.
12. *F. grandifrons*, transverse section in lower half of leaf. × 400.
13. *F. dealbatus*, part of stem with one leaf attached. × 25.
14. *F. dealbatus*, transverse section of leaf near the base. × 68.
15. *F. dealbatus*, transverse section of leaf at two-thirds the length of the vag. lam. × 400.
16. *F. usambaricus*, transverse section near base of leaf. × 255.
17. *F.* (*Conomitrium*) *capense*, transverse section near base of leaf. × 400.
18. *Polytrichum formosum*, transverse section of leaf. × 255
19. *Catharinea angustata*, transverse section of leaf. × 255.
20. *Pottia cavifolia*, transverse section of leaf. × 400.
21. *Barbula chloronotis*, transverse section of leaf. × 255.
22. *Tortula ambigua*, transverse section of leaf. × 400.
23. *Schistochila sp.*, leaf. × 25.
24. *S. alata*, transverse section of leaf in lower half. × 25.
25. *S. alata*, transverse section of another leaf. × 25.
26. *S. lamellata*, transverse section of leaf. × 25.
27. *Diplophyllum albicans*, leaf. × 25.
28. *Fissidens bifrons*, part of a sterile shoot. × 52.
29. *Fissidens bifrons*, leaf. × 150.
30. *F. floridanus*, leaf. × 25.
31. *F. floridanus*, areolation of a leaf at point *x* in Fig. 30. × 400.
32–35. *F. anomalus*, perichaetial leaves, in order from the outside. × 25.
36. *F. decipiens*, a perichaetial leaf. × 25.
37. *F. rivularis*, a perichaetial leaf. × 25.
38. *F. polyphyllus*, innermost perigonial leaf. × 25.
39. *F. decipiens*, perigonial leaf. × 52.
40. *F. serrulatus*, young leaf. × 25.
41. *Bryoxiphium norvegicum*, part of barren stem, with one leaf attached. × 25.
42. *Bryoxiphium norvegicum*, leaf from upper part of fertile stem. × 25.

Fig. 43. *Bryoxiphium norvegicum*, leaf from upper part of fertile stem, transverse section near apex. × 400.
44. *Bryoxiphium norvegicum*, transverse section near base of leaf. × 430.
45. *Bryoxiphium norvegicum*, transverse section higher up. × 400.
46. *Sorapilla Sprucei*, part of stem with one leaf attached. × 25.
47. *Sorapilla Sprucei*, perichaetial leaf. × 25.
48–52. *Sorapilla Sprucei*, transverse sections of leaf. × 400.
53. *F. taxifolius*, transverse section of the growing-point of the stem, showing the two-sided apical cell; segments numbered in genetic order. × 255.
54. *Mnium undulatum*, transverse section of the growing-point of the stem, with three-sided apical cell. × 255.
55. *Bryoxiphium norvegicum*, transverse section of a bud, just above the growing-point. × 255.
56. *F. taxifolius*, transverse section of a bud, just above the growing-point. × 255.
57. *B. norvegicum*, transverse section of young stem. × 255.
58. *F. taxifolius*, transverse section of young stem. × 255.
59–68. *F. aequalis.*
59. *a.* female plant, *b.* male plant, natural size.
60. Female plant. × 3.
61. Female plant, apex of stem with capsule. × 12.
62. Capsule. × 25.
63. Two peristome-teeth. × 150.
64. Leaf. × 64.
65. Apex of leaf. × 150.
66. Areolation of leaf. × 400.
67. Apex of stem of male plant. × 12.
68. Perigonial leaf and antheridia. × 52.
69–74. *F. nitens.*
69. *a.* sterile plant, *b.* female plant, natural size.
70. Sterile plant. × 12.
71. Female plant. × 12.
72. Leaf. × 25.
73. Areolation of leaf. × 400.
74. Transverse section of upper part of leaf. × 400.
75. *F. nitens*, var. *neglectus*, leaf. × 25.
76. *F. nitens*, var. *neglectus*, transverse section of margin of vaginant laminae. × 400.
77–80. *F. nigro-viridis.*
77. Plant, natural size.
78. Apex of stem. × 12.
79. Leaf. × 25.
80. Areolation of leaf. × 400.
81–91. *F. Nicholsonii.*
81. Fertile plant, natural size.
82. Stem of fertile plant. × 12.
83. Leaf. × 25.
84. Margin of vaginant laminae. × 255.

Fig. 85. Margin of superior and inferior laminae. × 400.
86. Capsule. × 68.
87. Transverse section of margin of vaginant laminae. × 400.
88. Base of fertile stem, showing the rhizautoicous inflorescence. × 25.
89. Male flower of same. × 52.
90. Leaf, with axillary male flower. × 25.
91. The same male flower. × 52.
92. *F. Ravenelii*, margin of superior and inferior laminae. × 400.
93. *F. anguste-limbatus*, areolation of leaf. × 400.
94. *F. subcrispus*, areolation of leaf. × 400.



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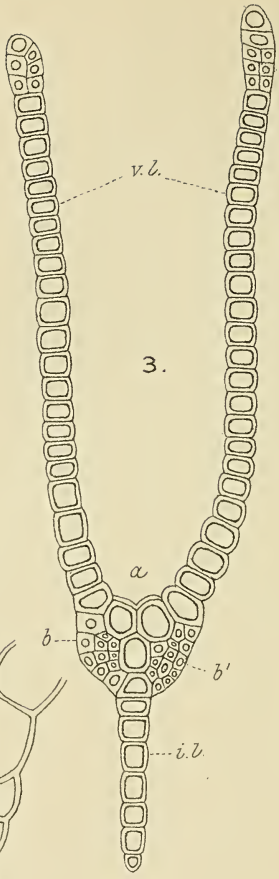

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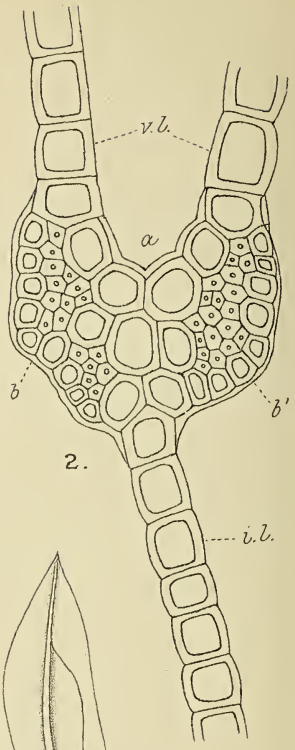

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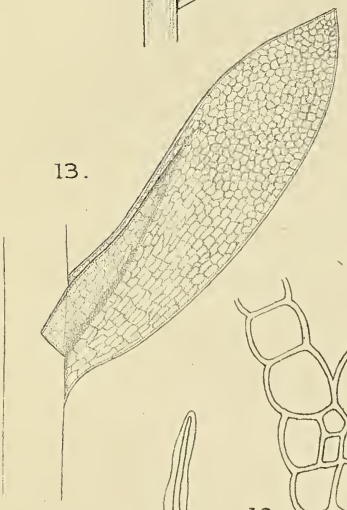

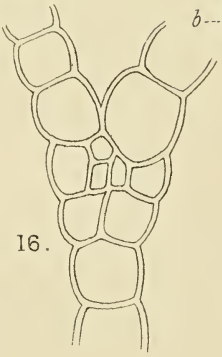

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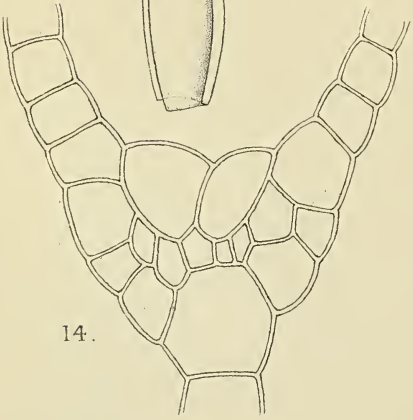

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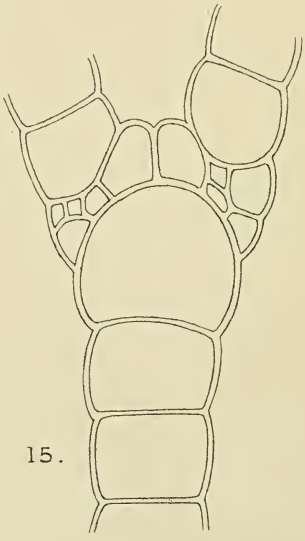

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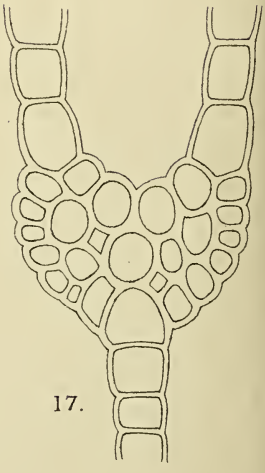

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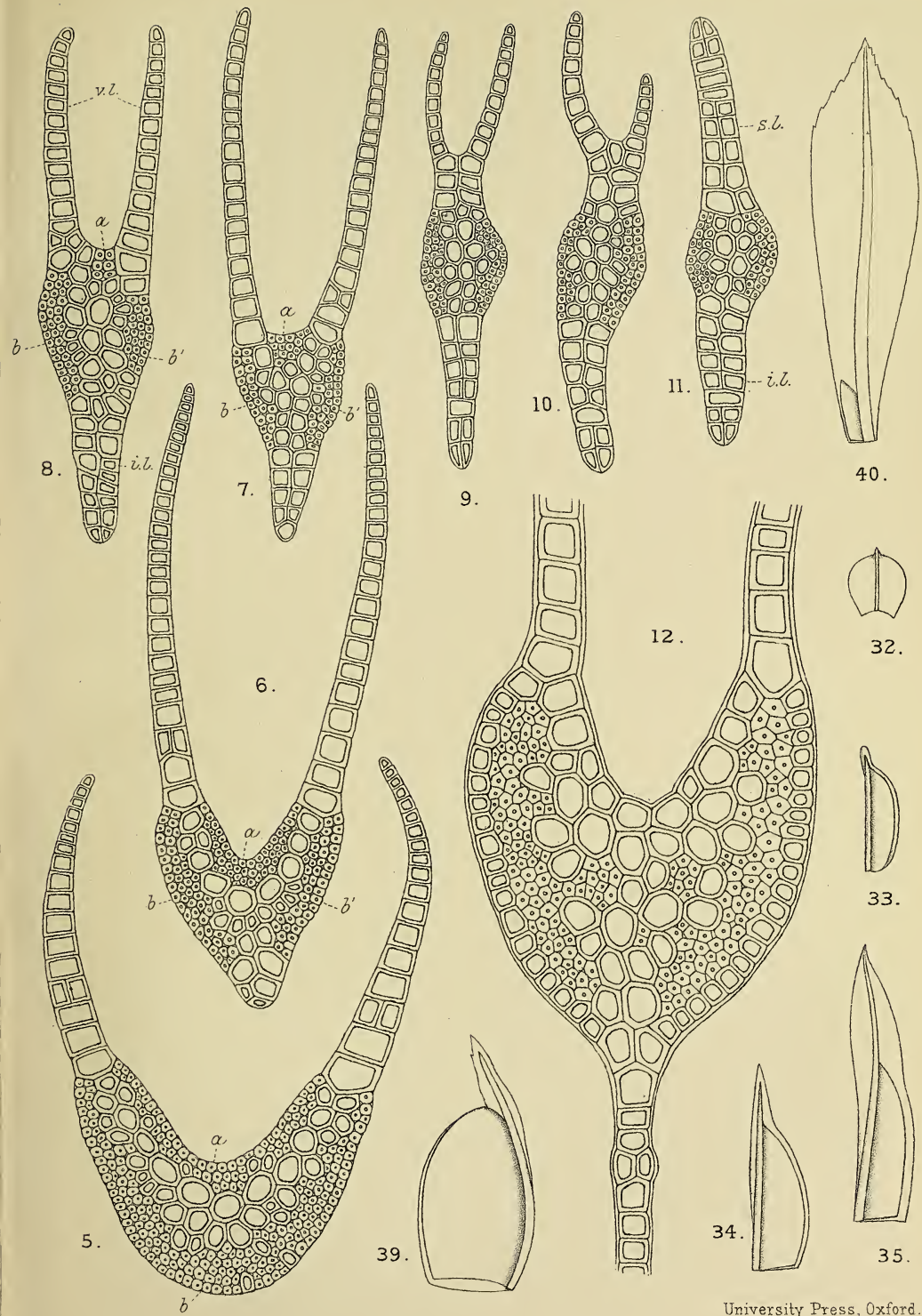

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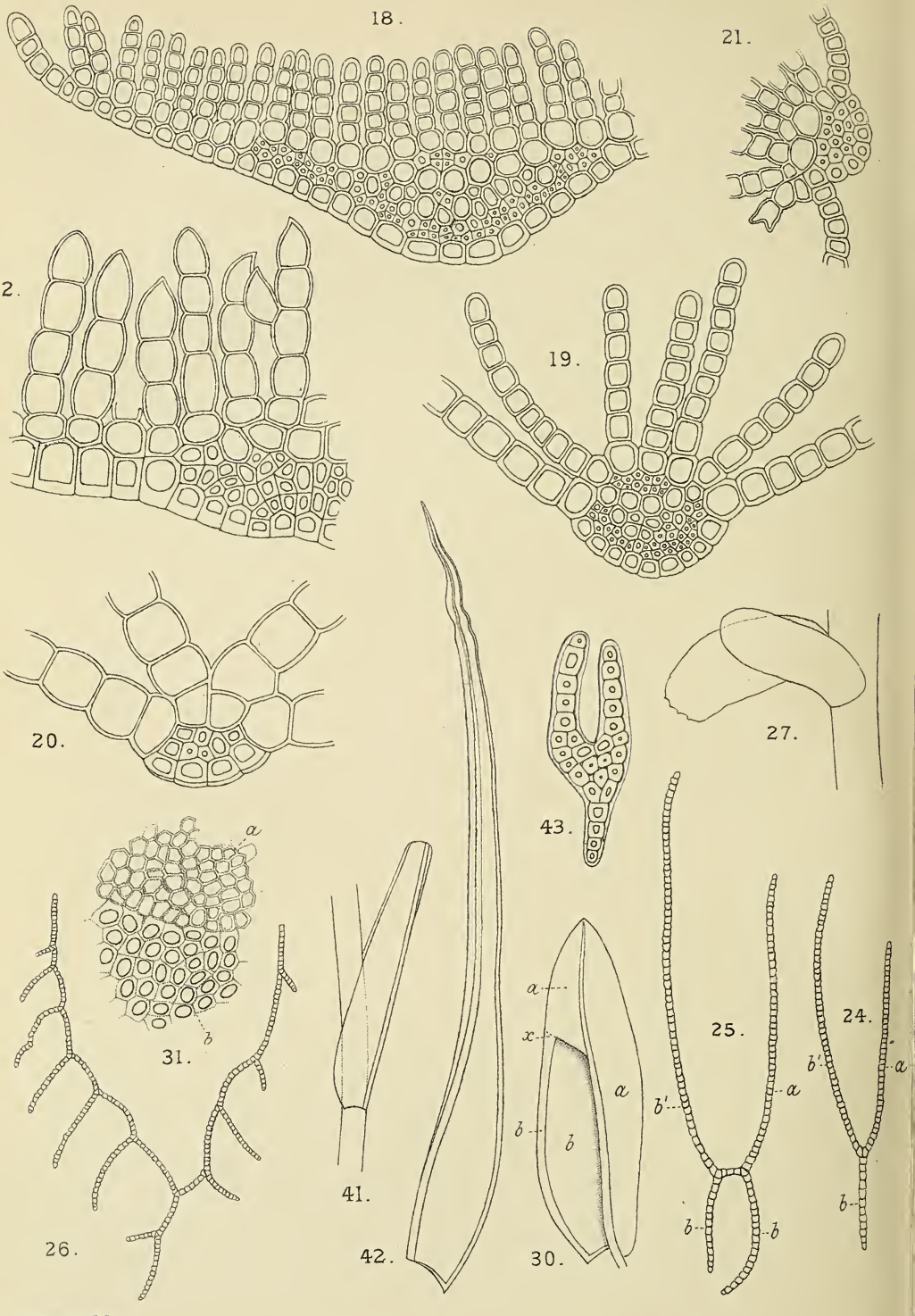

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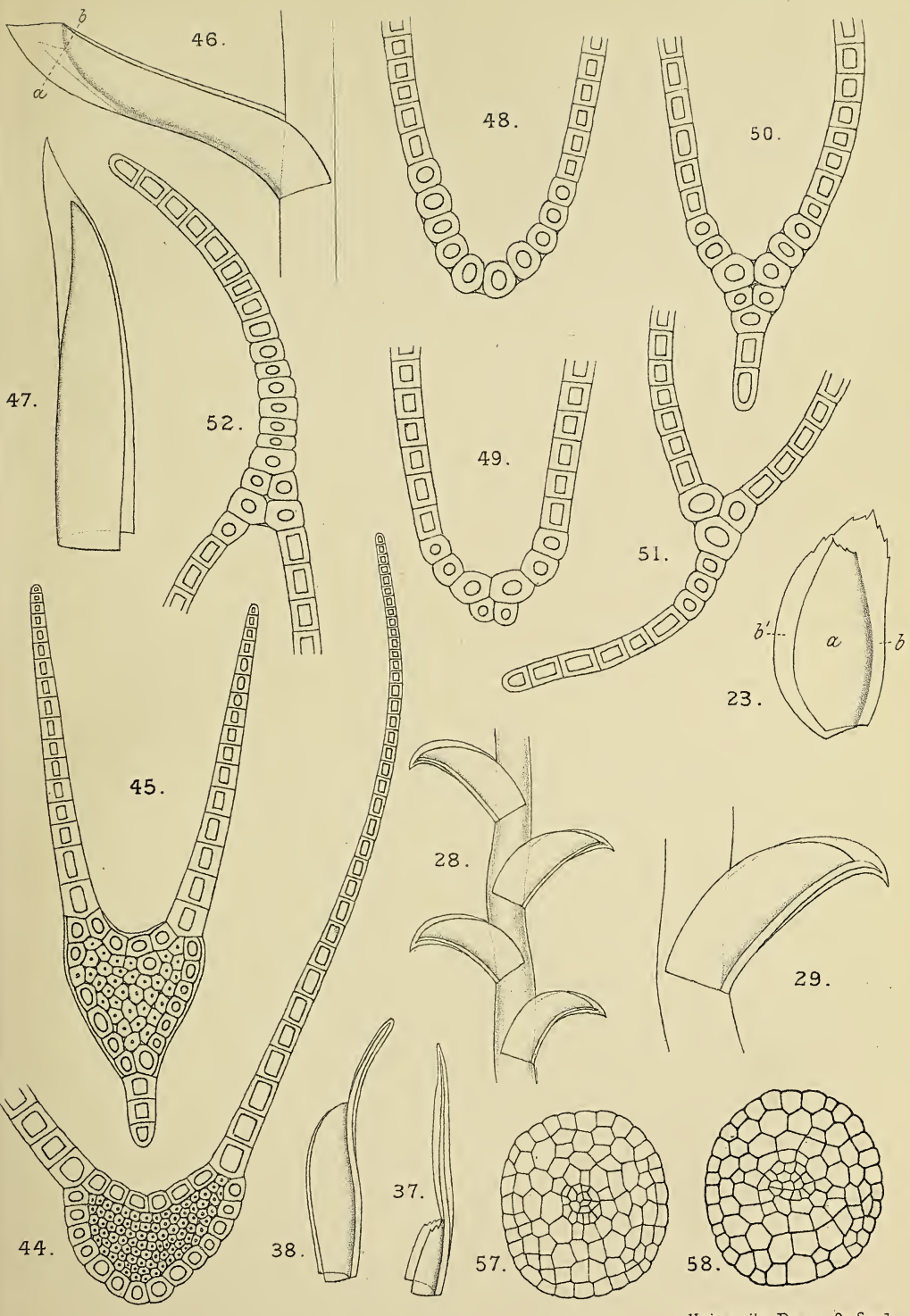

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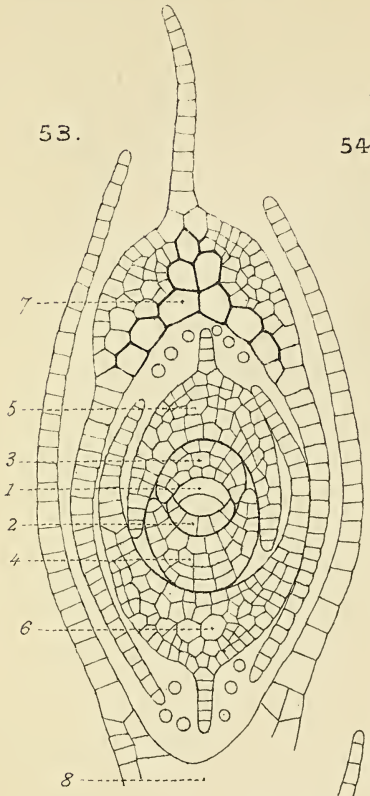

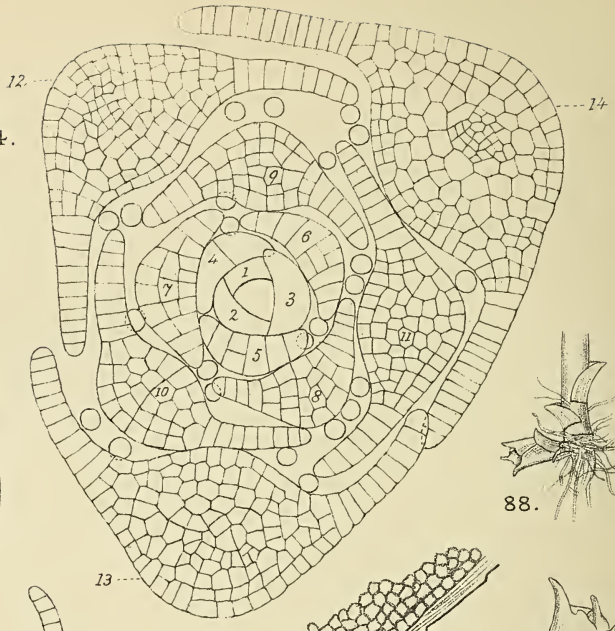

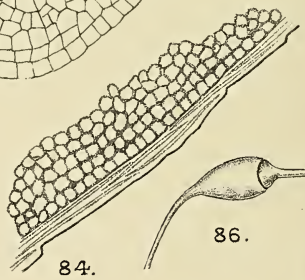

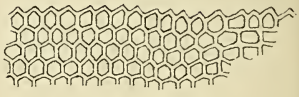

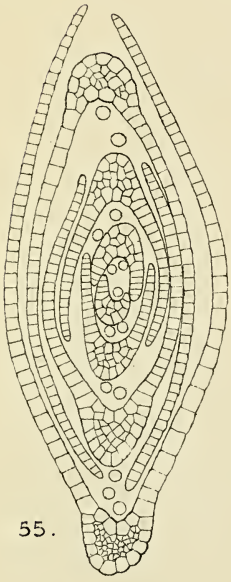

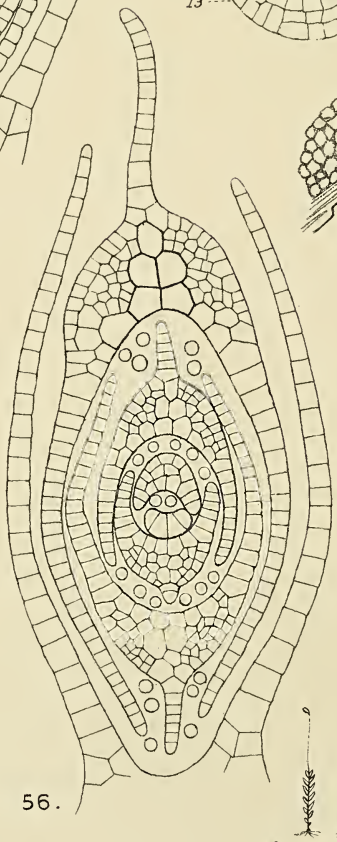

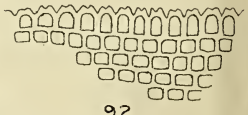

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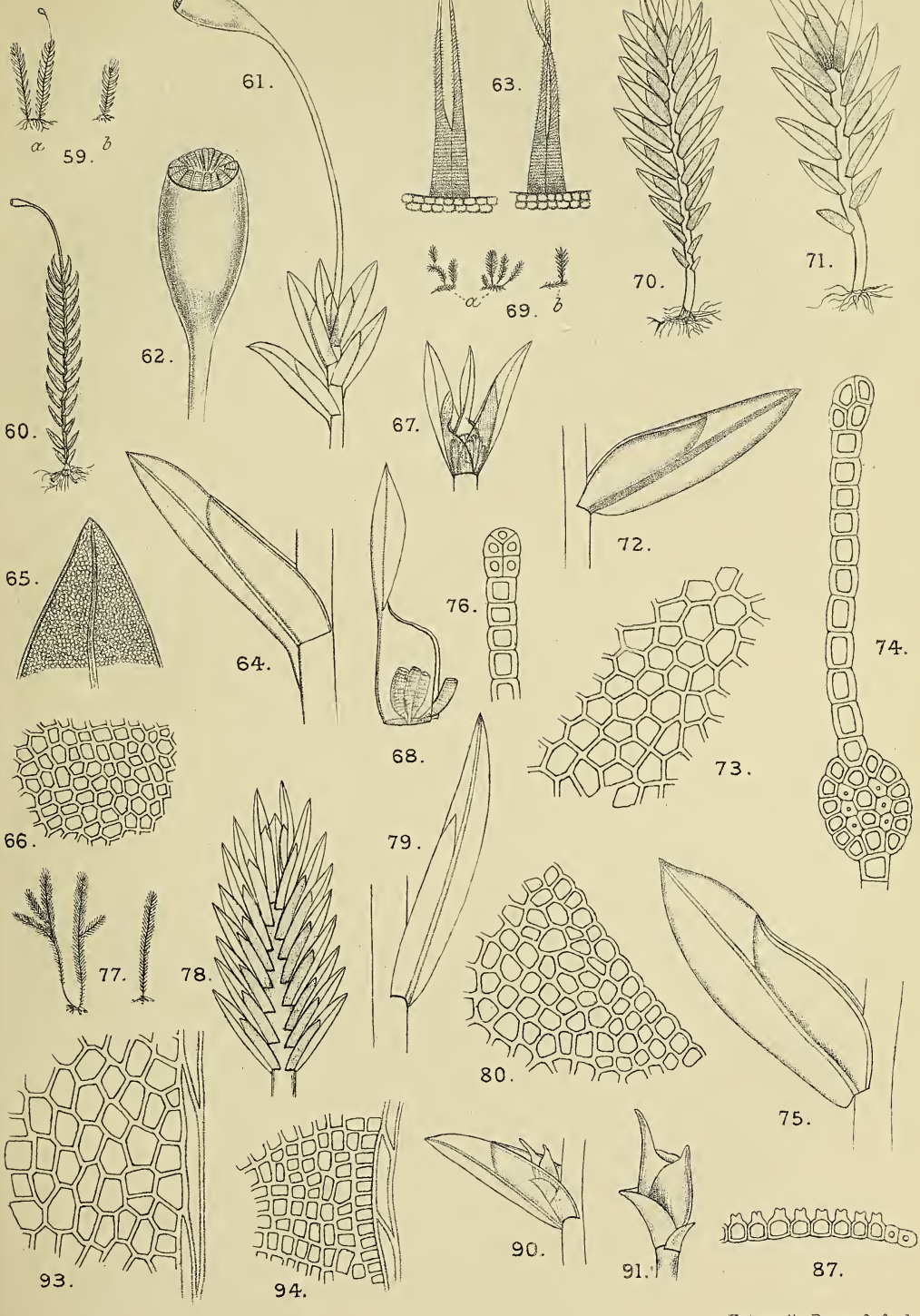

University Press, Oxford.

Observations on the Biology and Cytology of a new variety of Achlya americana.

BY

A. H. TROW.

With Plates VIII–X.

HARTOG'S ('95) paper 'On the Cytology of the Vegetative Organs of the Saprolegnieae,' and my observations on 'The Karyology of *Saprolegnia*,' ('95), appeared almost simultaneously. Hartog ('96) shortly afterwards published a brief criticism of my results, in which he expressed the view that all the species of the Saprolegnieae, or at any rate those examined by me, must have four chromosomes, and so conform to the case or cases investigated by himself, a view which one would hardly expect from an observer of such wide and varied knowledge and keen critical faculty; and it is even more remarkable to find that Zimmermann ('96), who, however, had not seen Hartog's paper, appears inclined to adopt it. One would imagine that the well-known case of *Ascaris megalocephala*, in which there are two varieties distinguishable only by the difference in the number of the chromosomes, would in itself be sufficient to indicate the fundamental weakness of such a criticism. The evidence furnished by the Liliaceae shows clearly enough that the number of chromosomes may vary not only from species to species, but even in the cells of the same individual.

[Annals of Botany, Vol. XIII. No. XLIX. March, 1899.]

A careful study of Prof. Hartog's work, the detailed criticism of which may be deferred for the present, convinced me that it would be necessary to examine a species of *Achlya*, and so acquaint myself at any rate with the two genera specially studied by this observer, before attempting to explain the discrepancies in our results. The genera *Achlya* and *Saprolegnia* agree with one another in nearly all their morphological features, with the exception of the order of formation, and the mode of dehiscence, of the sporangia. The genera can scarcely be distinguished, indeed, by reference to the mycelium and sexual organs alone. There are interesting differences, however, in the cytology of species of *Saprolegnia*, and it was not improbable that these would be accentuated in the genus *Achlya* and serve to explain the differences in the results obtained by the different observers—Hartog, Humphrey, Dangeard, and myself.

Other reasons than those furnished by Hartog's paper, and the discrepancies which have just been referred to, urged me to undertake a cytological investigation of a species of *Achlya*. Although the matter had escaped criticism, it was clearly desirable to follow the germination of the oospores and the nuclear divisions in the gametangia *in one and the same species* of Saprolegnieae, and even more so to determine accurately the nature of the chromosome-like body in the centre of the nucleus. Zimmermann's view as expressed in the following sentence was clearly untenable:—'Es dürfte aber wohl kaum zweifelhaft sein, dass der von Trow als Chromosom gedeutete Körper in Wirklichkeit als Nucleolus zu bezeichnen ist.' It was advisable, moreover, to put my surmise as to the meaning of the binucleate and uninucleate oospores of *Achlya americana* to the test of actual observation so far as possible.

Such a task could not be lightly entered upon—it was necessary to find a suitable species; to grow it for generations separated from other forms so that certainty might be attained as to its systematic position; to follow its life-history and every stage in the course of its development by direct

continuous observation, in part under the microscope; to find methods of fixing, imbedding, and staining, so as to secure well-stained serial sections of the organs which were to be submitted to detailed critical study; and finally to bring to bear upon the microscopic examination of the preparations the finest optical apparatus and the best technical skill available.

Prof. Hartog, no mean authority, states concerning one of the stages:—'the study of the nuclei and their behaviour at this period becomes enormously difficult; and it is only possible under the best possible conditions of preservation, staining, and microscopic apparatus.' My experience leads me to endorse this view, and it may be remembered that Dangeard completely failed to find the nuclei in certain stages.

It seemed clear, too, that work done on mixtures of species, and especially mixtures of genera, would be certain to lead to serious error. Accordingly it was decided to work with a single species of *Achlya* and to keep four definite points in view throughout; viz.: (1) the structure of the nucleus and especially its behaviour during karyokinesis, (2) the fate of the supernumerary nuclei in the oogonia during the development of the oospores, (3) the actual method by which fertilization is effected, and (4) the behaviour of the nucleus during the germination of the oospores.

A beginning was made in the summer of 1897 by Miss Dawson, who collected in the neighbourhood of Cardiff, and cultivated for a generation or two, a species of *Achlya*, which formed numerous sexual organs and was apparently very suitable for detailed cytological study. She adopted the methods of preparation which I had found most suitable in the study of *Saprolegnia*, but failed in the short time at her disposal—two months—to secure any satisfactory results in cytology.

In the autumn of the same year I commenced to work at this material myself. Fresh cultures were made in August, and upon these a preliminary study of the fresh material was instituted. Finally, about a dozen different methods

of fixing were tried, and the material imbedded in the manner found suitable in the case of *Saprolegnia*. Three months of hard work produced no more than one fact of any interest—that the young oospores contain two exceptionally large nuclei. The condition of the material was such as to render a detailed critical study impossible. The difficulty appeared to be entirely in passing the fixed material from weak alcohol into paraffin.

The size of the nucleus induced me to endeavour to overcome these special difficulties, and in June, 1898, they were successfully surmounted, and the record of the subsequent study, which almost completely occupied my time during the following four months, forms the main subject of this communication. It may be stated here that in order to avoid collapse and consequent distortion of the very sensitive organs of this plant, the greatest possible precautions must be exercised. One week was generally devoted to the fixing, washing, and dehydrating processes, and another to the gradual transference from absolute alcohol to paraffin. I was fortunate in having the skilled assistance of an attendant in carrying out these laborious processes. The amount of work is roughly represented by the fact that thirty-six pure cultures were successfully imbedded during this time.

It was only when the work was approaching completion, and after many generations of cultures had passed through my hands, that at last I was in a position to determine accurately the systematic position of the form chosen for study. In all the cultures two kinds of oogonia are to be found, the relative proportions of which, however, vary between wide limits. The one type has smooth pitted walls; the other is provided with protuberances or spines, due to outgrowths of the wall through the pits. These protuberances vary much in size—from those which are scarcely visible to others as long as the diameter of the oospores. For a long time I could not convince myself that I had not before me mixed cultures containing at least two species, due to some flaw in the method adopted for separation. For ten or more

times was the endeavour made to get rid of the spiny form, and in each case control-cultures were set up. The number of spiny oogonia certainly seemed to decrease, but they did not disappear. It was a source of much gratification later to discover oogonia of both kinds on one and the same filament grown under microscopic control in a moist chamber. It is possible that the decrease in number in the later generations was due to the process of selection I adopted. The infecting material used in these experiments was a single hypha bearing smooth oogonia.

The result of a long detailed study of the systematic position of the form under consideration leads me to conclude that it comes very close to *Achlya americana*, a species first recognized and described by the late Dr. Humphrey ('92) in his monograph on the Saprolegniaceae of the United States. It presents, however, several important points of difference, which it is not likely that Humphrey could overlook. In any case it needs accurate definition, especially in view of the controversies which seem to be inevitably associated with work on this group of plants. I propose to rank it provisionally as a distinct variety under the name *Achlya americana* (Humphrey), var. *cambrica*. The specific and varietal names will thus serve to call attention to one important feature in connexion with the plants—their geographical distribution; and the detailed description will not only serve to prevent misconceptions as to the nature of my raw material, but it will enable those botanists who are familiar with Humphrey's plant to relegate the forms to their true systematic positions.

The following is a detailed description of this variety:—

ACHLYA AMERICANA (HUMPHREY), VAR. CAMBRICA, VAR. NOV.

Mycelium, as developed on house-flies, with main hyphae about 1 cm. long, a maximum diameter at the base of 92 μ, and tapering gradually towards the apices, where they rather suddenly narrow to fairly sharp points.

Sporangia, terminal, cylindrical, scarcely thicker than the supporting hyphae, of a length generally varying from 250 μ to 368 μ, and of a breadth varying from 39 μ to 72 μ (average of six measurements—length=315 μ, breadth=56 μ); sometimes very small, producing in extreme cases no more than three or four zoospores; generally developed in the typical cymose order, *the main axis, however, frequently septate behind the oldest sporangium, each segment thus formed, of which there may be as many as ten, developing a short branch, the segment and branch together constituting a sporangium.*

Spores very numerous, averaging 13 μ in diameter, generally encysting at the mouth of the sporangium, occasionally, however, inside it.

Oogonia, on short unbranched stalks, which are about as long as the diameter of the oogonium, and of an average breadth of 10 μ; at first developed regularly in racemose order on the main hyphae; generally terminal and spherical, but not infrequently intercalary and barrel-shaped; of a diameter 31 μ to 85 μ (average of twelve measurements=60 μ), with a thick, pitted, *yellowish* smooth wall; *frequently, however, provided with blunt spines, which may exceptionally even reach the length of* 25 μ, *and are due to outgrowths through the pits.*

Antheridia, always present under natural conditions, *few in number*, produced on branched antheridial filaments of a diameter of 6·5 μ, which arise from the main hyphae side by side with the oogonial branches, or, as observed in a very few cases only, from the stalk of the oogonium; of very variable shape, but generally long and curved, and closely applied throughout to the surface of the oogonium, or opposite the pits only by means of outgrowths from the under surface; sometimes septate; of a maximum length of 65 μ and maximum breadth of 7 μ; emitting from points in contact with the pits one or more branched or unbranched fertilization-tubes of a diameter of 4 μ.

Oospores, one to twenty or more, mostly from three to eight (average of twelve cases=6); spherical, with a *smooth, very thick two-layered wall*; eccentric, of a *diameter of* 23 μ *to* 26 μ, and having an oil-globule of a diameter of 15 μ; germinating at once, and producing a long, thin, branched hypha, or one or more small sporangia, or passing into a resting condition and remaining capable of germination for at least four months.

Achlya americana may be regarded, as Humphrey points out, as the middle member of a group of three species—the *prolifera* group—all of which have eccentric oospores and a number of other characters in common. The members of the group are very easy to discriminate, for *Achlya prolifera* is a diclinous species with pitted oogonia, and *A. De Baryana* (Humphrey) = *A. polyandra* (De Bary) is an androgynous species whose oogonia are destitute of pits. The affinities are roughly expressed by regarding *A. americana* either as a pitted *A. De Baryana* or an androgynous *A. prolifera.*

The specific type as described and figured by Humphrey has obviously rather indistinct pits; the form I am familiar with has pits almost, if not quite, as well-defined as those of the *Ferax*-group of species of *Saprolegnia.* The American plants, too, apparently differ from those which I have examined in the greater number of antheridial branches and antheridia supplied to each oogonium, the colour of the wall of the oogonium, the structure of the oospore wall, and the size of the oospores. Other noteworthy points of difference have been indicated by italics in the description of the variety.

Having maintained this variety, in a series of pure cultures, under regular observation for a period of one and a half years, I have become intimately acquainted with its life-history. The whole course of development has been followed macroscopically dozens of times, and microscopically on several occasions. When the cytological work had been nearly completed, it appeared advisable, and even necessary, to follow the development, step by step, with great care, so as to be able to correlate as rigidly as possible the observations made on stained sections with those made on the living material. In consequence of these studies, notwithstanding the attention paid to the genus by previous observers, and especially by De Bary, I am able to make a number of additions to our knowledge of its biology.

Observations, Chiefly Biological, made on Living Material.

The normal course of development, from the germination of the zoospores to the maturation of the oospores, is completed in from ten to fifteen days.

If house-flies are thrown into a glass jar of one litre capacity, which has been nearly filled with water containing numerous zoospores, the following order of events can be easily followed with the naked eye. The mycelium may be detected as a fine halo around the flies at the end of the first period of twenty-four hours; at the end of the second day the mycelium is well developed; at the end of the third day it is full grown, and the first sporangia have made their appearance at the ends of the stoutest hyphae; at the end of the fourth day the oogonia are to be seen in various stages of development as lateral outgrowths of the main hyphae. The culture shows no further perceptible growth, but the number of oogonia increases from day to day, the majority, however, being present at the end of the fifth day. Fig. 1 illustrates its condition at this time, and the habit of growth as well. Microscopic examination reveals the fact that oogonia with fertilized eggs are abundant at the end of the fifth day, and that the first crop of ripe oospores appears on the tenth. A culture fifteen days old is made up of an exhausted mycelium and an abundant crop of oogonia with ripe oospores. Under favourable conditions the oospores may germinate at once. In most of the cultures at the end of three months a few ripe oospores still remain associated with the empty shells of those which have germinated and produced zoospores.

Such simple observations on the course of development are extremely important, as they do away, in most cases, with the necessity for a microscopic examination of the plants which it is proposed to fix. In order to obtain excellent material for the cytological study of the gametangia and

young oospores, it suffices to take a five-day culture and plunge it instantly into the fixing fluid.

So many changes take place during the development of the gametangia and gametes, and the maturation and germination of the oospores, that they need to be followed closely by direct observation of the living material under the microscope. The different stages are easily recognized, and their true sequence may be determined to a very great extent by observation and comparison. The determination of the actual sequence, however, and especially of the time involved during the complicated procession of events which lead up to the formation of oospheres, necessitated a resort to the method of continuous observation. A considerable amount of time was spent on this study, but the record of two observations will suffice to put us in possession of the fundamental facts.

Development of the gametangia and oospheres. A moist chamber culture was examined at 9 a.m. on October 7, and a hypha bearing three short branches selected for observation. These branches remained unchanged until noon, when they began to swell at their apices. Fig. 2 represents their condition at 12.30. The absence of a vacuole should be noted. The young oogonium marked *b* was selected for further study. At 2 p.m. the oogonium was nearly full grown; at 3.20 p.m. the antheridial branch began to develop, its place of origin being recognizable as early as 2.0 p.m. The antheridial branch came in contact with the base of the oogonium at 3.45 p.m. The antheridium was cut off by the appearance of a transverse wall at 6.10 p.m., and the basal wall of the oogonium appeared at 7.25 p.m. It appears to be the rule for the oogonium to be segmented off after the antheridium. The basal wall makes its appearance very suddenly as a thick membrane, separated by a narrow clear space filled with hyaloplasm from the dense granular peripheral protoplasm of the oogonium. The course of development is represented in Figs. 3–7. From 2 p.m. to 7 p.m. the vacuole in the oogonium suffered many changes. At first it was small,

eccentric, and communicated with a long tube-like vacuole in its stalk. It soon took up a central position, increased in size, and was apparently cut off from its continuation in the stalk. In all probability, though the axile vacuole of the stalk disappeared, the limiting layer of protoplasm persisted, as the narrow slit-like extension of the vacuole in the direction of the stalk suggests in Fig. 4. This slit appeared and disappeared several times, and the impression created was as if two bands of protoplasm were very slowly creeping up the stalk into the oogonium. Light patches were to be noted in the dense parietal layer of protoplasm of the oogonium, and these were possibly due to the presence of vacuoles. Although the hyphae finally almost completely emptied themselves into the gametangia, the peripheral mass of protoplasm did not increase as greatly either in quantity or density as might be expected. No doubt active metabolic changes were going on which led to alterations in the mass relations of the substances concerned. When the basal wall was produced, the vacuole was relatively small.

This oogonium was kept under continuous observation, being examined at intervals of ten minutes for the next seven hours without any very striking change becoming apparent. During this period, however, it was noted that the oil-globules, which at first were very small and numerous, became much larger, the cell-wall became thicker, the pits making their first appearance at 8.35 p.m., while the vacuole suffered many changes of form and gradually increased in size at the expense of the peripheral layer of protoplasm, whose density, however, did not very perceptibly increase. Irregular heapings of the peripheral layer were noted, once at the apex of the oogonium, another time on the side. The clear spaces in the parietal layer of protoplasm were seen to persist throughout the whole of this time. They were lost to sight at times, or perhaps actually disappeared. In one or two cases they appeared to fuse; in one very clear instance three of them came together in a row, joined up to form one, and the whole then disappeared. Some very curious vacuoles were seen, each

containing a solitary globule and surrounded by a single ring of similar globules. These were indistinguishable from the numerous oil-globules present, and attracted attention solely by their curious arrangement. Fig. 8 represents the state of the gametangia in the middle of this period. Finally, at 2.45 a.m. on 8/10/98, the protoplasm commenced to heap itself up at four points to form four oospheres (Fig. 9). At 3.15 a.m. these heaps were very obvious. The large oil-globules were at this time a very striking feature. They are more conspicuous in this species of *Achlya* than in any other member of the Saprolegnieae with which I am acquainted.

In the antheridium the most obvious structures were a few large granules. In addition were clear patches of protoplasm free from granules, which might possibly represent the nuclei, as they differed in refractive power from the surrounding protoplasm and were of the proper size.

Perhaps the most remarkable feature in the course of development so far outlined is the absence of the peculiar and striking vacuolated stage seen in species of *Saprolegnia*. In *Saprolegnia* the vacuoles are very large and obvious, and occur only for a relatively short period during the development of the oogonia. In *Achlya* the vacuoles are not conspicuous, and they appear to be present at all stages in the development of the oogonia up to that which De Bary described as *balling*. The observations having already been continued for a period of nineteen hours, they were of necessity interrupted. At 10 a.m. on the same day they were resumed, and a note made to the effect that the oogonium was provided with eggs and the fertilization-tubes had already reached them.

A second series of observations, completed before the one just described, enables me to trace the whole course of development in a single oogonium from the first stage in the formation of oospheres up to and including the germination of the oospores. At 10.45 a.m. on 21/9/98 the oogonium represented in Fig. 10 was brought under

observation. The commencement of oosphere-formation was noted at 12.40 p.m., two hours later, and continuous observations were made up to 4.45 p.m., when the fertilization-tubes had been lost to sight for some time behind the eggs—now presumably fertilized. Figs. 10 to 18 illustrate fully how the eggs are produced and the time occupied by each stage in development. It may be noted how the protoplasm heaps itself around certain centres, causing a thinning out over the intervening areas; how the oil-globules flow into the heaps thus produced, and project from their surfaces, leaving behind them a clear thin film of protoplasm; how when this film is ruptured the separate masses of protoplasm swell up; how they contract again, rounding themselves off until they appear an hour and a half after the first indication of their formation as perfectly spherical bodies densely filled with oil-globules and having a smooth external layer of hyaloplasm; and finally, how the fertilization-tubes grow out and place themselves in contact with the naked oospheres.

Neither in this case nor in that of many others examined have I been able to trace the entry of the fertilization-tube into the egg; it appears to grow past it, and on the side furthest removed from observation, possibly influenced thereto by the illumination from below. Protoplasm containing fine granules may be observed to pass slowly along the fertilization-tube, but I have never detected any rapid movement such as some observers insist must take place during fertilization.

Maturation of the oospores. The oospheres, after contact with the fertilization-tubes, surround themselves with a thin cell-wall, which gradually gets thicker and is apparently differentiated into an exosporium and endosporium. As development proceeds the oil-globules coalesce, until finally they form a single large eccentric one situated outside the protoplasm. A second wall is produced inside the first one, and this curiously enough appears to be separated from the outer one by a clear space—an optical delusion, as the study of sections proves. Each wall, outer and inner, is of consider-

able thickness, and has a double contour. The inner wall is a mass of reserve material. This thick double wall deserves special attention, as it apparently has not hitherto been noted in Saprolegnieae. It provides one of the chief difficulties in the preparation of suitable sections. Figs. 19 to 23 illustrate fully the maturation of the oospores in the oogonium, which we have already had under observation during the formation of the oospheres. The first eggs to ripen were those nearest to the fertilization-tubes. They took about $5\frac{1}{2}$ days for the purpose, the weather being very cold for the time of the year.

Germination of the oospores. The germination of the oospores was followed in the same oogonium. The two oospores nearest to the fertilization-tubes were noted six days after they had reached maturity to be already in course of germination. During this process the inner wall first becomes dissolved; the protoplasm increases in quantity; the oil-globule comes to occupy the central part of the spore, and is gradually decomposed and absorbed; the outer wall stretches, and its inner layers disappear; the oospore increases in size, and a vacuole is developed in the centre. Later, one or more germ-tubes make their appearance, and these as a rule soon stop growing, and produce each a small sporangium with from four to ten zoospores. Figs. 24, 25, and 26 illustrate these stages in germination, and from the times appended it is clear that germination may be completed in three days. It is noteworthy that the first oospore to germinate was the first to mature, and in all probability the one first touched by a fertilization-tube. An intermediate stage in the germination of the spores is represented in Fig. 27, in which three oospores have produced germ-tubes.

The foregoing observations enable us to arrange the appearances presented in serial sections either of oogonia or oospores in, at any rate, a rough chronological order. To anticipate somewhat, it may be said here that the order is sometimes difficult to make out. The difficulty is greatest where it would have been convenient for it to have been least. Between the stages represented by Figs. 7 and 9, representing

a period of seven hours, the nuclei in the oogonia undergo a division by a typical indirect method. The study of the living material scarcely enables us to arrange the sections dealing with these dividing nuclei in exact chronological order. We are reduced to the necessity of falling back upon analogy. We have to find a complete series of forms, and then to determine, by analogy with better known cases of nuclear division, which are the earlier and which the later stages in the process. The condition of the oogonium in the earlier and later stages of nuclear division is such that there can be no doubt in the main as to the true sequence in which the facts brought to light by sections should be arranged.

Special attention may be directed to the times necessarily occupied by the chief stages in the course of development. They are as follows:—

From the germination of the spores to the development of the gametangia	4 days
From the first appearance of the oogonial branch to the formation of the basal wall of the oogonium	10 hrs.
From the formation of the basal wall of the oogonium to the first indication of *balling*	7 hrs.
From the first indication of *balling* to the completion of egg-formation	1½ hrs.
From the completion of egg-formation to the completion of the growth of the fertilization-tubes .	1 hr.
For the maturation of the oospores	5 days
For the germination of the oospores, including the formation of the sporangia	3 days

The whole course of development may thus be normally completed in a period of thirteen days. The slight differences of temperature in the laboratory at different periods of the year do not make a very appreciable alteration in the time required for the completion of the whole course. In July, in hot weather, the maturation of the oospores takes about five days; in October, in cold weather, the maturation takes longer, but nevertheless not so much as six days.

The normal course of development, as outlined above, is not often adhered to. The conditions are seldom so favourable as to admit of the life history being completed in so short a period as thirteen days. In cultures four months old, for example, a number of the oospores may be found in the resting condition. In cultures six months old I have generally found nothing but the empty cases of the oospores. The external conditions which determine the germination of the oospores are no doubt checked and controlled by internal conditions; for, in oogonia on the same hyphae, in oospores in the same oogonium, even when kept in the same moist chamber in a single drop of water, noteworthy differences are almost constantly to be observed. I have seen in the same drop of water, in oogonia borne on a single hypha, kept under regular observation for several weeks, the following differences:—(1) The oospore germinates and produces a germ-tube which, instead of producing a sporangium, develops a small mycelium, as in Fig. 28, the branches of which are very delicate and of rhizoid-like character; (2) the oospore germinates and produces one or more germ-tubes, which terminate in small sporangia; (3) the oospore germinates, but fails to produce a germ-tube, and ultimately perishes; (4) the oospore germinates and produces a germ-tube, but fails to produce sporangia, and ultimately perishes; (5) the oospore germinates and ultimately produces sporangia, but the process of germination is effected in stages, with long pauses between, and thus sometimes extends over many days; and (6) the oospore may remain in the resting condition. As the external conditions could scarcely be more uniform than they are in a small drop of water in a moist chamber, much of this variability must be due apparently to differences in the constitution of the oospores themselves.

As it was of importance to me to secure cultures with large numbers of oospores undergoing germination at the same time, an endeavour was made to determine, to some extent, at least, the external conditions which were favourable to this process. In consequence of a verbal communication from

Professor Klebs, the effect of a high temperature was first tested: sixteen-day cultures were placed in a water-bath which was kept at a constant temperature of 29° C. in the dark for five days. There was no appreciable effect: not a single spore germinated. The same cultures, however, yielded valuable material six days later, after having been placed in a south window exposed to the full benefit of such sunlight as was afforded by a wet week in September. Cover-glass cultures were not checked in their development on being placed in this bath, so that we may conclude that this treatment had neither a stimulating effect upon the resting spores nor an inhibitory action upon those already in process of germination.

Another experiment which produced negative results was carried out as follows:—Eighteen-day cultures were kept at 0° C. in ice for fifty-two hours and then transferred to a warm well-lighted greenhouse at 65° F. to 70° F. for sixty-four hours. Here again exposure in the south window brought about free germination in the course of ten days. It appeared as if a combination of bright light and a high temperature would have a stimulating effect. Accordingly a sixteen-day culture was placed near a water-bath close to a very strong incandescent lamp: the temperature was maintained during constant illumination at 32° C. for forty-four hours: no spores germinated. On examination seven days later, after exposure in the south window a few germinating oospores only were found. Cover-glass cultures in a dimly lighted room gave the best results in germination, so that no experiments were set up to test the effect of bright light alone. A good supply of oxygen is probably a factor of great importance.

It may be noted further that the course of development, apart from the germination of the spores which has not yet been adequately tested, is perfectly normal in both ordinary or moist-chamber cultures whether these are exposed to, or protected from, the influence of light. In proof of this it may be stated that appropriate cultures carried out side by side with the external conditions exactly alike, but differing

with respect to illumination, were indistinguishable from one another at all stages of their development.

Time and opportunity were wanting for detailed investigation as to the conditions of germination. After a few weeks, too, the necessity which had urged me to examine the question disappeared, for excellent material with numerous oospores in all stages of germination was furnished by cultures which had been allowed to stand in small quantities of water in a south window. Such cultures would be relatively very well supplied with oxygen, for the continual variations in temperature inside the vessel in such positions would cause constant fluctuations in the volume of the air standing over the water and so aid thorough aeration. Really pure cultures, i. e. cultures free from *all other organisms* than the *Achlya* itself must, I fear, be used to secure perfectly satisfactory results as to the conditions of germination, and, of course, a much more rigorous method must be adopted throughout.

Induced apogamy. When engaged in following the course of development by means of cover-glass moist-chamber cultures, the method of cutting off hyphae from larger cultures and examining them separately was frequently resorted to. It was soon evident that this method offered a means of producing apogamous oogonia at will.

In normal cultures, the oogonia are *always provided* with antheridia. If a hypha bearing oogonia in all stages of development be cut off from the rest of the mycelium, transferred to a moist chamber and then examined at once, it is found that those oogonia provided with a basal wall have undergone no change of importance, but that those without one have suffered considerably. In the latter case the protoplasm of the oogonium under the conditions of the operation carried out by me, is in part carried down the stalk and a small mass of it even reaches the main hypha. The oogonium slowly recovers, most of the protoplasm passes back into it, but a little is frequently left behind and undergoes what may be called fatty degeneration, being converted to

a greater or less extent into fat-globules. The basal wall forms in due course and the oogonium produces its eggs in the normal manner. The antheridial branches, however, may under these circumstances be suppressed altogether, as in Fig. 29; or if developed they may grow past the oogonium without bending towards it; or they may even grow away from it altogether and attain a great length as if they had become converted into rhizoids. It is obvious that the oogonium must normally exercise a considerable influence in determining the nature and direction of growth of the antheridial branch. The determination of the nature of this influence must remain a subject for further study.

The eggs in such oogonia develop in every respect in a normal manner except that the time required for maturation is about doubled. Fertilized eggs mature in five days, unfertilized in ten days even when in oogonia on the same hypha in the same moist chamber.

It was noted in a large number of cases that oogonia such as that in Fig. 27, which in all probability were apogamous, possessed oospores which germinated in the normal manner and produced small sporangia. Two oogonia were kept under regular observation for a whole month, from 21/9/98 to 21/10/98. The eggs, two in number in each case, were produced on 21/9/98, they reached maturity in eleven days. Fig. 29 represents one of them on 4/10/98. This alone developed further—the other one became unhealthy. Fig. 30 represents a stage in germination. About 17/10/98, the whole culture which had been kept in good condition up to this time, became unhealthy, and on 21/10/98, when the germinating oospores were obviously dead or dying, it was destroyed. There can be little doubt that these unfertilized eggs produce oospores which are capable under normal conditions of developing sporangia and zoospores and giving rise to new plants.

The interest of this observation appears to me to lie in the fact that in a group in which apogamy occurs so frequently under natural conditions in some of its forms, it may be

induced artificially in others by recourse to unnatural methods. It appears as if apogamous development were a response of the organism to special external conditions. In forms like *Saprolegnia Thureti,* where De Bary after years of experience stated that he only once found an antheridium attached to an oogonium, it appears that the normal external conditions are very favourable to parthenogenetic development and very unfavourable for the development of antheridia. We may, however, expect to find that even in the case of *S. Thureti* a complete acquaintance with its conditions of life will enable us to secure cultures in which all the oogonia are provided with antheridia. The inherent power of producing antheridia is *proved by the single observation* of De Bary. It remains for future observers, following in the footsteps of Klebs ('97, '98), to determine the conditions of their formation.

Other effects of the Amputation of Hyphae. If stout hyphae are cut off just before sporangium-formation is about to take place, and are then placed in a drop of water in a moist chamber, with or without a supply of food, the development of the sporangium is stopped, and very numerous, thin branches appear at all points of the hyphae, and grow out in every direction as if seeking fresh supplies of nutriment. If, however, the basal wall of the sporangium was formed before amputation took place, the sporangium may or may not, according to the treatment it receives, ripen and liberate the zoospores. When hyphae bearing sexual organs are amputated, induced apogamy is not the only result. Young oogonia sometimes lose all their protoplasm. They are emptied to provide protoplasm for other organs whose development goes on unchecked. Occasionally young oogonia become converted into sporangia. The end opposite the stalk grows out a little, the usual beak-like protuberance appears and the spores are ultimately liberated in the usual way.

In other cases, oogonia may return to the vegetative condition and produce branches from any point, and these generally develop into fresh oogonia much as Humphrey

has described in the case of *Achlya americana*, without indicating, however, any reason for the anomaly.

Such observations as these could be extended greatly by any one who devoted himself specially to the work, and it is possible that in this direction a solution of the real difference between the asexual reproduction by sporangia and sexual reproduction by apogamous oogonia may be found.

Cytological observations on Serial Sections.

Methods. Before proceeding to indicate the results of the study of the cytology of this plant, it will perhaps be advisable to indicate the methods adopted by me in securing well-stained preparations in the form of serial sections. The fixing liquid used was a saturated aqueous solution of mercuric chloride, and it was applied hot as advised by Hartog and Humphrey. Differences in the preparations led me to investigate the effect of differences in the temperatures. Accordingly specimens were fixed in a solution of mercuric chloride maintained for twenty to thirty minutes at 55° C., 65° C., 75° C., 85° C. and 95° C. The differences in the results were not very great and it is possible that they were due in part to slight differences in the cultures. The solutions did not act uniformly on all the organs; as might be expected, the conditions for fixing young oogonia and ripe oospores cannot be exactly alike. The temperature of 55° C., however, appeared to give uniformly good results, and all of the figures are from material prepared at this temperature or that of 65° C.

The material was dehydrated by means of graded alcohols—30%, 50%, 70%, 90% and absolute—the transference from one to the other being made very slowly, drop by drop, and occupying, as has been said, the greater part of a week. The passage of the dehydrated material into paraffin was carried out in a similar manner, through the medium of xylol or chloroform, occupying the greater part of another week. The sections were cut of uniform thickness by a Jung-

microtome, double stained with gentian-violet and eosin, and mounted in xylol-balsam.

An attempt was made to secure uniformity of treatment throughout, so that the differences observed in the nuclei might be safely referred to inherent causes and not to the varying nature of the method of preparation. A very large number of successfully stained preparations were examined. Just as a systematist selects his type-plant after the examination of thousands of individuals, so in the main are the figures which illustrate the cytology of this plant, while drawn as accurately as possible under the camera from actual examples, nevertheless typical, as they have been deliberately selected to represent what may be regarded as the normal type.

An impression appears still to exist amongst some cytologists that sections, especially of such small objects as these oogonia and oospores are apt to be lost during the preparation of the slides, and that even if they are not, it is difficult and sometimes impossible to avoid mixing up the sections and so forming by a mental process a false picture of the object. Now, while diminution in size increases the difficulty of finding the series of sections which constitute the object, and involves an additional expenditure of time and patience, there should never be the least doubt in the mind of a competent observer as to whether his sections are really serial or not. If sections are loosened by accident, he should be in a position to put his finger, metaphorically speaking, on the vacant place. Even with such small objects as the oospores of *Achlya* any careful observer can be certain of the reality of his series even without recourse to the refinements of a mechanical stage and the measurement of the distance between the sections.

In the preparations which I have made and used for further study, I have never seen a loose or misplaced section, and have never been at a loss to locate any section of a series. It is fortunate that it is possible to attain certainty in this direction, for there can be no doubt that the oogonia and oospores are too thick and dense for clear pictures of their

contents to be obtainable by any other method than one which involves sectioning. Let us proceed to the examination of the actual facts revealed by the sections.

The zoospore (Fig. 31). The zoospore as seen in section is generally of circular outline and clothed with a very delicate cell-wall. When the fixing has been properly carried out there is no collapse of the protoplasm, and the cell-wall is practically invisible in balsam-preparations. The protoplasm is finely granular and vacuolated and contains a few microsomata of large size which stain deeply with nuclear stains and are in all respects like the similar structures already described by me in *Saprolegnia*. The nucleus is very large and spherical. It possesses a distinct nuclear membrane and what appears to be a central globular nucleolus with a spongy structure. This central body stains very deeply with nuclear stains, and appears to be kept in position by a number of fine threads which pass from projections on its outer surface to attach themselves to the nuclear membrane. The clear material through which the threads pass we may regard as nuclear sap or nucleo-hyaloplasm, the threads themselves as linin-threads, and the central body as containing both chromatin and nucleolar matter. As we shall see later, it resembles in its behaviour during karyokinesis, the so-called nucleolus of *Spirogyra* ; indeed a close study of the work of Mitzkewitsch ('98), and especially of his careful figures, has revealed quite a number of points of agreement. We cannot then describe it either as a nucleolus, as has been done by Dangeard, or as chromatin, or a chromosome, as has been done by Humphrey, Hartog, and myself.

The mycelium and sporangia. A study of the mycelium and its development from the germinating zoospores has revealed no fact of interest. The sporangia, however, are generally very large and contain both peripheral and central spores. In a transverse section we may find a central mass of four or more spores, occupying the place of the central cavity seen in sporangia of *Saprolegnia*, and surrounded by a parietal layer of spores. The spores frequently fail to escape,

and encyst within the sporangium. An oblique section of such a sporangium is shown in Fig. 32. I have not been able to definitely recognize nuclear divisions, direct or indirect, either in the germinating zoospores or in the mycelium, where nevertheless they certainly go on. I have still to search for them at the base of the main hyphae, where, according to Hartog, they take place. An endeavour to follow the fate of the nuclei at the apices of the main hyphae has hitherto been frustrated by the density of the protoplasm in that region and the abundance of the same microsomata which are seen in smaller numbers in the zoospores. Many sporangia were examined in all stages of development. The nuclei are easily traced at this time, and it can be safely concluded that nuclear divisions do not take place during the formation of sporangia.

Development of the gametangia and gametes. Karyokinesis. Young oogonia, as observed in stained preparations (Figs. 33, 34), are seen to possess a very delicate cell-wall which appears to be really adherent to the protoplasm. Shrinkage of the protoplasm which takes place to a slight extent is accompanied by a shrinkage of the cell-wall, so that one does not find even in bad preparations any gap between the cell-wall and the protoplasm. The outermost layer stains deeply with gentian-violet as if it were made up of microsomata. Time has not, however, permitted me to pay so much attention to this young cell-wall as the subject deserves. The mature cell-wall gives the reactions of cellulose very readily—as easily, for example, as those of *Spirogyra* and *Vaucheria*—and of course does not take up gentian-violet. The protoplasm exhibits foam-structure, although not so beautifully as in the species of *Saprolegnia* with which I am familiar, and at first almost completely fills the oogonium. The nuclei are very obvious and are apparently surrounded by a thin layer of protoplasm which stains deeply. One is tempted to regard this as kinoplasm, more especially as I have seen indications within it when well developed, of a fibrous structure. Provisionally, at any rate, we may regard the protoplasm as differentiated into *kinoplasm* with a fibrous structure and

trophoplasm with foam-structure. The nuclei moreover are preparing for division. The chief indications of this are the increase in size of the nucleus, the decrease in size of the central body, the increased prominence of the linin-threads, and the capacity for absorbing stains which is acquired by the nucleo-hyaloplasm. When the basal wall of the oogonium has made its appearance, these changes are very obvious, as may be seen by reference to Fig. 35, where it should be noted one nucleus is unusually large.

The antheridia differ from the oogonia chiefly in size, form, and the absence of a central vacuole. The nuclei in the antheridia, however, undergo exactly the same changes as those in the adjacent oogonium, as may be seen in Fig. 35 and succeeding figures.

As development proceeds, the cell-wall of the oogonium thickens, the protoplasmic layer gets thinner, the vacuole gets larger, and the nuclei undergo very important changes. The fat-globules which give rise to such characteristic appearances in the fresh material are, of course, absent from all the preparations, their places being indicated by clear vacuole-like, more or less globular, cavities. It is not possible to distinguish between such cavities and the small true vacuoles. It is difficult, moreover, to determine the substance which fills these true vacuoles, whether it be hyaloplasm or cell-sap; and so far as this research is concerned, it is impossible with the methods adopted. It may be regarded as certain, however, that in all stages of development from the formation of the basal wall to the development of the oospheres, the majority of the small clear spaces seen in the sections were occupied by fat-globules.

The changes in the nucleus, however, were for us of chief importance, and much effort was exerted to get clear views as to the true nature of the various appearances observed. The first indubitable evidence of a division of the nucleus by an indirect method was furnished by finding nuclei, chiefly in antheridia, but sometimes in oogonia, in the *spirem* condition. About thirty nuclei, one of which is represented in

Fig. 39, were carefully examined in this stage and it could be clearly made out that the skein was a very loose one, showing from four to five roughly parallel, but occasionally anastomosing strands in surface view. These strands were unequally thickened and their position could be shown, by following them to the margin of the nucleus, to lie immediately under the nuclear membrane, probably almost, if not quite, in contact with it. The centre of the nucleus was generally occupied at this stage by one or two small nucleolar fragments and a clear substance, staining a faint dusky-red, with the double stain. Although the size of the object deterred me from attempting to distinguish the longitudinal splitting of the coiled thread, careful attention was paid to the genesis of the skein, and the impression was gained of the chromatic material passing out from the central mass until nothing was left behind but the nucleolar fragments. If this view be correct, the nucleus of *Achlya* does not differ greatly from that of the higher plants. For its size it is relatively rich in chromatin and nucleolar matter, and the necessities of the case lead to an intimate mechanical mixture of these two nuclear substances.

The nuclei at this stage, and especially in those which immediately follow it, are very difficult to fix properly. They seem to collapse. In any section of an oogonium few nuclei can be recognized by definite morphological characters. Some of them, however, in almost every section were found suitable for study, and special attention being paid to them such results as appear in Figs. 36 to 40 come before us. Small bits of the oogonia are alone represented except in Fig. 39, where the nucleus of an antheridium appears as well. Figs. 36, 37 and 38 represent the nucleus in the *monaster* condition, Figs. 36 and 38 from the side, and Fig. 37 from the pole. Remains of the nucleoli are still to be seen at this stage. A fragment of a nucleolus is represented in Fig. 38. There was a similar fragment in the nucleus represented in Fig. 36, exactly behind a chromosome. Very few nuclei were seen in this stage and Fig. 36 represents the

clearest view obtained. At the poles of the spindle there were four small granules; two threads of the achromatic spindle could be traced from pole to pole, and to end in these granules; but no other threads were clearly seen. In the equator were two chromosomes which probably concealed two others, as there were indications of three in Fig. 38, and of four or five in Fig. 37. At this stage the nuclear membrane is still intact and the kinoplasm may occupy a lateral position. Fig. 39 represents a small portion of an oogonium in which the nucleus is in the *diaster* stage. The nuclear membrane still persists here, there are two pairs of daughter chromosomes and the threads of the spindle are seen to converge to one pole, but pursue a parallel course towards the other. Fig. 40 represents an end view of a similar nucleus.

Careful consideration of these figures and a large number of others, led to the conviction that in *Achlya* the achromatic figure is derived from the linin-network within the nucleus, as the nuclear membrane persists at any rate up to the diaster stage and there is no indication of the insertion of threads from the exterior. With such difficult objects of study it was impossible to obtain better results. It may be regarded, however, as proved that many, if not all of the nuclei in the oogonia and antheridia divide by an indirect method during the development of the gametes, and that the number of chromosomes in the nucleus is in all probability four, possibly varying between four and five.

Now comes the really difficult part of the study. Dismissing from the mind all preconceived ideas as to what ought to take place, bearing in mind—to anticipate a little—that each egg, when first recognizable as such, possesses one nucleus, we have to ascertain the fate of the supernumerary nuclei. A glance at the figures shows that many more nuclei are enclosed in the oogonia than are sufficient to provide one for each oosphere; countings of eggs on the one hand and nuclei on the other, show that on the average the number of the nuclei in an oogonium is ten times greater than the number of eggs to be produced.

At a stage in the development of the oogonium which just precedes that in which the *balling* of the protoplasm to form the eggs begins (Fig. 41), the fixing always produces a great shrinkage of the protoplasm, as if violent plasmolysis had taken place. Examination of the protoplasm leads to the conviction that it is neither injured nor distorted in the process, and a careful search for nuclei reveals the fact that there are in the oogonium about as many nuclei as will suffice to provide one for each egg—in this case *two*. The nuclei are recognized as in the last stages of division—the chromosomes are crowded together near the central point and the new nuclear membrane is apparently present. Figures have been seen which have led me doubtfully to infer that the new nuclear membranes may be formed whilst the old one is still intact. Numerous microsomata render the protoplasm highly granular ; and occasionally, as represented in the figure, we have in the large central vacuole, what we may regard as skeletons of fat-globules. These skeletons are very delicate spherical shells, almost invisible in some preparations, but generally very well seen in those stained with haematoxylin. On account of their position and size, most of them, of course, do not adhere to the slide and are washed away with the dissolved paraffin. Their presence in the sections is of some service as they help to fix the stage before us as a very late one in the development of the gametes, almost certainly later than that represented in Fig. 42. To this we may now turn. We note that we have here, as in the previous case, two nuclei in the last stage of division. We correlate them with those of Fig. 41 by the stainable nucleo-hyaloplasm. In addition to these, however, we have a number of groups of deeply stained granules. These granules are about as large as chromosomes, but the number varies in each group, four and eight being sometimes clearly seen. The grouping of the granules, moreover, is very irregular, and they appear to lie in a vacuole, giving one the idea of particles undergoing digestion. At first it was difficult to decide whether they represented cases of the segmentation of the skein

following upon the spirem-stage; but detailed study finally led to a positive and definite rejection of this view. As the matter may give rise to future discussion, it may be useful to give categorically my reasons for this. They are as follows:—(1) The very various and frequently fantastic arrangement of the presumed chromosomes; (2) the unstained matrix in which they lie; (3) the absence of a nuclear membrane; (4) the variation in the number of granules in the different groups; (5) the demonstration of the prophases of a typical indirect division at a much earlier stage in the development of the oogonium; (6) the presence of typical nuclei side by side with them in the same section and differing from them in structure; (7) the impossibility of tracing the earlier stages in their development; and (8) their very sudden disappearance as if dissolved by the protoplasm. The interval of time required for the passage from the stage represented in Fig. 42 to that in Fig. 41 cannot be much more than one hour. It may be concluded then, that in all probability, the supernumerary nuclei, multiplied to some extent at any rate, if not doubled, by indirect divisions, undergo digestion by the protoplasm.

The eggs now commence to form, and in the stages of *balling* it is easy to demonstrate in the centre of each mass of protoplasm a single nucleus. This nucleus is poor in chromatin and is still unprovided with a nucleolus, but has a distinct nuclear membrane and is surrounded by a dense mass of fine-grained protoplasm. It is at this stage, reproduced in Fig. 43, that the fibrous structure of this enveloping mass is alone to be clearly seen. The oil-globules are represented by the cavities in which they lay, and occasionally, in the case of those lying either partly or wholly in the vacuole, by the skeletons we have already described. Finally the eggs are produced.

All unfertilized eggs that have been examined in sections are of somewhat irregular shape, as, for example, those in Fig. 44. In the centre of each there is always a nucleus, perfectly well defined to one who has traced it through the various stages of karyokinesis.

Fertilization. In sections passing through oogonia with unfertilized eggs, fertilization-tubes may be seen in all stages of development. They contain nuclei which correspond exactly with those of the oospheres, except that they are somewhat smaller. Large numbers of sections were examined to try and elucidate the mode of fertilization, but only one section, that reproduced in Fig. 45, appeared to be capable of throwing any light on the actual process. In this case it was possible to trace the fertilization-tube without a break into an egg which was already surrounded by a delicate membrane. The figure, combined with what we know from observations made on living material, suggests that the fertilization-tube grows up to the egg, presses against it, indents it, stimulates it to the formation of a delicate cell-wall, and grows obliquely into the mass of protoplasm, carrying at its apex a single nucleus. Later stages, such as that in Fig. 46, tend to show that the wall of the tube within the oosphere breaks down, the nucleus, together with a small quantity of protoplasm, is liberated, and so comes to lie in the peripheral part of the egg. The cell-wall of the oospore is then completed, and the end of the fertilization-tube remains attached firmly to it. It is not easy to see the male gameto-nucleus while still at the periphery of the egg, but the female gameto-nucleus in the middle is very obvious, for it appears to be just at this period that the nucleolus makes its first appearance in it as a small deeply stained spherical granule near its centre. I have satisfied myself, however, of the presence of two nuclei in the egg *at all times* in this stage, one peripheral and the other central, and the peripheral one always close to the point of attachment of a fertilization-tube. There can be no doubt whatever that whether the view taken here as to the actual mode of fertilization be correct or not, eggs at this stage contain two gameto-nuclei of diverse origin, the one male, and the other female. In Fig. 46, drawn from one of many oogonia observed in detail, section by section, the two lower oospores each contain two gameto-nuclei; in the upper one, as shown,

there is a male gameto-nucleus, but the female gameto-nucleus was in the next section. With two of the male gameto-nuclei fertilization-tubes are associated; examination of the neighbourhood of the third in the adjacent section revealed the presence of one there also. The branching of the fertilization-tube is also seen.

We are now able to fix a maximum limit for the time required in karyokinesis from the earliest stages up to the reconstitution of the nucleolus. The time cannot be greater than ten hours.

Maturation of the oospores. Fusion of the gameto-nuclei. The male gameto-nucleus travels to the centre of the egg and places itself in contact with the female gameto-nucleus. Even from cultures five days old such preparations as that represented in Fig. 47 were obtained. The two nuclei are pressed closely together in one oospore, and they appear to have undergone partial fusion in the other. As a matter of fact they can only be recognized by their nucleoli. The male gameto-nucleus has the smaller nucleolus. At first I regarded this as distinct evidence of a very early fusion of the gameto-nuclei. Such preparations are uncommon, so that if this really represents a fusion it is probably a rapid one.

The detailed examination of older oospores from cultures six, seven, and eight days old, and in which the determination of the relative ages of the oospores is not difficult, led to the discovery of the fact that oospores, one and two days older than those just described, *always contained two gameto-nuclei*, while those three days older seen in eight-day cultures sometimes contained two and sometimes one. Fig. 48 represents oospores from an eight-day culture. At a glance, it is seen that the pairs of nuclei are in the resting condition, the central body is very large and the linin-threads are very evident. The nuclei in one oospore are approximated as if fusion were about to take place. Fig. 49 represents a zygote-nucleus in the act of formation; the central body and nucleus are both elongated, but the fusion is nearly complete. Fig. 50 repre-

sents uninucleate oospores from similar preparations of eight-day cultures.

The examination of many hundreds of oogonia and oospores in preparations from six, seven, and eight-day cultures leave little doubt in my mind that the approximation of the nuclei in the five-day culture preparations is temporary, and that the fusion does not take place until about three days later, when the nuclei have been for some time in the resting condition. It would be interesting to determine the conditions which regulate this long-delayed fusion of the gameto-nuclei. It appears to be of common occurrence amongst the Thallophytes.

During the three days which elapse before fusion takes place, the oospore undergoes important changes: the protoplasm contracts somewhat, and the cell-wall thickens; the fat-globules get larger, leaving behind them bigger cavities in the protoplasm, and little groups of microsomata make their appearance, recalling the much larger structures seen in the oospores of *Saprolegnia*. Fortunately, however, in *Achlya*, there is no structure present which prevents the accurate examination of the nuclei, and for this reason alone it is satisfactory that the study of the plant was carried to an end.

In cultures nine days old (Fig. 51) a new structure makes its appearance in the form of an additional thick inner wall showing a faint stratification. This wall is obviously built up of reserve material, as it disappears early in germination. Between it and the outer wall (in balsam mounts) there is always an empty space: this is no doubt due to the greater contraction which it undergoes during dehydration. If the sections are examined in water or in iodine solution, the outer and inner walls are in close contact, but are readily recognizable by the difference in colour and refractive power. Testing the sections for cellulose with iodine and sulphuric acid, the inner thick wall becomes a deep blue at once, and swells up quickly, frequently pressing out the protoplasm and completely filling the cavity; the inner layers of the outer wall swell considerably and become of a greenish blue colour, while the outermost layer retains the yellow colour

due to the treatment with iodine, and resists the action of the acid.

The inner wall then consists of cellulose, and is a mass of reserve material. The outer wall consists of two layers, a thick inner one, consisting of slightly modified cellulose, and disappearing during germination, and an outer thin one which is cuticularized and persists as a thin empty shell for months after germination has taken place. Other microchemical reactions lead to the same conclusions.

The nucleus is always found at rest in nine and ten-day cultures, and I have never been able to find more than one. The spaces in the protoplasm are very large, corresponding in size to the oil-globules present at this stage of development. No further changes take place in the oospore other than that the protoplasm diminishes in quantity, loses at the same time its vacuolated appearance, and takes up a parietal position.

Germination of the oospores. Karyokinesis. An examination of Figs. 52 to 59 will show almost at once the nature of the cytological changes which accompany germination. The nucleus first of all divides, and the division is repeated at the time the inner cell-wall is undergoing dissolution. Oospores with four nuclei fix badly for some hitherto unexplained reason, even when the nuclei are apparently in the resting condition, so that figures of them have not been drawn. The divisions proceed further, and oospores with eight nuclei are larger, have a thin cell-wall, and are completely filled with protoplasm, the large oil-globules having been completely absorbed. A central vacuole forms later, and the protoplasm takes up a parietal position. The number of nuclei increases to about twenty, and then one or more germ-tubes are produced. I have not thought it worth the trouble to follow the development of the small sporangia in detail. They have been seen in section several times, and they presented no peculiarities other than those associated with their small size.

Special attention was paid to the nuclear divisions, and although it was difficult to secure positive results, nuclei have

certainly been seen in all stages of karyokinesis. Figs. 60 to 70, which, it should be noted, are somewhat diagrammatic, having been drawn by free hand and magnified 2,400 diameters, illustrate the spirem-stage, which was very distinct, and spindles chiefly in metakinesis and diaster-stages. The four resting nuclei pressed closely together in Fig. 70, and which occurred near the apex of a germ tube are worthy of note.

An endeavour was made to determine the number of chromosomes, but the success attending it was scarcely commensurate with the effort. The chromosomes are so small and so close together that positive determinations were not possible. The conviction, however, was forced upon me in several cases that the number seen in a side view of the spindle was *four*, double that seen in the gametangia. Provisionally we may then regard the number of chromosomes in the gameto-nuclei as four, in the nuclei of the germinating oospores—the gametophytic mycelium that is to say—as eight.

It was not to be expected that the finer details could be seen so well in this case as in the gametangia, for the fixing fluid has to penetrate the thicker wall of the oogonium and the thick resistent wall of the oospores as well.

Criticisms and Replies to Criticisms.

Having made myself thoroughly acquainted with a species of *Achlya*, and solved the problems which presented themselves at the commencement of the work, I found myself in a position to examine critically the results obtained by Humphrey and Hartog.

On the evidence of Humphrey's ('94) own figures I had concluded, as a result of my work on *Saprolegnia*, that fertilization took place in *Achlya americana*. Humphrey's view that the uninucleate oospores were derived from the binucleate ones by fusion of the two nuclei was obviously correct; but

the inference that these two nuclei originated in the oogonium appears to have been based entirely upon hasty conclusions as to the impossibility of fertilization taking place. Humphrey's methods, moreover, were scarcely adapted to elucidate the difficult question of fertilization; and as he neither examined oogonia in the stage of *balling*, nor naked oospheres, a correct interpretation of the complicated phenomena which take place here was obviously out of the question. We need not then consider further his view, unsupported as it is by a single figure, that the supernumerary nuclei are got rid of by successive fusions. As, however, the nuclei in the oospores are easily identified, and they appear to agree closely with those of the var. *cambrica*, and especially in view of the evidence brought forward in this paper, it may fairly be concluded that fertilization takes place in *Achlya americana*. This being so, I venture to draw special attention to the fact that *fertilization is now known to take place in four* distinct forms of Saprolegnieae; viz. *Saprolegnia diclina*, *S. mixta*, *Achlya americana*, and *A. americana* var. *cambrica*.

Hartog's views deserve much closer attention, for he devoted himself almost exclusively to cytological studies.

To avoid tedious recapitulation of trivial points I will direct attention to those features alone which lead to real differences of opinion. It will be necessary in this case to consider the text of Hartog's communication separately from the figures which illustrate it.

As I have previously stated, the textual statements of Prof. Hartog do not differ greatly from my own. Bearing in mind that we were working probably with different material and certainly using widely different methods, the extent of the agreement, as to fact, is astonishing. Indeed, had Hartog been able to follow the division of the nucleus in the oogonium (as he did in the antheridium) and to recognize the nucleus in the naked oospheres, he would probably have never promulgated his remarkable theory of the 'multiple endogamous union of potential gametes.' It is so difficult to find a reason for a wholesale fusion preceded by an indirect

division. Why should the number of nuclei be doubled, when it is already ten times too great, if the final reduction in number is to be brought about by simple fusions, four times repeated? This question of wholesale nuclear fusions is one which we expect to see proved conclusively by actual demonstration. It is so improbable in itself at any stage in the life-history of a plant that we may be pardoned in the absence of such demonstration for unbounded scepticism as to its occurrence.

But Hartog's theory, even as a theory, has an unsound foundation. Consider what it means. The nuclei in question, surrounded by their protoplasmic masses—energids, let us say—are female gametes and very highly developed ones too. So far as external differentiation goes, the evolution of sex in the Saprolegnieae has been carried to an extreme. The theory, then, is that of the multiple endogamous union of highly developed female gametes in plants where very dissimilar male gametes are thoroughly well known. Imagine a botanist broaching as a theory the multiple union of *Fucus*-eggs to form a zygote and the universal incredulity with which it would justly be met, and we have some idea of the great improbability of Hartog's theory. Let us put the matter plainly:—a variation from the ordinary mode of fertilization so great as that propounded in this theory needs to be supported by evidence overwhelming in quantity and perfectly satisfactory in quality.

What is the evidence adduced by Hartog in support of his theory? It is furnished by the clusters of chromosome-like bodies represented in his Fig. 40, and in Fig. 42 of the present paper. His interpretation of these figures is that each granule is the representative of the central body of one of the original resting nuclei, and he states that the number of these granules varies from four to twenty: a figure with four granules represents four or more fused nuclei, one with twenty granules, twenty or more nuclei. The granules get fewer by fusing with one another, but as they do not get larger they must lose substance in some way which he has not yet

explained. The greatest objection to this view—and it is a fatal one so far as concerns my plant—is that the number of granules in a single section may be as large as the number of nuclei originally present in the whole oogonium.

Hartog himself had his doubts concerning the process, as the following quotations prove:—'Whether any of the nuclei disappear and dissolve into the protoplasm, as sometimes stated, it is, of course, impossible to tell; but I do not think so, though the idea had repeatedly crossed my own mind'; and, 'the size of the individual nuclein-masses when they occur in numbers at these late stages is enormously reduced.' The evidence all appears to tell in favour of the digestion of the supernumerary nuclei. It is, however, possible that fusions of these degenerate nuclei take place during digestion, and that before dissolution the chromosomes make themselves evident. It is worthy of note that Blackman ('98) appears to have seen similar figures in the degenerating nuclei of the tapetal cells of *Pinus silvestris*.

So far we have confined our attention to the textual statements of Hartog, but a careful examination of the figures in his paper raises doubts as to the suitability of the methods selected by him for the elucidation of such difficult problems in histology. Figs. 21 and 22, Plate XXVIII for example, represent two oospores of *Achlya*, one binucleate, and the other uninucleate. In each case the peripheral part of the oospore is occupied by dense protoplasm, the central portion appears in the form of a big vacuole, the nucleus or nuclei being suspended in the middle by fine strands of protoplasm. In *sections* of eggs of *Achlya* the central part is always the densest. Such differences as these are readily traced to the differences in the methods. The central vacuole figured by Hartog I have never seen in any oospore. It would be of interest for some one to test the validity of this explanation by actual observation, and such a research would be of value from another point of view, for the figures suggest that fertilization takes place in the species under consideration. The name of the species is not mentioned, but it could scarcely be

one of those examined by Humphrey or me. In all probability therefore it represents a fifth form in which fertilization takes place. As such it is well worthy of further examination, for much and possibly justifiable scepticism exists still as to fertilization in the Saprolegnieae.

We must conclude then, that, notwithstanding the great care with which Professor Hartog has investigated the cytology of the Saprolegnieae, he is not justified in building on so unstable a foundation such a theory as that of the 'multiple endogamous union of potential female gametes.'

Finally, we may refer briefly to the critical notes in the Annals of Botany in which Hartog adduces in support of his view the *solitary* case of a young oospore with two nuclei. This condition he ascribes to retarded fusion, *because he could not see an antheridium in connexion with the oogonium*, and *because in hundreds of other cases of oogonia with antheridia and fertilization-tubes in contact with them there were no binucleate oospores to be found.* With respect to the solitary oospore with two nuclei it may be said at once that I have never been able to absolutely prove the absence of an antheridium from an oogonium except by the method of following the development throughout on separate hyphae. In the case of oogonia taken haphazard I have great doubts as to the possibility of demonstrating the complete absence of an antheridium by Hartog's method.

However, it may be well to publish the fact that I have seen a single oospore in *A. americana* var. *cambrica* which contained three nuclei. There was no occasion, however, to explain this solitary monstrosity as a case of retarded fusion. It is not unusual to find two fertilization-tubes attached to one oospore. Why should not two male gameto-nuclei occasionally enter the egg and possibly fertilize it? Such cases are not infrequent in other plants. Why speak of retarded fusions before giving an absolute proof that unretarded fusions take place?

Again, the apogamous development of oospheres in one species of Saprolegnieae does not prove that fertilization does

not take place in another species, any more than the demonstration of four chromosomes in the nucleus of one species precludes the occurrence of one, two, eight, or any other number in the nuclei of some other species. Such evidence as that adduced by Hartog can be explained easily enough without reference to debatable theories.

May I suggest to those who believe in the disappearance of the supernumerary nuclei in the oogonium by nuclear fusions, that they should demonstrate to us the last stages in the process? It ought not to be impossible, it ought not to be difficult, to show naked oospheres with two or more nuclei. It would be especially convincing if preparations of the stage known as '*balling*' could be shown *with the nuclei*. It is at this stage, where the degenerate nuclei are almost or quite digested according to my view, and the single surviving nucleus is in the last stages of anaphasis but without a nucleolus, that the chief difficulties are met with. According to the views of Hartog and others such stages should be exceedingly easy to demonstrate, for the nuclei as they fuse ought surely to get bigger and bigger and more and more prominent. Until figures are forthcoming of this critical stage in the development of the oospheres it will be wise to ignore all criticisms as to the improbability of fertilization and the probability of nuclear fusions in the oogonium.

Significance of the Nuclear Divisions in the Gametangia.

A consideration of the nuclear divisions in the gametangia of species of *Saprolegnia* led me three years ago to a view of some theoretical interest which I expressed as follows :—'The sporophyte-generation of the higher plants probably owes its origin to the fusion of gametes whose nuclei did not undergo a reducing division. The doubling of the chromosomes acted as a stimulus to spore-formation and involved a halving of the chromosomes in the return to the undoubled condition of the nuclei in the gametophyte-generation.' The return to the original condition may under such circumstances be very well

explained by regarding it, as Strasburger has pointed out, as an atavistic phenomenon. Objections were, however, taken to Strasburger's view that reducing-divisions do not occur in the gametangia. The first direct evidence adduced in support of such divisions taking place was furnished by me in the case of *Saprolegnia mixta*. Since that time Farmer and Williams ('96, '98) and Strasburger ('97) himself have succeeded in demonstrating reducing-divisions (halving of the number of chromosomes) in a highly satisfactory manner in the gametangia of *Fucus*; and Williams has recently discovered them in *Dictyota*, making public his results at the meeting of the British Association at Bristol. We have good, if not excellent evidence that similar divisions take place in *Achlya*.

Strasburger, however, in the 'Cytologische Studien' ('97), if I apprehend his meaning correctly, suggests that these divisions in the gametangia of the Thallophytes, in part at least, may be best explained by regarding them as essential features in the differentiation of gametes; in the production of elements, that is to say, which have no independent power of germination, but whose capacity for further growth is limited by the necessity for a fusion in pairs. Such a view has no inherent improbability and it may well be accepted as highly probable. There is no reason, however, why such divisions should not, sometimes, at any rate, be reducing-divisions, and subserve two purposes at one and the same time.

Let us realize clearly the facts which need explanation in the present state of our knowledge regarding such divisions. In *animals* there are gamobia only; we have practically no sporobia. Reducing-divisions, so far as they have been elucidated, occur in the gametangia in oogenesis and spermatogenesis. In *plants* we have gametophytes and sporophytes. In plants higher in the scale than the Thallophytes we have a sharply marked antithetic alternation of generations. Whatever the origin of the sporophyte, whether by the intercalation of a new generation, or through the dimorphism of the gametophyte, we may regard the alternation as typically antithetic. In all these plants reducing-divisions,

so far as they are known, are confined to the sporophyte and to a stage in development which we may call sporogenesis. In the Thallophytes we have frequent cases of polymorphism, as in the Uredineae. Where the number of forms is reduced to two and these alternate with one another, we have what is frequently called an alternation of generations. Such instances are in reality, as a rule, only special cases of dimorphism of the gametophyte. They occur in *Cystopus*, *Cutleria*, *Achlya*, *Saprolegnia*, &c. Many of the Thallophytes are, however, monomorphic, and they may in that case be represented by gametophytes, as in *Fucus*, or by a plant-body with asexual reproduction alone, as in *Agaricus*. There may be a true antithetic alternation of generations in such forms as those met with in the Florideae and Ascomycetes. It is possible that the Uredineae present us with a specially interesting condition—that in which we may have three generations taking part;—viz. asexual gametophyte, sexual gametophyte, and sporophyte. Such, at any rate, is the logical consequence of the acceptance of De Bary's view as to the systematic position of this group of plants. Whether the alternation of generations is strictly homologous need not concern us greatly. Alternation of generations may have arisen independently several times as is clearly the case with heterospory, secondary thickening, sex, &c.

Strasburger's view that the thallus of *Fucus* may be a sporophyte deserves special attention. To my mind it is negatived from every point of view but that of karyology. We do not know enough of the nucleus in plants, however, to enable us to use it exclusively as a means for deciding complex phylogenetic questions. What we do know should make us very sparing of its use. Moreover, as we shall see, there is no necessity, even from the point of view of karyology, for such a topsy-turvy treatment of the Thallophytes. We may, upon the safer ground of comparative morphology, have no hesitation in regarding the plant-body of *Fucus* as a gametophyte. Reducing-divisions occur, as we have said, amongst Thallophytes, in the gametangia of *Fucus*, *Dictyota*, *Achlya*,

and *Saprolegnia*. In other Thallophytes, however, possibly in the Conjugatae (*Spirogyra*, *Closterium*, *Cosmarium*), no reducing-divisions take place in the formation of the gametes, but such, it is suggested, *may* take place during the germination of the zygote. In that case the Conjugatae would take rank, *in this respect*, with the higher plants.

In *animals* accordingly we have always one kind of gamete, provided with reduced nuclei. In *plants* we appear to have two kinds of gamete—one with reduced nuclei, in certain Thallophytes, and one with unreduced nuclei in a few Thallophytes and all the higher plants. Such being the case, it must be obvious that a reducing-division is not a necessary preliminary to fertilization. That which distinguishes a gamete from a spore is not the number of its chromosomes, whatever else it may be.

We are in all probability a long way from the final decision of the nature of the differences between gametes which will not germinate at all and soon perish; spores which germinate freely; and apogamous gametes which germinate readily, even after undergoing those divisions which are normally preliminary to fertilization, as, e. g. the normally apogamous oospores of *Saprolegnia Thureti* and the artificially produced apogamous oospores of *Achlya americana* var. *cambrica*. The differences, such as they are, remain to be discovered. In the Thallophytes apogamy is by no means an infrequent phenomenon, and it may well be due to the imperfect specialization reached by the gametes in these low forms.

Strasburger's chief difficulty in view of these complicated facts is to account for the occurrence of reducing-divisions *at different stages in the course of development*. He appears to assume that these reducing-divisions must be homologous, although by doing so he places all plants in contrast with all animals. As he is unable to conceive of a sudden development, such as the halving of the number of chromosomes in the reproductive cells of a potential gametophyte during the evolution of sex (or conjugation), he takes refuge, illogically, it seems to me, in the sudden evolution of some

attractive power in spores which has the property of converting them into fusing gametes. This unfortunately does not get rid of all the difficulties; for although the reducing division in sporogenesis is now readily explained by reference to atavistic phenomena, the case of *Fucus* can receive no valid explanation without regarding the plant-body as a sporophyte. One sudden development is as difficult to explain as another. A sudden evolution of attractive power is as difficult to understand as the sudden evolution of reduced nuclei. Moreover, it is always easier and better to explain the complex by the simple, the Lily by the Wrack, than *vice versa*. The gradual displacement theory, too, of Strasburger, is difficult, if not impossible, to conceive in detail. How, for example, is the boundary between the two generations to be crossed?

I venture to bring forward tentatively a view which appears to me to meet the case, and which in a less complete form appeared in the Annals of Botany three years ago. It appears to be certain that the number of chromosomes in the nuclei of an individual plant may be variable, as variable indeed at times as any other morphological character of a plant. The work of Dixon ('94) on *Pinus silvestris*[1] may be cited in support of this. We may further grant with Strasburger that a gamete becomes such by the development, very gradual probably, of a mutual attraction for other gametes. The behaviour of highly developed sexual gametes may be easily explained on such a hypothesis. When the ancestral asexual plants commenced to acquire sexual characters there was no hard and fast line between gametes and spores, and that is

[1] Blackman's ('98) results, indeed, may be brought forward to throw discredit upon Dixon's work. The vacillation of so expert and experienced an observer as Strasburger, however, and the careful figures and very positive statements of Dixon, must have some foundation in fact. That fact may well be the variability of the number of chromosomes in different individuals. Blackman's negative evidence has consequently little weight. It may further be noted that in the well-known *Lilium Martagon* the number of chromosomes is subject to variations, as was very clearly shown by Guignard. Moreover, nothing is more patent, in the records of recent research, than the uncertainty with which botanists declare their results as to countings of chromosomes.

still to some extent the case, as the study of apogamy teaches. Gametes were developed along two lines: on the one hand in close relation to a reducing-division, on the other without any such close relation to a reducing-division. The reducing-divisions in plants must then be regarded as having had two distinct origins at least, one of these *possibly* having an origin common with that which occurs in animals.

As sex, including conjugation, had apparently several independent origins in the Thallophytes, it is reasonable to expect such differences. Our wonder is excited more by the uniformity than the diversity of the results as they lie before us.

To guard against misconception, it may be expressly stated that, in forms like *Fucus*, *Dictyota*, and *Achlya*, I see no reason why the number of chromosomes should not have been a very variable one at the outset; whilst gametes with accurately reduced nuclei represented the most complete gametal development, and the law of the survival of the fittest led to the relative constancy in the number of the chromosomes which we now see. Certain apparent inconstancies in the number of chromosomes receive in this way a provisional explanation.

If these considerations have weight, there is no longer any necessity for a laboured reconstruction of well-grounded views as to the nature of Thallophytes to make them fit in with a theory of karyology. Moreover it becomes possible to explain the curious cases of apogamy so common amongst the lower plants.

Bearing in mind the variations met with in the number of chromosomes, it is not unlikely that the number of chromosomes in apogamous gametes may be doubled by growth, under the influence of definite external conditions. In plants like *Fucus* simple atavism would explain this. There is, however, no need for a resort to atavism. The difference in number observed in the chromosomes of the cells of the prothallus of *Pinus silvestris* must arise somewhere and somehow. The nucleus of the macrospore could not have more than one number. All the variations could scarcely

be referred to atavism. Why not refer them to the same category as other variations? The attempt might then be made, if not to refer them to their causes, at least to study them on the same lines as other variable characters. Let us at any rate keep our minds open to the probability that those external conditions which induce apogamy may be just those which bring about alterations in the structure of the nucleus. It is at any rate significant that in the typically apogamous oogonia of *Saprolegnia Thureti* we have an apparently unnecessary division in the gametangia, and a gameto-nucleus develops into a zygote-nucleus without fusion with another gameto-nucleus. There is too at least a probability that apogamy in the Ferns is brought about by alterations in a *group of cells* effected under the influence of certain external conditions. The changes in the nuclei are more likely to be brought about by growth in this case than by fusions.

Let us endeavour to keep in mind the probability that the same morphological elements may, in different plants, be correlated with diverse physiological functions, and in the same plant, and at the same time, have more than one type of work to do, and we shall be a little nearer to the elucidation of the meaning of reduced nuclei.

It is foreign to the purpose of this investigation to pursue these theoretical considerations further, but it may help to turn inquiry into a profitable channel if a sketch be appended to show the possible order of development of plant-forms according to this view.

Asexual forms without sporangia

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Asexual forms with sporangia

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Sexual forms. Reducing-divisions take place in the gametangia. No true alternation of generations:	Sexual forms. Reducing-divisions take place in sporogenesis. A true alternation of generations:
e.g. *Fucus*, *Dictyota*, *Achlya* and *Saprolegnia*:	e.g. Muscineae, Vascular Cryptogams and Spermaphytes:
possibly *Rhopalodia*, *Navicula* and *Brebissonia*.	*Cystopus* (?) according to Berlese ('97): possibly *Spirogyra*, *Closterium* and *Cosmarium*.

When we have full knowledge of the karyology of *Ulothrix*, *Oedogonium*, *Coleochaete*, &c., we shall be able to see how far this tentative view is justified. On Strasburger's view, for example, *Aglaozonia* cells should have twice as many chromosomes in their nuclei as the cells of *Cutleria*. On the view explained here the number should be the same.

Summary.

1. The nucleus in *Achlya americana* var. *cambrica* is bounded by a nuclear membrane, and possesses a central body of spongy texture. This contains chromatin and nucleolar matter. It is neither a nucleolus nor a chromosome. The space between the nuclear membrane and the central body is occupied by nucleo-hyaloplasm and traversed by fine threads of linin.

2. The nucleus undergoes divisions in the mycelium, and the nuclei produced in this way ultimately pass into the sporangia and gametangia.

3. Neither nuclear divisions nor nuclear fusions take place in the sporangia.

4. In the oogonia and antheridia many at least of the nuclei undergo division by a typical indirect method, the number of chromosomes being probably four.

5. No nuclear fusions take place in the gametangia.

6. The supernumerary nuclei in the gametangia are got rid of by digestion or simple degeneration.

7. In my opinion fertilization takes place. The naked egg has *one* central nucleus; oospores with very delicate investing cell-walls have two nuclei, one peripheral, near to a fertilization-tube, the other central; oospores three days old have one central large nucleus. The entrance of the male nucleus into the egg takes place when both gameto-nuclei are in the stage of anaphasis. The fusion of the gameto-nuclei takes place some time after they have assumed the resting condition.

8. The oospore may germinate as soon as ripe, or remain in the resting condition without loss of germinating power

for as long a period as four months. The single nucleus undergoes indirect divisions, and the number of chromosomes which appear during these seems to be eight. About twenty nuclei are produced by successive divisions. One or more germ-tubes are produced, and at the apex of each a small sporangium with from four to ten zoospores. A direct formation of a small mycelium from a germ-tube sometimes takes place.

9. A consideration of the chief facts of karyology, especially in the Thallophytes and in their relation to apogamy, leads to the conclusion that reducing-divisions in the different groups of plants are not all homologous. There is a true homology in the Muscineae, Vascular Cryptogams, and Spermaphytes. In the Thallophytes there are apparently two types of reducing-division which are not homologous.

University College, Cardiff,
November, 1898.

LITERATURE.

The following list includes the most important papers relating to the subject of this communication which have appeared in the last few years. Very complete bibliographies of the Saprolegnieae will be found in the papers by Humphrey and Hartog.

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FARMER, J. BRETLAND, and WILLIAMS, J. LL., '96: On Fertilization and the Segmentation of the Spore in *Fucus*; Proceedings of the Royal Society, Vol. lx, No. 361, p. 188.

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STRASBURGER, E., '97: Cytologische Studien; Berlin.

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EXPLANATION OF FIGURES IN PLATES VIII–X.

Illustrating Mr. Trow's paper on *Achlya*.

The figures were traced with the camera lucida and the finer details filled in by free hand. Most of the work in karyology was actually carried out with the aid of Zeiss's $\frac{1}{12}$ achromatic homogeneous immersion objective (N. A. 1·25) and compensating oculars. All the important results, however, were controlled by observations made with the 2 mm. apochromatic oil-immersion objective (N. A. 1·4). Artificial light was employed throughout for the study of the sections, which were of a uniform thickness of 7·5 μ.

Abbreviations:—*deg. n.*, degenerate nucleus; *f. g. n.*, female gameto-nucleus; *m. g. n.*, male gameto-nucleus; *f. t.*, fertilization-tube.

Fig. 1. Habit of the plant growing on flies floating on water. Nat. size.

Fig. 2. Sexual hyphae with young oogonia. Slightly magnified.

Figs. 3–9. Stages in the development of the same oogonium and antheridium kept under constant observation for about eighteen hours. × 540.

Figs. 10–26. Stages in the development of a second oogonium and antheridium kept under regular observation for fifteen days. The development of the oospheres and of the fertilization-tubes, and the maturation and germination of the oospores are represented in detail. × 540.

Fig. 27. An oogonium, probably apogamous, with three germinating oospores. × 540.

Fig. 28. An oospore, one of three in an oogonium, germinating and producing a mycelium without the intervention of sporangium-formation. × 170.

Figs. 29 and 30. An apogamous oogonium kept under regular observation for a month. Germination took place, but slowly. × 540.

Fig. 31. A group of four zoospores, the nucleus and outline alone represented in one. × 1,200.

Fig. 32. An oblique section of a sporangium. The spores had encysted within it, giving rise to a network of cell-walls. × 1,200.

Fig. 33. A median section of a very young oogonium at the stage corresponding to that of Fig. 2. × 1,200.

Fig. 34. A median section of an older oogonium at the stage corresponding to that of Fig. 3. × 1,200.

Fig. 35. A median section of an oogonium at the stage corresponding to that of Fig. 7. × 1,200.

Figs. 36–40. Small portions of sections of oogonia at the stage corresponding to that of Fig. 8. × 1,200.

Figs. 41 and 42. Median sections of oogonia at the stage immediately preceding *balling*. Fig. 42 about an hour earlier than Fig. 41. × 1,200.

Fig. 43. Median section of a small oogonium producing two eggs at the stage represented in Fig. 12. The young egg represented in median section is *uninucleate*, and the nucleus is surrounded by kinoplasm with fibrous structure. × 1,200.

Fig. 44. Section of an oogonium with two unfertilized eggs, the right-hand egg with a single nucleus; the nucleus of the left-hand egg was in the next section. An antheridium has produced a germ-tube, and a male gameto-nucleus may be seen in it near its apex. × 1,200.

Fig. 45. Section of an oogonium with four eggs, two of which are represented in outline only. The lower egg has a central female gameto-nucleus in which there is no nucleolus; the upper egg has a similar central female gameto-nucleus, and a peripheral male gameto-nucleus at the apex of the fertilization-tube which lies within the egg. × 1,200.

Fig. 46. Section of an oogonium with three binucleate oospores, the female gameto-nucleus of the upper one, however, was in the next section. The central nucleus is provided with a small nucleolus. × 1,200.

Fig. 47. Portion of a section of an oogonium from a five-day culture with three binucleate oospores, a part of one of which is represented in outline only. The gameto-nuclei are in close juxtaposition. × 1,200.

Fig. 48. Portion of a section of an oogonium from an eight-day culture with four binucleate oospores; the nuclei in the resting condition, and suggesting an approaching fusion. × 1,200.

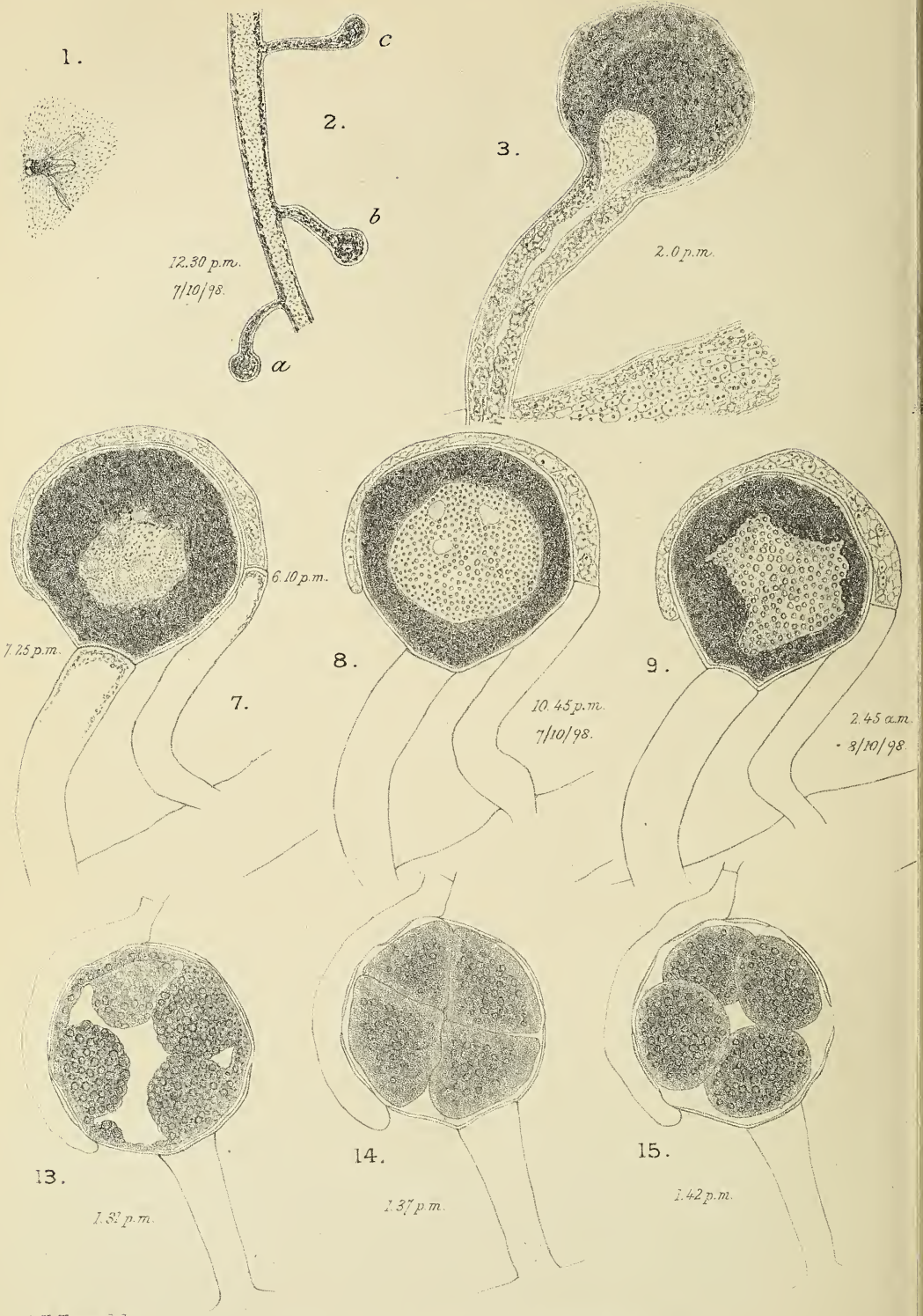
Fig. 49. A single uninucleate oospore from an eight-day culture. There are indications of an incomplete fusion. × 1,200.

Fig. 50. Part of a section of an oogonium from an eight-day culture with uninucleate oospores. × 1,200.

Fig. 51. Uninucleate oospore from a nine-day culture. × 1,200.

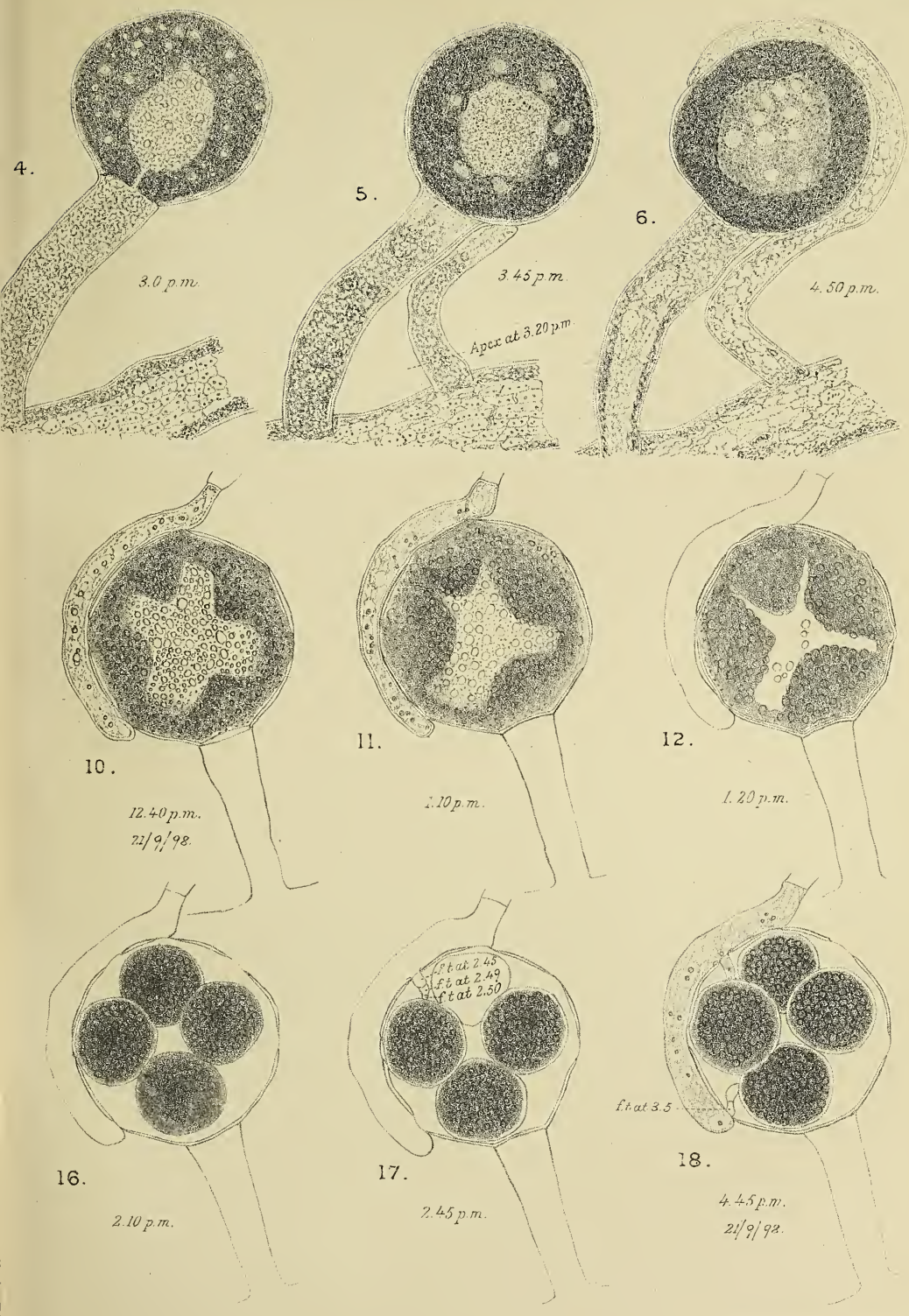
Fig. 52–59. Successive stages in the germination of the oospores. × 1,200.

Figs. 60–70. Nuclear figures from the germinating oospores. They all refer to the later divisions, being taken from oospores with at least eight nuclei. × 2,400.



A. H. Trow, del.

TROW — ACHLYA AMERICANA.



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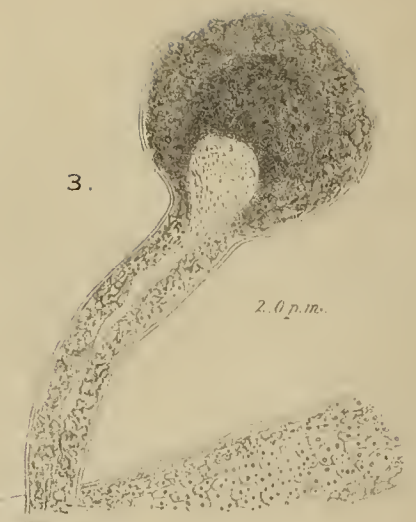

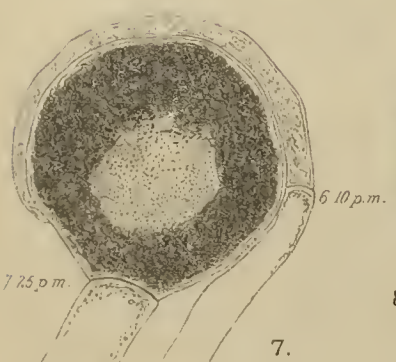

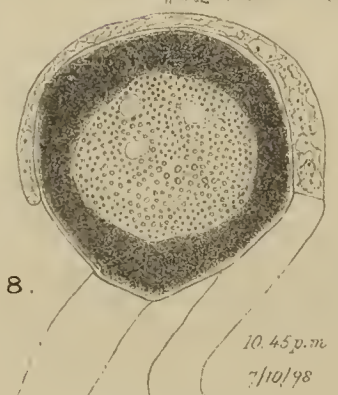

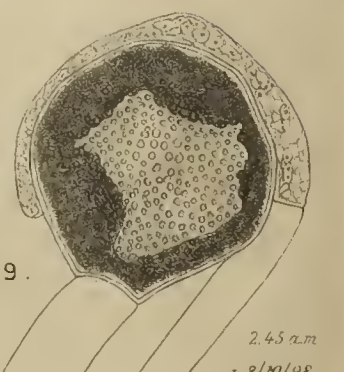

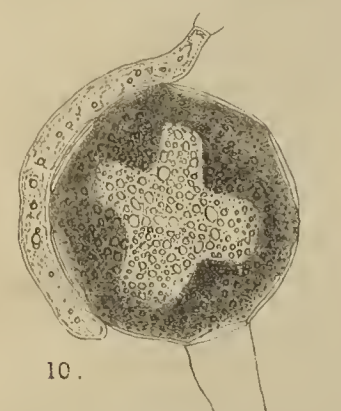

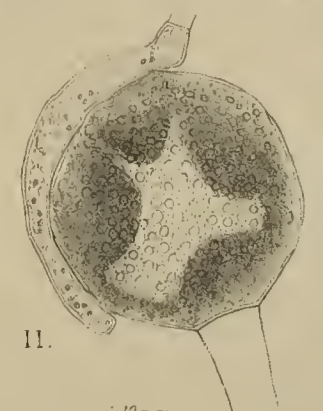

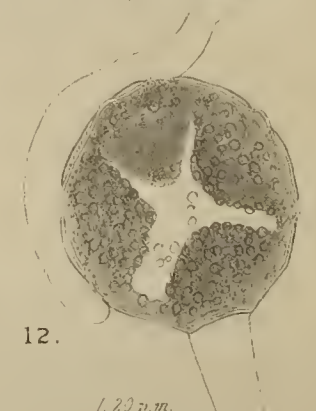

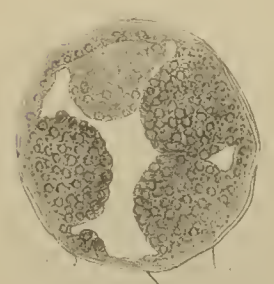

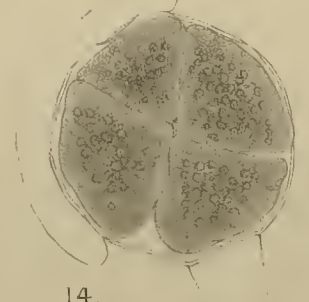

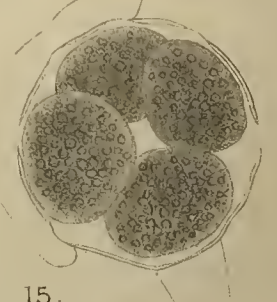

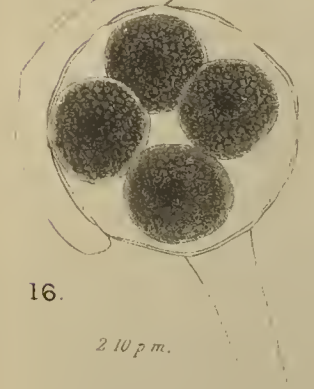

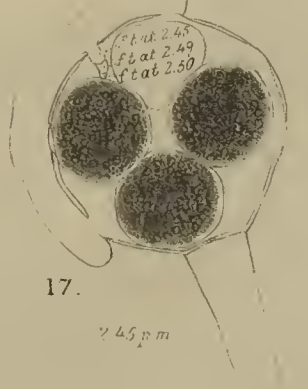

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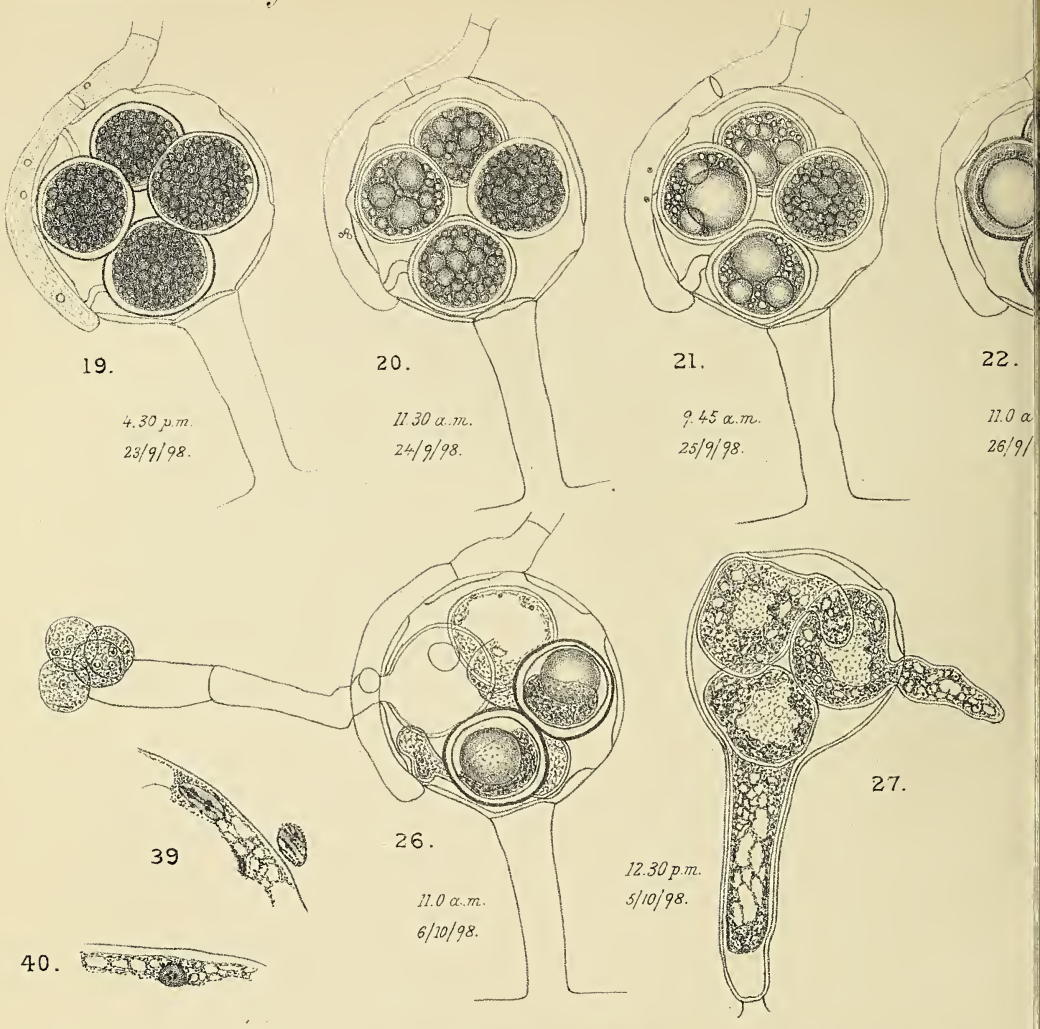

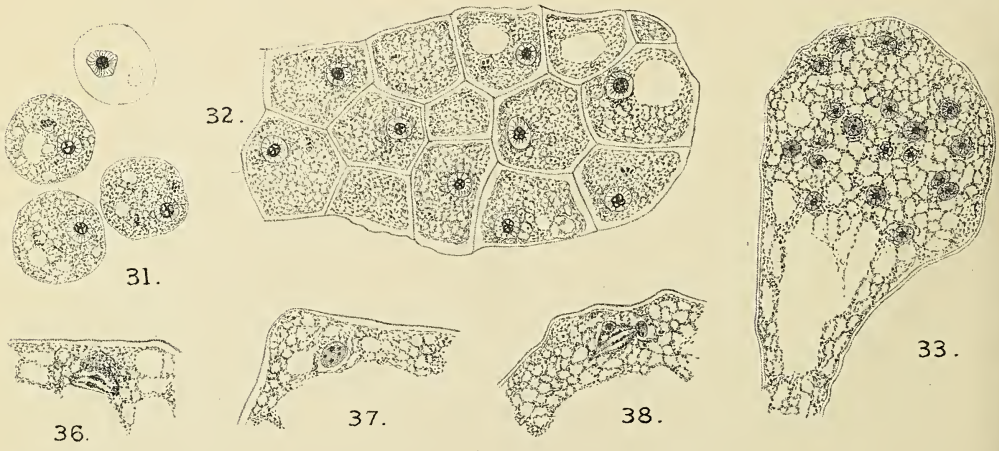

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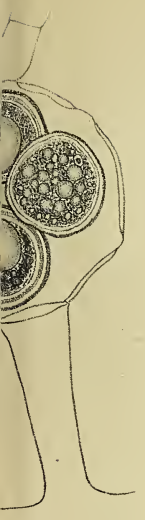

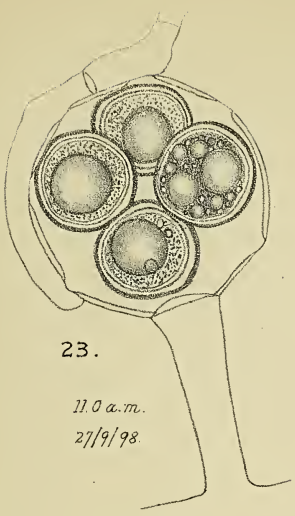

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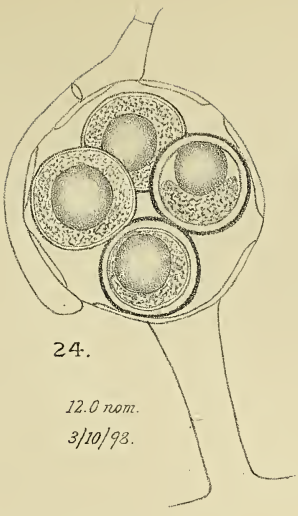

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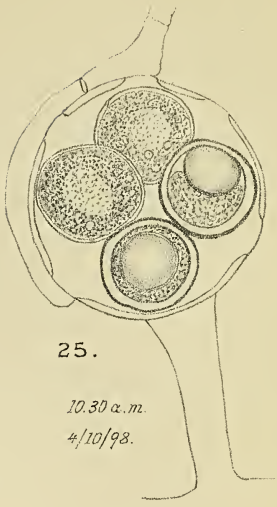

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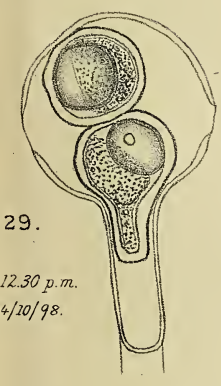

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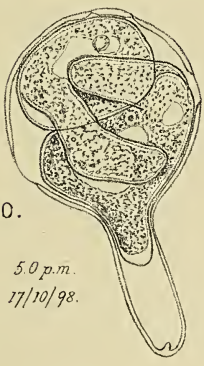

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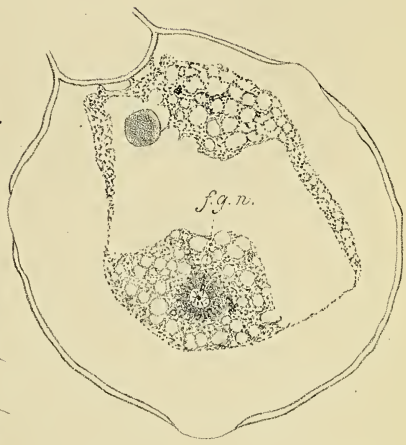

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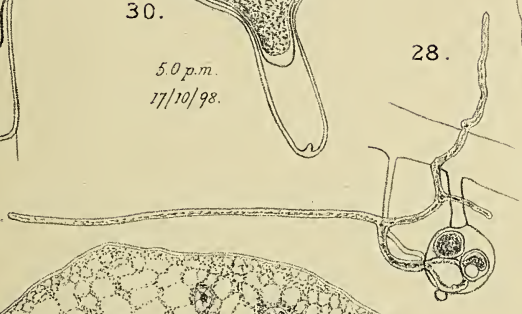

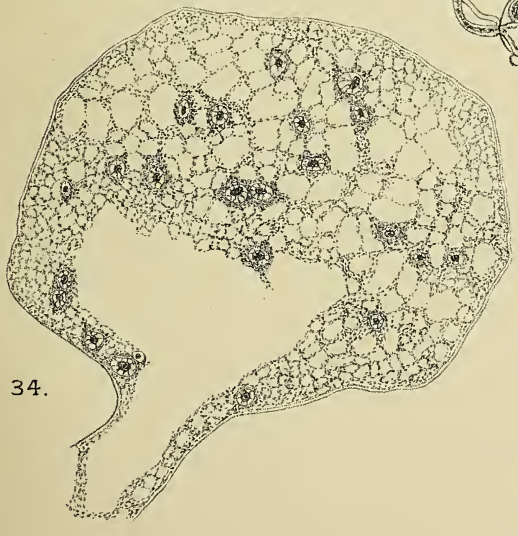

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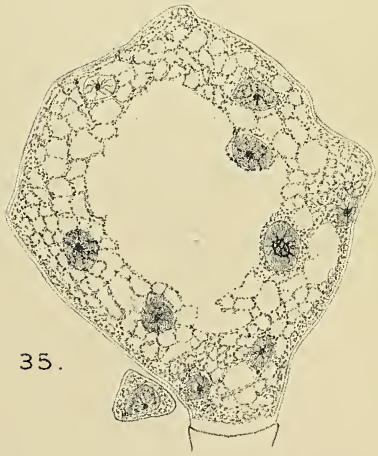

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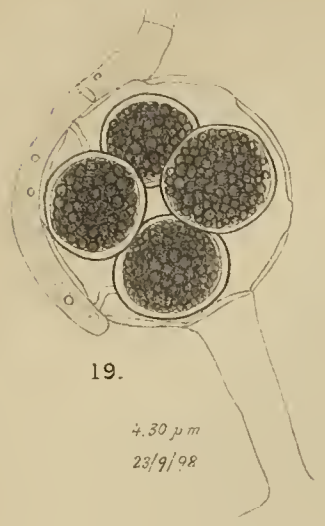

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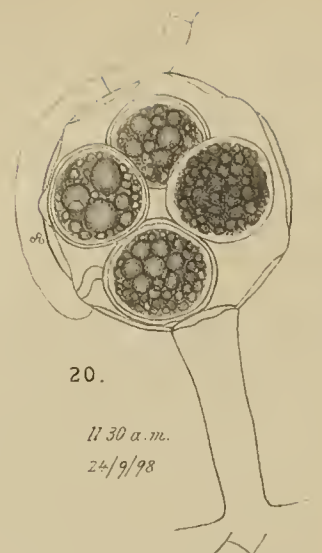

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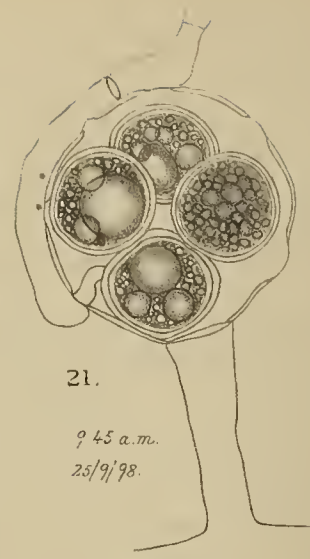

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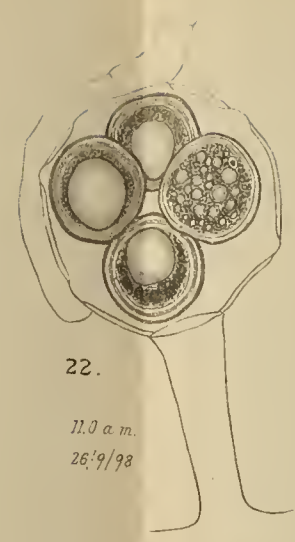

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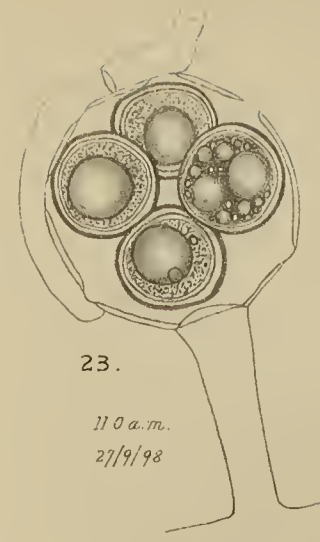

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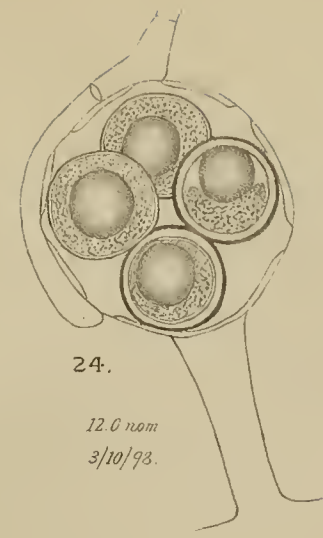

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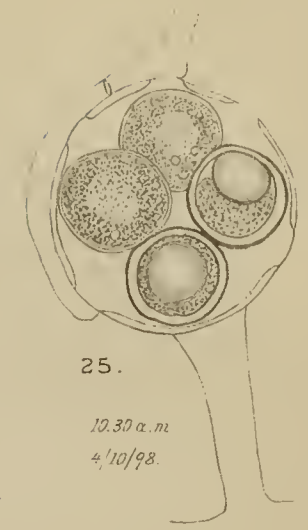

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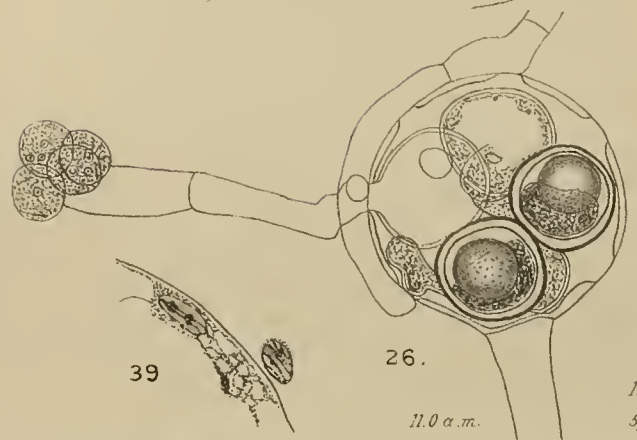

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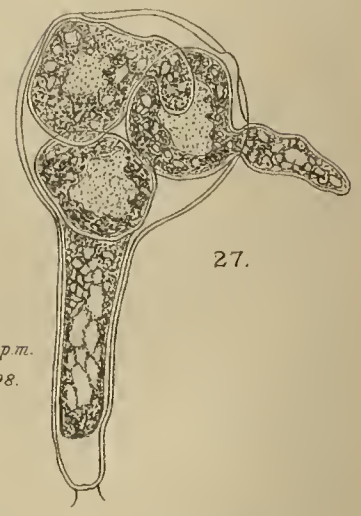

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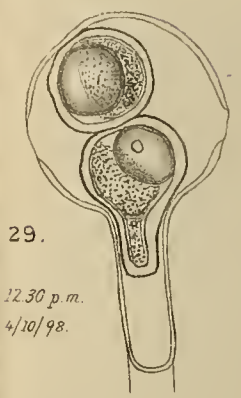

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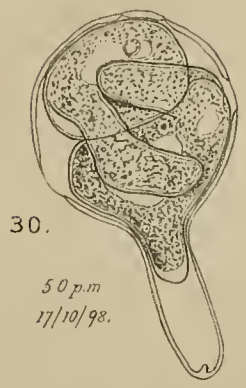

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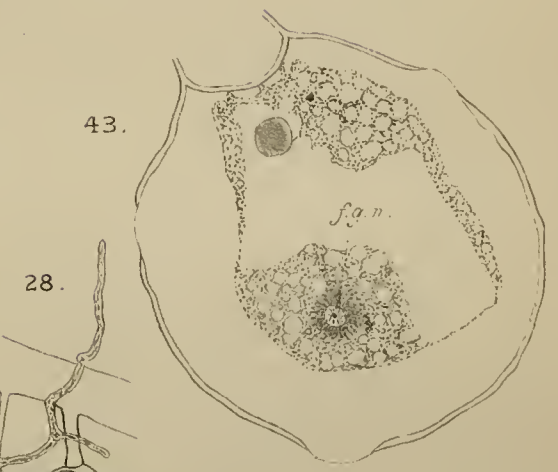

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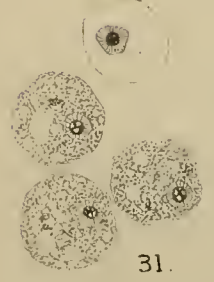

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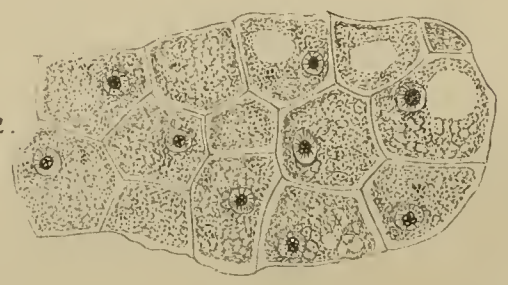

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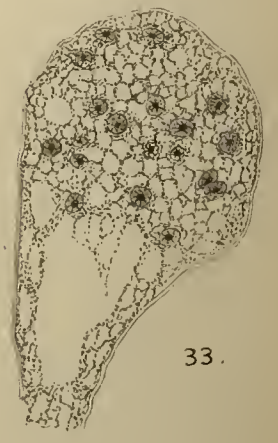

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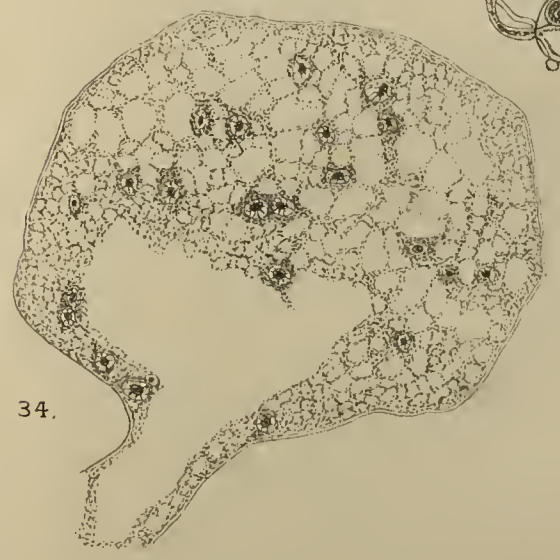

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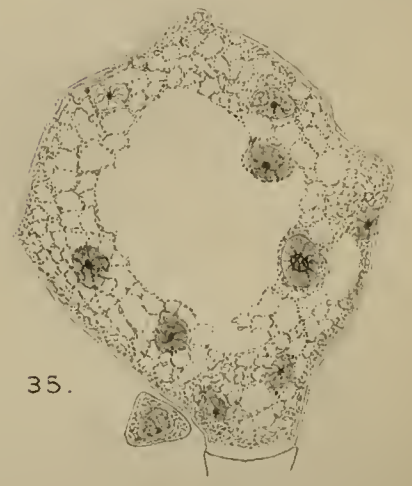

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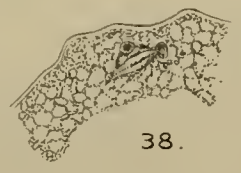

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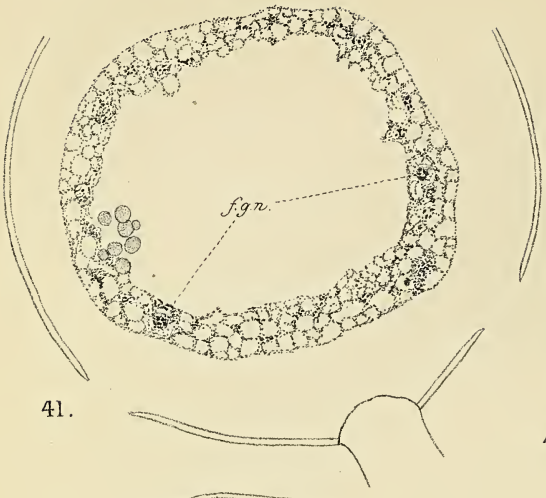

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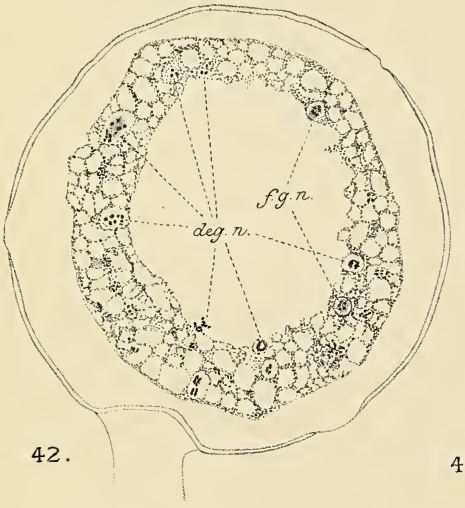

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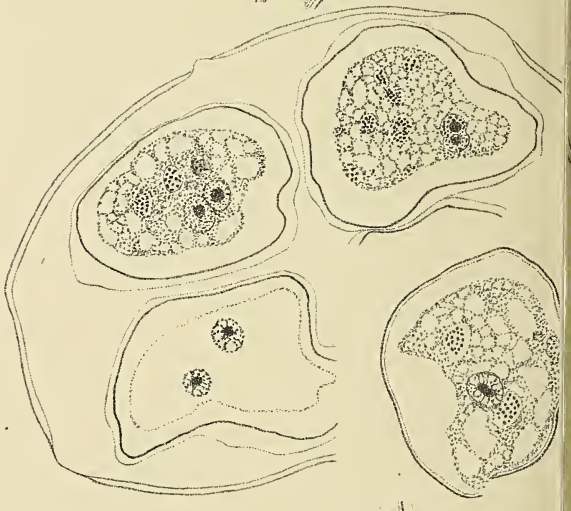

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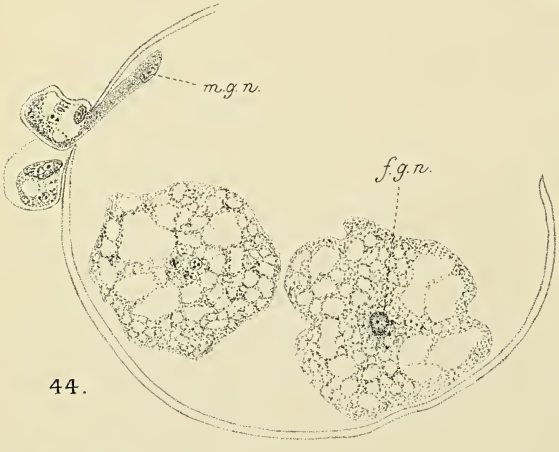

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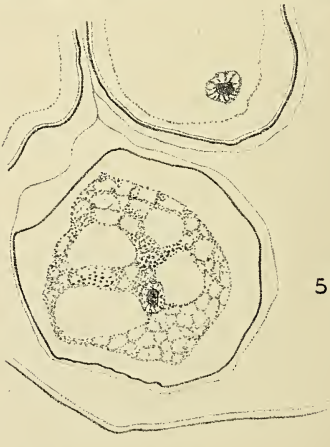

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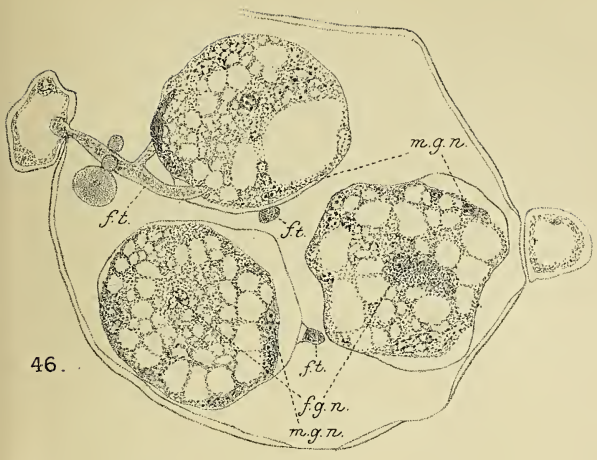

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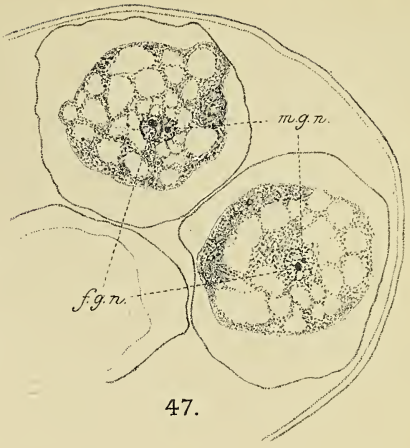

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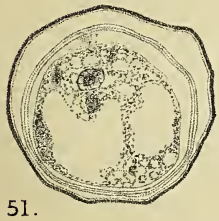

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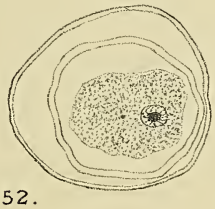

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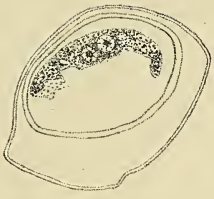

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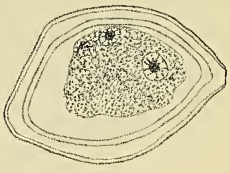

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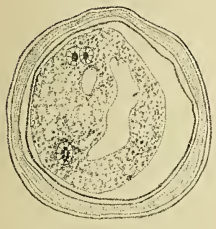

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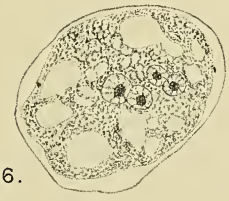

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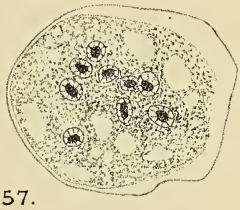

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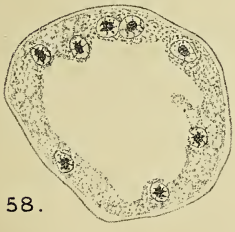

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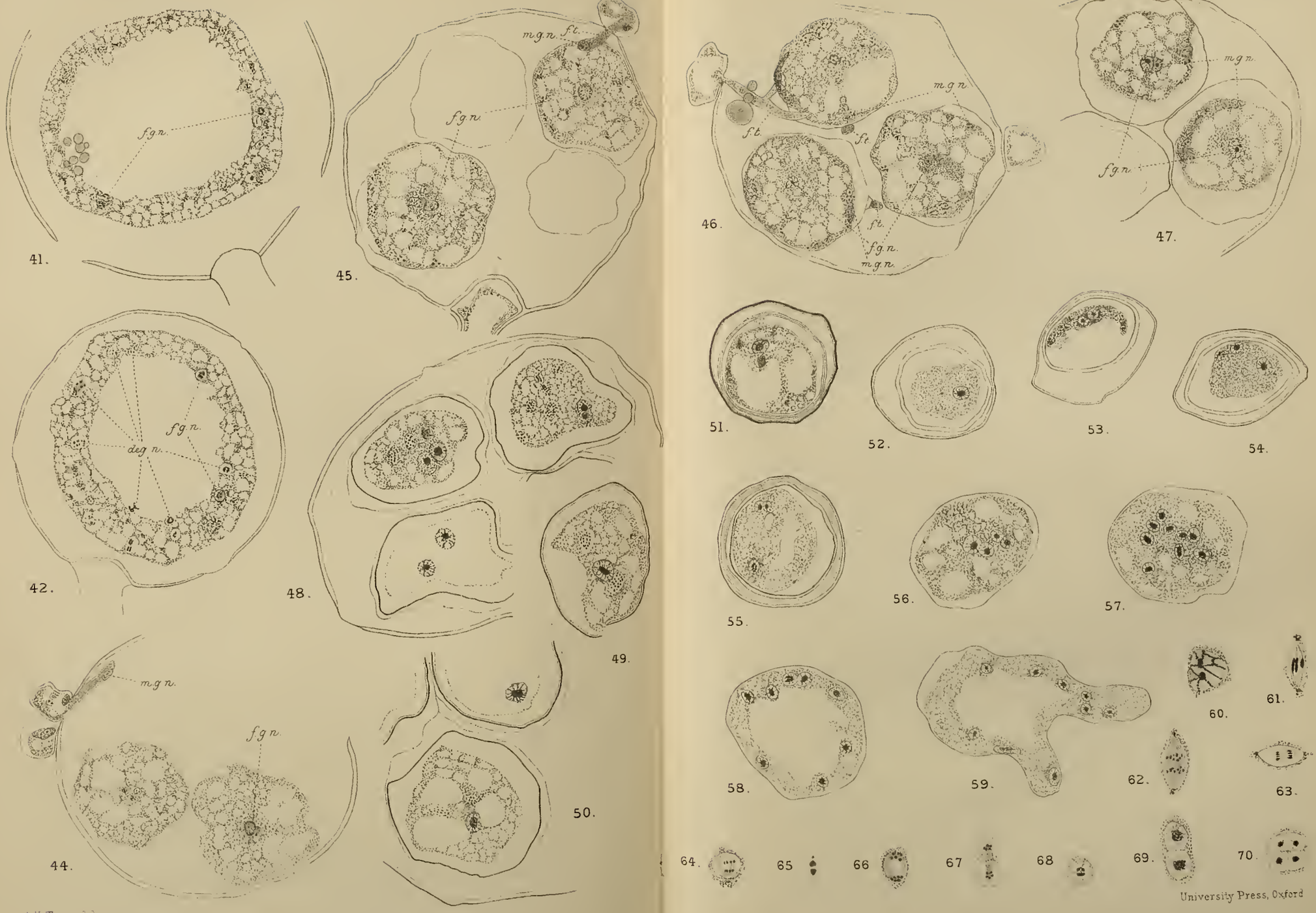

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NOTES.

ON PELARGONIUM RAPACEUM, JACQ.—We know a few plants such as *Collinsia*, of widely separated orders, the flowers of which are interesting in showing the adaptations for fertilization characteristic of the Papilionaceae; one which has more or less escaped notice is *Pelargonium rapaceum*, a native of South Africa. As a polypetalous plant it may indicate to us a course of evolution more parallel to that of the Papilionaceae than that of such a gamopetalous plant as *Collinsia*.

The plants which I have been able to observe were grown in the Royal Gardens, Kew, the roots having been received from Professor MacOwan of Cape Town. The following is a description of the floral mechanism as seen in them.

On the end of a common peduncle, 8–14 inches long, is produced an umbel of ten to twenty flowers: these mature in succession at short intervals, perhaps a third or a half of the total number being open at the same time, but, owing to the strongly marked protandry, in different conditions. The colour of the flower is primrose-yellow with short longitudinal red lines (honey-guides?) on the two upper petals. These two upper petals are bent back below the middle and represent the standard; the two lateral petals form wings, while the anterior petal is folded on itself to construct a keel. In the modification of the three lower petals, auricled and clawed at the base, lies the chief interest.

In the Papilionaceae it will be remembered that the keel is boat-shaped and so narrowed into its two claws that a very free movement of depression is permitted: this too is the case in *Pelargonium rapaceum*, the single claw—for here we have only one petal—forming a short curved spring. Again, in the Papilionaceae the narrow bases

of the wings permit the same freedom of movement, and their limbs, concave or hooked at the base, interlock with the keel: in *Pelargonium rapaceum* the wings are abruptly narrowed into a flexible claw and the concave base of the limb overlaps the keel in its lower part, so that depression of the wings may cause depression of the keel. The mechanism is faulty owing to a want of sufficient rigidity in the petals; but the resemblance in arrangement is most striking.

As the bud opens, it assumes a horizontal position, and the sepals become completely reflexed, the last to bend being the dorsal sepal. When these are out of the way, the two dorsal petals bend below the middle, assuming the position of the standard of a Papilionaceous plant, and the flower is ready for insect-visits. One day is passed in this the male condition, in which a slight depression of the keel causes the upturned dehiscing anthers to be exposed; at the end of it the anthers fall from the open slightly emarginate tip of the keel. On the next day the flower is neutral. On the third day the five stigmas are enabled by the elongation of the style to expand at the tip of the keel, where, some thirty hours before, the pollen was partly exposed. For three or more days the flower, if unfertilized, persists in this female condition, often losing, however, some of the Papilionaceous appearance by a displacement of the wings. In withering, the sepals return to the position they occupied in the bud.

At night the open flower is directed downwards by a nutation of the upper part of the pedicel; and this at the end of the male stage appears to facilitate the falling away of the dehisced anthers.

The dimensions of the parts of the flower are as follows:—posterior sepal 10 mm. × 2½, lateral 9 × 1½, anterior 10 × 2; posterior petals 15 × 3, the claw being 5–6 mm. long; wings 12 × 3, the claw being 1½ mm. long; keel 11 mm. long and 2 mm. deep (i.e. 4 mm. wide if flattened out), its claw equalling those of the wings; tube of the stamens 4 mm. long; spur (nectary) 18–20 mm. long and very narrow. These measurements show the zygomorphy of the flower, which appears to be specially adapted to the visits of butterflies.

In structure and mechanism the flower is certainly most suggestive of the Papilionaceae; and noting its imperfections—viz. want of rigidity in the limbs and of firm interlocking between the wings and keel, failure of the calyx to support the wings and keel necessarily weak at the base, and the imperfect concealment of the sexual organs when the keel is not depressed—we perhaps get a glimpse of a stage

in the evolution of such a perfect organization of that of *Trifolium* or *Cytisus*—a stage not to be found in our familiar Papilionaceous flowers. That a nearly actinomorphic genus such as *Pelargonium* should have produced this form is certainly a matter of interest.

I. H. BURKILL.

Royal Gardens, Kew.

ON MEDULLOSA ANGLICA: A NEW REPRESENTATIVE OF THE CYCADOFILICES [1]. By D. H. Scott, M.A., Ph.D., F.R.S., Hon. Keeper of the Jodrell Laboratory, Royal Gardens, Kew. —The existence of a group of fossil plants combining in their organization certain characters of the Ferns and the Cycads, has been recognized of late years by several palaeobotanists, as, for example, by the late Professor W. C. Williamson, Count Solms-Laubach, Mr. Seward, and the author. The convenient name, Cycadofilices, has recently been proposed by Professor Potonié to designate the group in question, which now includes several somewhat heterogeneous genera, among which *Lyginodendron*, *Heterangium*, and *Medullosa* may be mentioned.

Several species of the genus *Medullosa* (founded in 1832 by Cotta) have been already described, from the Permian and Upper Coal-measures of the Continent. They agree in the extraordinarily complex structure of the stem, which, as shown by Zeiller and Solms-Laubach, resembles in the ground-plan of its organization that of a highly differentiated Fern of the usual polystelic type, but with the addition of a zone of secondary wood and bast, sometimes reaching an immense thickness, developed around each stele. The mature stem thus acquired a Cycad-like character. The structure, however, has been extremely difficult to interpret owing to the comparative rarity and incomplete character of the specimens hitherto known.

No stem of a *Medullosa* has hitherto been recorded from this country, though specimens of *Myeloxylon*, now known to have been the petioles of *Medullosa*, are frequent in the calcareous nodules of the Lower Coal-measures.

The author has recently had the opportunity of investigating several excellent specimens of a new species of *Medullosa* from the Ganister Beds of Lancashire. These fossils are of special interest on

[1] Abstract of a paper read before the Royal Society, January 26, 1899.

several grounds ; they are considerably more ancient than any members of the genus previously described, they are the first English specimens recorded, they are preserved in a more complete and perfect form than any others at present known, and lastly, the greater simplicity of their structure causes the essential characters of the genus to stand out with greater clearness than in the more complex species. The specimens were discovered by Mr. G. Wild and Mr. J. Lomax, in material from the Hough Hill Colliery, Stalybridge. The sections have been cut, with the greatest skill and success, by Mr. Lomax, and are very numerous, about 100 sections, transverse and longitudinal, having been examined from one specimen alone.

The principal specimens are four in number, in addition to which other fragments have been included in the investigation. The species, which is very distinct from any form previously described, will be known as *Medullosa anglica ;* a diagnosis is given below.

The most complete specimen of the stem has a mean diameter of rather more than 7 cm., including the adherent leaf-bases. The others do not appear to have been very different in dimensions.

The large leaf-bases, to judge from the most perfect specimens, almost completely clothed the surface of the stem. They were decurrent, and confluent with the stem for a vertical distance of 13 cm. or more ; the diameter of the petiole, where it became free from the stem, being about 3 or 4 cm. The arrangement of the leaves was a spiral one, and in the only case where the phyllotaxis could be determined the divergence proved to be $\frac{2}{5}$.

In two of the specimens the external characters of the fossil are well shown. The external surface of the long leaf-bases is marked by a conspicuous longitudinal striation, the ribs (which would not have been so prominent during life) representing the fibrous strands of the hypodermal tissue. The habit of the stem, clothed with the long, almost vertical, overlapping leaf-bases, may have been not unlike that of some of the tree-ferns, such as *Alsophila procera.*

The vascular system of the stem consists of three (or locally four) steles, anastomosing and dividing at long intervals. Each stele has an elongated, somewhat irregular, sectional form, and is composed of a central mass of primary wood, surrounded by a zone of secondary wood and phloem. The primary wood, which is very well preserved, is made up of tracheides and conjunctive parenchyma, with the spiral elements (protoxylem) scattered near its outer margin. The secondary

wood consists of radial series of tracheides and medullary rays; the secondary tracheides bear multiseriate bordered pits on their radial walls; most of the primary tracheides are pitted in the same way, but on all sides alike. In the neighbourhood of the protoxylem-groups the tracheides of the primary wood are spiral or scalariform. The phloem is made up of elongated elements, presumably the sieve-tubes, forming a net-work, the meshes of which are occupied by the phloem-rays.

Each stele of *Medullosa anglica* shows the closest agreement in structure with the single stele of a *Heterangium*, so that the stem of this *Medullosa* might well be concisely described as a polystelic *Heterangium*.

The course of the leaf-trace bundles was followed very completely in consecutive series of transverse, and in longitudinal, sections. The leaf-traces leave the steles precisely in the same manner as in *Heterangium*. On becoming free the trace is a large concentric bundle, surrounded by its own zone of secondary wood and bast. As it passes obliquely upwards through the cortex, the trace loses its secondary tissues, and undergoes repeated division into a number of smaller bundles, each of which has collateral structure. These collateral strands have in all respects the same arrangement of their elements as the well-known bundles of *Myeloxylon*.

The base of the leaf received a large number of bundles, consisting of the ultimate branches derived from the subdivision of several of the original leaf-traces. This distribution of the bundles is peculiar and unlike that in any known plants of Cycadean affinities.

In a few cases accessory vascular strands, of concentric structure, recalling the cortical bundles of a *Cycas*, were found to the outside of the normal stelar system.

The stem formed a well marked zone of internal periderm. In one specimen the whole of the outer cortex, with the leaf-bases, had been exfoliated, so that in this case the periderm formed the external surface.

The leaf-bases and petioles present in all respects, as regards hypoderma, vascular bundles, and gum-canals, the characters of the *Myeloxylon Landriotii* of Renault, which was evidently not a species, but a type of leaf-stalk common to various Medulloseae. The petioles branched repeatedly, the finest ramifications of the rachis having a diameter of about 1 mm. only, but retaining in essentials the '*Myelo-*

xylon' structure. The leaf was thus a highly compound one; the structure of the leaflets associated with the rachis, agrees well with that of the *Alethopteris* leaflets, figured by M. Renault.

The roots, never previously observed in any species of *Medullosa*, were of triarch structure, with abundant formation of secondary wood and bast, and an early development of internal periderm, by which the primary cortex was thrown off. Developmental stages show that the periderm originated in the pericycle. The roots, which branched freely, were borne on the stem in vertical series, between the bases of the leaves. They were attached to pedicels, through which the vascular tissues of the roots were continuous with those of the stem. The author is indebted to Mr. J. Butterworth and Mr. G. Wild, for specimens which have thrown important light on the connexion between root and stem.

The full paper concludes with a short historical *résumé*, and a discussion of affinities.

Medullosa anglica, in the structure of its stem, shows unmistakable affinities with *Heterangium*, perhaps the most fern-like of the genera grouped under Cycadofilices. The new species is far simpler than any *Medullosa* hitherto described, for the steles are not only few but are uniform, showing no differentiation into a peripheral and a central system. The small central steles, called 'Star-rings' in other Medulloseae, are absent here. In these and other points the species agrees with the genus *Colpoxylon* of Brongniart, but as that genus is doubtfully distinct and its leaves are not known, it is not proposed to unite the English species with it.

In the structure of the petioles and the leaf generally, *Medullosa anglica* is as highly organized as any of the Medulloseae, and agrees closely with *M. Leuckarti*, the only other species in which the connexion between leaf and stem has been at all satisfactorily proved.

In the structure of the petioles, and of the roots, in the secondary tissues, and in the secretory canals, which occur throughout the plant, there are clear points of agreement with Cycads, though the primary structure of the stem was that of a Fern. The affinities in the latter direction come out more clearly in *Medullosa anglica* than in any of the other species as at present known.

The habit of the leaves, if as appears likely, they were of the *Alethopteris* type, must have been fern-like; but that in itself, as

the familiar example of *Stangeria* teaches, is as consistent with Cycadaceous or with Filicinean affinities.

When *Medullosa* thus combines, in a striking manner, the characters of Ferns and Cycads, the author is not disposed to regard it as having lain very near the direct line of descent of the latter group. It is more probable, as Count Solms-Laubach has suggested, that the Medulloseae represent a divergent branch, which has left no descendants among existing vegetation.

Medullosa anglica, sp. nov.

Stem vertical, clothed by large, spirally arranged, decurrent leaf-bases, perhaps cast off in old stems. External surface of leaf-bases longitudinally striate.

Vascular system of stem consisting of a few (usually three) uniform steles, somewhat elongated and lobed as seen in transverse section. Star-rings absent. Interior of each stele wholly occupied by primary wood.

Secondary wood and bast of moderate thickness, developed on all sides of the steles. Tracheides usually with bordered pits.

Leaf-traces concentric on leaving the steles, branching and becoming collateral in traversing the cortex.

Leaf-bases and petioles with the structure of *Myeloxylon Landriotii*, Ren.

Leaves highly compound.

Gum-canals abundant in the petioles and leaf-bases, and in the cortex, and around the steles of the stem.

Adventitious roots borne in vertical series, triarch, with secondary wood and bast, and periderm.

Stem with leaf-bases, about 7–8 cm. in mean diameter.

Petioles about 2·5–4 cm. in diameter at base, diminishing to about 1 mm. in the ultimate branches of the rachis.

Leaflets about 3 mm. wide.

Roots reaching 12 mm. in diameter.

Locality: Hough Hill Colliery, Stalybridge, Lancashire.

Horizon: Lower Coal-measures.

Found by Messrs. G. Wild and J. Lomax, 1892–98.

NEW FUCUS HYBRIDS.—Thuret, in the Études Phycologiques, describes his attempts to produce crosses between various members of

the Fucaceae. Hybrids were successfully obtained between *Fucus vesiculosus* ♀ and *F. serratus* ♂, but all the other experiments resulted in failure. The following additional observations may be of interest:—

1. Last November attempts were made by the writer to obtain hybrids between *Ascophyllum* and *F. vesiculosus*. When antherozoids of the latter were added to eggs of the former, no evidence of fertilization was observed: but the reverse cross (*Ascophyllum* ♂ and *F. vesiculosus* ♀) gave a limited number—about one in twenty—of segmenting oospores, while a much larger number became invested with walls. Similar results were given on two other occasions on which the experiment was tried. One of these lots, started Nov. 10, 1898, was allowed to grow, and at the date of writing (Feb. 11, 1899) the plants are quite healthy; they have long rhizoids, most of them are beginning to branch, and several have their first barren conceptacle forming.

2. Antherozoids of *F. serratus* were added to eggs of *Ascophyllum*. About a fourth of the number produced investing walls, a very few showed commencing rhizoids, but only two or three segmented, and even these soon stopped growing.

In view of the recent observations on the fertilization of *Halidrys*, the formation of an investing wall can safely be regarded as evidence of the entry of an antherozoid into the egg. It will be interesting to see whether the absence of segmentation in such an egg is due to the failure of the antherozoid to reach and fuse with the egg-nucleus.

3. Antherozoids of *Halidrys* were added to eggs of *F. vesiculosus*. They gyrated on the surface of the eggs exactly as they do on their own, but in smaller numbers and for shorter periods. Here no evidence of fertilization could be seen.

4. The circumstance which led to this series of experiments was the finding in the Menai Straits of a plant which in external characters seemed intermediate between an *Ascophyllum* and a *Fucus*, while the conceptacles were found to contain both antheridia and oogonia.

J. LLOYD WILLIAMS, Bangor.

Pseudo-Pleurococcus, Nov. gen.

BY

JULIA W. SNOW, Ph.D.

With Plate XI.

PLEUROCOCCUS VULGARIS, though one of the most common of all Algae and one that has received much attention, is also one about which there still exist widely differing views.

Klebs (5), Artari (1), and Gay (4) hold that *Pleurococcus vulgaris* is a perfectly constant form which reproduces only by means of vegetative division, and either exists as single cells or forms small cell-complexes more or less quadrangular in shape. This, according to these authors, is accidental, and division in other directions soon occurs, producing the normal small complexes.

Chodat (3), on the other hand, states that '*Pleurococcus* in the various conditions of its evolution may develop branches, filaments, sporangia with zoospores, gametes, and spores.' And under the name of *Pleurococcus* he would include 'not only the common form without a pyrenoid, but also the similar one with a pyrenoid (*Pl. simplex*, Artari), as well as such very different forms as *Cystococcus* and *Protococcus vulgaris*.' Further, he regards *Pleurococcus* as one of the Chaetophoraceae in a stage of degradation, owing to its existence as a Lichen-gonidium.

[Annals of Botany, Vol. XIII. No. L. June, 1899.]

That certain unicellular organisms are but phases of some higher Algae, assumed under some special condition, is now well recognized, and more and more is it evident that external conditions affect external characteristics. But it has been the result of my observation in all such cases, that the internal characteristics of a cell remain the same, however much external appearance may vary. Never have I observed the least variation in the internal organization of any species, although I have kept numerous unicellular and polymorphic forms in pure culture for many months at a time. With this in mind it is difficult to understand how Chodat can include under one species cells with and without a pyrenoid, and with either a parietal or a stellate chromatophore, as he does in the case of *Pleurococcus vulgaris.*

The view held by Klebs, Artari, and Gay with regard to *Pleurococcus vulgaris* is undoubtedly correct, as I have been able to verify in Klebs' own cultures and in numerous cultures of my own. There are, however, forms similar to that described by Chodat (2), which in their unicellular condition cannot readily be distinguished from *Pleurococcus vulgaris*, and which, under the influence of moisture, do produce filaments. But a close comparison of these forms shows differences which would prevent them from being classified with *Pleurococcus vulgaris.* Whereas in the latter species the chromatophore lines the whole inner surface of the membrane, in the corresponding small cell-complexes of the other forms the chromatophore is found in only a portion of the cell. *Pleurococcus vulgaris*, also, contains no pyrenoid, while in the filamentous forms which have come to my notice a pyrenoid is present. Chodat, however, found none in the form he studied.

One significant difference between these filamentous forms and *Pleurococcus vulgaris* is the response to cultivation. The latter form lives but a short time in Knop's solution of various concentrations, but under the very same conditions the forms which produce filaments grow well for any length of time.

In referring to this form described by Chodat (2), Klebs regards it as a *Stigeoclonium* which normally exists in a

protococcoid condition, and he believes it should be classified as such. Zoospores, however, have not been observed to be formed from the filaments, and on this account it would seem best to separate these forms from both *Pleurococcus* and *Stigeoclonium* and to create a new genus. As they resemble *Pleurococcus* so closely they might appropriately be called *Pseudo-Pleurococcus*. A description of two of these forms will here be given.

Pseudo-Pleurococcus botryoides, Nov. sp.

This was found on the bark of a tree near Ann Arbor, Michigan, in December '97, and formed a thick, dark-green, pulverulent covering, quite like that formed by *Pleurococcus vulgaris*. Microscopical examination showed it to consist of single cells and of parenchymatous masses varying in size from two cells up to clusters large enough to be easily distinguished by the naked eye (Figs. 1–6). The cells measure 6·5–7·8 μ in diameter. The chromatophore lines the membrane, but presents a somewhat irregular opening on one side. Opposite this opening lies the pyrenoid. The membrane is thin and gives the reaction for cellulose with iodine and sulphuric acid. The nucleus is single and lies near the centre.

Material directly from the bark was placed under cultivation in Knop's solution of various concentrations, and on agar mixed with the same solution. All of these media possessed more moisture than was present on the bark, and the result was that all the cultures began immediately to produce filaments, one arising from almost every superficial cell (Figs. 7, 8, 14, 15). Growth of the filaments continued for some time in all media alike; but after the Alga became accustomed to the stimulating effects of the moisture, development varied according to the condition. On agar, and in Knop's solution of ·4 % and 1 %, the filaments gradually began to assume the parenchymatous form; while in weaker solutions, such as ·1 %, the filaments continued to grow and to branch, until

large radiating clusters were produced fully 1–2 mm. in diameter. A small one of these is shown in Fig. 16.

The transformation from the filaments to the parenchymatous masses began by longitudinal division taking place in the filaments (Fig. 13), when both longitudinal and transverse division continued until all semblance of a filament was lost. In ·4% Knop's solution, if at any time during the transformation fresh nutritive solution, or even water, were added, the process was interrupted and new filaments were sent out from the rounded cells. In time these filaments also began to become parenchymatous; but each time that fresh liquid was added filaments were produced, so that by adding liquid and then allowing the filaments to become parenchymatous, the shape and the nature of the development could, within certain limits, be controlled.

Agar-cultures with little moisture seemed to reproduce best the conditions in nature. At first filaments were formed, but these soon passed into parenchymatous clusters of cells which afterwards became more or less disorganized; the cells became rounded and the connexion became less intimate, so that, by disturbance, disintegration of the clusters occurred; usually, however, two, four, or more cells remained united.

The development can be traced from a single cell. If a cell from bark be placed in liquid it will develop directly into a filament (Figs. 7, 8). In a ·4% Knop's solution, however, a cell which had already become accustomed to this solution was seen to develop directly into a parenchymatous mass with a tendency to disintegration (Figs. 9–12), and one instance was noticed under similar conditions where cells separated directly after division, thus causing the Alga to remain in a unicellular condition.

Strong nutritive solutions and also weaker solutions, providing the Alga has existed in these for some time, produce the same effect as existence in the atmosphere; that is, the parenchymatous and unicellular conditions occur, but change from the atmosphere to liquid always produces filaments.

In general we can say that when the form exists in a medium

where there is little moisture, the *Pleurococcus*-like condition is produced, the cells being either single or in small clusters; but when subjected to much moisture filaments are produced. This would seem to hold true also in nature, for even among the parenchymatous masses of cells taken from bark the form of the masses often suggested filaments, which had possibly been produced during a wet period, and afterwards transformed to the parenchymatous condition during a following dry season.

Whether we can regard this as a degenerate form of Alga which reverts to a filamentous form when placed in liquid media, is difficult to say. Might we not with equal right regard the filaments as a higher form which is being evolved from the lower by being placed under more favourable conditions?

PSEUDO-PLEUROCOCCUS VULGARIS, Nov. sp.

This form is characterized in its aerial existence by smaller cell-complexes than the preceding species, and in liquid media by branching less profusely (Figs. 17, 18). The mode of branching also is different. Instead of branches arising from any cell along the filament, their origin is terminal, two growing-points being developed at the apical cell.

The chromatophore does not occupy the whole cell, but lines only a portion of the cell-wall; and in the filamentous form often more than one chromatophore is present. The single cells are 6·5 μ in diameter; a pyrenoid is present; no blue colour was produced in the membrane either with chlor-iodide of zinc or with iodine and sulphuric acid.

The conditions governing form in this species are the same as in the preceding. In liquid culture-media filaments were produced, while on agar with Knop's solution total disintegration took place. The transformation from the filamentous to the unicellular condition also began by longitudinal division occurring in the filaments (Fig. 19). The process of disintegration, however, took place more rapidly and was carried farther than in the first species, for, within three weeks' time,

a filamentous cluster transferred to agar changed completely to the unicellular condition.

This form resembles *Pleurococcus vulgaris* greatly. It also resembles the illustrations of the form described by Chodat; but as this contains a pyrenoid, and none was found in the species he studied, it cannot be the same.

If these filamentous forms be regarded as a genus separate from *Pleurococcus*, the genus would be characterized as follows:—

Pseudo-Pleurococcus, Nov. gen.

Thallus, in the atmosphere either unicellular or forming parenchymatous masses of cells of various size; in liquid media filamentous. Cells 6·5–8 μ in diameter. Chromatophore parietal, but not completely lining the membrane. Nucleus single.

Pseudo-Pleurococcus botryoides, Nov. sp.

In atmosphere occurring as single cells, or forming dense clusters of various size; in liquid media profusely branched, the branches arising laterally from the filaments. Cells 6·5–7·8 μ in diameter. Chromatophore in the single cells reduced, pyrenoid present. On bark of trees near Ann Arbor, Michigan.

Pseudo-Pleurococcus vulgaris, Nov. sp.

In atmosphere occurring as single cells, or in cell-complexes of four or multiples of four; in liquid media branched, but not profusely so, the branches originating by the division of the apex of a terminal cell. Chromatophores line but a portion of the membrane in each cell of the filament; often two or more occur in a single cell. Diameter of cells 6·5 μ; pyrenoid present in the chromatophore. On bark of trees in Basel, Switzerland.

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3. Chodat, R.: On the Polymorphism of the Green Algae and the Principles of their Evolution; Annals of Botany, Vol. XI, No. 41, 1897.
4. Gay, Fr.: Recherches sur le développement et la classification de quelques Algues vertes; Paris, 1891.
5. Klebs, G.: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen; Jena, 1896.

EXPLANATION OF FIGURES IN PLATE XI.

Illustrating Miss Snow's paper on *Pseudo-Pleurococcus*.

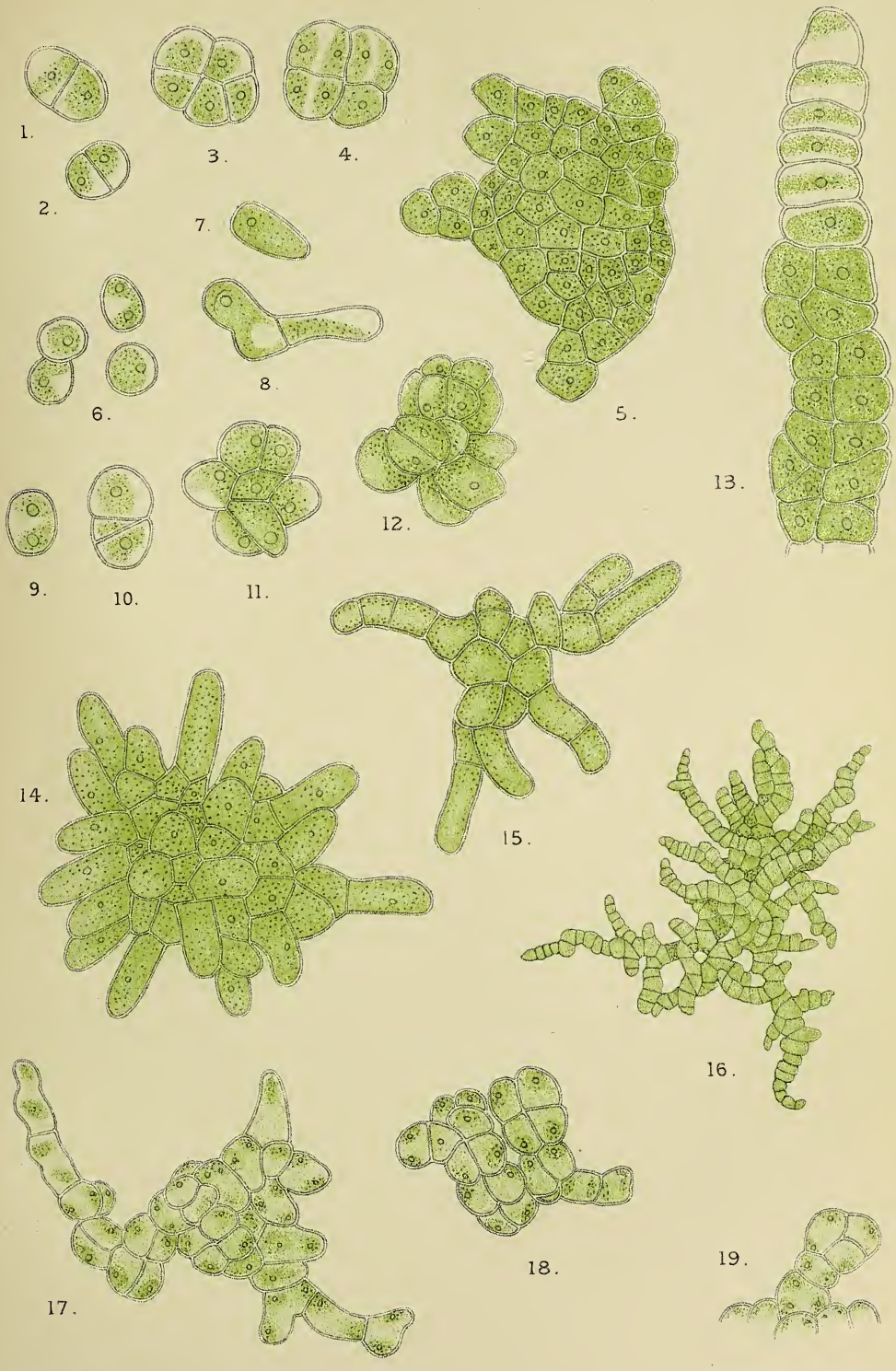
All figures were drawn with an Abbe camera. In all figures except Fig. 16 a 3 mm. Zeiss apochromatic objective and a No. 6 compensating ocular were used. In Fig. 16 the same ocular was used, and an 8 mm. lens.

Pseudo-Pleurococcus botryoides, Nov. sp.

Figs. 1–6. Different stages found on bark.
Figs. 7, 8. Development of unicellular stage into filamentous stage.
Figs. 9–12. Development of unicellular stage into parenchymatous stage.
Fig. 13. Development of filamentous stage into parenchymatous stage.
Figs. 14, 15. Development of parenchymatous stage into filaments.
Fig. 16. Filamentous stage from liquid medium.

Pseudo-Pleurococcus vulgaris, Nov. sp.

Figs. 17, 18. Clusters with filaments in different stages of development.
Fig. 19. Beginning of transition from filamentous to parenchymatous and unicellular stages.



Snow del. University Press, Oxford.

SNOW. — PSEUDO-PLEUROCOCCUS.

Thames Bacteria, III.

BY

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With Plates XII—XIV.

IN previous papers I have given some types of Bacteria from the Thames, selected as showing very definite characters, some of which were studied in detail. During my investigations of the Thames Flora, however, it was constantly found that certain well-known types of Bacteria are represented by varietal forms of considerable interest because the close and continued study of them might be expected to throw light on the vexed questions of species and fixity of type in Schizomycetes. Some of these groups of varieties were studied for long periods, and the results of such work on one well-marked group are here offered. I regard the forms described below as all belonging to one type; but since this breaks up very naturally into a series of yellow forms and one devoid of colouring-matter, it was judged convenient to regard them as forming two groups (VI and VII) in the survey given in the Fifth Report to the Water Research Committee of the Royal Society [1].

[1] Proc. Roy. Soc., Vol. lxi, No. 376, 1897.

[Annals of Botany, Vol. XIII. No. L. June, 1899.]

As will be shown below, some very remarkable biological characteristics have been determined for this type, which would doubtless repay more prolonged and closer study than I have accorded them.

That this type is related to a whole series of yellow pigmented Bacteria on the one hand, and of powerfully liquefying non-pigmented forms on the other, is rendered probable from the following results.

Group VI.

The Proteus Type.

Nos. 103, 34, 51, 61, 9, 9*a*, ι, φ, ζ, μ and θ.

In the present section I bring together a series of forms which include some 'species' already well known to water-bacteriologists, and best typified by *Proteus vulgaris* and its allies. Several authorities have already disagreed as to how far these do or do not liquefy gelatine—my studies lead to the conviction that the power of liquefaction varies with the conditions, both external and internal.

In his remarkable book entitled 'Ueber Fäulnissbakterien[1]' Hauser published in 1885 the results of his investigations into the bacteriology of three forms or species of *Proteus*, as he named them: these are *P. vulgaris*, *P. mirabilis*, and *P. Zenkeri*. Various criticisms have been directed against Hauser's naming of this new genus, and many observers have refused to take these forms out of the genus *Bacillus* on the evidence adduced: I do not propose to discuss this, merely remarking that the genus *Bacillus*, as at present defined, should depend on the formation of endogenous spores in the rodlets, whereas the literature teems with so-called species of *Bacillus*, in which no such spores have been observed. In any case Hauser seems to have hit on a good name, for the forms are evidently eminently protean in character, so far as peculiarities of colonies go.

[1] Leipzig, 1885, F. C. W. Vogel.

A more important point seems to be that no one has contradicted Hauser's main facts, which are the following:— these three 'species' occur together in putrefying flesh-infusions, and form rodlets in long filaments: the rodlets are about 3–4 μ long and 0·6 μ broad, or somewhat longer or shorter, *P. Zenkeri* being smaller and only about 0·4 μ broad. All motile: no spores known, but all endure drying up.

The principal characteristics as given by Hauser are the remarkable yellowish-brown and grey plate-colonies which consist of radiating mycelium-like, tendril- and hair-like filaments thinning out from a central mass of twisted tresses which in all but *P. Zenkeri* form zoogloea-masses. On the surface of the gelatine these spread out as 'wandering islets' of closely serried rodlets arranged in the most remarkable helicoid, tendril-like and irregular figures, which continually change their shapes. The first two forms liquefy the gelatine, but *P. Zenkeri* does not.

In stab-cultures the gelatine is liquefied rapidly by *P. vulgaris*, slowly by *P. mirabilis*, and not at all by *P. Zenkeri*. On agar, *P. vulgaris* rapidly spreads as a thin greyish layer, and on potato it forms a dirty slimy mass. Hauser gives no particulars as to the behaviour of the other two on these media. All these forms do best at 20–24° C., and grow quickly. They are also facultative anaërobian, and their products are toxic.

Zimmermann [1] found *P. vulgaris* and *P. mirabilis* in the river, and confirms Hauser's descriptions [2], adding particulars as to their behaviour in broth and on potatoes. The broth becomes turbid, and greyish flocks are deposited. Lustig [3] gives all three species, and quotes several observers as having found them in water. He also adds that *P. Zenkeri* does slowly and partially liquefy the gelatine after some time.

[1] l. c. pp. 66 and 68.

[2] Zimmermann says, however, that *B. mirabilis* does not liquefy 10 % gelatine, and does best at 24–30° C.: this suggests either great variability or that he did not keep his gelatine cultures long enough.

[3] l. c. pp. 89–91.

The first 'form' or 'variety' of this type which I have isolated has the following characters :—

No. 103 : **Proteus mirabilis** (Hauser).

This form is not common, but occurs in the summer. On the plates it consists of short rodlets 1·5 to 1·25 μ long by 1 μ broad, breaking down to cocci about 1 μ diameter (Fig. 4). It is quiescent, and no spores have been found.

In broth, however, it forms rods 3–6 μ long by 1 μ broad, breaking to shorter rodlets or joined into short filaments 10–12 μ long : all active, the filaments with gliding serpentine movements, the rodlets darting hither and thither. On potato the cocci are soon formed.

On plates at 18–20° C., the colonies are visible in twenty-four hours as zoogloeas spreading into very characteristic white spider-like and moss-like forms (Fig. 1) ; and under the $\frac{1}{3}$rd they are seen to consist of moniliform irregularly branched submerged zoogloeas, sending floating islands off on the surface, exactly as in No. 34 in principle, but more attenuated and divided (Figs. 2 and 3). To the unaided eye the figured colonies are quite white, or slightly yellowish in a few days: under the lens the zoogloeas are dull whitish ochre, and the floating islands transparent and difficult to see at first. As the culture ages, these islands become yellower and denser and are then easily seen. One curious feature is the rapid course of the growth: after the second day the colonies did not enlarge at all, apparently, but more careful examination showed that the tenuous films of floating islands were spreading over the surface. There was no sign of liquefaction in fourteen days.

In other plates the first signs are visible in 12–20 hours under the $\frac{1}{3}$ as isolated coils and filaments, tresses, &c., which imitate tendrils of varying degrees of fineness according to the number of filaments and the closeness of the coiling. These lie chiefly on the surface. The zoogloeas result from the same closely coiled up into skeins and balls.

Stab-cultures at 20°. In forty-eight hours the axis has developed outgrowths like root-hairs, and a tenuous film with a bluish sheen forms above (Fig. 6). In time the film spreads as a bluish-white layer above, but the very delicate root-hairs grow no further. No liquefaction in a month. The surface growth soon forms a continuous opalescent white layer, with a peculiar bluish cast on the surface.

Submerged cultures in gelatine show that it is very slightly anaërobic, but grows far better at the surface than in the depth.

Streak at 20°. A thin delicately fimbriated layer in 24–48 hours, spreading all over the surface in a few days, with the margins delicately cut like moss, and the whole film presenting an astonishing resemblance to a frosted window-pane. The layer is yellowish-white with iridescent edges and surface. No trace of liquefaction in a fortnight. There is, however, a slight growth in the gelatine just below the surface. The growth itself is matt, like ground glass, and soon acquires metallic iridescence on the surface like glass which has been buried (Fig. 5).

Agar at 35°. A yellowish-white streak with slightly spreading thin margins, cut and fimbriated, rapidly develops in 24–48 hours and then grows no further. It then remains unaltered for a month.

At 25° the growth is much better, as a delicate white film spreading all over in 24–48 hours, with radiating filaments from the streak forming moss-like outgrowths as on gelatine (Fig. 7). This also remains stationary after about 4–5 days, and appears unaltered in five weeks.

Potato. At 35° hardly any growth perceptible. At 25° a faint whitish film in 24–48 hours, but it does not spread. The potato acquires a bluish-grey hue in a week, and the thin dryish film then shows up a little clearer, but even in the best cases it is an indistinct grey-white film. It consists entirely of cocci, isolated for the most part.

Broth. At 35° rapidly turbid all through, and remains so for many days. No ring or veil, but a white deposit accumu-

lates. In a week this is fairly abundant and the liquid clears. At 25° the course of events is the same, but with more vigour and rapidity. An abundant white deposit, easily shaken up into the clear supernatant broth, which undergoes no change in colour. Even in 3–4 weeks there is no trace of ring or veil.

Milk. At 35° shows no change in a week, but in 2–3 weeks it is quietly peptonizing without any coagulation. At 25° no change in a week, but in three weeks shows signs of peptonizing without coagulum. The peptonization is very slow and in some cases does not begin—so far as the clearing of the milk shows—until the third week.

There can be little doubt that No. 103 is the form called *Proteus mirabilis* by Hauser[1] and *Bacillus mirabilis* by Zimmermann[2], and which has been found in water by a number of observers.

Tataroff[3] met with a variety which formed a dirty yellow colouring, but regards it as identical.

No. 34: **Type of Proteus** (Hauser).

This form has an interesting history. It was isolated in March, 1894, and kept in culture throughout 1895.

It showed at first no signs of liquefaction, but during culture a tendency to sink into, and slightly liquefy was manifested, and alterations of various kinds made themselves apparent in the characters of the colonies. The principal features when first isolated were as follows:—

Habitat. Thames, in March, 1894.

Morph. In gelatine it forms short active rodlets $3 \times 1\ \mu$.

Plates. Nothing visible even under the $\frac{1}{3}$ in forty hours, but visible to the unaided eye in ninety hours as thin, irregular, film-like plates with a bluish sheen when held up, contoured fimbriated edges, and a darker central spot (Fig. 8). The submerged colonies are yellowish,

[1] Hauser, l. c. [2] Zimmermann, l. c. p. 68.
[3] Tataroff, l. c. p. 19.

irregular and granular. No liquefaction, but in sixteen days the colonies are sunk in the gelatine, and open out with radial striations from the darker central mass, in some cases the radii appearing fimbriated (Fig. 9).

Streak. Begins to appear in twenty-four hours at 15°, and in forty-eight hours is very thin, white, slightly lumpy and dull. In seven days slowly sinking as a dull opaque white mass.

Stab at 15–18° C. Begins by forming a delicate arachnoid film on the surface, and very slender outgrowths like root-hairs with tufts and granules along the axis. In a week or two there is a striated membrane above (Fig. 10). In a month one-eighth of the gelatine is liquefied—not reaching the bottom of the tunnel—the liquid faintly turbid. A very copious white deposit on the flat base. The tunnel shows very faint root-hairs here and there.

In a year there is a clear yellow liquid, and cloudy white (yellowish) deposit.

Agar. Growth apparent in fifteen hours, but slow, as 2–4 days show. In a month a thin, glistening, slightly spread yellowish-white layer, with somewhat fimbriated margins.

Potato. Doubtful in three days, or else forms a thin film, the colour of the potato and almost invisible except as a wet patch. All grey. Same in a week.

Broth. Turbidity just perceptible in eighteen hours, but only slight on the fifth day; seventh day hardly turbid, but with a white deposit. Other cultures gave slight turbidity in forty-four hours, and in four days a considerable flocculent deposit, and nearly clear liquor: by the eleventh day the deposit was a little yellower.

When again brought into continued culture, in 1895, No. 34 gave the following characters, and clearly showed its nature as a feebly liquefying member of the *Proteus* type, allied to Nos. 51 and 103 respectively—in fact, midway between them.

It occurs as rods up to 10 μ long, breaking up to rodlets 1·5–2 μ × about 1 μ, or in couples or fours. In water it is more than 1 μ thick, and the rods about 2–3 μ long, and motile. The ends are apt to stain more deeply. Old broth-cultures give rods up to 10–12 μ, and longer, and about 1 μ

thick. Decolorized by Gram's method. Plate-cultures at 12–15° give typical colourless, contoured, typhoid-like colonies, growing very slowly.

Plates at 20° showed white flecks ½ mm. in diameter in two days. These have a spider-like appearance under the lens, and are composed of moss-like tufts of filaments and tresses from zoogloea-centres (Fig. 11 *a*). On the third day the corkscrew-like zoogloea-tresses radiate to the surface and form queer spider-like groups, and thin contoured fronds, up to 2 mm. in diameter (Fig. 11 *b*). Many colonies present dendritic or Moss-agate figures, but as the filaments reach the surface they flow out into flat, flame-like and delicately contoured lobes. No signs of softening on the fifth day (Fig. 11).

Stab-cultures at 12–15° give white dots in the tunnel, and an extremely thin hyaline frond-like film above in two days. In five days the film is still extremely tenuous, and the growth very slow.

In two days at 22° a beautiful and very delicate transparent frond, and irregular fine root-hair-like radiations from the tunnel. It grows in slightly acid gelatine also, but best in alkaline. On the sixth day the film is still exquisitely thin and transparent.

After six weeks the centre has sunk a little, but no liquid results: it is a dry hole 3–4 mm. deep and same diameter.

Streak at 20°. An extremely delicate thin and transparent film in two days, with whiter dots along the streak. In a week the film has spread nearly all over, but is scarcely visible at the transparent jagged edges, where it is flush with the gelatine and broad.

Agar at 34°. White spots along the streak in twenty-four hours. In forty-eight hours these have extended rapidly and are coalescing, like very thin scales, to a broad, rather waxy patchy streak. In four days it forms a very thin, flat, shining, yellowish film, with blue iridescent margins, and radiate zones. The streak thickens later to a shining, dirty-white, gum-like layer.

In some cases the growth at 34° is exactly like form 24 side by side, but the margins are a little thinner.

Potato. No visible growth, though possibly a transparent thin layer exists.

Broth at 35°. Shimmering turbid in twenty-four hours, and in forty-eight hours a slight deposit falls. In a week it is still slightly turbid, with flocculent, floating, serpentine masses, and traces of deposit. In a fortnight the liquid is clear and yellow, the flocculent deposit yellowish-white, and consists of rods 10–12 μ and longer, and about 1 μ thick. At 25° the progress is very similar. The turbidity increases in forty-eight hours, flocks and deposit as before. In a week it is still shimmering: in a month the liquid is clear, and an abundant yellowish-white deposit has formed.

Milk at 25°. No change in fifteen days, but in a month nearly all is peptonized and the turbid buff liquor is strongly alkaline.

Glucose solution at 25° gave no results.

Pathogenicity. According to Dr. Kanthack[1], a guinea-pig, after intra-peritoneal injection, suffered no effects whatever.

No. 51 : Type of Proteus vulgaris.

Much less common than No. 50, though among the common types in the Thames; is a powerfully liquefying, non-pigmented form with the following characters.

It occurs on the plates as short rodlets about $3 \times 0{\cdot}75\ \mu$ or a little thicker, and is motile. In broth the rods are chiefly $3 \times 0{\cdot}8$ or $4 \times 0{\cdot}8\ \mu$ or a little thinner, but filaments up to 30 or even 40 μ long occur, and have a serpentine motion. The shorter rods are often paired. The movement consists in a waggling about the centre of the rod, as well as forward motion, but not the rapid darting movements of No. 50.

[1] My late colleague, Dr. Kanthack, Professor of Pathology in the University of Cambridge, kindly examined many of these forms for me as regards their pathogenic properties.

Cultures in gelatine-drops gave positive results, and helped materially in further distinguishing this form. In a gelatine-drop made at 10.30 a.m., temp. = 21°, a short rod 3 × 0·75 μ was fixed at 11.15, and had (Fig. 16 *b*) divided at 11.50 into two rodlets each 1·5 × 0·75 μ: by 1 p.m. each had grown to 2 × 0·75 μ, and by 2.40 one was distinctly constricted and the other faintly so—each 4 × 0·75 μ, and by 3.30 each had again divided and four short rodlets now lay side by side. (See Fig. 16 *f*.)

By 6.15 p.m. there were about sixteen rods crowded into an oval colony measuring 10 × 6 μ, temp. = 20·5°; and by 10.30 next morning the colony was perfectly spherical, and 44 μ in diameter: the temperature had fallen to 18° during the night and was now 19° C. At 3 p.m. the colony was of the same shape, and with no sign of liquefaction or movement, and measured 52 μ across; temp. = 21°. At 9 p.m. the diameter was 56 μ, temp. = 19°, and no further progress was observed, the gelatine-drop having dried up somewhat.

In another case, in a broth-drop made at 10.30 a.m., the rodlets measured 3 × 1 μ or somewhat thinner, and moved with the characteristic waggling movement. Rapid division occurred, and in a few hours most of the rods measured 4 × 1 μ, and some had grown to filaments of 10–12 up to 30–40 μ, and with serpentine movements. On the second day most of the rodlets measured 1·5 to 2 × 1 μ, and on the third day most of them were so short as to be nearly cocci.

Plate-cultures at 18–20° C. showed colonies under the $\frac{1}{3}$ in twenty-four hours, the submerged ones being smooth in outline but irregularly oval or circular, yellowish, granular; and the emerged colonies coming out as thin, hyaline, faintly granular more or less circular films.

In forty-eight hours the colonies are visible to the eye as pale white circles with a central spot, and exhibit a curious crystalline appearance and bluish sheen when held up. Under the $\frac{1}{3}$rd each emerged colony is found to have a dense liquefying core of dark granules, whence radiate coils and tresses,

which straighten to rays as they approach the periphery and give the whole colony a curious resemblance to an *Acineta*. (See Fig. 12.)

But more careful observation—and easily overlooked, as it was by myself at first—shows that beyond the radiating tresses is an extremely tenuous and hyaline irregularly lobed coronal film on the surface of the gelatine, and formed by the radiating tresses as they emerge. Liquefaction rapidly sets in towards the end of the second day, and the plate is usually destroyed by the third day.

The liquefaction differs in character from that of most common liquefying Bacteria, and does not consist in a scooping out of the gelatine in circles and throughout its depth, but is universal over the *surface* of the plate only; even on the fourth day the lower parts of the film may be firm. Unlike the ordinary cases also, the watery liquid has a very slight odour, not decidedly unpleasant.

One of the most remarkable features about these plate-cultures at 20° and near that temperature, was the extremely rapid liquefaction that sets in towards the end of the second day, and for some time I was excessively puzzled by this phenomenon. Over and over again I left a plate looking quite 'safe' at 8 or 9 p.m., to find it liquefied all over next morning. Several times I have visited the plates at various hours between midnight and 4 a.m. with the same result; the plates looked quite 'safe,' with colonies only 4 or 5 mm. in diameter, and yet by 8 or 9 a.m. they had liquefied.

One point struck me, and eventually gave the clue to the explanation. When the liquefaction was observed carefully next morning, one saw that it was at first only superficial; the whole top-layer of gelatine was a watery liquid, but the gelatine below was still firm and had its colonies firmly imbedded.

Then—by an accident in the first case—I found that just before the sudden liquefaction, if the plates were held obliquely to the light of a lamp, a faint iridescent shimmer is to be discovered around each of the emerged colonies,

in the form of an irregular zone with jagged and indented edges (Fig. 13). More careful microscopic examination showed that this almost invisible zone is an extremely tenuous film of Bacteria arranged in coils and tresses and lying on the surface of the gelatine in curiously lobed, fimbriated, flame-like and other figures, extending round the more visible colony. In fact, what had hitherto appeared to be the periphery of the colony was only the periphery of the denser central parts, and this extended radially for long distances around as the tenuous lobed film, only *one* Bacterium deep in most of its parts. But this was not the most wonderful part of the phenomenon. When I had succeeded in seeing this film by careful focussing under the $\frac{1}{5}$rd it was possible to make out that its lobes are composed of the most curiously convoluted and doubled-up tresses of filamentous series of Bacteria. (See Figs. 19 and 22.) A very fair idea of the convolutions is got by saying that they are a good imitation of those obtained by Mr. Galton in his well-known finger-prints.

While looking at this convoluted coronal film by lamp-light one night, I found that the lobes at the extreme periphery perceptibly moved, and careful focussing soon showed that the whole system of this coronal film is in a state of creeping movement, and gradually extending itself radially over the surface of the gelatine.

If a lobe is carefully watched it is seen to put out processes like an Amoeba; these processes thicken up, or spread out, or thin out into fimbriae sometimes so fine as to be nearly invisible, and the Bacteria of the convolutions are also seen sliding one over the other. Or a piece of a lobe becomes constricted off, and moves freely away, over the surface of the gelatine; or two neighbouring pseudopodial processes curve over and fuse, and so on—the movements are so exactly like those of an Amoeba that no other term is applicable.

In Figs. 17–20 I have reproduced outline-drawings, and in Fig. 22 more detailed drawings, of some of these movements,

and they will give better ideas of their character than a lengthy description in words would do. In Fig. 17 two of the pseudopodia, as I shall term them for the sake of brevity, were sketched in outline (*a*) and their changes recorded for each succeeding minute (*b* to *e*). First, the lower thin tongue curved up towards and fused with the flame-like pseudopodium above; then it broke away from its basal connexion with the corona (to the right below), thickened up, and gradually fused laterally with the small blunt lobe near the × —a nearly fixed point throughout, and so converted the bay in which the × lies into a closed space; the changes during the next two consecutive minutes are seen in *d* and *e*.

Even more astounding are the changes seen in Fig. 18, where the changes of outlines (*a* to *n*) were drawn at intervals of half to one minute—practically as fast as I could record them, in fact.

The curved outline in which *a* is placed was the peripheral end of a blunt lobe of the coronal film, and just outside to its left was a serpentine free mass of the Bacteria. This latter was writhing like a worm, and in less than a minute broke in two (*b*), its distal part continuing to writhe freely, while the proximal one first joined the blunt lobe and then stretched out and again joined the free part (*c*). Again it broke and a free shorter part wriggled off (*d–f*), and this alternate breaking off (two pieces in *g*) and joining up again went on for a minute or two (*d–h*). At last the free curved end of the worm-like part curled over and made a loop (*i*, *k*), and it remained a constituent part of the larger pseudopodium, its further changes being sufficiently evident from *m* and *n*.

The series in Fig. 19 shows the somewhat slower changes observed in one of the broad and blunt lobes of the coronal film, the most marked alterations being observable in the outlines of the enclosed area in which the × is placed. *b* was drawn four minutes after *a*, *c* a minute after *b*, and *d* a minute later.

One of the most interesting cases is shown in the series

Fig. 20. At 8.20 p.m. the ring *a* was lying some distance away—to the left—from a peripheral lobe, not shown in *a* to *k*, and the outline sketches *a* to *h* were made at intervals of a minute.

It was evident that this ring was made up of a series of parallel, or nearly parallel, coiled series of Bacteria, and from 8.20 to 8.28 (under Zeiss B. occ. 4) these coils were revolving from left to right as one faces the ring, sliding one over the other more or less regularly and smoothly—as was quite clear by watching the notch-like free ends depicted in the inner and outer outlines. At the same time the flat mass of these coils was surging from side to side, sometimes widening, sometimes narrowing the ring. The movements of the ring as a whole had a curious resemblance to those of a smoke-ring—a vortex-ring. Between 8.28 and 8.31, however, the direction of revolution of the coils was *reversed*, as seen by the arrows in *i* to *l*.

I was curious to see how stable this ring was [for although it often thinned out dangerously at some part (*d* and *g*) it never broke] as compared with other free groups (e.g. those in Fig. 18), and should have watched it as long as possible, but at 8.39 the large lobe (to the right of *b*) referred to above had crept nearer and nearer, and between 8.41 and 8.42 it came into contact with and soon (*o*, *p*, *q*) fused with the ring, which now became merely a part of the lobe.

These illustrations will suffice to show how the progress of the coronal film is effected radially over the surface of the gelatine. The pseudopodia go on creeping outwards, and occasionally long tongue-like processes shoot out, and bits of these separate off as outlying free colonies, which may again join one to another or to the main corona as they wander over the surface, and *it is these outliers and the all but invisible coronal film which so mysteriously liquefy the surface of the gelatine.* In fact, at the very time I thought the visible colonies were extending so slowly that the gelatine could not liquefy before morning, the surface of the

latter was already covered with these wandering colonies and outliers, and with a tenuous all but invisible reticulate film of Bacteria.

The next question that arose was as to the mechanism of the movements of the pseudopodial-lobes, &c.; and to solve this I tried to cultivate the colonies in gelatine-drops under the $\frac{1}{12}$th immersion.

The series in Fig. 21 is a good one. The gelatine-drop was made at 11 a.m. and a rod *a* fixed: temp.=20° C. At 4 p.m. there were about eight rods in a clump *b*, and at 8 p.m. an oval colony $8 \times 4\,\mu$. At 9.30, the temperature having fallen to 19°, the colony measured $10 \times 8\,\mu$, and at 11.10 p.m. $12 \times 10\,\mu$: temp.=18.5°.

The temperature fell to 16° during the night, and at 8 a.m. the colony was circular and consisted of short rodlets closely packed and perfectly quiescent. Diameters $34 \times 32\,\mu$. At 9.30 a.m. it was a circle $36\,\mu$ diameter, temp.=17.5°, and through the day it slowly grew as follows :—

10.55 a.m.	$44\,\mu$	temp.=19·5°
12 noon	$59\,\mu$	20·5°
3.30 p.m.	$64\,\mu$	21·0°
8.40 p.m.	$72\,\mu$	19·5°

At 3.30 next day the hitherto granular circle was showing signs of differentiation (*e*). It was very opaque—really a sphere—but one could make out several curved filaments among the rest. At 6 p.m. the sphere was much clearer, and had evidently become looser, and the presence of numerous long curved filaments was observed (*f*), though no movement was discernible. I am strongly of opinion that the clarifying of these spheres is due to a slow increase of a slime between the cells, no doubt the swollen cell-walls. At 8.40 the clarification was more marked, especially in the middle, and slow writhing movements of the filaments and rods could be detected, the nature of which suggested the existence of a sort of zoogloea-slime as the medium in which they were occurring.

Just before 9 p.m. the softening gelatine gave way and the sphere began spreading itself out as a film, but I was unable to do more than satisfy myself that the rods and filaments *themselves* move.

Repeated attempts to bring the colony in the hanging-drop to the creeping stage observed in such perfection on the plates failed in some cases, evidently because the small drop of gelatine becomes exhausted before the colony is ready to spread, in other cases because the drop got diluted and too thick for focussing even with the E/4.

Eventually I abandoned this method in favour of another, the extraordinary success of which suggests its wider application in such researches as these.

I took a clean cover-slip, and sterilized it by holding it over the flame between talc plates, and spread a thin film of sterile gelatine on it and allowed it to set. I then lifted it by sterile forceps so arranged that one point held the middle of one edge and the other point the middle of the opposite edge; hence the cover-slip was so jammed between the forceps that when the latter were held vertical, the former was horizontal; and I then brought the sterile cover-slip, gelatine-side down, on to one of the creeping films on a two days' plate-culture. In fact, I made a 'Klatsch-präparat' of the creeping film, and after a few attempts obtained a very perfect specimen. The cover-slip was then fixed as the roof of one of my sterile culture-cells, and treated exactly as in the case of a hanging-drop.

After a few minutes at 20° C., I then saw under the $\frac{1}{12}$ immersion one of the most magnificent sights in the way of bacterial movements it has ever been my lot to witness.

The lobes and flame-like protuberances of the coronal film, as well as some of the looser bacterial aggregates from the centre of the colony, had been taken up and fixed by the gelatine to which they adhered in their relative positions: the impress of the film was particularly perfect, and after recovering from the shock, the movements of the lobes and wandering outliers went on in the now swelling film of

gelatine—taking up water-vapour from the cell—exactly as if no disturbance had happened.

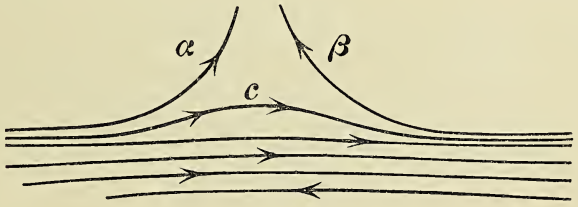
The movement consists in the sliding of the rods and filaments one over the other, and under the $\frac{1}{12}$ is far too rapid to draw in detail, whence I had to make line-sketches from which the figures were copied later.

The filaments are of various lengths, some being segmented very clearly into separate rods, others less clearly into longer segments. They are evidently growing and dividing as the active gliding movement continues, though of course it is impossible to trace that in detail. It is to be inferred, however, from the action of the film or lobe as a whole, for the filaments are laid flat in tresses, coils, &c., in one plane on the gelatine, and they evidently grow by intercalary growth and segment into shorter and shorter rods.

The worm-like segments glide over one another, as said, and by no means always in the same direction.

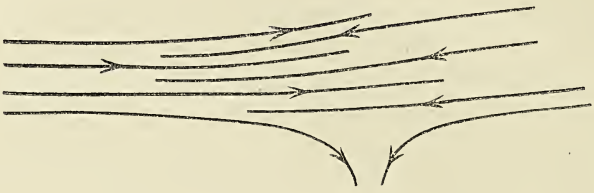
Of three parallel segments, for instance, any one may go for a time in one direction while the others are slipping past it in the opposite one. In the same sense, there is no order as to stoppage, reversal of movement, &c.

It is very interesting to see how fresh arrangements of the moving series are instituted. This may occur simply by an outside segment starting off on its own account, as it were, as *a* in the annexed diagram ;



or *a* may meet *β*, and form a new series, and in this case *c* will curve out into the increased space as indicated by the arrows.

Similarly when two series meet, the free ends may slip one between the other, as in the annexed diagram, or collisions



may occur. Sometimes such collision results in a bending on itself of one of the filaments, and it may snap, or a vortex may result, and the filament go round and round in one direction for a time as in the diagrams annexed, and so on with infinite varieties in the details of the movements.

Stab-cultures at 18–20° (see Figs. 14 and 15). Good growth in the tunnel in twenty-four hours, and a thin film has spread over the surface and liquefied the uppermost layer to a slime.

There is considerable variation as to the rate of liquefaction, however, some cultures forming a big 'pouch' in three days, while others only form a 'thistle-head' funnel in that time.

In gelatine, submerged, and the tubes put into pyrogallic acid and KHO in a closed bottle at 20° C., slow but perceptible growth occurred in the first twenty-four hours, and increased during the second day.

Agar at 20°. In forty-eight hours a broad layer, perfectly smooth and glistening, chalk-white, and with a bluish cast in oblique light. Slight white deposit.

Potato at 20°. A grey moist layer spreads all over in three days. It is much thinner than the flesh-coloured, raised, lumpy, and matt patch formed by No. 50, and totally different from it. In a week it is a little thicker, and spread all over as a wet grey-white layer.

Broth at 18–20°. Dense turbidity in 24–48 hours, and a white deposit falls. No ring or veil in four days: still very turbid.

At 33–35° a dense satiny turbidity in twenty-four hours, increasing during the second day. Still more turbid later, and with white flocks and deposit.

Milk at 35°. Separation of clot on third day. Distinctly but not strongly acid.

This form is pathogenic to guinea-pigs according to Dr. Kanthack.

No. 61.

No. 61, an imperfectly studied form, was evidently the same or very closely allied. There are doubts as to the pathogenicity, as the cultures were not successful. It seems, therefore, unnecessary to give details.

No. 9: **Proteus type.**

A not uncommon liquefying non-chromogenic *Bacillus* (old cultures of which, however, are orange-coloured) occurred as very short rodlets 1·5–2 μ long by 0·75–1 μ broad, hardly if at all motile, and often oval 1·5 × 1 μ or so, which developed plate-

colonies as follows. Gram's method decolorizes it, but it is easily stained by ordinary methods.

Plates at 12–15° C. Yellowish-grey, granular, circular discs, often with irregular edges, appeared in two to three days. On the fourth day liquefaction began, each colony being merely a zoned brownish circular disc about 1–2 mm. diameter, the edges of each colony being fimbriated or even with irregular tresses. Liquefaction very rapid on the fourth day, the small colonies—white to the unaided eye—retaining their circular shape as they float. Under the $\frac{1}{3}$ each was a grey disc, hollowing out in the centre, and with fimbriated edges (Fig. 24).

After-plates gave the same dimensions, i. e. ordinary round-ended rodlets $1{\cdot}5–4\,\mu \times 1\,\mu$. The thickness not uniform, but in some cases $0{\cdot}75–1\,\mu$.

After three months in culture, plates at 15–18° showed white-grey spots in three days, each sunk in a shallow lenticular depression. Under the $\frac{1}{3}$ the submerged colonies are moruloid, tawny, granular: as they emerge they thin out with anthrax-like tresses and fimbriated edges. On the fourth day they are about 1–4 mm. diameter across the lenticular depression, the ochre-coloured central eye being solid, or 1-zoned, and under the $\frac{1}{3}$ the zone is found to be due to clouds around the more solid centre. Others are masses of tresses, with fimbriated edges. They lift as a whole from the depression.

On the fifth day the lenticular depressions are coalescing: they measure 5–8 mm. in diameter. The central ochre-yellow eye resembles a sieve, and is composed of dense and less dense clouds. The fluid in the depression is turbid. In a week the plate is nearly liquid.

Stab-cultures at 12–15° C. Rapidly forms a thistle-head funnel, and liquefaction occurs and spreads to the sides. Repeated tough whitish veils form on the surface, and fall successively, and the resulting deposit is very shiny and tough. The top of the liquid may be clearer, or it may be more turbid above (Fig. 25). In a fortnight nearly all the gelatine is liquefied with a white ring and veil above: dense clouds fall

into the nearly clear liquid on shaking, and go to swell the dense yellowish-white deposit. In a month all is liquid, with a white ring and fallen veils.

Agar at 22° C. Shows a spreading, white, granulated layer in twenty-four hours, and in three days this is very white, and extends as a veil over the clear drainage. In six days the marked whiteness and granular appearance persist (resembling the growth on potato), and new veils, wrinkled, replace those deposited in the clear liquid.

At 34–35° the growth is also rapid, as yellowish-white spots confluent in twenty-four hours, and a veil and deposit already occur in the drainage. In two days it is an abundant white shining layer, with outlying dot-colonies. It consists almost entirely of paired rodlets 1–2 × 0.5 or 0.6 μ when stained, thicker before staining. It is more tawny than form No. 16. After two or three months the agar-growths are deep ochre-yellow.

Potato at 22° C. In forty-eight hours a dense chalky-white, granulated, spreading growth appears, becoming mamillated, and of a peculiar milk-white appearance later. No further extension after four or five days, but in three weeks the colour is dull, and a slight metallic iridescence is observable.

Broth. At 35° becomes rapidly turbid in twenty-four hours, and densely so in two to three days, when a white ring and deposit form. In a week an abundant white deposit has fallen and the liquid is still extremely turbid, but there are no distinct veils.

At 25°, in broth infected from agar-culture, the course of events is much the same, but definite veils form. In twenty-four hours a slight white greasy-looking ring and veil occurs, and from the third day onwards the thin veils fall successively. The dense turbidity and copious deposits are as before, the latter being very white, and even in a fortnight the turbidity is dense.

Milk, infected from an agar-culture, gave definite separation of the casein in forty-eight hours at 25° C., and by the sixth day much of the precipitated coagulum was dissolved,

the reaction being distinctly acid. In eleven days solution was rapid, and a chalky-white deposit had fallen.

Glucose solution. At 25° C. an infection from agar gave no results, except very minute floating flecks and deposit in eleven days, the liquid remaining otherwise quite clear.

No. 9 seems to be pathogenic to guinea-pigs according to Dr. Kanthack's report.

Attempts to revive No. 9 after a year failed.

Since this form presents several differences from the typical form, and suggests alliances with Groups VII and XIV, I set forth its characters in tabular form.

Number 9 (*Proteus type*), Figs. 24 and 25.

Habitat. The Thames.

Sizes, &c. Short rodlets 1·5–2 × 0·75 to 1 μ and hardly if at all motile. No spores observed.

Plates. At 12–15° yellowish-grey circular discs, edges irregular or fimbriated. Liquefaction rapid on the fourth day, the colonies floating and retaining their shape. Under $\frac{1}{3}$ each colony zoned, brownish, with tresses at edges.

Stab. At 12–15° rapid thistle-head funnel, and liquefaction, and repeated tough white veils form and fall. All liquid in three weeks or so, and a white ring and veil above, turbid liquid and slimy deposit of successively precipitated veils.

Agar. At 22° and at 35°: rapid growth as a very white layer of confluent granulated or circular colonies becomes markedly white and shining.

Potato. At 22° a peculiarly chalky- or milk-white granulated or mamillated spreading layer in forty-eight hours onwards. Becomes dull, and with slight metallic iridescence later.

Broth. At 35° rapidly and densely turbid in twenty-four hours, and in 2–3 days an abundant white deposit, and trace of white ring. At 25° the same, but more definite veils form and fall successively. Even after a fortnight the turbidity persists. The deposit is very dense and white.

Milk. At 25° separates in forty-eight hours, and in six days was rapidly dissolving the coagulum. Reaction distinctly acid. Chalky-white deposit in eleven days as solution proceeds rapidly.

Glucose. Merest traces of flecks and deposit in eleven days at 25° C. The liquid remained clear.

I am strongly inclined to the belief that No. 9 is a variety connecting the *B. Termo* type with the *Proteus* type, and if so it bears out a widespread suspicion among bacteriologists. The same applies to the following forms, the tabular summaries of which give the principal characters. Several of them appear to me to be weakened forms, but some had not been sufficiently studied before passing out of cultivation.

Number 9 a (Variety of Proteus type), Fig. 26.

Habitat. The Thames.

Morph. In six weeks, gelatine, practically cocci 1 μ in diameter, or short rodlets 1 × 1·5 μ fresh.

Plates. Nothing in forty hours even under $\frac{1}{3}$, but quite visible to the unaided eye in ninety hours, and slowly liquefying in 115 hours. By the sixth day the colonies float in a yellowish watery liquor, as balls of a grey-white colour and 1–2 mm. diameter.

Streak. In twenty-four hours at 15° an extremely thin white streak, and in forty-eight hours this is nearly flush with the gelatine-surface, very thin, matt, white. In seven days much slower and thinner than form A, but similarly white, flush and granulated. Rapid liquefaction in eleven days.

Stab. In a month the gelatine is half liquefied, with a very turbid liquor over a white deposit on the floor. Grows in acid gelatine. After some months a viscous clear yellow liquid and yellow-white very flaky deposit.

Agar. In five days at 20° a very thin dull white layer confined to the streak. In a week, a mere extremely thin film where the loop touched the agar.

Potato. In five days at 20° a very thin, wet, creamy-white film. In eight days extensive, thin, with matt edge, yellowish with metallic irridescence on the surface in the middle.

Broth. Faintest turbidity in eighteen hours, but white turbid in forty hours and with slight deposit. Very turbid in three days and with white deposit, but on the fifth day clearer above and only slightly

turbid. On the seventh day a thin veil, which breaks to flecks on shaking. In a month turbid above, and a slight ring and veil and yellow-white precipitate. It shows alliances with several other forms, and may be a weakened form of No. 9.

ι (*Variety of Proteus type*), Figs. 27 and 28.

Habitat. The Thames.

Morph. Extremely small and active rods 2–3 × 0·75 to 0·5 μ.

Plate. Yellow-grey, slightly sunk, zoned and radiate circles, just visible as points under the $\frac{1}{5}$ in forty hours, visible to the unaided eye in ninety hours. Gelatine completely liquefied in 115 hours.

Streak. Slowly scoops and liquefies the gelatine with a white deposit.

Stab. Very slow growth as white, leafy, frondose colonies, and dots in upper part only. In a month it has liquefied $\frac{1}{3}$ of gelatine, and forms a white deposit on the flat base: liquid perfectly clear.

Agar. Merest film or nothing visible on the fifth day, and hardly visible in three weeks. After being some time in culture, a rapid white streak extending in fifteen hours; a milk-white broad streak in two days; spreading, yellow-white, and thicker in four days. Thin, yellowish, and extended in fourteen days. In a month a thin white layer not spread much. Recalls forms φ and ζ.

Potato. Yellow-buff wet film, not unlike form π but more waxy. At 20° nothing appears, or a mere wet film in three to seven days, and even in eleven days a mere trace is seen as a faint white film.

Broth. At 35° no trace in a month, and another attempt gave nothing in a week. At 25° the broth becomes turbid and has a whitish deposit.

Milk. Precipitates casein in five days. Alkaline reaction. Solution later; nearly all gone in three weeks, liquor yellow. All clear in a month.

Glucose. No results.

Several points (e. g. its behaviour on potato) suggest its resemblance to π, which may be a stronger form.

Agar suggests resemblances to forms φ and ζ, and the negative results on broth suggest φ and γ.

On the whole, colony ι seems to be one of the *Proteus* group, probably a very weak form.

Variety ϕ, Figs. 29, 30.

Habitat. The Thames.

Morph. Short swinging paired rodlets $2 \times 0{\cdot}5\ \mu$.

Plates. Trembling liquefying area with grey-white centre and dots around, but in further cultures the one or two colonies visible under $\frac{1}{8}$ in 115 hours are still invisible in six days. In ten days the gelatine is liquefying and the feeble colonies running together and white.

Streak. Slow white colonies sinking and scooping the gelatine. White flecks.

Stab. 15–18° C. It begins by forming a faint film on the surface and faint clouds in the tunnel. In fourteen days an eighth of the gelatine is slowly liquefied and a dense white-yellow deposit forms: liquid turbid. Tiny dots in tunnel. In a month three-fourths of the gelatine is liquefied and clear, white deposit resting on the solid base. In a year a very slight deposit, brownish liquid.

Agar. Thin white film. Hardly visible in fifteen hours. White wet patches in two days. Shining milk-white layer in five. On the second day a yellow-white streak; spreading on the fourth, as a thin white layer. On the fourteenth day still extremely thin and slight: little growth in a month—white flecks in drainage.

Potato. Nothing in three weeks. Other cultures have a thin white streak first seen on the seventh day.

Broth. Failed in all cases.

Milk. Precipitate in twenty days, alkaline. In three weeks it shows peptonization, but slow. Yellow liquor. Liquor nearly clear in a month. Nearly clear yellowish-brown liquor and clots.

Glucose. Nothing.

I cannot understand the negative results in broth. The whole behaviour suggests a very weak form.

The behaviour on agar joins ϕ, ζ, and ι perhaps. It seems more like ζ in some respects, but the milk- and broth-cultures are sharply different.

On the whole I think it is an extremely feeble form of *Proteus*.

Variety ζ, Figs. 31–33.

Habitat. The Thames.

Morph. Non-motile rods to nearly cocci. Rodlets often paired. Fairly large, $1 \times 0{\cdot}5\ \mu$.

Plates. Grey, granular, dense and structureless colonies, just visible in forty hours. Slow and non-liquefying—at least at first, but (see streak) they slowly liquefy later.

Streak. Grey-white, spreading, and eventually scoops and sinks, slowly liquefying—white.

Stab. White points in tunnel. In fourteen days a thistle-head and yellowish-white precipitate, and dots in tunnel. In a month half-liquefied, turbid, copious white deposit. Grows also in acid gelatine. In a year a brownish-yellow liquid, clear, and very glairy deposit, pale brown.

Agar. White along the streak, or greyish, often hardly visible in three weeks. Growth visible in fifteen hours, and forms a white mesentery in two days: waxy and very white in four; but even in fourteen hardly spreads beyond the streak. In a month it is little spread. White flocks in drainage.

Potato. Nothing in four days: in seven a very thin wet white film. In three days at 20° are formed white flecks, wet, and exactly the colour of the potato. In seven days a thin dew-like colourless wet film.

Broth. Soon clouds, and deposits a white precipitate. Very turbid in eighteen hours and same to third day. On fifth day not very turbid, slight deposit, no veil. In a month no veil, turbid throughout, and yellow-white deposit.

At 20° C., distinctly turbid in forty-four hours, and in four days turbid but no veil: white deposit. Similar in seven days, and on the eleventh day there is still no veil, only yellowish-white flocculent deposit, &c., as before.

Milk. No visible change in seven days. Acid reaction.

Glucose. No change in a week.

Perhaps μ and ϕ are connected, but they are slower in broth.

Agar suggests the union of ϕ, ζ, and ι, and broth connects ζ, μ, and θ more or less.

I am inclined to regard ζ as a form of *Proteus*; while the potato-cultures strongly support this, the microscopic characters do not help us. On the whole it is probably a weakened form.

Variety μ, Fig. 34.

Habitat. The Thames.

Morph. Extremely short and small rods: nearly cocci: no motion: 1 to 1·5 × 0·75 μ.

Plates. Crenate circular colonies, orange-yellow and sinking. Nothing visible even under $\frac{1}{3}$ till ninety hours, and very slow at 115 hours. In 140 hours small colonies, yellowish and granular, are visible, the largest being $\frac{1}{20}$ mm. In seven days the largest is 1 mm. finely granular greyish-yellow circles. Slowly liquefy.

Streak. Soon sinks and forms a yellow eye. Liquefies slowly: pale and dull yellow.

Stab. Rapid dots in tunnel in forty-eight hours. In three days a slight depression (thistle-head) with a dark eye and cloudy liquid: deposit yellowish and dense. In fourteen days one-eighth gelatine liquefied, and a funnel forms with rusty yellow-white deposit, and dots in tunnel. Resembles ζ but is more orange. In a month one-fourth liquefied, but not to the bottom of the funnel; very turbid, a yellow clot rests on the solid base. In a year the liquid is brownish with a slight dirty-white deposit.

Agar. White layer in twenty-four hours. Yellowish-white broad wet streak in five days. Cloudy-white and slightly mesenteric.

Grows in fifteen hours. In two days a thick, wet, white mesentery, and in four days copious yellowish-white wet mesentery. In fourteen somewhat buff, thick and wet.

In a month it is white with a buff tinge [1], thick, wet, not much spread, and forms a somewhat buff deposit in the drainage.

Potato. Nothing, or an invisible film in three days at 20° C.; a thin slightly buff streak in six days. Nothing in three weeks at 15° C.

Broth. Traces first visible on the third day; on the fifth slightly turbid with traces of white deposit. Same seventh day. More on tenth. In a month densely turbid, and yellowish-white deposit: no veil.

Milk. Very faintly alkaline in twenty days, but no visible change in a month.

In a year a brown liquid with white clots, like cream clots.

Glucose. Doubtful traces of deposit.

This form seems, in some respects, to connect this typical *Proteus* group with the yellow species considered under the next type.

Colony θ, Fig. 35.

Habitat. The Thames.

Morph. Very small rodlets $1 \times 0{\cdot}5\,\mu$, or nearly cocci on old gelatine.

[1] Yellower than β.

Plates. Mycelium-like very thin, circular, radiating colonies, with very transparent wedge- or chisel-shaped fimbriae at margins, and liquefying in centre. Two days' growth at 15° shows a dark eye and faint zone. All liquefied in seventy-two hours.

Streak. Rapidly scoops and liquefies at 15° C., and forms a very white turbid mass. All liquid in ten days, with white turbidity and deposit.

Stab. At 15° yellow-white dots in tunnel. Rapidly spreads above as a yellow-white, radiating mycelium-like layer with an eye. In five days an eighth of the gelatine is liquefied, turbid. In three weeks half is liquid, very turbid, white deposit. In a year a red-brown liquid and dirty deposit.

Agar. In twenty-four hours at 20°, yellowish-white, wet, thin, confined to the streak, and even in seventeen days there is only the thin yellow-white streak.

Potato. In three days at 20°, wet, yellowish-grey, spreading. In six days spread all over and very wet. In seven days very like form *v* but rather yellower.

Broth. Turbid in twenty hours at 20°, very turbid in forty-four hours, and more so in sixty-eight hours. On the fourth day a white deposit, and still very turbid in a week: no veil, but deposit very white on eleventh day.

All the characters seem to point to this being a weakened form of *Proteus.*

Group VII.

Colonies 7, 17, 23, 31, Ⓑ, K, and *B. arborescens.*

The Yellow Proteus Type.

Among the commonest Bacilli, or at any rate rod-like Schizomycetes, to be found in the Thames at all seasons, are a series of forms of which the following are representatives isolated and cultivated by me.

While their differences in detail are not to be overlooked, they present a number of peculiar characters in common so strongly marked that there can be little doubt they must

be brought together as a group of allied varieties, or if you will, 'species.'

They all agree in forming the peculiar tenuous cloudy colonies on plates, composed of coarse and fine radiating and much interlaced tresses and filaments, like an extremely fine mycelium sunk in the gelatine. The coarser of these masses and tresses are in the condition of zoogloeas, i. e. the rods and filaments are held together by their swollen cell-walls. They also agree in liquefying the gelatine more or less rapidly.

The filaments of which the tresses are composed are always very thin—about 0·5–0·75 μ—and break into rodlets about 4–6 μ long, which may again break up into shorter ones 1–2 μ long. These are either quiescent or only slightly motile, and no spores could be obtained in any cultures.

Another point of general agreement is the gradual assumption of a more or less pronounced ochre-yellow to chrome-yellow colour in the older plate-cultures, and other gelatine cultures; though, as we shall see, there is great variation as to the extent and depth of this.

The growths on agar differ considerably in small details, but they agree in being more or less yellow, and in failing at temperatures above 25° C.

On potato also, while great differences were observed as to the depth of colour and vigour of growth, all agree in forming some shade of yellow, and in refusing for the most part to grow above 25° C.

In broth again, with differences in detail they agree in not growing above 25°, and in forming a shimmering turbidity and more or less yellow deposit.

Similar resemblances in milk. No peptonization or, as a rule, marked coagulation occurs, but a faint acid reaction occurs in all.

It is for these reasons that I propose to unite all these forms under a common type, which for further reasons which will come out as we proceed, I term the *Yellow Proteus* type.

In order to make clear the details to follow, I begin with

the description of No. 23, a form studied very carefully and for long periods—I have kept it under continuous observation for some months—with interesting results.

No. 23: **B. radiatus** (Zimm.). Figs. 36–42.

This form is very common in the river at all seasons apparently.

It occurs on the separation-plates as slightly motile rods 2–6 μ × about 0·6 μ. After three months in culture the plate-colonies gave similar measurements. No success was obtained with Gram's method, though it stains readily by ordinary methods, and impression preparations are remarkably beautiful.

Plates at 12–15° gave ochre-yellow colonies, more or less regularly circular, and often surrounded by a sort of yellow halo (Fig. 36). The central part opens out like a star, and the clouds around are found to consist of floating or 'wandering islets' of much coiled and curved filaments or series of rodlets in a zone of liquefaction.

Plates at 20°: in two days there were circular bluish-grey clouds 10–15 mm. diameter, but so tenuous as to be nearly invisible. Under $\frac{1}{3}$ these give the appearance of central tresses breaking up into flocculent granular masses. Liquefies. (Fig. 37.)

Plates at 18–21° in forty hours show the characteristic bluish-grey clouds, circular and very indistinct, which under the $\frac{1}{3}$ exhibit coils and tresses radiating like mycelial strands from a dumb-bell-like or spider-like centre, with serpentine tresses running about: the whole hardly yellowish. On the third day the circles are about 5 mm. diameter, and pale ochre with bluish margin; granular, opaque and zoned, and beginning to run and sink. The centre opens out into reticulated or sieve-like clouds of granules; the margin has radial tresses like striae. All is composed of tresses of filaments falling into cloudy aggregates of rodlets (Fig. 37 *d* and *e*). On the fourth day liquefaction is commencing, and most of the

colonies are irregular cloudy aggregates; others still preserve the zoned and sieve-like appearance to some extent. Colour more yellow and colonies sinking or running. In a week the circular colony (15 mm. diameter) is composed of bright ochre cloudy masses floating in the lens of liquefaction, and this is surrounded by a nearly colourless zone of radiating tresses in the still solid gelatine, and with floating 'islands' on the surface around.

The most extraordinary feature in all these colonies, however, is the formation of the primary tresses—the serpentine central tresses referred to above. These are in reality compact zoogloea-masses composed of numerous twisted tresses, the walls of which swell and hold the whole together, forming the curious dumb-bell-shaped or jointed or serpentine masses referred to. The thinner and thinner tresses which radiate out from these become more and more tenuous as the filaments remain isolated and break up into rows of segments or free Bacilli.

Later on one finds that many of these at first almost free filaments can also become intertwined to serpentine zoogloea-masses, and the Figs. [36–38] give examples of the various ways in which this can come about: it appears to depend on the submergence in the gelatine.

The explanation of this phenomenon in detail is difficult, but there can be no doubt that these zoogloea-tresses are the same as those figured by Hauser in his monograph on 'Fäulnissbakterien,' as seen in the various forms of *Proteus*, and as those described on pp. 207–214 above.

The following observations throw more light on this subject.

A rodlet isolated in 5 per cent. gelatine was fixed at 20° under the $\frac{1}{12}$ immersion at 9.20 a.m. (Fig. 39 *a*). At 11 o'clock it had grown to nearly three times its original length, and divided once (Fig. 39 *b*). At 11.50 each of the two rodlets had slipped a little past the other, and one of them had divided again (*c*). At 12.30 there were four rodlets, two still in connexion, the other two free (*d*). At 5 p.m. a small

elongated colony of about 20–25 rodlets (perhaps more behind; it was impossible to count accurately) had formed, and some were slowly wandering off (Fig. 39 *e*). At 10 p.m. the initiation of a colony as a beautiful double-fan or constricted bundle of parallel or intertwining filaments breaking up into rodlets was established (*f*), and next morning this appeared as an extremely tenuous cloud of slowly wandering rodlets, filaments and tresses of the types Figs. 36 and 37 *b*. Some of these filaments intertwine to curious serpentine zoogloea-tresses, like those shown in Fig. 36 *b*, the frayed ends of which go on emitting rodlets (Fig. 37 *e*).

Tracing the colony of No. 23 further in gelatine it begins as coloured mycelium-like tresses, radiating offshoots in all directions from the one or more denser coiled and serpentine central zoogloea-tresses, and in forty-eight hours the whole of the circular and extremely tenuous cloud-like colony is built up of the radiating strands branching from the central zoogloea-mass (Fig. 37 *b*).

On the third day in 10 per cent. gelatine the marginal strands become closely serried, and radiate like flames (see Fig. 37 *d*); or if the surface of the gelatine is suitable, these marginal filaments grow out on the moist surface as coiled islets, or compact contoured films.

In this way the different marginal zones shown in Figs. 36–38 are produced, the last-mentioned thin filmy borders (e. g. Fig. 37 *d*) being very iridescent.

The central portion of the colony breaks up into cloudy masses arranged more or less like a network, and very often this more or less membranous part looks just like a sieve, fine or coarse, as the case may be (Fig. 38). The differences in colour, iridescence, zoning, &c., are entirely due to the rate of liquefaction and attendant pigmentation, the amount of flowing of the contoured margin, and the mode of breaking up of the tresses into radiating spokes, networks, sieves, or mosaics, according to the distribution of the clouds of segments into which they disintegrate.

The behaviour of the colonies of No. 23 in 5 per cent.

gelatine is suggestive. Plates kept at 15–18° C., for forty hours, showed the characteristic clouds consisting of the fimbriated mycelia with snake-like zoogloea-tresses as centres. On putting the plate under the Zeiss C, in Sachs' box at 20° C., the characteristic swarming islets described above (p. 209) were seen on the surface and their movements traced, an example being given in Fig. 40, where the changes are recorded at intervals of one minute. These islets, which are composed of the slender rods or coiled filaments, closely arranged side by side, are seen to change shape, advance and retreat, form new connexions or break away from old ones, in a manner so suggestive of the movements of an Amoeba that one might almost take them for something of the kind. They are exactly as Hauser describes them. The whole plate is liquid in five to six days. One point of difference between Hauser's *Proteus* and this one is that the movements *do* occur on the moist surface of 10 per cent. gelatine, though very much more slowly than on 5 per cent. gelatine.

Stab-cultures at 12–15° C. begin with the development of yellow dots in the tunnel and a film above.

In five days the latter is sinking, and is a circular yellow colony, spreading like a yellow star from a central eye; the dots in the tunnel are larger. The colour is brighter, more decidedly yellow than No. 7, but they are much alike. In three weeks about one-tenth of the gelatine is liquid, turbid, and with yellow floating flocks, and a yellow deposit on the flat floor: the tunnel is somewhat wider. In two months half the gelatine is liquefied, and an ochre deposit lies on the flat floor below the turbid liquor. Almost the only distinction between this and No. 7, is the floating yellowish flocks, forming an imperfect veil.

At 18–21° a bluish-white circular film and cloudiness all round the axis in forty-eight hours, and sinking just beginning at the yellow eye (Fig. 41). In three days the faint cloudiness pervades the upper part of the gelatine, and a cloudy yellowish film above covers all; liquefaction supervenes by the fifth day, no cloudiness is left, and the clear liquid in

a thin layer extends to the sides, with a pale-yellow cloudy mass above; the axis being hardly visible—faded away.

At 24° C. it rapidly liquefies above in 48–60 hours, with yellow-white clouds.

Streak at 15° C. In forty-eight hours a scooped grove, along which the mass slides as a viscid yellow and whitish mass. Fimbriated thin edges to the groove, like No. 7. On the third day the colour is ochre to orange, very like No. 7. In fourteen days the resemblances to Nos. 7 and 17 are pronounced: turbid liquid (half liquefied), slight veil, flecks, and abundant orange-yellow deposit—rather paler than in No. 7. In twenty-two days, four-fifths is liquid, turbid, and with an ochre deposit. This deposit consists of cocci and rods up to 6 μ long. Floating flecks. Submerged in gelatine it will not grow, indicating that it is aërobic.

Agar at 34° C. No signs of growth.

At 25° C. slow growth. In five days an abundant dirty-white deposit, and a smooth, thin, nearly white film on the agar. This becomes yellowish-white, and the colour and thicker growth alone distinguish it from No. 7.

At 22° the growth is somewhat better. A pale yellowish, cloudy and iridescent film spreads in three days, and a yellowish-white deposit forms. The filmy edges of the growth are diffuse and very iridescent, greenish: the drainage becomes densely buff-white, turbid. When scraped with a needle the growth is dirty-white.

At 12–15° on agar a wet yellowish or dirty-white streak forms in two days, with the edges fimbriated. It seems to grow more rapidly than at 25° C. This becomes a dirty-white shining gum-like layer all over. No growth under anaërobic conditions in fourteen days at 22–25° C.

The growth on agar is decidedly thicker and whiter than either No. 7 or No. 17, but otherwise similar, and the curious faint iridescence with slight tinges of green to pink in various lights is marked. The abundant deposit is flocculent, nearly white. The broad streak-layer has thin jagged edges: colour yellowish, but far paler than No. 7 in eight days.

Potato. At 34° no growth. At 25° a yellow, thin, diffuse watery patch is formed in two or three days; this remains pale—hardly visible as more than wetness in places—and in a week has scarcely spread.

At 22° the growth was thicker and eventually more orange and wax-like: in a week it resembled the corresponding growth of No. 7, but more ochre-yellow in hue, spread, and bright like wet gum.

Broth at 25° C. Slightly turbid in forty-eight hours. In a week hardly turbid, but an abundant flocculent yellowish-white deposit has fallen. On the ninth day there is a flocculent incomplete veil, and falling flocks. In a month the floating veil is easily shaken down, and the greyer colour of the deposit distinguishes it from No. 7.

No growth at 35°, but at 18° it is as good as at 25°.

The old cultures turn the broth sherry-colour, but the deposit remains greyish-ochre, not orange as in No. 7.

Milk at 25° C. No change observed for three weeks, when the reaction was acid. In a month there are doubtful traces of clotting, but no true separation occurs.

Glucose at 25° C. No results.

Not pathogenic to guinea-pigs according to Dr. Kanthack's report.

No. 23 was revived in June from an agar-culture thirteen months old, which had remained quiescent from May 23 to June 24 of the following year. It came up on the plates very white and rapidly liquefied on the fourth day at 20°. The spider-like zoogloea-masses, characteristic fringed edges, and flame-like films on the surface of the gelatine suggested the type, and further cultures confirmed its identity, including the yellow colour.

The *Stab-cultures* gave a thistle-head funnel, very slowly liquefying at 20°, but in all other respects to type.

Streak-cultures, side by side with the stab, gave rapidly scooping streaks, and in five days an abundant ochre growth and deposit formed in the liquefying gelatine.

On *agar* and *potato* the cultures were normal; except that

on potato the very deep orange waxy appearance was developed forthwith at 25° C. In broth the growth stops below 35° C.

In spite of minor variations there could be no doubt of the identity, and the characters obtained undoubtedly strengthen the resemblances between this form and No. 7.

If Zimmermann's *B. radiatus*[1] is compared with my No. 23, it will be found that the coincidences are so numerous that they point to identity. He gives the thickness as about 0·65 μ, the rods being slightly motile. He notes the whitish-bluish-grey colour, and a 'root-like' central mass; the branching to finer and finer twigs of this root-like central mass (my central zoogloea-tresses) and the general resemblance to a mycelium. Most of his details, including liquefaction on the fourth day, the yellow colour, and radiate bundles, come into accord with my description, the slight discrepancies being easily referable to differences in growth.

The stab-cultures will also bear close comparison, though we have each employed different modes of expressing the similar events.

The same is true for the agar-cultures, and we have both noticed the greenish hue. On potato also, Zimmermann's 'ochre-yellow, often tending to red-brown' seems not inconsistent with my 'eventually more orange.'

Similarly with the broth-cultures, he also notes the falling flecks and yellowish-white deposit. The temperature and other conditions, and even the failures with Gram's method, agree, and the whole impression I get from Zimmermann's description leads to the conviction that we are both working with a *Proteus* form, either identical or closely allied and belonging to the same type.

No. 7: **B. radiatus** (Zimm.). Figs. 43–45.

A peculiarly indistinct colony frequently made its appearance on the isolation plates, like a faint mycelium sunk in the gelatine. This was found to consist of radiating rows

[1] Zimmermann, l. c., p. 58.

of rods, each about 3–4 μ long by 0·5 or 0·6 μ thick, in series. Each rod 4 μ long seemed to consist of four segments, so that we may take the unit-rodlet as about 1 × 0·5–0·6 μ. Old gelatine-cultures give cocci.

Plate-cultures at 12–15° yielded variable colonies of the mycelioid and branched zoogloea- and arborescent forms (Figs. 43 and 44) so common in *B. arborescens* and its allies, and comparable with those of No. 23. After being in culture some months, plates at 18–21° C. showed the tenuous bluish, hardly visible, circular clouds (like No. 23) in forty-eight hours, and these on the third day are about 5 mm. diameter. On the fourth day 8–10 mm., very tenuous and cloudy, and with offsets like nebulae around (Fig. 43 *a*).

Under the $\frac{1}{3}$ any group of these offsets is seen to be composed of extremely fine, curved, reticulated and branching colourless tresses, radiating from serpentine, yellowish, central, stouter, curved and branched zoogloea-tresses (Fig. 43 *b*). On the fifth day the colonies are 15–20 mm. in diameter, very grey and cloudy, and spreading into the gelatine, which softens. On the sixth day the ochre-yellow colony opens into sieve-like mottled nets, with thin contoured iridescent margins (like No. 23).

After passage through broth, the cultures at 20° (see Figs.) go through the serpentine zoogloea stage, to dendritic and mycelium-like forms, exactly as in *B. arborescens*, and in six days liquefy and become more and more ochre-yellow. These colonies fall to pieces and float before the sieve-like stage is reached.

Stab-cultures at 12–15° showed in two or three days as a yellowish or ochre film, spreading like a spider's web, or like irregular stars over the surface, while ochre dots appeared in the tunnel (Fig. 45). The gelatine liquefies, and in a week the yellow film has sunk, one-tenth of the gelatine being liquefied and somewhat turbid, and an ochre-yellow precipitate lying on the flat floor. Growth is very slow in the tunnel, cloudy masses occupying its narrow calibre. In two months half the tube is liquefied, and a bright orange deposit lies on

the flat floor, under a nearly clear yellow liquid. The liquefaction does not quite reach the bottom of the still slender tunnel, but it gradually extends to the bottom of the tube in the course of three or four months.

At 18–20° the thin film above is rapidly developed in forty-eight hours, with a yellow liquefying centre, and the axis is surrounded with a pale cloudiness as in No. 23 under the same conditions. On the third day yellow cloudy liquefaction sets in above, and cloudiness throughout the solid gelatine, exactly as in No. 23; and so also on the fifth day, i. e. the axis fades, and a watery layer with yellowish cloudy masses occurs all over the top.

At 24° rapid liquefaction as a cloudy yellowish mass above, and clouds in the gelatine.

Streak at 15° C. Rather rapid ochre-yellow streak, scooping in forty-eight hours and with a delicate fringed filmy border to the scooped channel. The orange mass slides down into the viscid liquid at base. In fourteen days nearly all is liquid, bright orange deposit and a turbid liquid exactly like No. 17, but with no veil. All liquid in twenty-two days: deep orange deposit.

At 23° the behaviour is almost exactly the same—rapid scooping of a channel off which the mass runs, leaving a thin fringe of radially arranged film attached to the gelatine. On the third day the action is somewhat slower than at 15°, but in longer cultures (fourteen days) it is a little more rapid: otherwise exactly the same. All liquid in twenty-two days, and deep orange deposit, consisting of cocci only (examined in water).

Agar at 34° C., showed no growth.

At 25° the growth is also slow and thin. In seven days a scarcely visible film, smooth, glistening and transparent, and only visible owing to its faint iridescence, and yellow colour when viewed edge on: a yellowish deposit in the drainage. Even in fourteen days the layer does not thicken, but on passing the loop over one collects a yellow mass.

At 22° a similar thin orange film grew, and orange deposit.

In about a week the glassy layer can be made out to be orange-yellow, and the deposit is deep orange. Older cultures, kept a month or more at 12–15°, show a more distinct growth —still thin, but more opaque and evident, and scraping off as a thin orange paste.

After being three or four months in culture, this form became so weak that it hardly grew at all on agar at 15°, 25°, or 30° if infected from agar; if, on the other hand, infected from old gelatine-cultures it grew very well at 15° as a glassy film to type.

The failures are not due to aëration; no growth was got in anaërobic conditions in fourteen days at 22–25° C.

After cultures through broth and again isolated on gelatine, however, the agar-growths at 18–21° were as good as ever: yellow gummy layers in forty-eight hours with pale lilac iridescence and spreading all over. This lilac iridescence is sometimes very marked about the fourth day. In eight days the lilac tinge has gone, the thickish layer is ochre-yellow, gum-like with jagged thin edges, and deposit yellow. Much more yellow than No. 23.

On *Potato* at 22° C. it often refuses to grow, but in some cases a slight golden-yellow patch forms, and in a few cases this became a dull orange fairly thick patch in seven days. It does not spread, and becomes dull, dry and wax-like. At 25° the best growths were very slight, pale golden-yellow, hardly extending beyond the streak. No growth could be got on potato at 34° C.

Broth at 25° C. Slightly turbid in forty-eight hours. In a week a slight turbidity and faint ochre deposit. In a month the same shimmering turbidity and orange deposit. No growth at 35°, but at 18° it is as good as at 25°. The colour of the deposit deepens to red-orange, and the broth becomes sherry-colour.

Milk at 25° showed no signs in fourteen days, but traces of separation were observed on the fifteenth, and in three weeks a stiff clot had formed; the reaction faintly acid. In a month all the casein was down as a coagulum below a straw-coloured

liquid; but no signs of peptonization could be detected in six weeks.

Glucose at 25°. No results beyond a very faint shimmer about the ninth day, and an extremely slight, hardly white deposit.

No. 7 was found by Dr. Kanthack to be non-pathogenic to guinea-pigs.

No. 7 was revived from an agar-tube thirteen months old (May 28 to June 14 of following year), but it was so weak[1] that I could not get the plate-colonies beyond the stage of golden-yellow circular colonies of the λ type—minute points not more than 0·5 mm. diameter just sinking into the non-liquefied gelatine in fourteen days, at 20°, and then growing no larger; liquefaction never took place.

It certainly appears that here we have a weakened liquefying form becoming non-liquefying. If we compare No. 7 with No. 23 the following points of difference come out.

No. 7 breaks up to shorter rodlets. In the plate-colonies there are slight differences of colour and rate of liquefaction and pigmentation only.

In stab-cultures also the differences are chiefly as to depth of colour, and No. 23 forms a veil.

Agar-cultures of No. 7 differ little in colour and thickness, and it appeared somewhat weaker, and this feebleness appeared on potato also.

But revival in broth was easy at first. In broth No. 23 differs by its veil and greyer deposit.

In milk No. 7 slowly coagulated but did not peptonize, and in this is distinct from No. 23.

These differences can hardly be insisted on as distinctive, and I therefore regard No. 7 as the same as No. 23, and both as identical with Zimmermann's *B. radiatus*.

I have already referred to the connexion between these forms and the *Proteus* of Hauser, and this is strengthened by the evident preference for peptones and broth as against

[1] i. e. I infer it is weakened, by its behaviour in gelatine, and because no attempts to invigorate it by broth-cultures succeeded.

carbo-hydrates. As we shall see, also, several other varieties exist which behave similarly.

No. 31 : **Yellow Proteus Type.** Figs. 46–48.

This form was often met with in the Thames. It occurs on the plates as rods 2–4 $\mu \times 0{\cdot}5$ or $0{\cdot}6\,\mu$, or as coiled filaments of rodlets 2–3 μ long and about $0{\cdot}7\,\mu$ (in water) thick. Other measurements give 2–6 $\times$ 0·5 to $0{\cdot}6\,\mu$.

Plates at 12–15° give white circles with star-like central portion, opening out to arborescent or sheaf-like branched forms, with 'wandering islets' and rapidly liquefying.

At 18–21° C., the colonies appear on the fourth or fifth day as translucent, circular, contoured, colourless or bluish fronds with a yellowish central boss; the edges extremely tenuous, and the central parts varying to spidery and contoured figures. In a week the colour becomes pale-ochre, and liquefaction begins; the colonies 10 mm. or so in diameter forming flocculent mottled and flame-like masses in the liquefied depression. All the stages from the contoured type (Fig. 47 *a*) to the forms with mosaic-mottled clouds are to be traced (Figs. 46, 47, and 48).

Plate-cultures after passage through broth show in twenty-four hours as spider-like colonies. In forty-eight hours all stages of the type are seen: the submerged yellowish ovals and the spidery-like zoogloeas with tails. They emerge as thin contoured plates, colourless, and with wandering ringlets (see Figs. 47 *b* and *c*). On the fourth day they open out as pale ochre sieves, like No. 23, and liquefy (Fig. 48).

Stab-cultures at 12–15°. A sieve-like flecked yellowish-white membrane grows above, and floats in a week on the softening gelatine as a clouded patch: yellowish dots in the tunnel. About the ninth day the dirty-white to yellow-ochre cloudy growth is sinking, and in a fortnight it resembles No. 23. In two months half the tube is liquefied, rather turbid, and has a floating flecked veil.

At 18–21°, in forty-eight hours a yellowish-white small

sinking spot above, and dots in tunnel. No cloudiness. On the third day a shallow funnel of liquefaction above: no change in tunnel. Thistle-head in five days, white flocculent mass; axis with yellowish-white dots, sword-shaped.

At 24° also much slower than Nos. 7 and 23.

Streak at 15°—very slow, merely shows bluish-white in forty-eight hours.

At 23° also slow: mere streak in forty-eight hours.

Agar at 34°. No growth. At 25° a thin, smooth, yellowish-white layer, pinkish in some lights, covers the agar in five days, and an abundant yellowish-white deposit falls. It is like No. 23.

At 22° rapidly forms a thin, glassy, yellowish-white film with a greenish cast. In three days the fimbriated edges are remarkably iridescent: yellowish precipitate in the slightly turbid drainage. In four days, a greenish-white glistening layer—the green sheen only in some lights.

The layer, when scraped off with a loop, is dirty-white.

Exactly the same difficulties as with No. 7 occur as regards old agar-growths losing power. No growth under anaërobic conditions in fourteen days at 22–25° C.

At 18–21° a greenish-white flecked layer with pale lilac iridescence in some lights, rapidly spreads in forty-eight hours. The curious faintly marbled or flecked appearance was sometimes marked about the fourth day; it has a slight iridescence of greenish and lilac. In eight days, much like No. 23, but edges less jagged, and green to lilac iridescence still marked. Yellow-white deposit.

Potato at 30° C. No growth. At 25° a greyish film in twenty-four hours becoming drier and not spreading. The growths on potato at 22° varied considerably according as the medium was alkaline or not. On ordinary potato a pale ochre patch formed in forty-eight hours, and this spread but little, as a dry small ochre streak with a whitish rim: on alkaline potato a thin gummy ochre film formed in forty-eight hours, and in three days this was flowing all over, with lilac margins. This pink-lilac hue spreads as the

yellow, wet, flowing mass runs over the potato, and in a week the general tone is more and more golden-yellow, displacing the ochre and the pink. All wet and gummy-looking.

Broth at 25° C. Slightly turbid in twenty-four hours. In a week a faint white ring, slight turbidity, and abundant yellowish-white deposit. In nine days a dense white free veil, no ring and the liquid turbid. Resembles No. 23. In old cultures the liquor is sherry-colour and the deposit pale ochre.

Milk at 25° C. No visible alteration in three weeks, but the liquid is acid. In a month the casein is partially separated.

Glucose at 25° C. No result.

Dr. Kanthack found it was not pathogenic for guinea-pigs. I found it impossible to revive No. 31 from agar-tubes a year old.

On comparing No. 31 with No. 23, the following are the only differences to be noted.

The sieve-like film on the surface of stab-cultures—not essentially different in type from the stellate film of No. 23.

The very slow streak-cultures at both 15° and 23° C.

The pink hue on potato-cultures is a difference, though it did not persist. Here again we cannot insist on the differences, though they appear in parallel cultures, and I therefore place No. 31, with Nos. 23 and 7, as mere varieties of the same form—*B. radiatus* (Zimm.) and belonging to the *Proteus* group.

No. 17: **B. ochraceus** (Zimm.). Figs. 49–53.

Morphology &c. Composed of very short rods $1 \times 0{\cdot}5\,\mu$ in rows as stained. Fresh preparations show pairs of rodlets $2 \times 0{\cdot}6$ to $0{\cdot}7\,\mu$, or long series, i. e. filaments, segmented into rods about $1 \times 0{\cdot}6\,\mu$. Old gelatine-cultures give cocci about $0{\cdot}6 \times 0{\cdot}5$ to rods 2–$1 \times 0{\cdot}6\,\mu$, all quiescent: old agar-cultures give all cocci or very short rodlets about $1 \times 0{\cdot}7\,\mu$.

On plates at 12–15° very small yellow colonies, which in three to four days float in a viscous liquid, as circular, yellow,

granular firm dots, pale and very transparent under the $\frac{1}{3}$. These are apt to stick to the glass (Fig. 49).

On re-sowing and obtaining less crowded plates, the colonies form yellow-eyed, sinking circular colonies, with spider-like or dumb-bell-shaped zoogloea-mass in centre, and floating islets around by the end of a week. Most of the surrounding clouds or islets are spider-like zoogloeas (Fig. 50).

In three weeks the gelatine is softened, and the colonies pale yellow. Sour smell. Plates of No. 17 after being some months in culture give, at 18–21°, colonies just visible in forty hours, under $\frac{1}{3}$ these are moruloid, or spiky, or spider-like zoogloea-forms, yellowish.

On the third day (see Figs. 50 and 51), while most of the colonies are as above, but larger, others emerge as plates 1 mm. diameter with spider-like zoogloeas in the centre. On the fourth and fifth days the small yellow dots present similar features, with much variation in details, and the yellow colour is more pronounced; some are still moruloid or gland-like, and submerged; others are cloudy centres with outlying nebulae, and consist of much branched, dendritic, zoogloea-tresses; others are contoured plates as before, with zoogloea in the centre. In the course of two or three days more liquefaction sets in, and the floating cloud-like colonies are yellow with a deep chrome-yellow central eye, reminding one of a weak form of No. 35.

The film is more frondose at the edges, however, and sinking begins about the fourteenth day, and a yellow frondose floating mass remained above, like egg-yolk.

Stab-cultures at 12–15°. Begins by forming an extremely thin yellow film above, and pale yellow dots in the tunnel in three days. In six days the yellow centre is surrounded by a bluish or pale purple iridescent border: slimy and viscous to the needle.

At 18–21° a slight sinking yellowish spot is formed above in forty-eight hours, and short root-hairs along the axis (Fig. 52 *a*). Small funnel in three days. In five days rings around the axis may replace the root-hairs (Fig. 52 *b*).

At 24° rapid spread above and root-hairs along the axis, producing a cloudy appearance all the way down the latter.

In two months the liquefaction has proceeded one-fourth down the tube, the viscid liquid being slightly cloudy. All else as in the type, except the floating yolk-like scum.

Streak at 23°. A very slight yellow streak shows in forty-eight hours. On the third day it is sending pale-yellow clouds into the gelatine, i. e. sinking, but is still only a narrow wet streak. In a fortnight nearly all is liquid, and evenly turbid. Thin yellow floating veil, and orange deposit. In nineteen days all the liquid is nearly clear. Deep orange deposit, of quiescent cocci, and floating flecks composed of rods up to 6–10 μ long.

Agar. No growth at 34–35° C. But at 22° there slowly forms an extremely thin pale-yellow, shining, gum-like film, all over the surface in three to five days, with a slight deposit in the nearly clear drainage, and with bluish iridescence at the margins. The growth stops sharp at the clear drainage. In five weeks the ochre layer is little thicker, but the liquid is turbid and has a copious deposit.

At 25° the faint, filmy, nearly white streak formed in twenty-four hours, becomes a thin canary-yellow smooth layer, and floating flecks and a yellow-white deposit form in the turbid liquor in a week. The growth was better from old gelatine (Fig. 53).

At 18° C., the agar-cultures were exactly like No. 23, except that the amount of deposit in the drainage (in eight days) was much smaller.

Potato at 22°. Extremely slow ; in a fortnight only a small ochre patch, duller than No. 18 and far slower.

Broth at 25°, infected from agar, was shimmering in twenty-four hours. And in fourteen days was exactly like No. 18, with a yellow deposit at the base of the turbid liquor. No growth at 35°, but equally good at 18° and 25°. The deposit turns more orange-yellow and the liquor is of a clear sherry-colour : the orange hue is far less deep than in No. 7, however.

Milk at 25°, infected from agar, gave no results in fourteen days beyond acid reaction, and a *trace* of perceptibly yellow deposit.

Glucose at 25°, infected from agar, gave doubtful shimmering in twenty-four hours. In fourteen days no further results, except a very pale ochre deposit as in the type.

No. 17 was found by Dr. Kanthack to be non-pathogenic for guinea-pigs.

All attempts to revive No. 17, from cultures a year old, failed.

If we compare Zimmermann's *B. ochraceus* with No. 17, the resemblances come out strongly. In sizes and measurements they agree very well, and the capsule-like envelope he found might well be due to the zoogloea investment.

Zimmermann observed movements, 'slow and pendulous,' however, but could detect no cilia. His comparison of the warted young colonies to *Viel-beinigen Gliederthiere* when the prominences become marked, is very suggestive, and taken with the other characters points to strong resemblance, if not identity.

The only essential difference in the stab-cultures is that a floating yolk-like mass not mentioned by him is formed in my No. 17.

On agar the general resemblances are obvious, but in my cultures the growth was much stronger, while on potato they seem alike, and the same may be said of the broth-cultures. As regards temperature and air-requirements, the slow growth and slow liquefaction, failure to obtain spores, and general characters of the yellow pigment, the two descriptions agree.

I think it extremely likely therefore that my No. 17 is identical with Zimmermann's *B. ochraceus.*

If No. 17 is compared with No. 23, the following differences are noteworthy.

The rodlets are much shorter, and even break up to cocci. But we must not overlook the fact that equally short rodlets and cocci occur in No. 7. On crowded plates liquefaction was rapid, and old cultures have a sour smell. On the whole

the type of plate-colony is the same, but the zoogloeas are more condensed, and the colour more golden or chrome-yellow than No. 23.

The chief differences in stab-cultures were the root-hairs in early stages, and the deep chrome-yellow yolk-like veil; the streak-cultures also liquefied more rapidly.

The agar-cultures were also much brighter yellow in hue, but the growth on potato was feebler than the type.

In broth, the brighter colour and absence of veil distinguish it.

It seems likely, in view of all we know, that the more condensed growth on the plates and the deeper colour go hand in hand. If this is so, there may be no sufficient reason for removing No. 17 from the *Proteus* type. I have already shown that No. 17 and Zimmermann's *B. ochraceus* are closely allied, and they must go together.

That these forms are closely allied to Zimmermann's *B. radiatus*—see Nos. 23 and 7 above—will scarcely be denied; and this being so, the position of all these forms in the *Proteus* type seems justified.

B. arborescens (Frankl.). Figs. 54–62.

This form is by no means rare in the Thames, and was described by Frankland in 1889[1], but it is evidently merely a form-variety of the group we are considering, as the following study of it shows.

It exhibits very neatly the connexion between the liquefying and non-liquefying conditions, and I have no doubt it can be modified much more than I have succeeded in modifying it. There can be little hesitation in connecting it with Zimmermann's *B. radiatus* and *B. ochraceus* on the one hand, and with the *Proteus* type and allies on the other.

Habitat. Fairly common in the Thames, especially in the winter.

Morph. Non-motile rods, about 5 or 6 μ long by 0·5–0·6 μ broad, often in pairs, or associated in irregular chains. No traces of spore-formation observed.

[1] Zeitschr. f. Hygiène, B. VI, p. 379.

Plate. Extremely variable according to the conditions. The colonies begin as yellowish granular disks or ellipses. These grow out radially or at the ends into branched zoogloeas and fimbriated tufts, or may elongate into snake-like zoogloea-forms branching at the two ends: thus, according to conditions, there are formed in from two to four days, circular, dumb-bell-shaped, irregularly branched arachnoid or serpentine zoogloea-colonies, ochre-yellow in colour, and running into delicate filaments at the margins or ends, which ramify copiously (Figs. 54–57). To the unaided eye these colonies appear as delicate, mould-like, faintly iridescent growths, or as more obvious, ochre-yellow, somewhat zoned, oval or round colonies. At low temperatures, especially when the plates are exposed to light, the emerging colonies grow very slowly as thin, frondose, typhoid-like, irregular plates, marked with striae, and almost colourless, except at the thicker centre, where there is an irregular yellowish mass (Fig. 62): the submerged colonies under such conditions are dull yellow, very irregular in outline, and marked with constrictions. The mycelium-like, more delicate and branched form (Fig. 56) rapidly liquefies the gelatine completely in 24–48 hours, the dumb-bell-shaped and zoned circular colonies (Fig. 55) only liquefy slowly and incompletely after four or five days. The frondose plates (Fig. 62) show no signs of liquefaction even after a week.

Streak. At 15° C., an ochre-yellow, streaky and flecked growth is formed, which slowly liquefies the gelatine to a glairy fluid. In ten days half the tube is liquefied to a viscid fluid, in which a yellow deposit forms.

Stab. In two days at 15° C., a thistle-head funnel of liquefaction is formed, with greyish dots in the tunnel, and an ochre-yellow somewhat stellate mass floating on the liquid above. As the funnel approaches the sides of the tube and deepens (four to five days) the colonies in the tunnel show slightly yellow, and ochraceous flecks are deposited at the bottom of the liquefied gelatine above. In two to three weeks the gelatine is liquefied one-eighth down, a thick yellow deposit forming below the liquefied portion. (Fig. 58; cf. also Fig. 45.)

Agar. In twenty-four hours at 20° C., the streak is yellow, with thin, glistening, frondose spreading margins, which are whiter and slightly iridescent. In three days (Fig. 59) the broad and more frondose expansion is a brighter ochre-yellow, thicker, glistening, with delicately fimbriated, thin, irregular, iridescent margins.

Potato. In twenty-four hours, at 20° C., forms a deep golden-chrome spot. On the third day the colour is still richer, the surface waxy, but rather dull, growing slowly. In seven days a thick waxy mass, red-orange, especially in the centre, surface rather dull, margins striated (Fig. 60).

Broth. Slight turbidity in twenty hours, at 20° C., and this increases during the second and third days. On the fourth day a yellow deposit is formed, but no veil above. In ten days or so the turbidity diminishes and a copious yellow deposit has formed below.

Milk. Slowly peptonized, without previous coagulation.

Requirements as to air. Aërobic.

Temperature. Grows slowly at 10° C., fairly rapidly at 15° C., more so at 20° C.

Liquefaction. Sometimes hardly, or even not at all observable. In other cases fairly rapid.

B. arborescens shows obvious resemblances both to No. 23 and to No. 17, and the forms allied to them, and there can be little doubt as to the propriety of placing it here. But the principal interest attaching to *B. arborescens* is the experimental proof of the extreme variability of the colonies according to conditions.

When the temperature is very low, or the *Bacillus* has been exposed to unfavourable conditions for some time, e. g. exposed to light at 10–12°, or in very old gelatine-tubes, its feeble growth is expressed by slowly emerging as transparent, contoured, thin plates, like a feeble colony of *B. coli-commune*, only that one can still recognize the dense spider-like or star-fish-like zoogloea in the depth (Fig. 62). The fine contours are due to the much curved parallel filaments on the surface of the gelatine (cf. Fig. 11): these lie closely side by side and remain so because no liquefaction occurs, and the segments are therefore held in place flat on the surface of the gelatine.

When the plates are exposed to sunlight for three or four hours, at ordinary temperatures, taking care that the gelatine does not melt; or if the sowings are made from tubes of water exposed to the sun; the type of colony produced is more like

that of No. 23, i. e. slowly growing circular colonies with an ochre-yellow or leather-yellow central spot, and sometimes a zone of the same colour, and with very evident radial structure. On magnifying this, one finds densely branching and interwoven filaments radiating and crossing one another from a central zoogloea of the arborescent or serpentine type. The extreme tips of the irregularly radiating strands are very thin, and give the fimbriated appearance shown in Fig. 57 (cf. Fig. 37). These colonies slowly liquefy the gelatine, and each lies for some time in its own lenticular circle of liquefaction.

When, on the other hand, the colonies are developed from Bacilli in full vigour, e. g. after passing through broth and not exposed to light, they appear in forty-eight hours or so as serpentine zoogloeas with thin fimbriated branches from the ends, and at once liquefy the gelatine throughout, in the manner typical for a *Proteus*, without developing into formed visible colonies. This is because the Bacilli separate off and wander all over, and liquefaction is so vigorous that no condensed growths are formed after the first (Fig. 56, and cf. Fig. 44).

There is no doubt that in these experiments the form of colony is dependent on the conditions to which the Bacilli have been exposed, because the latter have their powers of liquefaction, and therefore of nutrition and growth, affected. Unfortunately it is impossible to so arrange matters that only one factor at a time is varied in the environment, and the experimental proof of the action of each individual factor is therefore wanting. We have here, however, ample proof that it is important to keep the conditions as constant as possible in plate-cultures.

Sanfelice[1] had already suggested that *B. arborescens* is a variety of Hauser's *Proteus*, as is also *B. aquatilis* of the same authors. My studies of the yellow liquefying forms which I have classed as Group IX in my fifth report to the Royal Society[2], suggest that Zimmermann's *B. fulvus*,

[1] Lustig, l. c., p. 85.

[2] Roy. Soc. Proceedings, Vol. lxi, p. 419.

B. subflavus and *B. ochraceus* are also connected by this form to the true *Proteus* type.

Having regard now to all the facts, there can be little doubt that my Nos. 7, 23, and 31 are allied to the *Proteus* type ; and if we admit this, and bear in mind that they all become yellow as the cultures progress, the same is true of Nos. 17, K, and *B. arborescens*, in spite of their assumption of the yellow pigments at so early a date in their development. My own conviction is that we have in Hauser's three 'species' of *Proteus*, and in my six forms 7, 17, 23, 31, K, and *B. arborescens* a set of varieties of the same species, the definition of which rests on their agreement in size and form, mode of growth, formation of zoogloeas and tresses, method of liquefaction, requirements as to temperature and so on, but that they vary in detail as to rapidity of growth and liquefaction, and consequent differences in the extent and appearance of the colonies, and as to the intensity of pigmentation, which may well be assumed to vary with the above.

But if this is so, we shall have to go further, and to regard Frankland's *B. aquatilis* as belonging to the same series—it may well be a merely weaker variety of his *B. arborescens*—and Zimmermann's *B. radiatus* also, for this is almost certainly identical with my No. 23.

The most serious difficulty here is as regards No. 17, for it shows unquestionable resemblances to Zimmermann's *B. ochraceus*. If these are also identical, then we shall have to consider the question of a possible (indeed probable) alliance between this *Proteus* group and the whole of the golden-yellow liquefying forms which I have brought together as Group IX.

The following varietal forms of this type may be sufficiently characterized by diagnosis as below.

Varieties K *and* (B). Fig. 63.

Habitat. In the Thames in 1893–4.

Morph. Slender inactive rods 3 to 4 μ × 0·75 μ.

Plate. Circular, radiated and zoned colonies with a yellow eye: arachnoid. Soon fail.

Streak. Soon sinks and forms yellow-chrome flocks. In a month half the gelatine liquefied with an ochre-yellow deposit.

Stab. Rapid in twenty-four hours, cloudy along the axis. In fourteen days no growth visible on the surface, but 'root-hairs,' cloudy and mycelium-like processes round the axis. In a month the same, but with slender cloudy outshoots into the gelatine.

Agar. Merest film or nothing appears up to the fifth day, and hardly any certain growth in three weeks. In a month a very slight buff-whitish deposit forms in the drainage: no streak visible.

Potato. Nothing visible for certain in three days at 20°: in a week a thin, yellowish, filmy streak, nothing further.

Broth. Faintly turbid on the third day, but others show nothing till the seventh day, when a faint turbidity and white deposit are seen. In a month slightly turbid, and a brittle slight veil and ring, and faint yellowish deposit. Others show a doubtful ring.

Milk. No change in a month visible, though perhaps slightly acid in twenty days.

Glucose. No results.

This appears to be an enfeebled form of this type, and it soon failed in the cultures.

EXPLANATION OF FIGURES IN PLATES XII, XIII, AND XIV.

Illustrating Professor Ward's paper on Thames Bacteria.

PLATE XII.

Group VI. The Proteus Type.

Number 103.

Fig. 1. Plate-colonies twenty four-hours at 20° C. Natural size.

Fig. 2. A small colony under the $\frac{1}{3}$, showing zoogloeas and floating islands.

Fig. 3. Young colonies from a plate-culture twenty hours at 20°, showing the origin of the coils, tresses, zoogloeas and islands.

Fig. 4. Cocci from a gelatine-culture three weeks old, $\frac{1}{12}$ imm.

Fig. 5. Streak-gelatine-culture at 20°: *a* after two days; *b* three weeks.

Fig. 6. A two-days' stab-culture at 20° C., showing 'root-hairs,' and moss-like film above.

Fig. 7. Agar-culture twenty-four hours old at 25° C.

Number 34.

Fig. 8. Plate-colonies at the ordinary temperatures (15–18° C.): *a* in ninety hours, *b* in 115 hours, under the $\frac{1}{3}$. In both cases the smaller colonies are submerged.

Fig. 9. The same in sixteen days, at beginning of liquefaction.

Fig. 10. Stab-cultures at 15–18° C., fourteen days.

Fig. 11. Plate-colonies of strong cultures at 20° C.: *a* on the second day; *b* on the third day; both under $\frac{1}{3}$, natural size. *c* and *d* two of the colonies on the fifth day, under the $\frac{1}{3}$.

Number 51.

Fig. 12. Colonies of No. 51 in forty-eight hours: *a* natural size, showing the almost invisible tenuous film *b* and *c* under the $\frac{1}{3}$; in *b* the surrounding film was too tenuous to see; in *c* only part of the margin is shown.

Fig. 13. A plate-colony of the largest size on the second day at 20° C. Natural size; the tenuous film just visible.

Fig. 14. A stab-culture after a week at 18° C.

Fig. 15. A stab-culture, more vigorous, after three days at 18° C.

Fig. 16. Successive divisions of a rodlet isolated in gelatine: *a* at 11.5 a.m.; *b* 11.50; *c* 1.0 p.m.; *d* 1.50; *e* 2.40; *f* 3.30; *g* 6.15. (See text, p. 206.)

Fig. 17. Outlines of the pseudopodial films from a two-days' plate at 19° C.: *a* to *e* drawn at intervals of one minute. Under $\frac{1}{3}$.

Fig. 18. Similar series at intervals of $\frac{1}{2}$ to one minute, $\frac{1}{3}$.

Fig. 19. Similar series: *a* at 8.4; *b* at 8.8; *c* at 8.9; *d* at 8.10, $\frac{1}{3}$.

PLATE XIII.

Fig. 20. Similar series: *a* at 8.20; *a* to *h* at intervals of one minute; *l* at 8.39; the rest at intervals of a minute. The arrows mark the direction of rotation, $\frac{1}{3}$.

Fig. 21. Culture under E/4 in a hanging drop of gelatine, at 16–21° C.: *a* the isolated rodlet at 11 a.m.; *b* progeny at 4 p.m.; *c* at 8 p.m.; *d*. at 9.30. The resulting colony measured $10 \times 8\,\mu$. At 11.10 it measured $12 \times 10\,\mu$; at 8 a.m. next day $34 \times 32\,\mu$; at 9.30 it was a sphere $36\,\mu$ diameter; at 10.55 = $44\,\mu$, and so on. See text, p. 211. At 3.30 it was a sphere too large to measure, and *e*, *f*, *g* show the further changes under the $\frac{1}{3}$; *e* 3.30; *f* 6 p.m.; *g* 8.40 p.m.

Fig. 22. Details of movement of the filaments: *a*-*c* under $\frac{1}{12}$ imm.; *d* under E/4.

Fig. 23. Rods, filaments and zoogloea-clumps from advanced liquefying colony, $\frac{1}{12}$ imm.

Number 9.

Fig. 24. Plate-colonies of No. 9 at 15–18° C.: *a* on the third day under $\frac{1}{3}$; *b* on fourth day, natural size; *c* the same under $\frac{1}{3}$; *d* on the fifth day, natural size.

Fig. 25. Stab-cultures at 15–18° C.: *a* in forty-eight hours; *b* on the fifth day; *c* on eleventh day.

Fig. 26. No. 9 *a*. Plate-colonies as isolated: *a* in ninety hours, submerged under $\frac{1}{3}$; *b* in 115 hours, natural size; *c* the same magnified.

Variety ι.

Fig. 27. A colony under the $\frac{1}{3}$ as seen on isolation plate.

Fig. 28. Plate-colonies under the $\frac{1}{3}$: *a* in ninety hours; *b* in 115 hours.

Variety φ.

Fig. 29. A large colony as first isolated. Natural size.

Fig. 30. Plate-colonies: *a* in 140 hours; *b* in ten days.

Variety ζ.

Fig. 31. Plate-colonies at 12–18° C.: *a* in forty-two hours; *b* in ninety hours; *c* in 115 hours; *d* on sixth day. In *a*, *b* and *c* the small colonies are submerged. In *d* the small colony is natural size.

Fig. 32. From a ten-days' plate at ordinary temperature: *a* submerged under $\frac{1}{3}$; *b* emerging (natural size); *c* the same under $\frac{1}{3}$.

Fig. 33. From a six-days' plate: *a* submerged under $\frac{1}{3}$; *b* emerged, natural size.

Variety μ.

Fig. 34. A plate-colony as isolated.

Variety θ.

Fig. 35. A two-days' plate-colony. Natural size.

Group VII. The Yellow Proteus Type.

Number 23.

Fig. 36. Plate-colonies of No. 23 on a five-days' culture at 12–15° C., showing the great variety in type: *a*, *c*, *d* natural size; *b* part of the tenuous margin of *a* under the $\frac{1}{3}$.

Fig. 37. Plate-colonies at 20° C: *a* two of the very tenuous colonies after two days; *b* one of them under the $\frac{1}{3}$; *c* colonies on the third day, natural size; *d* part of the margin of a colony; *e* one of the strands in *d* more highly magnified.

Fig. 38. Plate-colonies on third day under a lens to show structure, at 20° C.

Fig. 39. Culture in five per cent. gelatine-drop, $\frac{1}{12}$ imm.: *a* at 9.20; *b* at 11.0; *c* at 11.50; *d* at 12.30; *e* at 5.0; *f* at 10 p.m. The rods feebly motile. See p. 227.

PLATE XIV.

Fig. 40. Swarming islets drawn at intervals of one minute, in five per cent. gelatine; order = *a–g*.

Fig. 41. Stab-cultures of No. 23 at 18–21° C.: *a* in forty-eight hours; *b* in a week.

Fig. 42. Plate-colonies from a nine-days' culture after lying dormant a year: *a* natural size; *b* under $\frac{1}{3}$.

Number 7.

Fig. 43. Plate-colonies of No. 7 on the fourth day at 18–20° C.: *a* natural size; *b* one of the outlying groups under the $\frac{1}{3}$.

Fig. 44. Colonies from a forty-eight-hours' culture-plate under the $\frac{1}{3}$.

Fig. 45. Stab-culture four days at ordinary temperature.

Number 31.

Fig. 46. Colonies from a six-days' plate at 18–21° C., natural size.

Fig. 47. Similar colonies under $\frac{1}{3}$ from a forty-eight hours' plate : *a* emerged ; *b* submerged ; *c* ' floating islands ' more highly magnified.

Fig. 48. A colony from an eight-days' plate, natural size.

Number 17.

Fig. 49. Plate-colonies of No. 17 at 18–21° C., for three days : *a* natural size ; *b* an emerged colony under $\frac{1}{3}$; *c* submerged colony under $\frac{1}{3}$.

Fig. 50. The same on the fifth day : *a* an emerged colony, natural size ; *b* a part of one of its marginal outlyers ; *c* a submerged colony under $\frac{1}{3}$.

Fig. 51. A colony twelve days at 20° C., natural size, liquefying.

Fig. 52. Stab-cultures at 18–21° C. : *a* on third day ; *b* on fifth day.

Fig. 53. Agar-culture one month at 25° C.

B. arborescens.

Fig. 54. A typical plate-colony of *B. arborescens* isolated from the river, two days at ordinary temperatures (15–17° C.) : *a* natural size ; *b* under the $\frac{1}{3}$.

Fig. 55. *a* A slowly grown plate-colony on sixth day under $\frac{1}{3}$; *b* another colony on sixth day, and *c* the same on eighth day, both under $\frac{1}{3}$.

Fig. 56. Quickly grown colony in dark at 20° C., forty-eight hours, under $\frac{1}{3}$. In this condition they rapidly liquefy the plate.

Fig. 57. Colonies retarded by light, four days old : *a* natural size ; *b* under $\frac{1}{3}$.

Fig. 58. Stab-cultures at 15° C. : *a* and *b* after two days ; *c* fifth day ; *d* three weeks.

Fig. 59. Agar-culture three days at 20° C.

Fig. 60. Potato-culture seven days at 20° C.

Fig. 61. Rodlets from gelatine-cultures.

Fig. 62. Colonies from a seven-days' gelatine-plate in the dark at low temperatures (6–12° C.), showing retardation and complete change of form.

Fig. 63. Plate-colonies of variety (B) as isolated : *a* natural size ; *b* the same under $\frac{1}{3}$; *c* natural size ; *d* the same under $\frac{1}{3}$.

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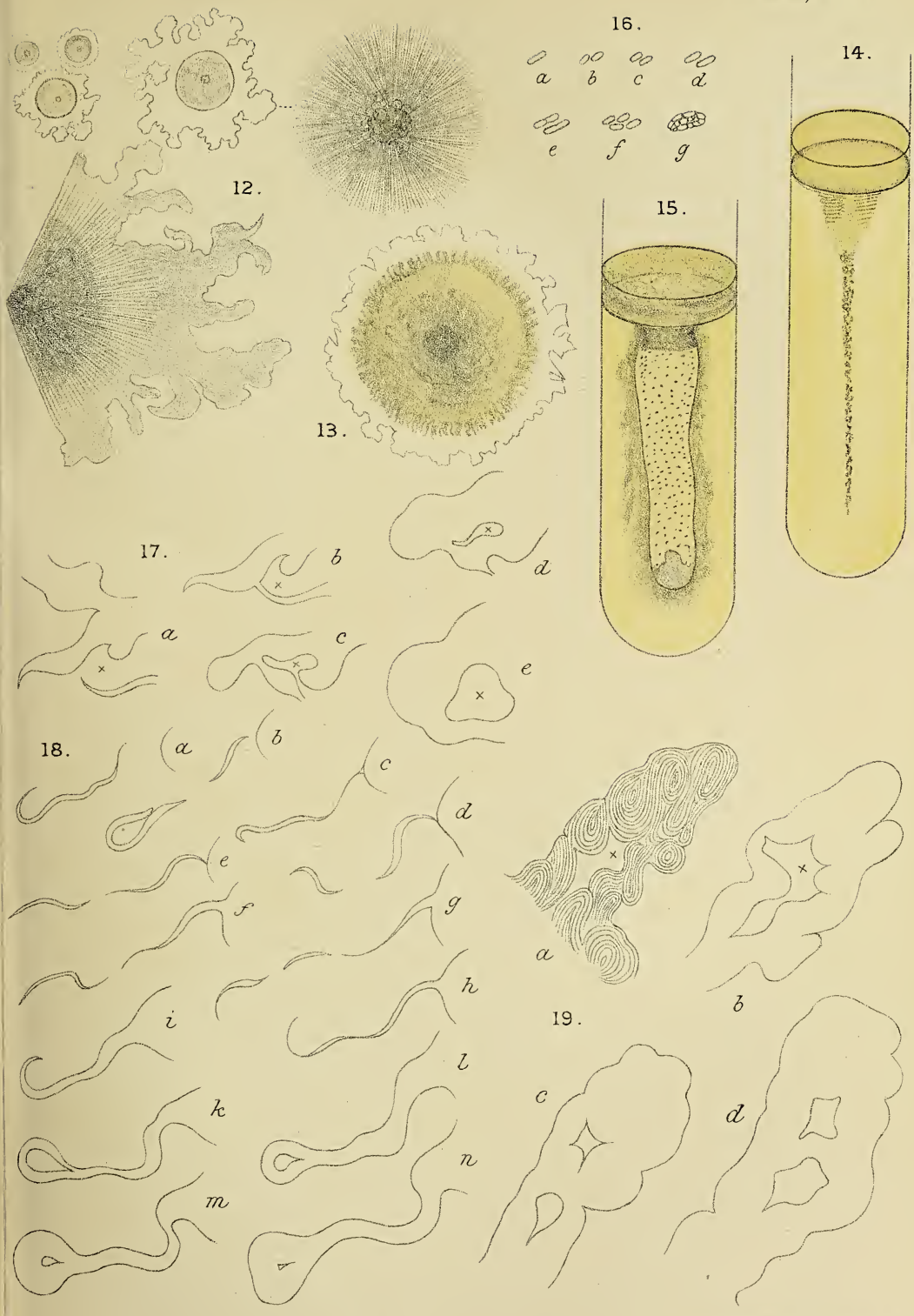

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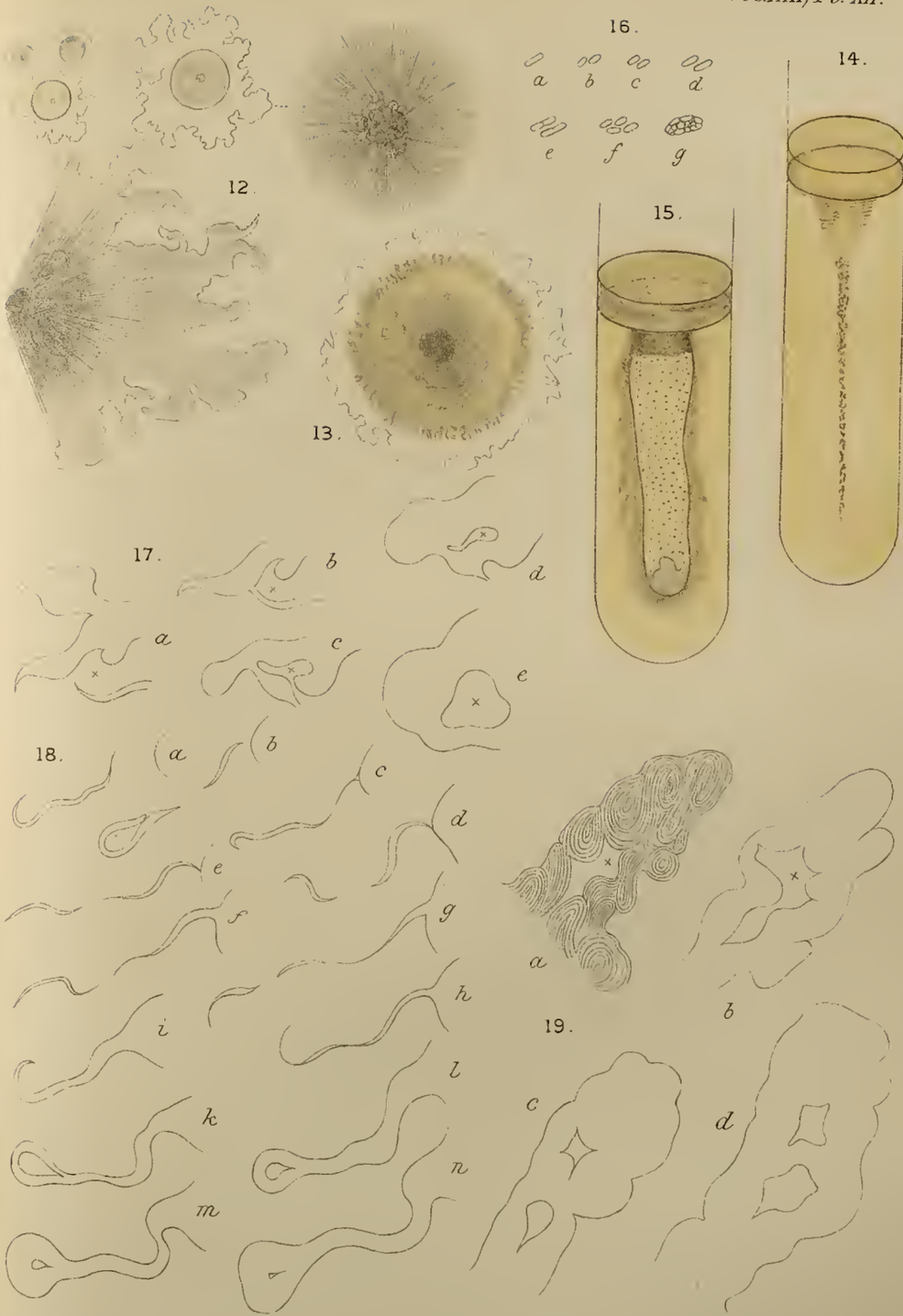

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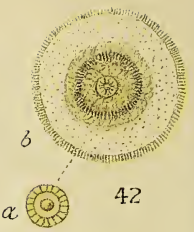

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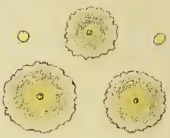

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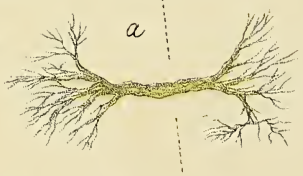

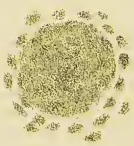

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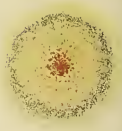

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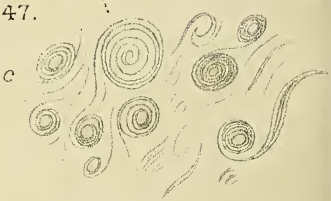

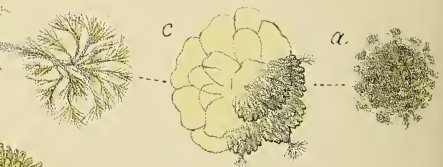

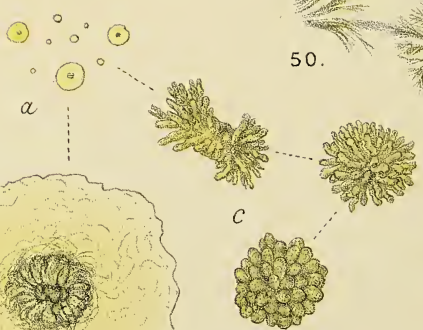

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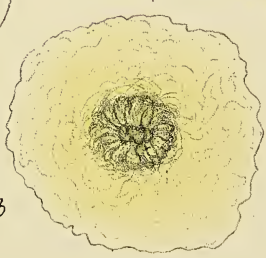

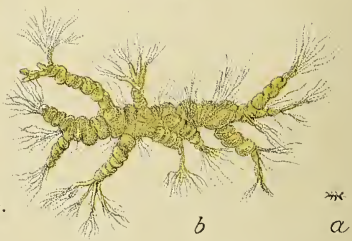

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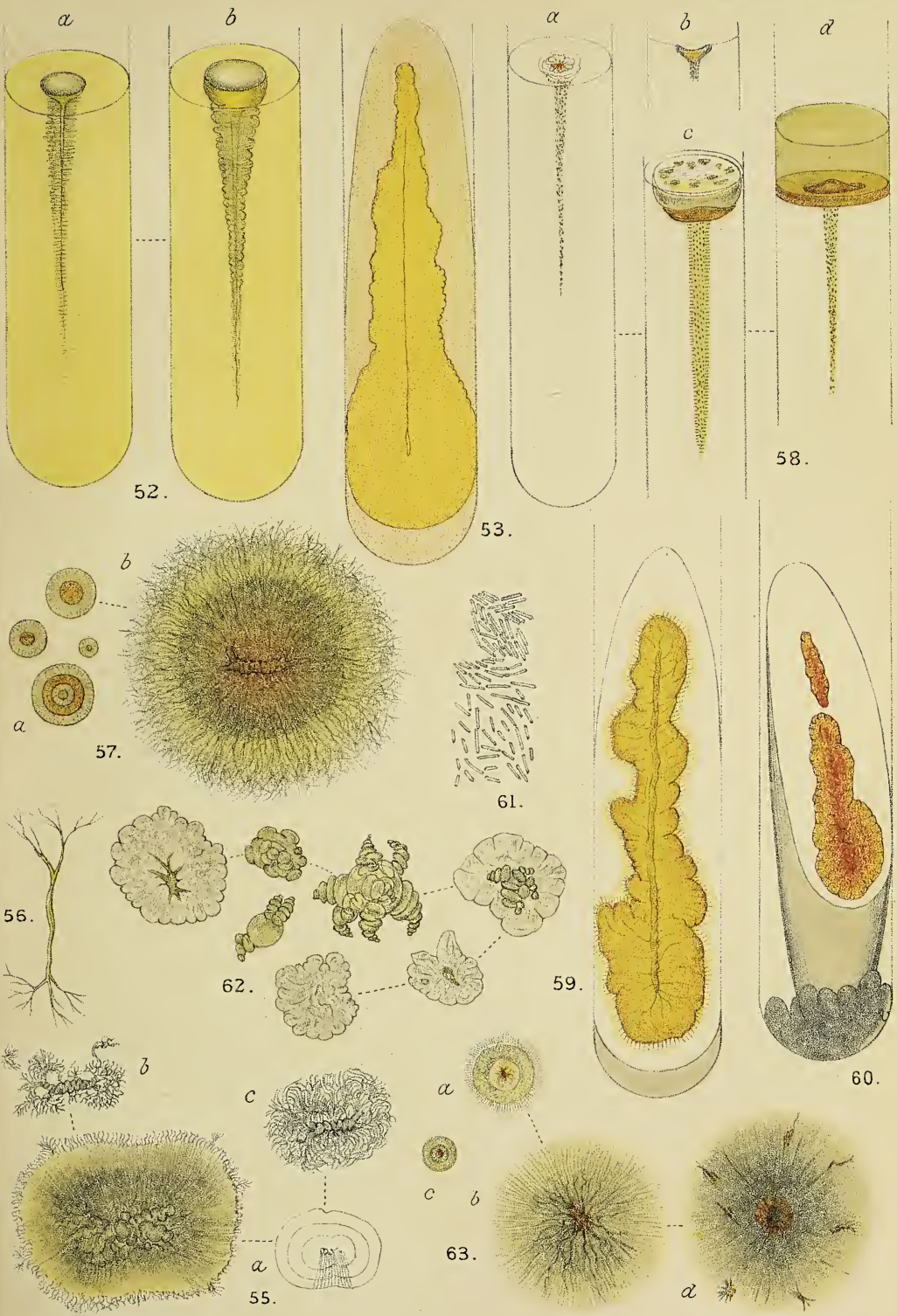

University Press, Oxford.

On Actinococcus and Phyllophora.

BY

OTTO VERNON DARBISHIRE,

The Owens College, Manchester.

With Plate XV and seven Figures in the Text.

Credidi enim et etiamnunc credo, tubercula illa nihil aliud esse quam parasiticum quid . . .—LYNGBYE, Tentamen Hydrophyt. Dan., 1819, p. 11.

IN 1893 Schmitz published a paper, in which he discussed at some length the *Actinococcus* question. He maintained that all so-called forms of fructification of *Phyllophora Brodiaei* (Turn.) J. Ag. which he had so far been able to examine, belonged in reality to a different Floridea growing parasitically on the former species (5, p. 371). This paper was shortly after reviewed by Gomont, who expressed his full agreement with the views held by Schmitz; so that the true nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. still remained to be found.

Doubts had long been felt with regard to the true nature of the so-called nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. The few lines quoted at the head of this paper were written by Lyngbye in 1819 in describing these very same nemathecia. In the beginning of 1894 the author of this paper gave

[Annals of Botany, Vol. XIII. No. L. June, 1899.]

a preliminary account of some observations on the anatomy and development of the Baltic species of *Phyllophora* Grev., in which Schmitz' assertions concerning the parasitic nature of the nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag. were discussed and the accuracy of his conclusions was doubted (1, p. 47). A more detailed account of the author's work on the Baltic Phyllophorae was published about a year later, but unfortunately Schmitz died in 1894. In the second paper just mentioned the author again expressed it as his opinion that the so-called nemathecia of *Phyll. Brodiaei* did really represent the true tetrasporic fructification of this Floridea (2, pp. 2 sq., 23 sq., 36).

The subject has not been worked at by algologists since, and Schmitz' theory has therefore naturally been most generally accepted. Kolderup Rosenvinge alone seems to have adopted an opposite view (4, p. 33).

Since commencing work on the anatomy and development of *Phyllophora* in 1892, the author has devoted much time to the examination of all forms of fructification found on it. Up to 1896 opportunity was however wanting for dredging and examining fresh material during the months of September and October, owing to the author's absence from Kiel during that time. Practically all the material used in these investigations was collected in the Baltic near Kiel. The observations carried out up to this point indicated that the one conclusion to be drawn was that the so-called (*Actinococcus*) nemathecium of *Phyll. Brodiaei* was really the genuine tetrasporic fructification of that plant.

In 1896 for the first time specimens of *Phyll. Brodiaei* were dredged and preserved at all times of the year. This was continued up to September, 1898, when the author left Kiel. The development of the plant in question was followed out, and as a result the author has accepted Schmitz' view of the parasitic nature of *Actinococcus*, the correctness of which view, however, Schmitz unfortunately was not able to establish, as he did not succeed in observing the entrance of the parasite into the host.

The following is an account of the anatomy and development of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv., which forms the 'pseudo-nemathecium' of *Phyll. Brodiaei* (Turn.) J. Ag.

ACTINOCOCCUS SUBCUTANEUS (Lyngb.) K. Rosenv.

Almost at every time of the year small, more or less spherical, dark reddish bodies are found on the flat expansions forming the thallus of *Phyll. Brodiaei*. They are

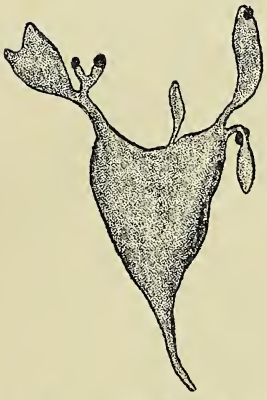

Fig. 1. Nemathecia of *Actinococcus roseus* (Lyngb.). Kold. Rosenv. on *Phyllophora Brodiaei* (Turn.) J. Ag. Nat. size.

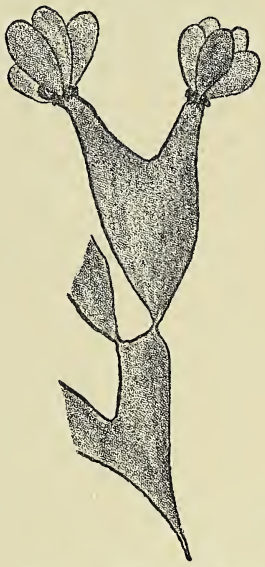

Fig. 2. Nemathecia of *Actinococcus roseus* (Lyngb.). Kold. Rosenv. on *Phyllophora Brodiaei* (Turn.) J. Ag. Nat. size.

sessile on the young shoots at the apex of the thallus, but often appear to be stalked, this appearance being produced by the shoots on which they grow (Figs. 1 and 2) being at first rather narrow.

These red bodies are the nemathecia of *Actinococcus subcutaneus* (Lyngb.) Kolderup Rosenvinge, the Floridea mentioned above as growing parasitically on *Phyll. Brodiaei*.

The young shoots of the latter Alga are not usually much modified by the presence of the parasite, but are on the contrary as a rule well developed.

In the Baltic sea small detached portions of the thallus of *Phyll. Brodiaei* frequently occur lying on the sea-bottom.

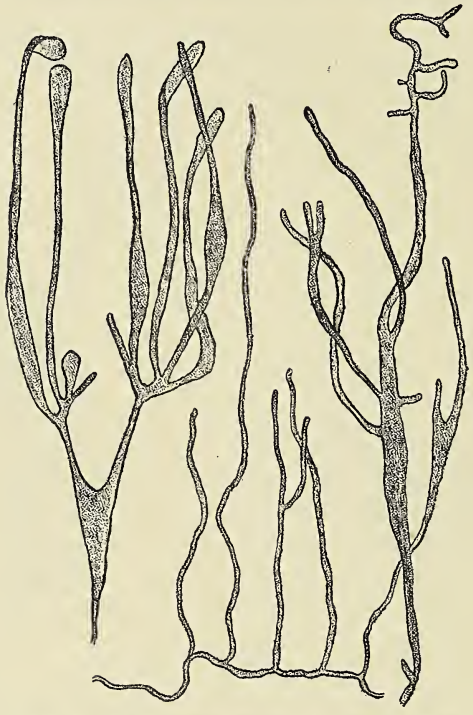

Fig. 3. *Phyllophora Brodiaei* (Turn.) J. Ag.
Sterile, Baltic forms. Nat. size.

They are characterized by being very narrow, elongated and always sterile (Fig. 3, *forma ligulata, elongata, &c.*, of various authors). They therefore never develop antheridia or procarpia. Furthermore they are never attacked, at any rate not successfully attacked, by the germinating spores of *Actinococcus subcutaneus*. This parasite can only enter the host when the male (or the female?) organs of the latter are present. It has been known to the author for some

time that the antheridial cavities of *Phyll. Brodiaei* often accompanied the presence of *Actinococcus subcutaneus*, but only during the last year has it become possible to explain definitely the significance of this appearance.

As the presence of the antheridial cavities is so intimately associated with the relationship of the two red Algae which form the subject of this paper, it might be useful to recall the structure of the former (2, p. 29).

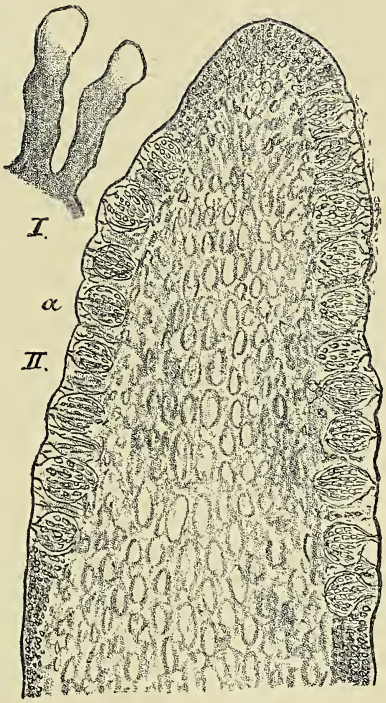

Fig. 4. *Phyllophora Brodiaei* (Turn.) J. Ag. I. Two spermophores. × 10 diam. II. Longitudinal section of spermophore, showing the antheridial cavities in the cortical layer. × 200 diam.

The antheridia of *Phyll. Brodiaei* are developed in the cortical layer of the spermophores, the latter being shoots more or less modified temporarily for the production of the male organs (Figs. 4, 5). They are slightly flattened near the lighter coloured apex, attaining a length of about 3 mm., being rarely broader than 0·5 mm., and they are borne on the apical margin of the flattened vegetative thallus. In the cortex of such a spermophore, close to its apex and not further down from it than 1·0–1·5 mm., we find the small cavities which contain the antheridia. These cavities are flask-shaped and communicate with the exterior by a small ostiole. Their height is 24–34 μ, their breadth about 20 μ. From the flat bottom of the flask-shaped cavity arise a number of 2, 3 or even 4-celled antheridia, which produce the single male cells or spermatia,

at their apex (Fig. 5). The spermatia pass out of the cavity through the ostioles, which measure about 6–10 μ across.

It is not necessary to describe the carpophores of *Phyll. Brodiaei,* on which the female organs are borne (vid. 2, p. 32, Figs. 46, 47). I have not been able to ascertain definitely whether our *Actinococcus* can enter its host by means of the opening caused by the projecting trichogyne. Very probably it does, as I have seen *Actinococcus*-bearing shoots of *Phyllophora,* in the cortical layers of which could be seen what were apparently remains of undeveloped carpogones. Antheridia and procarpia, moreover, do not occur on the same plant.

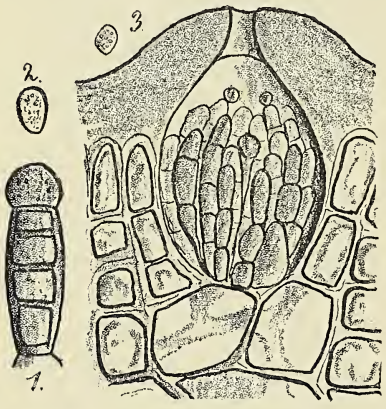

Fig. 5. *Phyllophora Brodiaei* (Turn.) J. Ag. 1. 4-celled antheridium with a spermatium at its apex. 2. Single spermatium. 3. Antheridial cavity with ostiole at its apex. × 1,000 diam.

In the autumn it is possible to observe the entrance of *Actinococcus* into *Phyll. Brodiaei* by the small ostioles of the antheridial cavities. The spores (tetraspores or carpospores) which ultimately give rise to the nemathecia of *Actinococcus subcutaneus* germinate on the surface of the host about this time.

The immediate product of germination seems to be a small heap of perhaps 4–8 cells, one of which always comes to be near an ostiole leading to an antheridial cavity. The antheridial cavities are developed in large numbers and very close together (Fig. 4, II). A filament is then formed, which passes into the host-plant through the antheridial ostiole (Plate XV, Fig. 1).

It is worth while perhaps to draw attention to a former figure which was intended to show the origin of the nemathecium of *Phyll. Brodiaei* (2, Fig. 31). It shows the nemathecium arising from the lower cells of the cortex or

the outer cells of the medulla, the filaments in question being drawn darker, to show them up better. The drawing in itself is correct though misleading. It only represents a portion of the initial filament of the nemathecium, which in the other adjoining sections, had they been preserved, would have been seen to originate from cells just outside one of the antheridial cavities. Not unfrequently the spores seem later on to be drawn down into the antheridial cavities, and thus no trace of their external origin is left.

After entering the host by the ostiole of the antheridial cavity, the parasite immediately branches (Plate XV, Fig. 1), the branches at first forcing their way into the internal medullary portion of the thallus of the host. Very soon a differentiation takes place in the primitive thallus thus developed. In between the cells of the medulla the filaments of the parasite branch and some of them grow out in the direction of the cortex. These form the shoot-part of the plant, the former acting as root-portions. The filaments do not enter any cells of the host, but simply force their way in between them along the middle lamella. In some way, however, they become connected with the cells of the medulla of the host-plant, secondary pits being formed between the cells of both organisms.

The normal cells of the medulla of *Phyllophora* as a rule contain large quantities of starch. This store of food-material is the chief source of food for the parasite. The parasite therefore first of all takes possession of this starch, and in older plants we find that the starch of the host has disappeared from the cells of the medulla, the cells of the parasite now being filled with starch. For this reason it is easy to differentiate the two organisms by adding iodine, when the parasite will turn dark blue, the cell-contents of the host remaining unstained.

From the filaments, which are found in the medulla of the host, arise, as already mentioned, the shoot-filaments. They grow in a direction chiefly towards the flat surface of the spermophores, which are infested by the parasite. The

filaments soon reach the cells of the cortex, and passing out between the filaments of this layer, they branch outside the host, and gradually the nemathecium of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv. is formed on the external surface of the host-plant. Numerous filaments arise from the root-portion, and by repeated branching the internal vegetative portion (intramatrical filaments) and the external portion (extramatrical filaments) assume large dimensions (Plate XV, Fig. 2). The external filaments form a mass of radiating rows of cells, often branched at the base, which in the end give rise to the tetraspores (Fig. 6).

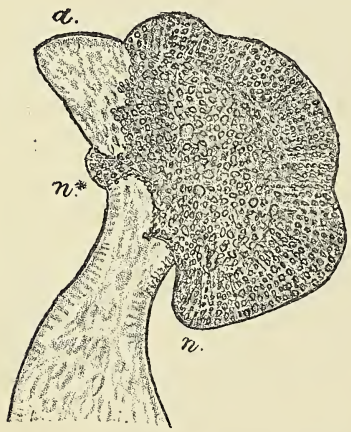

Fig. 6. *Actinococcus roseus* (Lyngb.) Kold. Rosenv. The shaded portion represents the parasitic nemathecium; the lighter portion is the thallus of the host-plant. *n.* large primary nemathecium; *n**. smaller secondary nemathecium just forming. × 40 diam.

The parasite is unable to pierce the outer covering of the host, when entering the latter. It can only attack the latter through the antheridial ostioles. On the other hand this outer coat is easily pierced when the same filaments are passing out to form the nemathecia. In this case they have a firm backing in the solid structure of the medulla of the host-plant. The apex of a filament which is passing through the outer wall of the latter seems to affect the surrounding substance in a peculiar way. This is otherwise quite homogeneous in structure, but in places, where it is being pierced, it seems to become vacuolated. It appears to be corroded in some way by the attacking filament of the parasite (Plate XV, Fig. 3).

A large number of filaments pass out of the tissue of the spermophore within a certain limited space (Plate XV, Fig. 4). On the outside of the spermophore they form dark-reddish

bodies, the nemathecia of *Actinococcus subcutaneus* (Fig. 6). They are all joined together in a common gelatinous substance, in which they branch; the branches finally radiating outwards in a direction from the centre of the whole parasitic tissue.

At first as a rule these nemathecial bodies are formed only on one side of the flattened spermophore. Often, however, we see filaments arising from the intramatrical tissue of the parasite, which pass out on the opposite side of the spermophore (Fig. 6). When a second nemathecium is formed, it may be separate, if it is produced exactly opposite to the first: if it is produced close to the first nemathecium and next to it, the two frequently coalesce.

Ultimately, owing to the almost pseudo-parenchymatic development of the intramatrical tissue of the parasite, it is no longer possible in older specimens to distinguish between the filaments of the parasite and the ordinary tissue of the cortex and medulla of the host. As already mentioned, the effect of adding iodine is to show up the cells of the parasite more clearly, as the latter has absorbed all the starch out of the cells with which it has come in contact. It is easy to make out the shape of the starch-grains of *Phyllophora* (2, p. 22, Fig. 26 *n*, 8–10), but impossible to detect any definite structure and shape in the case of the very minute elements composing the starchy mass found in the cells of *Actinococcus subcutaneus*. Those parts of the primitive thallus of the latter, which are presumably most actively growing, as, for example, the apices of the filaments of the shoot-portions, contain no starch or only very little indeed. The root-filaments are usually completely filled with starch.

The internal cells of the whole parasitic cushion vary much in size (2, Fig. 30). The smallest are barely 6 μ in diameter, the largest measure 60 by 30 μ. The latter do not always belong to the parasite, but often form part of the tissue of the host. They are more or less round, the cells of the parasite being slightly elongated as a rule. The latter are connected with one another by fine cytoplasmic strands. The cells of

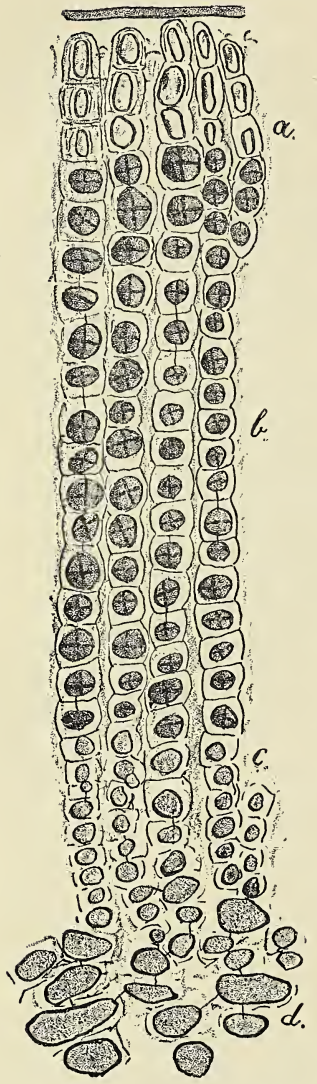

Fig. 7. *Actinococcus roseus* (Lyngb.) Kold. Rosenv. Vertical section of outer layers of nemathecium: *a.* sterile outer cells of nemathecial filaments; *b.* tetrasporangia; *c.* sterile inner cells, and *d.* the cells of the parasitic cushion. × 300 diam.

Phyllophora also possess numerous pits, by means of which their cytoplasm is continuous. Only on rare occasions is it possible to make out any connexion between the cells of the parasite and the host. In cases where the parasite has been growing in the host for some time, the filaments of the former often may grow a considerable distance down into the tissue of the thallus of *Phyllophora* in order to absorb more food-material for the cells of the tetrasporic fructification.

The extramatrical filaments gradually form the nemathecium of the parasite. The outermost, radially disposed filaments gradually develop into tetrasporangia. Each cell of these usually unbranched fertile threads, gives rise to four tetraspores, with the exception of the two to four apical cells (Fig. 7). The inner and lower cells of the fertile filaments also remain sterile. The whole fertile nemathecial layer is in itself about 150–200 μ deep. The sterile apical cells contain some clear, frothy cytoplasm and a distinct, though as a rule very much reduced, rhodoplastid. They are usually larger and longer than the tetrasporangia.

The tetraspores are formed by cruciate division, the first cross-wall formed being placed at right

angles to the long axis of the fertile filament. The spore mother-cells measure 12 × 16 μ, the four nuclei being formed before the formation of cell-walls gives rise to the four tetraspores.

The tetraspores themselves are spherical in shape, and when escaping at maturity they measure 10–12 μ in diameter. They are surrounded by a very thin membrane, their cell-contents consisting of a very finely divided rhodoplastid and numerous starch-grains.

On arriving at maturity the nemathecia break up, the tetraspores are set free and presumably germinate. It is impossible as yet to say what actually is the fate of these tetraspores of *Actinococcus subcutaneus*, after they have been set free from the parasitic nemathecium. Do they germinate on another host and there form sexual plants with antheridia and procarpia?

The tetraspores ripen in December and January and shortly after are discharged from the nemathecia, some of the latter apparently continuing to vegetate for some time. With the appearance of the spermophores in the following autumn, we again find the parasite entering the host-plant. What have the spores been doing in the meantime? From what has already been said, it is not unlikely that what we see germinating on *Phyll. Brodiaei* in the autumn is really a carpospore.

It has been possible to follow out the germination of the tetraspores of our species of *Actinococcus*. Briefly the results obtained were the following (2, pp. 25–27, Fig. 32, 33). The tetraspores, derived from the nemathecia of *Actinococcus subcutaneus*, were sown on sterilized and purified parchment-paper. The whole was put in a small glass vessel filled with filtered seawater. Several cultures were started. The spores germinated, and in parts remained in a living condition for nearly two years. The products of germination took the form of small protonema-like organisms. The largest of these consisted of uniserial filaments and larger aggregations of cells, which at the time I took to be the rudimentary basal attachment-disks of *Phyll. Brodiaei*: it consisted altogether of

upwards of 250 cells. As yet it still remains to be seen what actually becomes of these products of germination. Possibly they would normally have attacked a new host, be this *Phyll. Brodiaei* or some perfectly different plant, on which ultimately the carpospores would possibly be formed. The carpospores perhaps, one might almost say probably, germinate on *Phyll. Brodiaei*, and eventually give rise to the nemathecia of *Actinococcus subcutaneus*. It is not to be wondered at that the parasite should be able to live separately for nearly two years in an artificial culture, when it is borne in mind that its cells contain rhodoplastids. The long filaments are probably searching for a suitable substratum, whereas the larger aggregations of cells represent portions of the thallus which are chiefly assimilating. On the other hand, the form of the plants resulting from the experimental germination of the tetraspores of *Actinococcus* may be due to the very abnormal conditions under which germination took place.

In discussing the question a short time ago with Professor Reinke, the latter suggested as a possibility, which ought not to be dismissed prima facie, that *Actinococcus* might really be an asexual generation of *Phyll. Brodiaei*, growing parasitically on the sexual generation. Although this is not absolutely impossible, it is not very probable that it represents the true state of affairs. I need only recall to mind the fact that the contents of one antheridial cavity at least are destroyed by the parasite entering the host.

The foregoing paper was written for the purpose of definitely settling the nature of the pseudo-nemathecia of *Phyll. Brodiaei* (Turn.) J. Ag., of the *parasiticum quid* of Lyngbye, of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv. and *Act. roseus* Ktz., all of which represent the nemathecia of *Actinococcus subcutaneus* (Lyngb.) K. Rosenv.

Other species of *Phyllophora* Grev. and of other closely related Gigartinaceae and other species of *Actinococcus* Ktz. and allied genera, are about to be examined by the author. It is a remarkable fact that the nemathecia of *Phyll. Brodiaei* have not yet been found, although this species is as common

as *Phyll. membranifolia* (G. and W.) J. Ag., the nemathecia of which are frequently met with, even in the Baltic. It is necessary that this point also should be elucidated.

There are frequently found growing on specimens of *Phyll. Brodiaei* in the Baltic, and more rarely in other seas, certain structures, which have been called 'Traubenkörper' (1, p. 9). Tetraspores, antheridia and procarpia are found on these, but they never apparently attain to maturity. Schmitz also mentions these peculiar bodies, referring them provisionally as a new species to the genus *Actinococcus* Ktz. (5, p. 380). Kolderup Rosenvinge has described a new species of *Ceratocolax*, which apparently embraces the two organisms just mentioned, and to which he has given the name of *Ceratocolax Hartzii* K. Rosenv. (4, p. 34).

The following is a brief description of the genus *Actinococcus* Ktz. and species *Actinococcus subcutaneus*. It is my intention to give a complete list of synonyms, literature and exsiccata in a later paper; as also a discussion of the systematic position of this plant.

Actinococcus Ktz.

Literature: (1) Darbish., Beitrag, p. 7, &c.; (2) Darbish., Phyllophora-Arten, p. 36; (3) Gomont, p. 2, &c.; (4) Kolderup Rosenvinge, p. 33; (5) Schmitz, Actinococcus, p. 392.

Thallus parasitic on other Florideae, the vegetative portion consisting of filaments branching in the interior of the host-plant (intramatrical portion), and fertile filaments forming a cushion of parasitic tissue on the external surface of the host (extramatrical portion of the thallus). Antheridia and procarpia unknown. Tetraspores formed by cruciate division in radially disposed rows of tetrasporangia.

Actinococcus subcutaneus (Lyngb.) K. Rosenv.

Literature: (1–4) The same as of the genus; (5) Schmitz, pp. 369–379.
Synonymy: Actinococcus roseus Ktz.

Thallus (asexual plant at least) parasitic on the young spermophores of *Phyll. Brodiaei* (Turn.) J. Ag., which it

enters through the ostiole of the small antheridial cavities. The intramatrical portion consists of longish cells in uniserial filaments, which branch in the medullary tissue of the host, forcing their way along the middle lamellae of the cells. The extramatrical portion is formed by filaments arising from the intramatrical tissue which break through the cortex and give rise, on the external surface of *Phyllophora*, to a parasitic cushion of radially disposed rows of cells, which eventually develop into tetrasporangia. The outer and innermost cells of these fertile filaments remain sterile. The tetraspores are formed by cruciate division. Antheridia and procarpia are unknown. It is also not yet known what becomes of the tetraspores, and whether the spores which give rise to the parasitic nemathecia on *Phyll. Brodiaei* (Turn.) J. Ag. are tetraspores or carpospores.

In conclusion I have to express my thanks to the 'Kgl. Ministerial-Kommission z. Untersuchung d. deutsch. Meere in Kiel' for permission to make use of the *clichés* for the illustrations in the text of this paper.

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EXPLANATION OF FIGURES IN PLATE XV.

Illustrating Dr. Darbishire's paper on Actinococcus and Phyllophora.

Actinococcus subcutaneus (Lyngb.) K. Rosenv.

Fig. 1. Longitudinal section through the apical portion of a spermophore of *Phyllophora Brodiaei*. The dark cells stained with iodine represent the cells of the parasite, which has entered the host through the ostiole of the antheridial cavity (at *a*). The parasite is branching in between the cells of the host. A differentiation is already visible in the former into a root and shoot portion. × 250 diam.

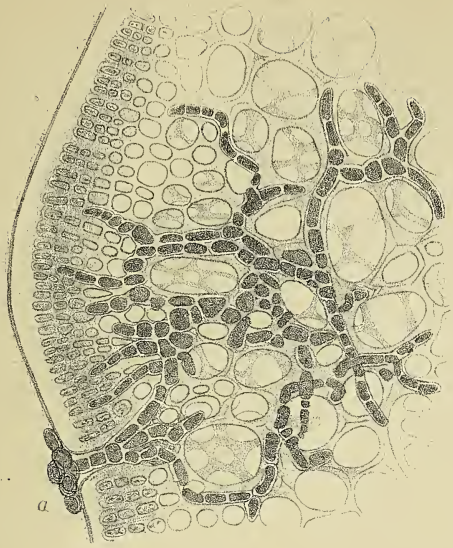
Fig. 2. General view of a longitudinal section through the apex of a spermophore of *Phyllophora Brodiaei*, which has been successfully attacked by the parasite, *Actinococcus subcutaneus*. The intramatrical filaments, stained dark blue with iodine, are clearly seen. From these arise the extramatrical filaments, which have passed out of the tissue of the host, through the cortex. They have formed a nemathecium, only part of which is seen in the drawing. To the left of the section, at *a*, are seen four small antheridia-lined cavities. × 170 diam.

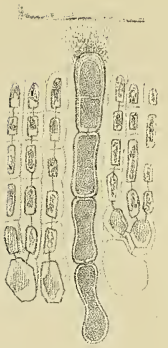
Fig. 3. A single shoot-filament of the parasite breaking through the cortex of the host. The outer covering of the latter is apparently undergoing some change. × 400 diam.

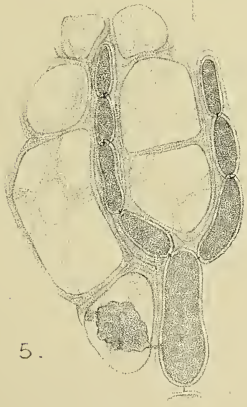
Fig. 4. A bunch of shoot-filaments of the parasite forcing their way through the cortex of the host. They are much branched. × 400 diam.

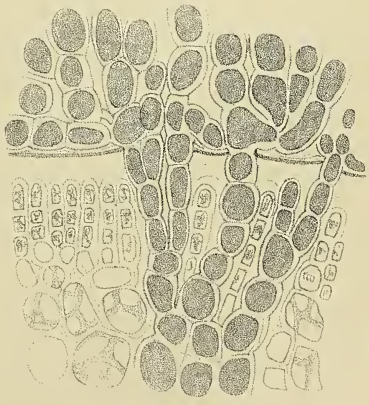
Fig. 5. A branching filament of the parasite forcing its way between the medullary cells of the host-plant. The arrow points in the direction of the cortex. × 400 diam.

Fig. 6. Section through the basal attachment of an older nemathecium, showing clearly the boundary between the host and the parasite. × 400 diam.









O.V.Darbishire del

University Press, Oxford.

DARBISHIRE.— ACTINOCOCCUS AND PHYLLOPHORA.

The Possible Function of the Nucleolus in Heredity.

BY

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STRASBURGER, I believe, was the first to suggest that the nucleus is the bearer of the hereditary qualities of the organism [1]. Subsequent observation and deduction have certainly gone far to establish this theory, and now the majority of biologists believe, it may be with some mental reservation, that the hereditary substance is transmitted in the nucleus, or more precisely in the chromatin-thread of the nucleus [2].

The view advanced here, and urged merely as a tentative suggestion, is a slight extension of the generally accepted theory. According to it the hereditary substance or germ-plasm is to be regarded as completely contained within the chromatin-elements (chromosomes) during nuclear division, as is at present generally held; but during the resting-stage of the nucleus, it is suggested as probable that the hereditary

[1] E. B. Wilson (The Cell in Development and Inheritance, p. 5) says that Haeckel expressed this view as early as 1866, but that O. Hertwig, Strasburger, Kölliker, and Weismann almost simultaneously identified the nucleus as the vehicle of inheritance in 1884–5.

[2] Wilson, l. c., p. 257.

[Annals of Botany, Vol. XIII. No. L. June, 1899.]

substance is distributed between the chromatin-thread and the nucleoli. This distribution is so arranged that the units of hereditary substance, idioblasts (Hertwig) or pangens (De Vries), which determine the attributes of the cell in which the nucleus is actually situated, are located in the chromatin-thread, while the inactive or dormant idioblasts are contained in the nucleolus or nucleoli.

As will be seen, this view involves no differential division of the germ-plasm during cell-division such as Weismann assumes, but is in accordance with the views on heredity held by De Vries, Hertwig, and Driesch. If, however, we wish to express it according to Weismann's theory of the germ-plasm, we may regard the chromatin as containing the active, the nucleolus the inactive or latent determinants, of which, even according to Weismann, there must be some in the nucleus[1].

Hertwig[2] says, 'In order to establish the hypothesis that the nucleus is the transmitter of the elemental germs, four points have to be considered :—

'1. The equivalence of the male and female hereditary masses:

'2. The equal distribution of the multiplying hereditary mass upon the cells which are derived from the fertilized ovum :

'3. The prevention of the summation of the hereditary masses :

'4. The isotropism of protoplasm.'

These criteria may also be used to test the proposed view of the hereditary function of the chromatin and of the nucleoli.

In recent years the opinion that the substance of the nucleoli is distributed along the chromosomes during the early stages of karyokinesis has been steadily gaining ground. The evidence in favour of this view is chiefly based on observations of the simultaneous change in the amount of nucleic acid in the chromatin and nucleoli. When the amount

[1] Germ-plasm; Eng. trans., p. 103.

[2] The Cell; Eng. trans., p. 345.

in the latter decreases, that in the former increases, and vice versâ. For a discussion of the matter, which to be at all complete would have to be very lengthy, I will refer to Hertwig's [1] and Wilson's [2] standard books on the cell. Here it may be stated that we have the authority of Wendt, Flemming, and Hermann for this view; while A. Zimmermann, in his 'Morphologie und Physiologie des pflanzlichen Zellkernes,' also quotes additional references in its favour which seem to render this view very probable if not certain.

If we assume this distribution of nucleolar matter among the chromosomes and the integral division of the hereditary substance in the latter during the karyokineses taking place in the ontogeny (in contradistinction to the differential division assumed by Weismann), then the first condition as to the male and female hereditary masses is assured.

But if we look to the chromatin alone and leave the nucleoli out of count as bearers of hereditary substance, the equivalence of the two masses is not so apparent, but often seems to be contradicted by observation. Thus it is frequently observed that the male nucleus immediately before fertilization is a compact mass of chromatin, while the female nucleus has but little chromatin, and is possessed of a large nucleolus. In many cases this difference equalizes itself after the male nucleus enters the ovum. Its chromatin is then apparently reduced and nucleoli appear [3]. But in other cases the difference persists up to the moment of fusion. Thus, according to Farmer's [4] and Strasburger's [5] figures, the amount of chromatin in the sperm-nucleus of *Fucus* appears to be much greater than that in the nucleus of the ovum. But the large nucleolus of the latter equalizes the amount of hereditary substance in the two nuclei if we admit the hereditary function of the nucleolus. The same may be stated regarding the

[1] Hertwig, l. c., p. 206.

[2] Wilson, l. c., p. 92.

[3] Cp. Guignard, Nouvelles Études s. l. Fécondation, Figs. 76, 81.

[4] Cp. Farmer and Williams, Phil. Trans. Roy. Soc., 1898, B, Figs. 20, 21.

[5] Cp. Strasburger, Jahrb. f. wiss. Bot., xxx, Taf. xviii, pp. 267, 246, 286.

sexual nuclei of some Ferns. A reference to Campbell's [1] and Shaw's [2] figures will bear this out.

It will thus be seen that if we do not assign hereditary functions to the nucleoli of the sexual nuclei we cannot in all cases assume the equivalence of the male and female hereditary masses.

The view which has been just expressed with regard to the behaviour of the nuclear substances (chromatin and nucleoli) during karyokinesis, necessitates that Hertwig's second condition also should be fulfilled. The hereditary mass being composed of the nucleoli and chromatin is, according as it multiplies, equally distributed by karyokinesis among the cells derived from the fertilized ovum.

We next come to the third condition laid down by Hertwig, which must be fulfilled by the hereditary substance. According to this, in order to prevent the summation of the hereditary masses resulting from the periodic fusions, there must be a corresponding periodic elimination of hereditary substance.

Regarding the chromatin as the sole hereditary substance, investigators have believed that this elimination is effected by means of the so-called 'division with reduction' which has been supposed to be of general occurrence during the formation of the sexual cells. During this division the chromosomes of the daughter-nuclei are supposed to arise by the transverse division of the chromosomes of the mother-nucleus, instead of by longitudinal division. In this manner half the number of idioblasts (hereditary units), which are supposed to be placed in series along the chromosomes, are distributed to one of the resulting nuclei and half to the other.

The form of the chromosomes found in these divisions in animals appears to make it doubtful whether the cleavage of the chromosomes is longitudinal or transverse. The long chromosomes found in the karyokineses giving rise to the sexual nuclei of plants leave little room for such uncertainty, and all investigators, with one exception, are agreed that no

[1] Campbell, Mosses and Ferns, Fig. 211.

[2] Shaw, Fertilization of *Onoclea*, Annals of Bot., Sept. 1898.

such 'reducing-division' occurs in the case of the higher plants at least [1].

If then we are to look for reduction of the hereditary mass we are justified in searching for its elimination under some other form than the chromosomes. By assuming the location of some of the idioplasm or germinal substance in the nucleoli the nuclear processes during the development of the sexual cells become intelligible. During these processes in plants all observers agree that an extrusion of nucleolar substance occurs; while in several cases a similar extrusion has been recorded during the formation of the polar bodies in animals [2]. Thus we may suppose that the redundant hereditary substance is extruded in the nucleoli during the development of the sexual cells, or, sometimes, by means of a reducing division of the chromatin (where such occurs), or by both.

The fourth condition, with regard to the isotropism of protoplasm, affects Strasburger's nuclear hypothesis and the proposed extension of it equally. It will be sufficient to refer to Hertwig's discussion of it (l. c., p. 354); and to mention that Wilson, who accepts the nuclear hypothesis, does not completely endorse Hertwig's view on this matter [3].

From the foregoing considerations it would appear that the proposed view regarding the hereditary functions of the

[1] Strasburger held for some time that in the first division of the mother-nucleus both transverse and longitudinal division occurred, and that no cleavage took place in the second division. He has since convinced himself that this does take place. Vide Strasburger and Mottier, Berichte d. Deutsch. Bot. Ges. 1897, p. 331.

Since the appearance of this last paper of Strasburger's, W. Belajeff has published a note (Ber. d. D. Bot. Ges., 1898, xvi. 2) in which he revives the opinion that no longitudinal cleavage of the chromosomes takes place in the second division of the pollen-mother-cells. It seems, however, as if his figure of this division was taken from a later stage when longitudinal cleavage was complete. Indeed, I have little doubt, both from my own observations (vide On the Chromosomes of *Lilium longiflorum*, in Notes from the Bot. School, Trinity College, Dublin, 1896, Figs. 13 and 14) and from Strasburger's and Mottier's figures and results (quoted above), that longitudinal fission actually does occur in this karyokinesis. L. Guignard (Arch. d'Anat. Micro., 20 mars, 1899) also maintains that no longitudinal cleavage takes place in the second karyokinesis; but it is not a 'reducing-division,' inasmuch as a double longitudinal cleavage occurs in the first.

[2] Wilson, l. c., p. 95, quoting Haecker.

[3] Wilson, l. c., p. 312 ff.

nucleoli and chromatin, when judged by Hertwig's criteria, has no inherent objections; while with regard to the equivalence of the male and female hereditary masses and the prevention of the summation of hereditary substance, I venture to think it offers some advantages over the generally received hypothesis, of which it is an extension.

As additional support of this view some other phenomena may be quoted. Thus the diminution of chromatin and the relatively large amount of nucleolar substance observable in the nuclei of mature tissues is just what we would expect, if the chromatin-thread contains the hereditary substance which actually decides the properties of the mature cell, while the nucleoli contain the other—inactive in that special cell—hereditary properties of the species. That these latter must be present in the nuclei of mature cells is rendered necessary by the observed phenomena of regeneration [1].

The relatively small quantity of chromatin and the large amount of nucleolar substance in mature tissues seems to have been already remarked upon by Rosen [2]. He, however, believes that the nucleolus increases in importance with the gradual diminution of the chromatin; whereas, according to the proposed view, it contains the dormant idioblasts, or hereditary units, gradually increasing in number with the specialization of the cell. The chromatin, on the other hand, as the cell specializes, is reduced in quantity until the idioblasts representing the special properties of the mature cell are alone present in its substance. It may not, perhaps, be out of place to point out that the great surface of the thread-like chromatin, compared with the small surface exposed by the spherical but much more bulky nucleolar substance, favours the view that the chromatin may be composed of the active idioblasts of the nucleus. Although this statement of the relative amounts of chromatin and nucleolar substance holds good in a number of cases, yet there are undoubtedly instances in which there

[1] Hertwig, l. c., p. 346; and 'Präformation oder Epigenese?' Eng. trans. p. 47: see also Wilson, l. c., p. 311.

[2] Rosen, Cohn's Beiträge, 1895, p. 305.

is neither an absolute nor a relative increase of nucleolar matter accompanying the specialization of the cell. Nor is this contrary to what might be anticipated. For it is very probable that only a few of each kind of inactive idioblast would be present in the nucleus, while those which are active, or about to spring into activity, would be multiplied. This uneven multiplication would of course lead to an apparent reduction in number of latent idioblasts and an increase in number of active hereditary units. There is a second source of inaccuracy which may at present enter into any endeavour to estimate the number of idioblasts from the amount of hereditary substance, and which should not be lost sight of. Whether we regard the chromatin alone, or the chromatin and the nucleoli as the bearers of the hereditary properties, in all probability there is much extraneous matter present besides the actual vehicle of the properties of inheritance in this so-called hereditary substance, and varying conditions may lead to varying amounts of this extraneous matter being present. As bearing out this statement it may be mentioned that it has been shown by Liebermann, Altmann, Malfatti, and Kossel, that the percentage of albumin in the chromatin of nuclei is very various[1].

Another nuclear phenomenon, which has been brought into prominence recently by numerous observers, is rendered, I think, more intelligible by the proposed view of the properties of the nucleoli and chromatin. I refer to the great attenuation and elongation of the nuclear thread during the 'Dolichonema' stage (Rosen)[2] which immediately precedes synapsis in the formation of the reproductive cells.

During this stage the nuclear (chromatin) thread becomes enormously long and thin; while the nucleoli fuse together into one, which then becomes reduced in size. At the beginning of synapsis the nucleolus is much reduced, and in fixed specimens there appears a 'sickle'-shaped (rather concavo-convex) body in contact with the inner surface of the

[1] Wilson, l. c., p. 241.

[2] F. Rosen, Beiträge zur Kennt. d. Pflanzenzellen; Cohn's Beiträge, vii. 2, 1895.

nuclear membrane. In all probability, as Humphrey and Strasburger [1] have pointed out, this body is formed as a precipitate from the nuclear fluid. Zimmermann [2] has shown that its substance is derived from the nucleolus. In fact the attenuated chromatin-thread is at this stage immersed in a nuclear fluid containing an exceedingly large amount of nucleolar substance suspended [3] in it; and we may regard the elongation of the thread and this synaptic stage as a process for distributing all the idioblasts or bearers of the hereditary properties with great uniformity along the chromatin-thread, so that the nuclei of the reproductive cells which arise from the nucleus under consideration may be all equally possessed of these properties. The undisintegrated or persisting portion of the nucleolus of this nucleus, being fragmented and extruded in the karyokinesis immediately following this stage, most probably contains that portion of the hereditary mass which is to be got rid of, and which is not to appear in the succeeding generation nor again in the phylogeny of the species.

The behaviour of the sexual nuclei during fertilization also indicates the hereditary function of the nucleolus, and it would appear that if we attribute hereditary properties to the chromatin of nuclei there are observations which compel us logically to assign these properties to the nucleolus also. Thus not infrequently in the higher plants, both the male and female nuclei contribute nucleolar matter to the nucleus of the oospore [4]. The most striking observation in this respect is, however, that which has been made on the fertilization of *Fucus* by Farmer and Williams and by Strasburger [5]. In this Alga the sperm-nucleus is very rich in chromatin before fusion with the nucleus of the ovum, and no nucleolus is

[1] Humphrey, Nukleolen u. Centrosomen; Ber. d. D. Bot. Ges., 1894, p. 108.

[2] Zimmermann, Morph. u. Phys. d. pflanz. Zellkernes, p. 69.

[3] According to the view here urged the *solution* of the nucleolus does not take place, but rather its subdivision into idioblasts, which may be supposed to be suspended in the nuclear fluid after the disappearance of the nucleolus.

[4] Guignard, Nouv. Ét. s. l. Féc., Figs. 80, 81.

[5] Farmer and Williams, Fertilization of *Fucus*; Phil. Trans. B., 1898, Figs. 20, 21. Strasburger, Jahrb. f. wiss. Bot., xxx; Taf. xviii, pp. 246, 267, 286.

visible in it. After fusion a second large nucleolus appears in the nucleus of the ovum, presumably derived from the chromatin of the sperm-nucleus, as indeed the authors quoted state. If this be true, and if this nucleolus does not represent some of the hereditary substance contributed by the male nucleus, we can no longer admit the equivalence of the male and female hereditary masses in this case; but we should be forced to believe that the female nucleus contributes much the greater share of hereditary substance to the embryo. As was seen above, a comparison of the amount of chromatin in the male and female nuclei before fusion led to a contradictory result.

The transformation of nucleoli bodily into nuclei in the formation of the spores of *Saccharomyces* recently observed by Wager[1] appears to me to be an exceedingly strong argument in favour of the view here put forward. This observation I have been able lately to confirm by the discovery of a completely similar phenomenon in the development of the ascospores of *Tuber aestivum*. The spores of this Ascomycete are multinucleate. The formation of the spores takes place as follows:—The nuclei formed in the ascus are large and contain very finely granular chromatin, in which are imbedded a few nucleoli which are easily stained with the acid anilin-dyes. Round four or five of the nuclei, and in close proximity to their membranes, the cell-walls of the ascospores are developed. When thus enclosed the nucleus enlarges until it completely fills the cell. During its enlargement the finely granular chromatin disappears, whilst the nucleoli increase in size and number until there are about eight in the spore. Simultaneously with their increase in number and enlargement, their colour-reaction changes, and from fixing the acid anilin-dyes, the nucleoli come to exhibit an affinity for the basic stains. They then lose their completely homogeneous appearance and pass into a state resembling the dense minute nuclei of the hyphae of the Fungus, but they are larger and more granular than these latter.

[1] Wager, Ann. of Botany, Vol. xii, Dec. 1898.

In this manner, when the ascospores are approaching maturity, each is possessed of a number of nuclei derived from the nucleoli of its original nucleus. In the process there is probably at first a transference of chromatin into nucleoli, and afterwards a bodily transformation of these latter into nuclei. The first action is parallel to that described by Farmer and Williams in the fertilization of *Fucus*[1]; the latter is the converse of this, and is similar to that discovered by Wager in the spore-formation of Yeast.

In all these cases, if we admit the hereditary function of the chromatin, it is scarcely possible to deny it to the nucleoli.

[1] L. c., p. 632.

The Prothallus of Lycopodium clavatum, L.[1]

BY

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With Plates XVI and XVII.

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IN no group of the Pteridophyta has information regarding the life-history been accumulated more slowly than in the case of the Homosporous Lycopodiaceae. Both sexual and asexual generations are now known, however, in several species of the genus *Lycopodium*. Had the prothalli of these been found to resemble one another as closely as is the rule in the other genera of Vascular Cryptogams, comparatively little interest would attach to the investigation of the remaining species. But among them several distinct types of prothallus

[1] Since the manuscript of this paper was completed, an important monograph by Professor Bruchmann (Ueber die Prothallien und die Keimpflanzen mehrerer europäischen Lycopodien; Gotha, 1898), dealing with the prothalli and young plants of *Lycopodium clavatum*, *annotinum*, *complanatum*, and *Selago*, has been published. The facts contained in this are considered along with the other knowledge we possess of the gametophyte in this genus, in the concluding portion of the present paper, but the earlier part has been left untouched, save by the addition of a few notes referring to Bruchmann's observations on the same species. I may therefore take this opportunity of pointing out that the description of the facts, though founded on independent observations, must for the most part be regarded as confirmatory of the account previously published by Professor Bruchmann.

[Annals of Botany, Vol. XIII. No. L. June, 1899.]

are known, associated with differences in the young sporophyte. The possible systematic importance of these characters of the gametophyte in determining the relationship of the species of *Lycopodium* to one another has been pointed out by Dr. Treub, to whose investigations our entire knowledge of the life-history of tropical species is due; the subject will be further discussed below.

The present paper deals with the prothallus of *Lycopodium clavatum*, a preliminary account [1] of which was read before Section K of the British Association last year. During some weeks spent at Clova in Forfarshire, where all the British species of *Lycopodium*, with the exception of *L. inundatum*, can be found in abundance, the soil in which the plants grew was repeatedly examined in the hope of finding prothalli, but without success. In July of last year, however, a number of young plants of *L. clavatum* were found growing among a patch of *Racomitrium lanuginosum* on a rock shaded by a few trees in Glen Doll. Since the presence of the 'foot' rendered it certain that these plants had been borne on prothalli, the thin layer of peaty soil underlying the moss was examined with great care, every part of it being gently crumbled down. In this way seven prothalli were found, three of which bore young plants of various ages. Careful examination of the ground for a considerable distance around failed to disclose any more young plants, nor were old plants of this species to be found near the spot.

External Form and Structure of the Prothallus.

The size and general appearance of the prothalli and young plants, upon the study of which the following description is founded, will be evident from Fig. 1, which is reproduced from a natural-size photograph of a prothallus seen from above, and of one of the smaller plants; the foot of the latter is still recognizable though the prothallus to which it had been attached had disappeared. Although the material did not

[1] British Association Report, 1898, p. 1050.

allow of the development of the prothallus being followed, the specimens differed from one another in size and shape, and probably illustrate changes of form associated with the later stages of growth ; all conclusions drawn from the comparison of a small number of prothalli must, however, be tentative.

The smallest of the prothalli, which is represented as seen from above in Fig. 2, measured 4 mm. in length by 3 mm. in breadth. It was an almost flat plate of tissue about 0·5 mm. thick, of a dirty white colour. Numerous rhizoids of considerable length projected from the under surface, especially around the margin ; they were absent from the upper surface, which was lighter in tint than the rest of the prothallus. From external examination an apical[1] region could not be distinguished, though in this and all the other prothalli a median plane dividing them into equal and similar halves could be recognized. In this longitudinal direction the prothallus was slightly curved, the upper surface being convex. Owing to the lateral margins being slightly bent up, this surface was also concave from side to side. It will be seen that these curvatures increase in larger and presumably older specimens, but it is of interest to note that in a prothallus of such relatively considerable size they were so slight. In the prothallus represented in Fig. 3 both these curvatures were much more marked. Owing to the increase of the longitudinal curvature, the ends were approximated to one another; while the transverse curvature gives rise to the trough-like form which will be apparent from an examination of the figure. Each of the lateral margins exhibited a secondary fold rather nearer to one end. As in the younger prothallus, the rhizoids, which were here much shorter, were more numerous near to the margin. Another prothallus, which resembled this in shape, save in the absence of the secondary folds, was found. Two

[1] This difficulty is explained, as Bruchmann has shown, by the flattened structure being preceded by a vertical, radially symmetrical stage. The longer diameter of these old prothalli has thus no relation to the true longitudinal axis. On re-examination of serial sections of my prothalli, the conical projection from the under surface, which is all that remains of the earlier stage, was to be found, though small and inconspicuous. It is not visible in any of the figures.

others had a more complicated outline. One of them is represented from the side in Fig. 4, which shows that the increase of the longitudinal curvature has brought the two ends into actual contact, while a single secondary fold is visible. Each lateral margin of the similar prothallus represented in Fig. 1 showed two such secondary folds.

The resemblance in form and habit which these prothalli of *L. clavatum* presented to those of *L. annotinum*[1] rendered it probable that the structure would be similar in the two cases. As will be seen, this similarity was found to exist even in points of detail. In Fig. 5 part of a vertical section across the lateral margin of the prothallus shown in Fig. 3 is represented; the corresponding sections of the ends presented a similar arrangement of tissues. As will be seen from the figure, a number of layers of tissue can be distinguished which agree, in relative thickness and in the characters of the cells composing them, with the layers described by Bruchmann in *L. annotinum*[2]. This distinction of layers appears to stand in relation to the localized distribution of an endophytic Fungus, the characters of which will be described toward the end of this paper. The slightly flattened cells of the outermost layer (*a*, Figs. 5, 6), which bounds the prothallus on the under surface, are quite free from the Fungus, with the exception of some of those prolonged as rhizoids. The outer layer of the free walls of these cells gives the reaction of cuticle; this is especially marked at the base of a rhizoid. The latter structures are as a rule simple extensions of superficial cells, but in some cases the main part of the rhizoid is separated by a transverse wall from the basal portion. Above the lower limiting layer comes a band of cells three or four deep (*b*, Figs. 5, 6), which are extended parallel to the surface of the prothallus. These cells, as well as the succeeding single layer (*c*, Figs. 5, 6), which consists of more or less regular palisade-shaped elements with their long

[1] Fankhauser, Bot. Zeit., 1873, p. 1; Bruchmann, Bot. Centralblatt, xxi, 1885, p. 23.

[2] Loc. cit., 1885.

axes at right angles to the surface, are filled with the mycelium of the Fungus, which is here entirely intracellular. In consequence of this they appear darker than the other regions of the section. Above the layer of palisade-cells comes a region (*d*, Figs. 5, 6) about eight cells deep, from the cell-cavities of which the Fungus is absent. These cells are smaller than those of the preceding layer, but are not infrequently elongated in the same direction. Their walls appear thicker than those of the rest of the prothallus, especially at the angles. Throughout the whole of this region the mycelium of the Fungus can be traced within these thickened walls, but never penetrating into the cavities of the cells. The latter contain a large quantity of starch, which is also present, though less abundantly, in the tissues above, but is practically absent from those in which the mycelium is intracellular. Whether, as in *L. annotinum*, oil was also stored in this region was not determined. The appearance of the four layers of tissue described above is shown in greater detail in Fig. 6. The region of the prothallus above the layer last described usually forms more than half its thickness (*e*, Fig. 5). The cells composing it are thin-walled and possess a scanty protoplasmic lining. Those situated more internally are of large size, but those surrounding the antheridia or archegonia are much smaller. The latter form a fairly definite layer (*f*, Fig. 5) on the upper surface, the origin of which, from the superficial layer of cells at some distance from the growing region, can be followed in Fig. 5. This uppermost layer may be distinguished as the generative layer, while the large-celled tissue beneath may be compared to the cushion of a Fern-prothallus. The prothallus is thus seen to consist structurally of six more or less clearly defined regions, the four lowest of which are, as their structure indicates, concerned with the nutrition of the organism, while the uppermost layer contains the sexual organs.

Great difficulty was experienced in tracing the origin at the growing regions of the different tissues, the mature condition of which has now been described. It may be stated at the

outset that the formation of new tissue is not confined to one end, but takes place all round the edge of the prothallus. Leaving aside for the moment the structure of the two ends, the results obtained from the study of sections across the lateral margin may be first considered. The merismatic region to which growth can be referred is situated at the margin of the prothallus toward the upper surface ; its position is indicated by a cross in Fig. 5. Toward the lower surface the tissues of the vegetative half of the prothallus become differentiated, while to the other side the cushion-layer is formed. When the latter, in cases where the sexual organs are numerous and near to the margin, attains a considerable thickness, a marginal groove is the result, which can be recognized in Figs. 5, 8, and 9. In these cases the initial region occupies the upper side of the lower lip of the groove. Periclinal divisions sometimes occur in the superficial cells of the merismatic region, but it is very doubtful whether these cells can be considered to be initials. The comparison of a number of sections rather suggested that the meristem is imperfectly stratified. The superficial layer can be traced on the one hand into the limiting layer of the under surface, the cells of which undergo anticlinal divisions only. On the other side this layer gives rise at a greater or less distance from the margin to the small-celled generative layer with the sexual organs. Below the superficial layer in the merismatic region are a few layers of fairly large cells, which can be traced on the one hand into the band of longitudinally extended cells (*b*), and on the other into the cushion-layer (*e*). Still deeper in is a small-celled meristem, from which the storage-layer (*d*) and the layer of palisade-cells (*c*) are differentiated. This differentiation is complete at a very short distance from the meristem, the whole appearance suggesting that the addition of new cells to the permanent tissue is very slow. This explains the difficulty that was experienced in following the course of divisions in the meristem.

The vertical sections through the ends of the prothalli presented considerable differences in the various specimens

examined, and also when the two ends of any one prothallus were compared. In the prothallus represented in Fig. 4, no difference could be made out between sections of either end and those across the lateral margin; in all these regions the appearance of the tissues and the succession of archegonia showed that growth was proceeding. But in the other prothalli the sections of the ends differed from each other and from those across the lateral margin. The nature of these differences will be gathered from Fig. 7 *a* and *b*, which represent the sections through the ends of a prothallus which resembled the one in Fig. 3; the crosses indicate the points in relation to which the tissues seemed to be arranged. The insufficiency of material will not permit of any more detailed statement being made, but the existence of such differences is of interest with regard to the appearance of symmetry presented by these old prothalli.

Although the details of the merismatic regions will require to be investigated in younger prothalli, the facts of which an account has just been given suggest the explanation of the main changes of form exhibited by these old prothalli. Starting from a flat plate of tissue (Fig. 2), the growth of which is proceeding all round the edge, irregularities of growth would cause the lateral margins to assume the folds seen in Figs. 1, 3, and 4, while the main curvatures of the prothallus may find their explanation in differences in rapidity of growth between the upper and the lower sides of the marginal meristem. A much larger number of prothalli would, however, be needed to enable this to be traced in detail.

Antheridia and Archegonia.

From what is known of the distribution of the sexual organs on the prothalli of other species of *Lycopodium*, it might have been anticipated that archegonia and antheridia would have occurred in numbers upon the same prothallus. It is of course impossible to draw any general conclusion as to the distribution of the sexes in *L. clavatum* from the few

prothalli investigated: but the striking fact results from the detailed study of complete series of sections of six prothalli and the external examination of another, that a practically complete separation of male and female sexual organs on old prothalli of similar size and form may occur. Of the seven prothalli six were female and only one male. No archegonia were found on the latter, but on two of the female prothalli a few antheridia were seen among the much more numerous archegonia. In the absence of continuous observations on developing prothalli it was impossible to be certain whether the same prothallus passes through a male and a female stage, or if at an earlier stage both kinds of sexual organs do not co-exist [1].

The archegonia and antheridia occupy corresponding positions: both are confined to the upper surface of the prothallus. In the specimen represented in Fig. 2, the archegonia were scattered over the slightly projecting cushion, the youngest being found nearest to the edge, a distribution that is explained by the marginal growth. In older prothalli the central region bore only old and often almost unrecognizable sexual organs, while the functional and developing archegonia and antheridia were found near the margin, the part of the cushion on which they were situated projecting as a more or less prominent ridge. The position of this ridge will be evident from Figs. 8 and 9, which represent vertical sections across the margins of the prothalli shown in Figs. 4 and 3 respectively. As the figures show, the central region of the upper surface of these prothalli bore only obscure remains of antheridia or archegonia, while on the marginal ridge a succession of these organs can be traced, the youngest being nearest to the marginal groove. In the case of the male prothallus (Fig. 3) this ridge was only present within the lateral margins, while in the largest female prothallus (Fig. 4) the appearance of a vertical section of either end was in-

[1] Bruchmann's observations (loc. cit., 1898) show that as a rule archegonia and antheridia occur together on the prothallus. He does not mention any cases of their separation in old prothalli such as those described in the text.

distinguishable from the section across the lateral margin (Fig. 8). So far therefore as the evidence afforded by these specimens goes, the archegonia and antheridia appear to be formed in regular succession following the marginal growth of the prothalli.

The antheridium is developed from a single superficial cell which divides by a wall parallel to the surface (Fig. 10). In the outer of the resulting cells only anticlinal divisions occur, the free wall of the antheridium being only a single layer of cells in thickness; from the inner by repeated divisions the small-celled tissue, which gives rise to the spermatozoids, is formed (Figs. 11 and 12). The mature antheridium projects very little if at all from the surface of the prothallus. The large ellipsoidal mass of spermatocytes is wholly enclosed within the generative layer of the latter; its form and size will be evident from Figs. 13 and 14, which represent longitudinal and transverse sections of almost mature antheridia. The cells adjoining the spermatocytes are usually more or less flattened, but do not present any distinctive characters. The development of the spermatozoids resembles that in other Vascular Cryptogams, but was not followed in detail. The opening of the antheridium appears to be effected by the breaking down of a small cap-cell, which, with the cells surrounding it, originates from the outer of the two segments into which the mother-cell of the antheridium divides. A sufficient number of sections in the required direction were not available to determine the succession of divisions in this cell, but, as the examples figured (Figs. 15 and 16) show, there is probably some variation in this respect. The spermatozoids were not obtained.

The archegonium also is developed from a single superficial cell which divides by two periclinal walls into a series of three, which are recognizable by their dense protoplasmic contents (Fig. 17.) From the outer of these the neck arises; the other two form the central series of cells. A basal cell does not appear to be present. Older stages, in which the neck as yet projects but little above the surface, are shown in Figs. 18 and

19. In the latter, however, the series of central cells is complete. In the mature archegonium, the neck usually projects considerably from the surface (Fig. 20), though only the upper portion of its wall is a single layer of cells thick. The lower portion appears to be produced by the surrounding cells of the generative layer keeping pace with the enlargement of the archegonium at maturity, and is not derived from the outermost cell of the young archegonium (Fig. 17); this region, which is as a rule more than one layer of cells thick, is thus not strictly comparable to the archegonium-neck in other Vascular Cryptogams. In the archegonium represented in Fig. 21, this division and growth of the cells of the generative layer has on one side not been confined to the cell immediately adjoining the archegonium. The venter of the archegonium and the lower portion of the canal are surrounded by a layer of small cells cut off from those of the generative zone. These cells have dense protoplasmic contents, and their nuclei are frequently to be found against the wall which bounds the canal of the archegonium (Figs. 20 and 21). Within the venter is the large ovum, and the canal is occupied by six to eight canal-cells, seven being the number most frequently observed (Figs. 20 and 21); the lowest of these was not distinguished with any certainty as a ventral canal-cell, since the material was insufficient to allow of the succession of divisions in the two inner cells of the young archegonium being traced. Transverse sections of the young archegonium-neck show that it is typically composed of four rows of cells (Fig. 22), though subsequent divisions in one or more of these may increase the number. In sections a little lower down, the wall of the neck-canal is found to be more than one layer thick, while still lower down the ovum is found embedded in the prothallus. These relations will be sufficiently apparent from the longitudinal section (Fig. 20). The details of the opening of the archegonium-neck were not observed.

The Young Plant.

It has been mentioned already that three of the prothalli bore young plants. These were of different ages, but none were young enough to afford information as to the embryology. Figs. 23 and 24 represent two of the prothalli. The one in Fig. 23 is seen from the end, and shows a young plant, the leafy stem of which had already attained a considerable length, while the first root is still unbranched. In this and another example the plants occupied a position on the marginal ridge corresponding to the younger archegonia in Fig. 8. Here, as in *L. annotinum*, the plants remain attached to the prothallus, which, however, had ceased to produce sexual organs, until they have attained a considerable size. The peculiar form of the prothallus represented as seen from above in Fig. 24, appears to have been due to an injury at an earlier stage of development. One-half appears to have been partially destroyed and had ceased to grow, while from the opposite margin a new growth of semicircular outline had proceeded; the light colour of the latter contrasted with the older portion, which was dark brown in tint. Two of the last-formed archegonia must have been fertilized about the same time, for the young plants were almost exactly similar in form and size. The longer cylindrical body projecting from the ruptured archegonium-wall is the shoot, the scattered scale-leaves of which were inconspicuous, while the primary root is recognizable as the short conical projection at the base of this. It may be noted that the orientation of these two plants with regard to the edge of the prothallus is the same.

No structure comparable to the protocorm of *L. cernuum* was recognizable on external examination of the young plants, and its absence is further shown by the outline drawings in Figs. 25 and 26, which represent sections through the young plants in Fig. 24. In Fig. 25 the section passes in the median plane of the first root, but owing to the shoot being curved to one side, fails to follow the latter for any length;

while in Fig. 26 the section is in the median plane of the shoot, but at right angles to the median plane of the root. The two sections are thus in planes at right angles to one another.

The superficial layer[1] of the foot was characterized by the dense protoplasmic contents of its cells. The extent of this layer, which is possibly concerned in the absorption of organic substances from the prothallus, is indicated by the shading in Figs. 25 and 26; a small portion of a similar section is represented in detail in Fig. 27. These figures and Fig. 28 also show the irregularity of outline of the foot and the thickness of the outer walls of its limiting layer of cells; these outer walls stained very deeply with Bismarck-brown.

The structure of the region of attachment to the prothallus of the older plant shown in Fig. 23 is drawn in detail in Fig. 28. It was ascertained on comparing this section, which is approximately median as regards both shoot and root, with the rest of the series that the vascular system does not extend into the foot; the central cells of the latter, however, seem to be arranged so as to facilitate the conduction of absorbed substances towards the vascular strand. The absorbent layer, though still recognizable, did not present the characteristic appearance seen in the younger plant (Fig. 27). This may be associated with the fact that the plant at the latter stage was devoid of chlorophyll and wholly dependent on the prothallus, whereas the absorbent function of the foot would be of secondary importance in the case of a plant with a shoot-system capable of assimilation. It may be noted that in the specimen represented in Fig. 28 the structure of the foot is not uniform, the region just below the insertion of the root being composed of smaller, thinner-walled cells with more abundant contents; whether this difference possesses any significance is however uncertain.

The large foot persists for a considerable time after the prothallus has disap peared, and wasrecognizable in much

[1] Bruchmann (loc. cit., 1898, p. 44) ascribes to this peripheral layer of the foot the main part in the growth of the latter; the arrangement of the cells in my specimens did not however seem to support such an interpretation.

older plants than that represented in Fig. 1. Its position with regard to the main axis of the plant is variable. The differences probably depend on the inclination of the surface of the prothallus upon which the plant was borne, the young shoot and root of course tending to grow vertically upwards and downwards respectively. Figs. 29 and 30 represent two extreme examples. In the former the position of the foot is distinctly lateral, the surface of the prothallus having in all probability been almost vertical; while in the specimen represented in Fig. 30, which was probably inserted on a horizontal surface, the foot is in the same line as the axis of the shoot, the root arising at the base of the latter. For a variable distance above the position of attachment to the prothallus, the stem bears only small colourless scale-leaves which higher up pass by gradual transitions into ordinary green foliage-leaves (Fig. 1). This doubtless depends on the thickness of soil and moss through which the shoot had to grow before reaching the light.

The Endophytic Fungus.

It has been mentioned above in the general description of the prothallus that its lower half harbours an endophytic Fungus, the distribution of which within the cells of the layers marked *b* and *c* and between those of the layer above (*d*) was described. It will be sufficient in this place to refer to Fig. 6, with regard to which it should, however, be noted that the intercellular mycelium is more uniformly distributed than was demonstrable in the portion of the section drawn. When thicker sections are mounted in Schulze's solution, the filaments of the Fungus swell somewhat and take on a purplish colour; it is then seen that the mycelium is present throughout the entire thickness of the storage-layer.

The cells containing the Fungus, although their protoplasmic body was occupied by closely packed hyphae, appeared to be perfectly healthy. The nucleus presented a normal appearance (Fig. 32), though occasionally it may be altered in

shape by the pressure of the adjoining hyphae. It was usually found close to the vacuole, but sometimes against the cell-wall. No indication of septa in the hyphae has been seen, though a large number of sections have been examined with this object in view. Their infrequency may therefore be considered to be probable; though owing to the difficulty of examining a long filament of the Fungus, their absence cannot be definitely asserted. Small oval nuclei occur at intervals in the filament. The intercellular hyphae resembled those found within the cell-cavities.

The appearance of the endophyte was found to present differences according to the region of the prothallus in which it occurred, and differences of a similar nature were noted when fertilized prothalli were compared with those in which marginal growth continued. The important factor common to both these cases appears to be the age of the tissue in which the Fungus is found.

Taking first the case of an unfertilized prothallus, the appearance of the Fungus may be described as it is seen in a section on passing from the margin towards the more central regions of the prothallus. For a short distance from the small-celled meristem at the margin, the tissues are free from Fungus. In the corresponding cells a little further from the margin, fungal filaments are found in the protoplasmic body, running for the most part parallel to one another. None but these vegetative hyphae are present in this region, and both the cells and the mycelium appear to be quite healthy. Passing to the cells slightly further from the margin, in addition to the ordinary hyphae, bodies of peculiar form are found in them; the nature of these will have to be discussed later, but they may be termed *multinucleate vesicles*. A number of these are to be seen in the portion of a vertical section represented in Fig. 6. They were found to be most numerous in the band of tangentially extended cells, but they occur occasionally in the cells of the palisade-layer; they have not been observed between the cells. Early stages of these vesicles are represented in Fig. 31 *a* and *b*, and

an isolated older vesicle, in which a number of nuclei are already present, in Fig. 31 *c*. The vesicle appears to originate by the swelling of the end of a hypha, and at first contains only one nucleus. Intermediate stages have been seen between these small swellings and the large vesicles containing many nuclei. The connexion of the larger vesicles with the mycelium is usually not apparent (Fig. 32); but by examining series of very thin sections, examples such as that represented in Fig. 33 can be found which show that they are borne on hyphae. Whether or not a septum is present between the cavity of the hypha and that of the multinucleate vesicle could not be definitely determined. On passing to the older cells of these layers, the vesicles are seen to increase in size, the number of nuclei within them to become greater, and their wall to thicken somewhat, though it never attains any great thickness; the protoplasmic body fills, or almost fills, the cavity of the vesicle. Up to this stage the mycelium appears to be healthy, its nuclei staining readily, as does the nucleus of the cell of the prothallus.

But in the region succeeding this, about the same distance from the margin in all the prothalli examined, a change in the appearance of the mycorhizal tissue occurs. The cells with their nuclei still appear healthy, and the latter stain as well as before; but the fungal filaments have lost the regular arrangement they exhibited before and, what is still more significant, show merely the stained wall, the nuclei, and presumably the other contents, having disappeared. The multinucleate vesicles persist in a healthy condition in fairly old cells, their nuclei staining as before. But besides these vesicles of normal appearance, more or less collapsed ones were found, some of which were empty while others had a portion of their contents remaining. These, however, were difficult to detect, though there is no reason to doubt that this tissue formerly resembled that nearer to the margin, in which numerous multinucleate vesicles occur. It has been found impossible to arrive at any definite conclusion as to the way in which the disappearance of the contents of the vesicles comes about.

No perforation in the wall has been seen in any specimen, and it is quite possible that the contents of these dilatations of the mycelium are absorbed in the same way as from the filamentous portion. On the other hand, it is not impossible that in some instances at least the contents may escape into the cell-cavity. In this connexion another characteristic of the region of mycorhizal tissue in which the vesicles are mostly empty must be referred to, although its significance is still doubtful. There are frequently to be found in the cells of this region small round or oval bodies, the walls of which stain deeply with Bismarck-brown ; a cell containing these spore-like bodies is represented in Fig. 34. They may be distributed through the mycelium, with which they sometimes appear to be connected, or may form a more or less compact group. Though appearances suggestive of an origin of these 'spores' from the contents of the multinucleate vesicles have been seen, it has been found impossible to demonstrate any connexion between the two structures. The question must therefore be left open. In the case of prothalli the marginal growth of which had ceased, the changes described above as affecting the older mycorhizal cells extend to those close to the margin until in these also the contents of the hyphae have disappeared ; the nuclei and protoplasm of the vesicles persist longer.

The multinucleate vesicles described above appear to be the same as the 'Sphaeromen' described by Bruchmann[1] in the prothallus of this and other species of *Lycopodium*. The considerable differences between the above description and that given by Bruchmann may be accounted for by the fact that the methods employed in the present investigation have demonstrated the contents of these bodies more clearly. They appear also to agree closely with the 'vesicules' found by Janse[2] in a large number of examples of mycorhiza. The organs of the endophyte, termed by that author 'sporangioles,'

[1] Bot. Cent., xxii. 1885, p. 312, and loc. cit., 1898, pp. 19 and 23.
[2] Annales du Jard. Bot. Buitenzorg, xiv. p. 53.

have not been observed in the case of the Fungus inhabiting the prothallus of *L. clavatum*.

Neither the structure of the mycelium nor the presence of the multinucleate vesicles, the nature of which is uncertain, afford sufficient indication of the group to which this endophytic Fungus belongs. The appearance is quite consistent with the position, as one of the Peronosporeae, which has been commonly assigned to the similar endophytes found in the prothalli of other species of *Lycopodium*; but fuller knowledge of its reproductive organs and life-history is needed before the question of its systematic position can be settled.

The Fungus comes into relation with the surrounding soil by means of hyphae which are present in many of the rhizoids. One or more hyphae, the continuity of which with the mycelium in the underlying tissue can be traced, may be found running almost parallel within the cell-cavity of a rhizoid (Fig. 35). In passing through the basal wall of the latter, the hypha is surrounded by a short sheath derived from the perforated cell-wall. The presence of similar sheaths has been described and discussed by Jeffreys[1] in the case of the Fungus inhabiting the prothallus of *Botrychium virginianum*; but there the sheaths follow the hypha as it passes *inwards* through the outer cell-walls. In *L. clavatum*, on the other hand, the sheaths project *outwards* into the cavity of the cell which bears a rhizoid (Fig. 36). This difference is not without meaning, for it affords indirect evidence that the hyphae penetrated from the interior of the prothallus into the rhizoid, and are therefore not to be regarded as infecting hyphae. Probably after passing along the rhizoid, the filaments of the Fungus penetrate its wall and ramify on the soil, as has been described for *L. annotinum*[2].

In concluding this account of the prothallus of *L. clavatum*, one or two biological points which can be inferred from the study of its structure may be referred to, though they can at

[1] Trans. Canadian Institute, 1896–7, p. 273.

[2] Bruchmann, loc. cit., 1885.

present only be suggested as possibilities. The flattened and still more the trough-like form which these older prothalli present may be an adaptation to facilitate fertilization. Whether this be the case or not, it must, in cases where both kinds of sexual organs are present on the upper surface of the same prothallus, serve this purpose by arresting the water percolating through the peaty soil and causing it to run over the surface of the prothallus before sinking deeper. The complete absence of chlorophyll shows that the prothallus is a total saprophyte, and, as is almost invariably the case in the higher plants, a Fungus is found living as a symbiont in the tissues. The few prothalli found did not unfortunately permit of a determination of the relation which the two organisms bear to one another; such a comparatively simple example of mycorhiza presents a problem of great interest. We may however assume with probability that the Fungus acts in some way as an intermediary in rendering the organic material, absorbed from the soil by the numerous rhizoids, and possibly by the hyphae which extend to the soil, available as food for the prothallus. The appearance of the mycelium in different regions of the prothallus further indicates that at a certain stage the whole of the contents of the Fungus are made use of by its host. Since the young plant is not inhabited by the Fungus, it must be wholly dependent on the prothallus until it reaches the light. This, owing to the depth at which the prothalli grew, does not take place until the plant has attained a considerable size, a fact which may be put in relation with the large foot which the embryo possesses.

Comparative Remarks.

In this portion of the paper an attempt will be made to gather together the facts that are known regarding the gametophyte of *Lycopodium*, and to consider their bearing on the relationship of the species included in that genus to one another and to other groups of Vascular Cryptogams. The full account of the prothallus of a number of European

species given by Bruchmann in his recent work has rendered this more possible: but it will be evident that all that can be done at present is to estimate the direction in which the facts appear to point; further investigation both of the sporophyte and the gametophyte is needed before definite conclusions can be drawn.

A natural classification of the Bryophyta and Pteridophyta, in which both sexual and asexual generations attain considerable complexity and more or less complete physiological independence, must present special difficulty. The characters of both generations must be taken into account in ascertaining affinities; and in both, recent adaptations to the conditions of life must be distinguished from long-inherited characters which indicate common descent and the degree of relationship. While the chances of error are increased, however, the additional evidence available in the case of those plants, neither generation of which is rudimentary or extremely reduced, cannot be neglected. In the Pteridophytes the classification has been founded almost entirely on the characters of the highly developed sporophyte, the differences in the sexual generation affording additional evidence in the recognition of the larger subdivisions. The resulting arrangement would in most cases be little affected by taking the characters of the gametophyte further into account, mainly because the latter exhibits such ready adaptability to its environment; while those of its characters which may be regarded as of morphological importance, are for the most part closely similar throughout the large groups.

The genus *Lycopodium* appears, however, to be to some extent exceptional among Vascular Cryptogams, since in it the sporophyte conforms closely to a general type, while great differences exist among the gametophytes. Thus the possible use of the characters of the sexual generation in arranging the species of this genus cannot be overlooked. The examination of an arrangement of the species of *Lycopodium* based on the external characters of the sporophyte, such as that given in Baker's Fern-Allies, shows clearly that some

at least of the characters upon which the systematist is forced to depend are not such as render it probable that the groups distinguished by their means will be those expressing common descent. So slight are these differences that it may be said that there is nothing in the external characters of the sporophyte to suggest that *Lycopodium* is not a natural genus, the species of which have come to differ somewhat in habit from one another.

All that need be said here with regard to the geological history of the genus, which is unfortunately imperfectly known, is to point out that there is sufficient evidence to show that even in Palaeozoic times Lycopodineous plants of the general habit and size of *Lycopodium* and *Selaginella* existed along with the larger Lepidodendreae. The comparative anatomy of the stele of the existing species of *Lycopodium* is at present too little known to be available as an indication of relationship. It may be hoped that the investigations which are at present being made on this subject will afford a valuable means of checking the conclusions as to affinity drawn from the study of the gametophytes.

At present our attention will be confined to the evidence afforded by the gametophyte which is now known in *L. cernuum* [1], *inundatum* [2], *salakense* [3], *clavatum* [4], *annotinum* [5], *complanatum* [6], *Selago* [7], *Phlegmaria* [8], *Hippuris* [9], *nummulariifolium* [9], and *carinatum* [9]. Before those of *L. complanatum* and *L. Selago*, which have only recently been discovered, were known, three types unconnected by intermediate forms appeared to exist ; these had been termed the *cernuum*, *Phlegmaria*, and *annotinum* types. The further information regarding the earlier stages of the prothalli of *L. clavatum* and *annotinum* given by Bruchmann, and his description of the prothalli of *L. complanatum* and *Selago*, necessitates

[1] Treub, Ann. Jard. Bot. Buitenzorg, iv. p. 107 ; viii. p. 1.
[2] Goebel, Bot. Zeit., 1887, p. 161. [3] Treub, Ann. Jard. Bot. Buit., vii. p. 141.
[4] Bruchmann, loc. cit., 1898.
[5] Fankhauser, loc. cit. ; Bruchmann, loc. cit., 1885 and 1898.
[6] Bruchmann, loc. cit., 1898. [7] Ibid.
[8] Treub, Ann. Jard. Bot. Buit., v. p. 87. [9] Ibid. vii. p. 146.

a reconsideration of the importance to be attached to the types of gametophyte within the genus *Lycopodium*. This has been done briefly by Bruchmann, with whose conclusions, however, the author is unable to entirely agree. He recognizes five distinct types, represented by the prothalli of *L. clavatum* (i), *L. complanatum* (ii), *L. Selago* (iii), *L. inundatum* (iv), and *L. Phlegmaria* (v) respectively, and expresses the opinion that the differences distinguishing them warrant the subdivision of *Lycopodium* into a corresponding number of genera. After enumerating the main characters in which the above types agree and differ from one another, Bruchmann thus sums up his conclusion:—'It follows from the above facts that the groups of *Lycopodium* characterized especially by means of their sexual generation do not stand in close relationship to one another, especially not such as one would expect in species of plants which have found their position together in one genus. This knowledge leads to a separation of the Lycopodiums into groups, or still better into genera, to which it would be quite in place to give new names. There arise as many groups as the sexual generation allows types to be distinguished. Thus, for example, the six European species would be separable into four groups (genera), of which only those of the types i. and ii. are represented by two species each, those of types iii. and iv. by one species each. These still existing [groups of] Lycopodiums, now poor in species, are the much reduced survivors of a family of plants which in earlier time played a prominent part, and the origin of which from a common stock cannot be gainsaid; still, their very considerable differentiations point to a long course of independent evolution, and thus to a separation at a very ancient period [1].'

It must be admitted that this view is a quite possible one; but when all the facts bearing on the question are considered, another mode of regarding the diversity in the sexual generation of *Lycopodium* appears to be more probable. Whether this will prove to be the case or not can only be seen as our

[1] Bruchmann, loc. cit., 1898, p. 108.

knowledge of the life-history is extended to other species, but it appears advisable to state it. In order to do so it will be necessary to briefly discuss the characteristics of the *Lycopodium* prothalli now known. It would be tedious to compare them point by point in every case, but it may be stated that in drawing conclusions the germination of the spore, form and structure of prothallus, position of meristem, position and structure of the sexual organs, and the embryogeny have been taken into account. Further, the results have been put into relation with the habit of the prothallus and that of the sporophyte. Those prothalli which belong to the type of *L. cernuum* will in the first place be considered, and those of the other species afterwards compared with this type.

The prothallus of *L. cernuum*, with which those of *L. inundatum* and *L. salakense* agree in all important respects, is a small body of cylindrical form which grows upright on the surface of the soil. On the germination of the spore an oval or spherical mass of tissue (the primary tubercle) is formed, from the surface of which one or more short filaments originate. By the further development of one of these the cylindrical portion of the prothallus is formed. Sometimes the primary tubercle can be recognized at the base of the cylindrical portion of a prothallus which has attained its full size. In other specimens these two regions are not to be distinguished on external examination. The latter state of affairs is the usual one in *L. inundatum*, while in the other two species the tubercle is as a rule recognizable. The merismatic region of the young prothallus is not at first localized. Ultimately, however, a zone of merismatic tissue can be distinguished extending completely round the upper part of the cylindrical portion of the prothallus, but not terminal. As the cells of the zone become converted into permanent tissue, they contribute on the lower side to the growth of the cylindrical portion; while above the meristem green expanded lobes are formed, at the bases of which the sexual organs are situated. These lobes may be well-developed structures, as in *L. cernuum* and *L. inundatum*, but in *L. salakense* they are

rudimentary or entirely absent. Thus, in the fully developed prothallus of this type, three regions can be more or less clearly distinguished: the primary tubercle, the cylindrical portion, and the terminal region bearing the sexual organs and assimilating lobes. Since development proceeds from a zone between the two latter regions, the youngest lobes and sexual organs are to be found towards the periphery next the meristem.

The structure of the prothallus is very simple, and exhibits little differentiation of tissues. Chlorophyll is most abundantly present in the terminal lobes and the upper portion of the cylindrical part, while the rhizoids are borne on the lower portion of the latter. The more internal cells are somewhat longer than those of the superficial tissue, but are not markedly elongated in the direction of the longitudinal axis. An endophytic Fungus is almost constantly present; this occupies the cavities of the outer cells of the primary tubercle, and extends between the internal cells of this region and into the cylindrical portion. Both antheridia and archegonia take origin from a single superficial cell. The former hardly project from the surface of the prothallus, and the free portion of their wall presents a triangular cover-cell. The spermatozoids developed from the large mass of spermatocytes are biciliate. The mature archegonium consists of a short projecting neck of four rows of cells, and of the central series of ovum, ventral canal-cell, and a single neck canal-cell.

The embryo at an early stage consists of the suspensor, which usually remains unicellular, and of two tiers of cells borne on this. The small foot proceeds from the whole of the tier adjoining the suspensor; the terminal tier gives origin to a small tuber-like structure (the protocorm), which becomes attached to the soil by numerous rhizoids, and to the first leaf. As the protocorm develops, other leaves of simple form and structure are produced from it. Not until a number of these have appeared is the apex of the stem differentiated; this grows into the leafy shoot of the *Lycopodium* plant, the first root arising exogenously from the protocorm near to it.

There are several reasons which appear to indicate that the type of prothallus, the main characters of which, as described by Treub and Goebel, have been summarized above, is in some degree a primitive one. Whether it is to be regarded as primitive in relation to the other types of *Lycopodium* prothallus is a further question that will be considered later. In the first place the prothalli of the *L. cernuum* type possess chlorophyll, and are thus capable of assimilation, though the presence of an endophytic Fungus probably indicates that some degree of saprophytism is possible in addition. But more direct evidence is supplied by the form of the young sporophyte. The parts of this which have been mentioned, viz. protocorm, leaves borne on the protocorm, leafy shoot and exogenous first root, afford when taken together characteristic marks which may fairly be used for phylogenetic purposes. They are repeated exactly, not only in all young plants arising from the fertilized ovum, but in those which originate vegetatively. These are known in the case of *L. inundatum* to arise from leaves separated from the young sporophyte, and in *L. cernuum* from the 'root-tubercles,' the further development of which agrees with that of a young protocorm. Further, a close correspondence can be traced between the development of plants of *L. cernuum* from the root-tubercles and the vegetative propagation of *Phylloglossum Drummondii* [1], which may on other grounds be regarded as a primitive form. Even in the absence of any information as to the gametophyte in this genus, this resemblance affords important evidence that the form of the young plant of *L. cernuum*, &c., is not to be regarded as recent and adaptive, but as possessing an important phylogenetic bearing.

The prothalli and young plants of *L. Selago*, *complanatum*, and *clavatum*, and those which present the type of *L. Phlegmaria*, may now be compared with the *L. cernuum* type. It will be convenient to deal in the first place with the external form, sexual organs, &c.; then to consider the differences of anatomical differentiation; and lastly to compare

[1] Bower, Phil. Trans., 1885, p. 675.

the embryos and young plants. The question upon which this comparison may throw light may be stated at the outset. All the other *Lycopodium* prothalli known differ considerably from the *L. cernuum* type. Is there anything to indicate that they have been derived by modification of a form of gametophyte similar to that of *L. cernuum*, or do their characters rather suggest that, owing to the similarity of the mature sporophytes, a number of groups of species derived from independent stocks of ancient Lycopodiaceous plants have been grouped together in the genus *Lycopodium*? The latter conclusion, which practically amounts to regarding the genus as an artificial one, is that at which Professor Bruchmann arrives. It may be as well to add that this is distinct from the question whether the differences in the gametophyte are such as to justify the separation of the species of *Lycopodium* into several *closely related* genera.

The prothallus of *L. Selago* may be taken first, since it is in several respects less specialized than those of the other species. In form it may resemble the *L. cernuum* type, being a short, upright cylindrical body, growing by means of a merismatic zone beneath the terminal region on which the sexual organs are borne. Being as a rule situated a short distance below the surface of the soil, this prothallus is devoid of chlorophyll, which is, however, developed in considerable amount on natural or artificial exposure to light. Leafy expansions are absent from the terminal portion, which may be distinguished as generative in distinction to the lower vegetative half. The germination of the spore is not known, but no distinct region comparable to the primary tubercle of *L. cernuum* is recognizable in the fully grown prothallus; in uninjured specimens this terminates below in a conical point, the structure of which may in some respects be compared with the primary tubercle. Besides this form of prothallus, the resemblance of which to the *L. cernuum* type will be evident, elongated cylindrical forms which originate by growth becoming localized in one portion of the merismatic zone are found. The interest of these will be seen in connexion with

the *L. Phlegmaria* type of prothallus. As in *L. cernuum*, the sexual organs of *L. Selago* are confined to the terminal region, the youngest being found nearest to the merismatic zone. Associated with them, however, are found numerous hairs (the paraphyses) consisting of a single row of cells; these structures, which are known in all the saprophytic *Lycopodium* prothalli, are not found in those of the *L. cernuum* type. The sexual organs themselves do not appear to differ fundamentally from those of that type, the antheridia being closely similar, while the archegonium has a slightly longer neck, the canal being occupied by a central series consisting of the ovum and not more than six canal-cells.

Thus as regards external form and sexual organs, the prothallus of *L. Selago* might reasonably be regarded as having originated by modifications in relation to its wholly saprophytic mode of life from a type of gametophyte resembling that possessed at the present day by *L. cernuum*, &c. The prothalli of the other European species, which are all subterranean saprophytes, present greater differences in these respects, which can be mentioned more briefly. Those of *L. clavatum* and *annotinum* are, in a young stage, not unlike that of *L. Selago*. As the activity of the merismatic zone between the vegetative and generative halves proceeds, however, the upper portion of the prothallus becomes greatly extended horizontally, the conical portion of the young prothallus appearing as an insignificant projection from this flattened part which later becomes more or less irregularly folded. The description in the earlier part of this paper will show the similarity of arrangement of the sexual organs in this species to that in *L. Selago*. It must be added to this, however, that paraphyses develop when the prothalli are grown at the surface of the soil; under such circumstances chlorophyll is also formed. It has also been seen that the increased length of the archegonium-neck in this species is mainly due to a growth of surrounding cells; the central series consists of ovum and seven canal-cells.

The prothallus of *L. complanatum* (with which that of

L. alpinum appears to correspond closely) does not become extended horizontally in this manner. It maintains an elongated conical shape, and the strongly convex generative region bears antheridia resembling those of the other species, and archegonia which have still longer projecting necks than those of *L. clavatum*. Here also the true neck is not very long, and the increased length is almost entirely due to its being elevated upon a 'false neck' of considerable height. Besides the ovum, 8–14 canal-cells are present.

There is considerable uniformity in the appearance of the prothalli of the other tropical species at present known, *L. Phlegmaria*, *carinatum*, *Hippuris*, and *nummularifolium*, the main differences being in the thickness of the cylindrical branches, of which they consist. These ramify in all directions through the dead bark in which the prothalli grow. Thicker branches, on the upper surface of which the sexual organs are situated, are also found. This form suggests comparison with the modification of the prothallus of *L. Selago* referred to above, which was seen to arise by the localization of growth in one part of the merismatic zone. The early stages of the *L. Phlegmaria* prothallus are not known, and evidence is wanting to show whether they ever are radially symmetrical with a merismatic zone; but the growth of the branches which bear the sexual organs shows a distinction of upper and lower surfaces comparable to that seen in *L. Selago*. The sexual organs themselves, which are accompanied by well-developed paraphyses, also resemble those of the latter species, the projecting archegonium-neck being entirely a product of the outer segment of the archegonium-mother-cell; the central series consists of ovum and 3–5 canal-cells.

It will be evident from the above description of the external form and the sexual organs of the known *Lycopodium* prothalli that they cannot on these grounds be separated into types unconnected by intermediate forms. The similarity in ground-plan of the prothalli would appear rather to indicate that they are all more or less profound modifications of a type not unlike that of *L. cernuum*. The two forms of prothallus

found in *L. Selago* give the clue to the more specialized saprophytic types which, in the deeper-growing subterranean species, retain the radial symmetry while becoming modified in shape. On the other hand, the type of prothallus growing in rotting wood has lost the radial symmetry, and consists of cylindrical but more or less clearly dorsiventral branches. The variability of several characters, such as the presence or absence of leafy lobes, the distinctness of the primary tubercle, and the passage from radial to dorsiventral symmetry within the limits of the *L. cernuum* type, when taken in conjunction with the varieties of form of the prothallus of *L. Selago*, appears to justify such a view as that suggested above. The differences between the archegonia in the saprophytic forms are all such as suggest later modifications rather than deep morphological distinctions.

The importance to be attached to the differences in anatomical differentiation in the vegetative regions of these prothalli has now to be considered. The generative half of the prothallus consists in every case of a parenchymatous tissue into which the endophytic Fungus does not penetrate. In *L. cernuum*, &c., the lower half has been seen to be almost equally undifferentiated save by the distribution of the endophyte, which occupies the cavities of the outer layer of cells of the primary tubercle and penetrates between the cells of the internal tissue. In the case of all the prothalli in which the oldest region adjoining the spore is known, the endophyte is present in the limiting layer of cells of this region only ; as suggested above this may be regarded as the representative in these prothalli of the distinct primary tubercle of *L. cernuum* and *salakense*. In the vegetative portion of the prothallus of *L. Selago*, which has been seen to approach those of the *L. cernuum* type most closely in external form, the structure is correspondingly simple. The cells of the limiting layer, with the exception of those prolonged as rhizoids, are free from Fungus. This is found within the cells of a broad band of tissue internal to this, the cells of which show no alteration of their form. This tissue

surrounds a central strand of elongated cells which broadens out above where it is continuous with the cushion of the generative half. In this prothallus the endophyte is never found between the cells, and the reserve materials are stored within the cells occupied by its hyphae. The differences presented by the other subterranean prothalli almost entirely concern the band of tissue in which the endophyte is found; in all of them there is a central region of more or less elongated cells, and the external limiting layer is free from the mycelium. In *L. complanatum* a few layers of oval cells containing the Fungus within their cavities are present within the latter, while between them and the central strand is found a single layer of cells enormously elongated at right angles to the surface; the mycelium extends between these cells, but is absent from their cavities which contain reserve materials. The differentiation of the corresponding region of the prothallus of *L. clavatum* has been described in the earlier part of this paper. In this type several layers of oval cells with intracellular mycelium are found within the limiting layer, then a single layer of palisade-cells also containing the endophyte, while next to the central tissue is a broad band of smaller cells stored with reserve materials between which the hyphae extend. Finally, in the case of the prothalli of the *L. Phlegmaria* type, the endophyte is absent from the outermost layer, but is found in the cavities of most of the cells within this; in the thicker branches a central strand of elongated elements can be recognized. This simplicity of structure, which is associated with the thin cylindrical branches of which these prothalli consist, finds its nearest analogue in the much thicker dorsiventral branches of the prothallus of *L. Selago*, the tissues of which are, however, more sharply differentiated.

In considering these structural differences it must be borne in mind that they concern the vegetative region of the prothallus, those tissues which there is reason to believe are occupied in the assimilation of the absorbed plant-food and its storage in the form of starch and oil. Our ignorance of

the physiological processes taking place in such organisms as these renders any explanation of the differences in the arrangements of tissue in the several species at present impossible; but as regards their morphological value, they may fairly be considered as comparable to variations in the details of arrangement of the assimilating tissue of leaves in closely related plants. The furthest degree of separation of the types that they justify us in assuming is that these types represent independent modifications of less differentiated prothalli, something like those of *L. cernuum*, which have enabled them to become wholly saprophytic. Such close similarity in the arrangement of the various layers as is seen in *L. clavatum* and *annotinum* on the one hand, and in *L. complanatum* and *alpinum* on the other, probably indicates comparatively recent origin of these pairs of species from ancestors the prothalli of which were already adapted to saprophytic life. The close relationship here indicated by the gametophyte is borne out by the similarity of the sporophytes of the species named. With regard to the *L. Phlegmaria* type of structure, the most probable view would appear to be that, along with the reduction in thickness of the branch, the structure had remained or become greatly simplified. The dorsiventral cylindrical branches of the prothallus of *L. Selago* afford a suggestion of how such a state of things may have come about, whether the *L. Selago* type be regarded as in any way related to that of *L. Phlegmaria* or not. It will be evident that if the complexity of structure of some of the saprophytic forms of *Lycopodium* has arisen as an adaptation to the mode of life, it cannot be regarded as evidence of the descent of the *Lycopodium* gametophyte from highly differentiated ancestors as Bruchmann suggests[1]. The similarity of the regions into which the tissues harbouring the mycelium are divided in the *Lycopodium* prothalli to those which Janse[2] has been able to distinguish in the roots of many plants containing a mycorhizal Fungus, affords considerable support to the point of view advocated above.

[1] Loc. cit., 1898, p. 111.

[2] Ann. Jard. Bot. Buit., xiv. p. 53.

Lastly the species of *Lycopodium* with saprophytic prothalli must be compared with the *L. cernuum* type in respect to their embryology, before the attempt is made to estimate the phylogenetic significance of all the resemblances and differences that have been mentioned taken together.

A well-developed protocorm is as yet known only in those Lycopods the prothalli of which belong to the *L. cernuum* type, though indications of it have been recognized by Treub in some of the young plants of *L. Phlegmaria*. It is thus confined to those species whose prothalli grow on the surface of the soil, with which the young plant soon comes into direct relation by means of the rhizoids of the protocorm, and thus early becomes independent of nutritive supplies from the gametophyte. In all the other cases known as yet, the prothalli being buried in the soil or in rotting wood, the young plants have to attain a more or less considerable size before they are capable of assimilation and become independent of the reserve materials in the prothallus. The larger size of all the saprophytic prothalli may be put in relation with this need. The very young embryo in all the Lycopods of which the embryology has been followed in detail, exhibits a stage in which it consists of a suspensor and two tiers of four cells each. Slight differences in the succession of the divisions by which this result is brought about are known, the two tiers being sometimes separated by the second division in the cell from which the embryo will be formed, in other cases by the third; little importance can, however, be attached to such a distinction when the similarity of the result is borne in mind. From the whole of the tier of cells adjoining the suspensor the foot is derived in all cases. In *L. Phlegmaria* and the other species with this type of prothallus, the foot remains comparatively small, though much larger than the same structure in *L. cernuum*. From the terminal tier there originate the stem-apex, the first leaf, and the first root; in some examples, as mentioned above, the appearance of the root is delayed, and a rudimentary protocorm can be recognized. By the elongation of the

hypocotyledonary stem the first leaf is brought to the light, and is capable of carrying on the work of assimilation; it is almost immediately followed by the second leaf, which is apparent before the embryo has broken through the prothallus. The embryology of *L. Selago* agrees in detail with that of *L. Phlegmaria*, save in the period of formation of the wall separating the two tiers; no appearance of a protocorm is recorded for this species.

The main differences then between the embryology of these species with saprophytic prothalli, which grow a short distance below the surface of the substratum, and that of the *L. cernuum* type, are accounted for by the absence of a protocorm. The reasons given above in favour of the protocorm being a primitive organ, taken together with the similarity between the *L. Selago* prothallus and those of the *L. cernuum* type, support the view that this stage has been omitted from the development of these young plants. Some direct evidence in favour of this is further afforded by the occurrence of a rudimentary protocorm bearing rhizoids in *L. Phlegmaria*[1]. While the absence of a protocorm stands in relation with the subterranean habit of the prothallus, the comparatively small foot must be put into relation with the fact that the first leaf is able to reach the light, and by its assimilation renders the young plant independent of the prothallus.

There remains for consideration the type of embryology presented by *L. clavatum*, *annotinum*, and *complanatum*, which all agree in this respect. The early segmentation is the same as in *L. Selago*, and here also the suspensor and two tiers are recognizable. The stem-apex, first leaves, and first root originate from the tier furthest from the suspensor; this, however, remains for a considerable period as a small-celled meristem in which no differentiation of members is apparent. Before this takes place, the foot, derived from the middle tier, has undergone great enlargement so that the embryo for a time is almost spherical. With the appear-

[1] Treub., Ann. Jard. Bot. Buit., viii. p. 32.

ance of the rudiments of the members of the young plant in the region derived from the terminal tier, another difference of detail is manifest. For in this case the apex of the stem is first apparent, followed by a pair of leaf-rudiments the position of which is not constant; these are succeeded by a second pair at right angles to the first. Later the first root appears. These differences seem to stand in relation to the depth at which the prothallus grows. The young plant, on account of this, is dependent on the prothallus until it has attained a much greater size than in *L. Selago* and *L. Phlegmaria*. This explains the need for the large spherical foot with its special absorbent layer. Further the arrangement of the first leaves covering the apex serves the purpose of protecting the latter on its way to the surface.

The known forms of *Lycopodium* prothallus having thus been compared with one another, the evidence which the resemblances and differences between them afford as to the relationship of the species of that genus must now be considered. In doing this it is necessary to bear in mind that the problem is complicated by the need of adaptive modifications of the prothalli to the diverse conditions under which they live. In the preceding pages the relation that appears to exist between the conditions of existence and particular characters of the prothallus and young plant has been repeatedly referred to. The importance to the species in the Vascular Cryptogams of satisfactory adaptation of the gametophyte to its environment can hardly be overestimated. For the establishment of the sporophyte in a new situation is entirely dependent on the gametophyte having been able to come to maturity and produce sexual organs, so that the localities of the plants are determined more by their suitability for the gametophyte than for the sporophyte, except in so far as the latter is spread by vegetative propagation. Since the struggle for existence tells largely upon plants in their attempts to seize upon fresh situations, it becomes all-important in the case of a Vascular Cryptogam that favourable modifications should take place in the prothallus. It is thus

on *a priori* grounds quite comprehensible that a genus of this group, which is known to have had a long geological history, might retain the spore-bearing generation but slightly modified, and owe its survival almost entirely to the adaptations of the gametophyte to new conditions. The uniformity of the sporophyte in the genus *Lycopodium* suggests that this has actually been the case with regard to it, and, as has already been shown, the main differences between the prothalli can be put into relation with the conditions under which they live. These considerations appear to lead to some such view of the relationship of the species of *Lycopodium*, of which the life-history is known, as the following.

In the *L. cernuum* type the primitive form of prothallus and young plant has been most completely retained. From forms like these others have been derived, in which the gametophyte is adapted to the saprophytic mode of life. In all of these the protocorm is undeveloped, and changes in form of the prothallus, &c., have taken place. In those in which the first leaf is able to reach the light, whether the prothallus grows in rotting wood or just below the surface of the soil, the chief difference in the embryology is the absence of a protocorm, and the foot remains small. Under this head come the *L. Selago* type and the *L. Phlegmaria* type which may be looked upon as somewhat similar independent derivations from the primitive form. Further, several types of prothallus adapted to life at a considerable depth below the surface have probably arisen from the primitive type independently of one another. In the embryos of these the foot has attained much greater development and the first leaves arise in pairs protecting the apex. This includes the *L. clavatum* and *L. complanatum* types which differ mainly in the structure of the prothallus. On this view several biological types would be recognized that may or may not characterize related groups of species.

If the habit of the sporophytes, the prothalli of which are known, be now taken into consideration, a further confirmation of this view will be obtained. For of the twelve species the

three which present the *L. cernuum* type, are terrestrial plants not specially attached to soil containing humus. The plants of the *L. Selago, complanatum,* and *clavatum* types of prothallus live for the most part on moors or in open woods, places where the soil is rich in humus. The rarity of young plants, unless in exceptional circumstances due to human interference, is an index of the difficulty encountered before the spore can succeed in developing into a prothallus, a disadvantage only compensated by the capacity for vegetative reproduction of the sporophyte by a creeping habit or by means of bulbils. Lastly, all the species which possess the *L. Phlegmaria* type of prothallus are epiphytes.

It would be strange if the genetic affinities of the species of *Lycopodium* were found to coincide exactly with these biological divisions. More probably when all the facts are known such a parallelism will be found to be disturbed by instances of independent adaptation to similar conditions. Only the investigation of the remaining species can confirm or disprove the correctness of this mode of regarding the differences of the gametophyte and young plant; it appears to the author to be indicated on the imperfect data we at present possess. Until further facts are available its general discussion as one possible interpretation of the facts will be sufficient. It follows from such a view that, while a complete critical study of the gametophyte may afford valuable assistance in determining affinities within the genus *Lycopodium*, the characters of the prothallus cannot be safely taken by themselves, since the variations in them have been shown to stand in such close relation to the conditions of life, and especially to the saprophytism of certain species. The characters of the less modified sporophyte must be used to check those derived from the gametophyte and should have greater weight. On these grounds it seems inadvisable to subdivide the genus *Lycopodium* as suggested by Bruchmann.

It may be hoped that the discovery of the prothallus in *Phylloglossum*, *Psilotum*, and *Tmesipteris* will cast further light on the relationship of the existing Homosporous Lyco-

podiaceae. But any serial relationship between the few surviving groups is highly improbable. This is at least equally the case when *Lycopodium* and *Selaginella* are compared, where such direct connexion is frequently assumed to exist. While these two genera are rightly grouped together, there appears to be no sufficient evidence for the view that they constitute a direct series. In connexion with this, Bruchmann justly points out the 'far-reaching separation' between them. With that author's further conclusion, I am however unable to agree. He says, 'according to such comparison of the two families of *Lycopodium* and *Selaginella*, their mutual relationship does not appear to be a close one, and in my opinion the distinction between the Selaginellas and the Gymnosperms would be less than that between the Selaginellas and Lycopodiums[1].'

The relationship of *Lycopodium* and *Selaginella* is none the less clear because the connexion lay far back in the history of the Lycopodiaceae. For all that we know to the contrary, these two genera may have persisted with but slight modifications since Carboniferous times, having escaped the extinction which was the fate of all the larger forms. That the Coniferae arose from some heterosporous forms, whether Pterictophyta or more primitve Gymnosperms, about that period may be regarded as probable; but little or nothing points to a close relationship between *Selaginella* and that group, though the life-history of the former aids us in picturing the passage-forms from the Vascular Cryptogams to the Conifers.

The only other group of Vascular Cryptogams that need be referred to is the Ophioglossaceae. Without entering into the general question of the relationship between that family and the Lycopodiaceae which has been suggested[2] on grounds afforded by a detailed comparison of the sporophyte, it may be pointed out that the fuller information regarding

[1] Loc. cit., 1898, p. 110.

[1] Bower, Studies in the Morphology of Spore-producing Members: II. Ophioglossaceae. London, 1896.

the gametophyte recently obtained in both groups does not afford support to such a common origin. For the resemblance between the prothallus of *Lycopodium clavatum* for instance and that of *Botrychium virginianum*[1], which appears at first very striking, seems to be entirely homoplastic and to stand in relationship with the subterranean saprophytic life. It is in form and texture, position of the sexual organs, and the large foot of the embryo, that the likeness exists; while the prothalli differ in symmetry, in the details of the sexual organs, in the spermatozoids, and in the embryology, points which when taken together must be regarded as of great weight. The resemblances between the prothalli of these two plants are of interest, as indicating how an appearance of affinity may follow from the modifications related to subterranean life of the prothallus, and afford an additional reason why the species of *Lycopodium* which possess similar saprophytic prothalli should not, on that evidence alone, be regarded as closely related and forming a sub-genus.

The differences between the prothalli of the great groups of Vascular Cryptogams are indeed so striking, even when we confine the comparison to those which live in similar situations, as to be quite in accord with an independent origin of all those groups from Algal or Bryophytic forms. Within any one group, and especially within a narrow circle of affinity such as the genus *Lycopodium*, aid may doubtless be obtained from a consideration of the characters of the gametophyte. The critical attitude which has been taken up in the preceding pages towards the immediate use of the gametophyte for the purpose of subdividing the genus *Lycopodium*, is not intended to imply any doubt of the value of such characters, but to contribute to the separation of homoplastic from homogenetic resemblances, which must form the preliminary to any such use of the gametophyte in groups where it has become adapted to several different kinds of environment.

[1] Jeffreys, loc. cit.

EXPLANATION OF FIGURES IN PLATES XVI AND XVII.

Illustrating Mr. W. H. Lang's paper on The Prothallus of *Lycopodium clavatum*, L.

PLATE XVI.

Fig. 1. Photograph of a prothallus of *Lycopodium clavatum* and of a young plant free from the prothallus. Natural size.

Fig. 2. The smallest prothallus found, seen from above. × 7.

Fig. 3. Prothallus bearing numerous antheridia on ridges just within the lateral margins: *a* from the side, *b* from above. × 7.

Fig. 4. The largest prothallus found, seen from the side. The two ends are in contact. × 7.

Fig. 5. Part of a transverse section of the prothallus in Fig. 3 including the margin: *a–f*, the layers of tissue described in the text; × position of the marginal meristem. The shaded cells are those within which the endophytic Fungus was present. × 80.

Fig. 6. Lower portion of a similar section of the same prothallus including cells of the layers *a*, *b*, *c*, and *d*. × 375.

Fig. 7 *a*, *b*. Median sections through the ends of a prothallus to show the difference of outline that may exist between them, and for comparison with sections across the lateral margin: × position of meristem. × 25. In this and in the other outline figures of sections through the prothallus (Figs. 8, 9, 25 and 26) the darker shading indicates the region in which the mycorhizal Fungus is intracellular, the lighter shading the layer in which it is intercellular.

Fig. 8. Vertical section across the margin of the prothallus in Fig. 4 to show the marginal ridge and succession of archegonia. × 35.

Fig. 9. Similar section of the prothallus in Fig. 3 showing the succession of antheridia on the marginal ridge. × 35.

Figs. 10–12. Stages in the development of antheridium; the cells which give rise to the spermatozoids are shaded. × 530.

Fig. 13. Longitudinal section of an almost mature antheridium. × 375.

Fig. 14. Transverse section of an almost mature antheridium. × 375.

Figs. 15, 16. Arrangement of cells forming the free part of the antheridium-wall. The triangular cell in the centre has broken down to allow of the escape of the spermatozoids. × 375.

PLATE XVII.

Figs. 17–19. Stages in the development of archegonium. × 375.

Fig. 20. Longitudinal section of a mature archegonium. × 375.

Fig. 21. Similar section of a mature archegonium in the development of which the divisions in the generative layer forming the 'false neck' are seen to extend to adjoining cells on the right-hand side of the figure. × 375.

Fig. 22. Transverse section of the neck of an opened archegonium. × 375.

Fig. 23. Prothallus bearing a young plant, seen from the end. × 7.

Fig. 24. Prothallus, the irregular shape of which is probably the result of an injury, bearing two young plants of similar age; from above. × 7.

Fig. 25. Section of one of the young plants in Fig. 24 in the median plane of the root. × 25.

Fig. 26. Similar section of the young plant in Fig. 24 in a plane at right angles to that of the root. × 25.

Figs. 25 and 26 demonstrate the absence of the protocorm; in both the extent of the absorbent layer of the foot is indicated by shading.

Fig. 27. Portion of the foot of one of the young plants in Fig. 24, showing the absorbent layer of cells bounding it; these are seen to be densely filled with protoplasm. × 200.

Fig. 28. Section through the region of attachment of the young plant in Fig. 23 to the prothallus in the plane of root and shoot. The tissue of the prothallus is uniformly shaded. × 80.

Figs. 29, 30. The region of attachment to the prothallus of two young plants to show the differences in form and position of the foot. × 7.

Fig. 31 *a*, *b*, *c*. Stages in the origin of the 'multinucleate vesicles' from the ends of hyphae of the mycelium within the cells of the prothallus. × 1,000.

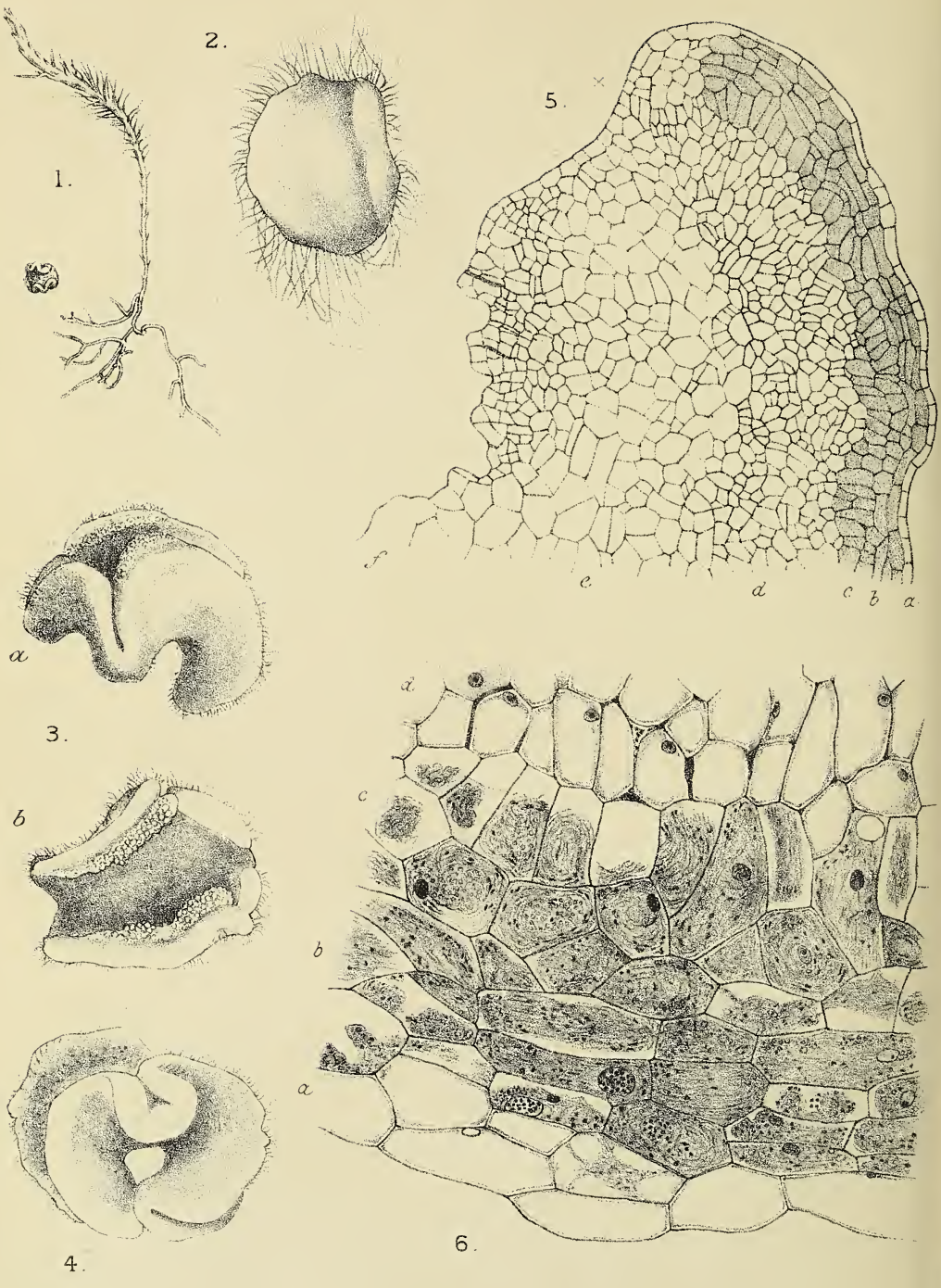
Fig. 32. Cell from layer *b* of the prothallus, showing a multinucleate vesicle, close to which the nucleus of the cell is seen presenting a healthy appearance. × 1,000.

Fig. 33. Cell from the same layer, showing coarser hyphae, two of which are seen to expand into multinucleate vesicles. × 1,000.

Fig. 34. Older cell from the same layer, showing the disorganized mycelium in which bodies resembling spores are visible. × 1,000.

Fig. 35. Basal portion of a rhizoid in optical section, showing two hyphae passing into the cavity of the rhizoid from the tissue beneath. × 375.

Fig. 36. Section through the basal portion of a rhizoid, showing the entrance into its cavity of two hyphae which receive short tubular sheaths on passing through the wall. × 750.



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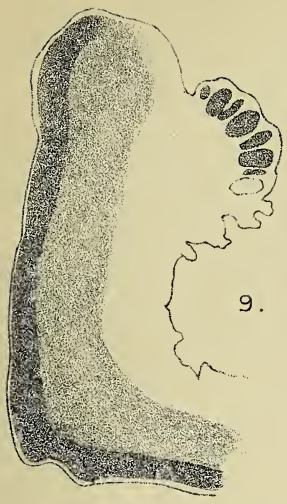
LANG. — LYCOPODIUM CLAVATUM.



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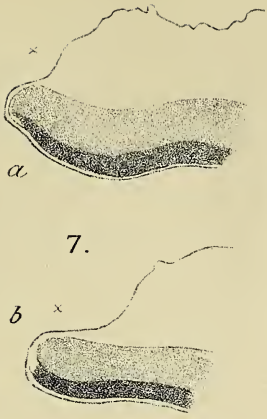

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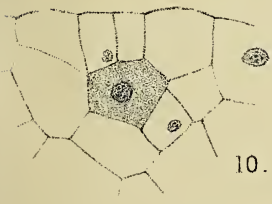

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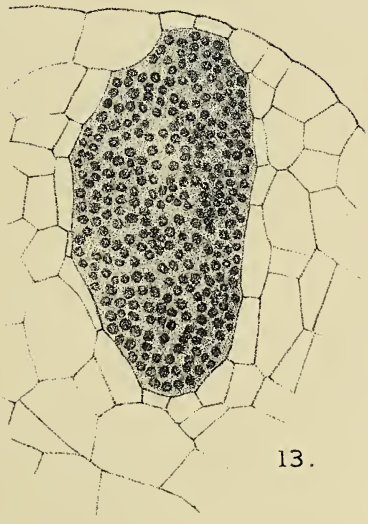

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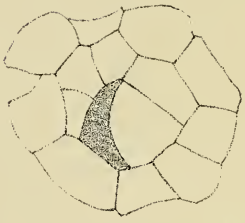

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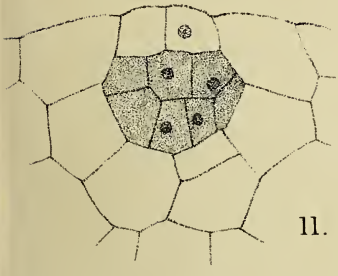

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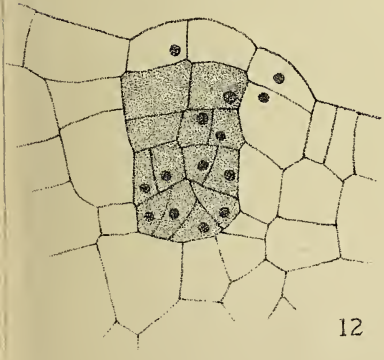

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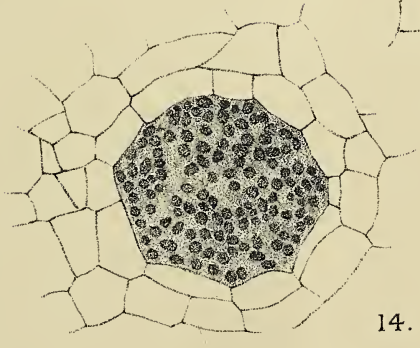

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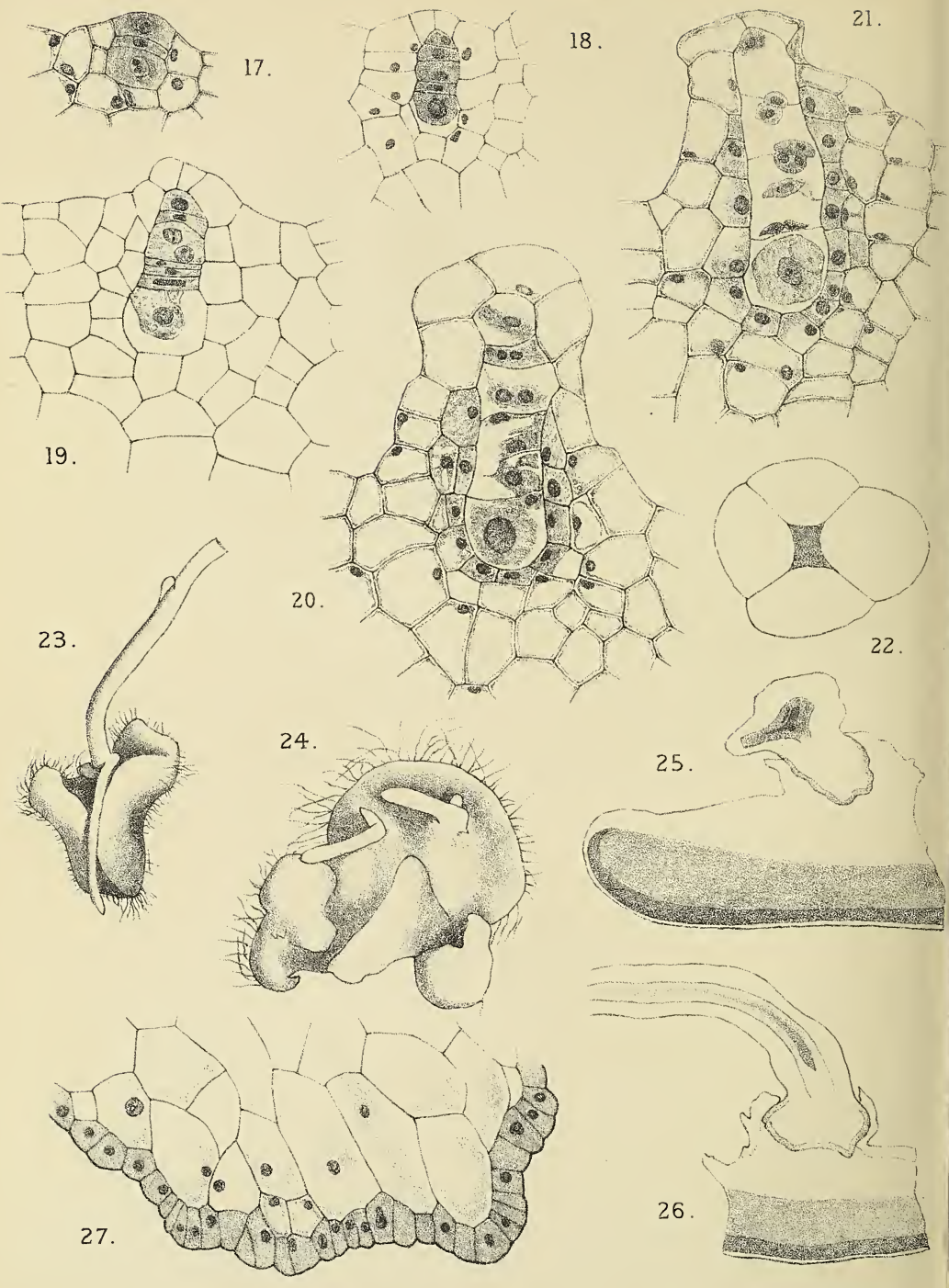

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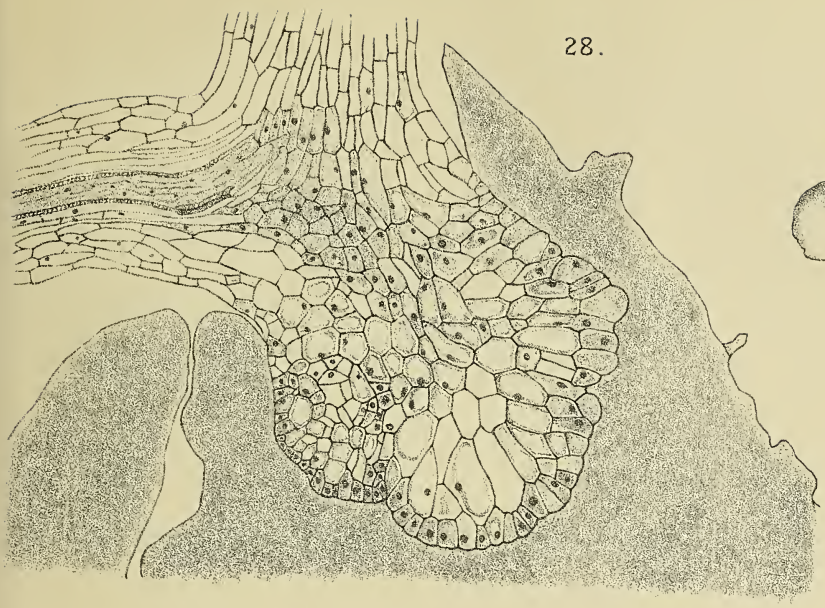
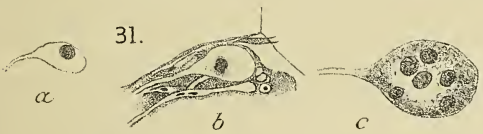
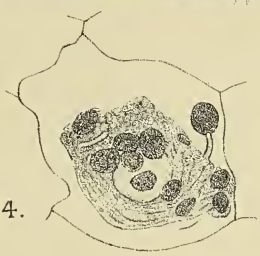
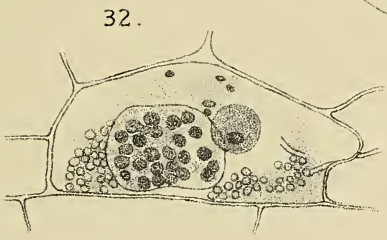
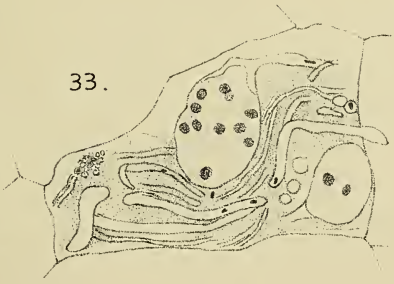
LANG. — LYCOPODIUM CLAVATUM.

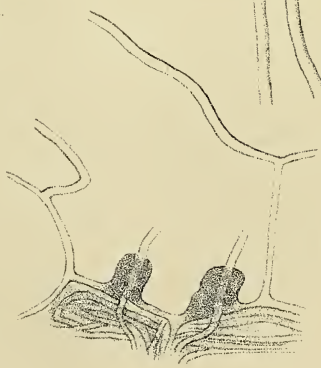












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NOTES.

THE STRUCTURE AND AFFINITIES OF MATONIA PECTINATA[1].—By A. C. Seward, F.R.S., University Lecturer in Botany, Cambridge. The genus *Matonia* has long been known as an isolated type among existing Ferns. It is represented by two species, *M. pectinata* R. Brown and *M. sarmentosa* Baker, both confined to the Malayan region. *Matonia* has not hitherto been examined anatomically, and its reference by several writers to an intermediate position between the Cyatheaceae and Gleicheniaceae, is based on the structure of the sorus, which, in the small number of the sporangia and in its circular form, resembles that of the latter family, while the presence of an indusium and the position of the annulus afford connecting links with Cyatheaceous Ferns.

In *Matonia pectinata* the frond has a characteristic pedate habit, with numerous long pinnae having slightly falcate linear segments, practically all of which appear to be fertile. The sori are circular in form and indusiate, each consisting of about eight large sporangia with an oblique incomplete annulus, containing sixty-four tetrahedral spores. The dichotomously branched rhizome, which grows on the surface of the ground, is thickly covered with a felt of multicellular hairs, and gives rise to long-stalked fronds from its upper face, and a few wiry roots which may arise from any part of the surface of the stem.

The full paper deals more especially with the anatomical structure of *Matonia pectinata*. The material which rendered the investigation possible, was generously supplied by Mr. Shelford of the Sarawak Museum, Borneo, to whom the author wishes to express his hearty thanks.

The stem is polystelic, and of the gamostelic type; there may be two annular steles, with the centre of the stem occupied by ground-tissue; or in shorter branches of the rhizome a third vascular strand may occupy the axial region. Each stele consists of xylem-tracheids

[1] Abstract of a paper read before the Royal Society, March 9, 1899.

and associated parenchyma, surrounded by phloem composed of large sieve-tubes with numerous sieve-plates on the lateral walls, and phloem-parenchyma; an endodermis and pericycle surround each stele, and in the case of the annular steles these layers occur both internally and externally. At the nodes the outer annular stele bends up into the leaf-stalk, and a branch is also given off from the margin of a gap formed in the inner annular stele; the axial vascular strand may or may not be in continuity with the meristele of the leaf. The petiole is traversed by a single stele, similar in shape to that of certain Cyatheaceous Ferns; towards the top of the leaf-stalk the stele alters its form, and gradually gives off separate U-shaped branches to supply the pinnae.

The most interesting feature in the structure of the pinnules is the marked papillose form of the lower epidermal cells. The roots have a triarch stele enclosed by a few layers of thick brown sclerous cells.

In structure *Matonia pectinata* presents points of agreement with several families of Ferns, on the whole approximating more closely to Cyatheaceae than to any other family; but the peculiarities are such as to fully confirm the conclusion previously drawn from external characters that *Matonia* should be placed in a separate division of the Filices.

After comparing the structure of the Malayan species with that of other genera, the paper concludes with an attempt to give an account of the geological history of the Matonineae. The genera *Laccopteris* and *Matonidium* are dealt with at some length, and reference is made to other Mesozoic Ferns, which may probably be included in the same group.

The data furnished by an examination of palaeontological evidence lead to the conclusion that in *Matonia* we have a survival of a family of Ferns, now confined to a few localities in Borneo and the Malay peninsula, and represented by two living species, which in the Mesozoic epoch had a wide geographical range, being especially abundant in the European area.

STUDIES IN THE MORPHOLOGY OF SPORE-PRODUCING MEMBERS: IV. LEPTOSPORANGIATE FERNS.—By F. O. Bower, Sc.D., F.R.S., Regius Professor of Botany in the University of Glasgow[1]. The characters used in current classifications

[1] Abstract of a paper read before the Royal Society, April 20, 1899.

of Ferns need strengthening. In recent years the more detailed knowledge of the prothallus has been used for this purpose; but while not denying its value in certain specific cases, the author holds that the vegetative development of the prothallus is an uncertain guide to a general classification. On the other hand the archegonium is so uniform in its character that it gives little help; the comparison of the antheridium is, however, a useful aid, though not sufficiently varied to serve in detail[1]. Accordingly the sporophyte must be the main basis. Its vegetative organs have lately been largely used for systematic purposes by Christ[2]; but the same objection holds here as in Phanerogams to the use of these as characters of first rank for comparison. An attempt has therefore been made in this memoir to strengthen the characters derived from the sorus by a fresh examination of its details, and certain of its features will now be used for purposes of general comparison, which have hitherto received too little attention; they are—

1. The relative time of appearance of sporangia of the same sorus.
2. Certain details of structure of the sporangium, including its stalk.
3. The orientation of the sporangia relatively to the whole sorus.
4. The potential productiveness of the sporangium as estimated by its spore-mother-cells, and the actual spore-output.

Observations of these features extending over all the more important living genera, coupled with data of habit and the characters of the gametophyte as collateral evidence, have led the author to divide the Homosporous Ferns thus:—

Simplices	Marattiaceae	Eusporangiate
	Osmundaceae	
	Schizaeaceae	
	Gleicheniaceae	
	Matonineae	
Gradatae	Loxsomaceae	Leptosporangiate
	Hymenophyllaceae	
	Cyatheaceae	
	Dicksonieae	
	Dennstaedtiinae	
Mixtae	The bulk of the Polypodiaceae	

[1] Heim, Flora, 1896, p. 355, &c.
[2] Die Farrnkräuter der Erde, Jena, 1897.

These divisions are primarily based on the order of appearance of the sporangia in the sorus, the Simplices having all the sporangia of the sorus formed simultaneously, the Gradatae having them disposed in basipetal succession, and the Mixtae having the sporangia of different ages intermixed. But it is found that other important characters run parallel with these: thus the Simplices and Gradatae have an oblique annulus (where definitely present), the Mixtae (with very few exceptions) have a vertical annulus. None of the Mixtae have been found to have a higher spore-output per sporangium than sixty-four, but this number is exceeded by some of the Gradatae, and large numbers are the rule in the Simplices. The Simplices and Gradatae have relatively short thick stalks, the Mixtae usually have long and thin stalks. The orientation of the sporangia in the Simplices and Gradatae is usually definite, in the Mixtae it is indefinite. The receptacle is often elongated in the Gradatae, but not in the Simplices or Mixtae. The sum of these characters, which for the most part run parallel to one another, appears to give a substantial basis to the classification.

Evidence as to the transition from type to type has been collected. In the case of the transition from a simultaneous to a successive sorus it does not amount to a demonstration: but it is specially pointed out how slight a step it is from such a sorus as that of *Gleichenia dichotoma* to that of an *Alsophila*: that given a basal indusium and marginal position, the similarity of sporangial structure and dehiscence between *Gleichenia* and *Loxsoma* is suggestive; as also the sporangial structure and high spore-output in *Hymenophyllum*. Though we may recognize these lines of similarity, they do not focus upon any one genus as the actual transitional link from the simultaneous to the basipetal. But the transition from the basipetal to the mixed sorus can be followed in detail; intermediate steps are seen in the Dennstaedtiinae, while the fully mixed type is seen in the closely allied *Davallia*. Probably this is only one of several such lines of transition from the basipetal to the mixed type.

It is shown that a biological advantage would be gained by the suggested transitions. In the Simplices the few sporangia are large, and, arising simultaneously, make a demand all at once on the nutritive resources of the part. In the Gradatae the smaller sporangia are produced in succession upon an elongating receptacle, and the drain on the part is spread over a longer period. But with the assumption of

the mixed character the drain may be spread over an equally long time, while, as the elongated receptacle disappears, the surface from which nourishment can be derived is enlarged, and the distance through which it has to be transferred is shortened. Thus it appears biologically reasonable that the succession should be as suggested.

It is shown how the various types of dehiscence and the action of the annulus stand in close relation to the orientation of the sporangia, and to their arrangement in the sorus. Thus the position of the annulus, which has played so important a part in classification, has been placed upon a footing of adaptation.

Estimates of numerical output of spores per sporangium have been made with a view to illustrating the relation of the Eusporangiate and Leptosporangiate Ferns in this respect. The estimated output in the Marattiaceae has been shown to be high [1]; that of the Polypodiaceae is sixty-four or less. The result of numerous countings is to show that, of all Leptosporangiate Ferns, *Gleichenia* approaches most nearly to the Marattiaceae (*Gl. flabellata* may produce over 800 per sporangium); *Osmunda* may have over 500, and *Lygodium* 256. The most interesting results were derived from the Hymenophyllaceae, in which *Hym. tunbridgense* may have over 400, while species of *Trichomanes* may produce as few as thirty-two per sporangium. These results, when taken with those derived from the filmy *Todeas*, make it seem probable that the filmy habit is a condition leading to reduction of output per sporangium, and indicate that the Hymenophyllaceae are a derivative series of reduction.

A most important commentary upon the classification proposed is derived from comparison of the antheridia, which Heim [2] found to be the most dependable part of the gametophyte for comparative purposes. He recognizes two types according to their dehiscence: the one type includes, with the exception of two genera of Schizaeaceae, our Simplices and Gradatae, while the other includes the Mixtae. I can only regard this correspondence of parts, so aloof from one another as the antheridium and the sporangium, as establishing the relations of the Simplices and Gradatae upon a firmer footing; the facts also give substantial support to the distinction of the Gradatae and Mixtae.

The effect of the observations and comparisons in this memoir is rather confirmatory of the current classifications than disturbing. The divisions suggested would supersede those of Eusporangiatae and

[1] Studies, No. 3, p. 60.

[2] Flora, 1896, p. 355, &c.

Leptosporangiatae, though these terms would still be retained in a descriptive sense. If the sub-orders Osmundaceae, Schizaeaceae, and Marattiaceae be transferred from the end of the 'Synopsis Filicum' to the beginning, and grouped with *Gleichenia* and *Matonia*, we have the Simplices before us. The Gradatae include the Cyatheaceae, Dicksonieae (excl. *Dennstaedtia*), Hymenophyllaceae, and Loxsomaceae, sequences probably of distinct descent, and, in my view, derivative from some prior forms such as the Simplices; and in the arrangement of Sir William Hooker they hold a position following on the Gleicheniaceae. The family of Dennstaedtiinae, founded by Prantl to include *Dennstaedtia* and *Microlepia*, also has its place here, but it leads on by intermediate steps to undoubtedly mixed forms such as *Davallia*, *Cystopteris*, *Lindsaya*, and the Pterideae. But this sequence is already laid out in this order in the Synopsis, and it illustrates one at least of the lines along which mixed forms are believed to have been derived from the Gradatae. No attempt has been made to follow the natural grouping of the Mixtae into detail, or to test the arrangement of them in the Synopsis. Sufficient has, however, been said to show that the systematic divisions of the Ferns now proposed fall in readily with the system of Sir William Hooker, notwithstanding that they are based upon details of which he cannot have been aware.

The Effect of Centrifugal Force upon the Cell.

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With Plate XVIII.

THE subject discussed in the following pages is one which has received little or no attention at the hands of either the botanist or zoologist. So far as is known to the writer there exists, as yet, no published account of any extended study dealing primarily with the effect of centrifugal force upon the living contents of the cell. It is true that centrifugal force has been extensively used by investigators in studies on geotropism, but in almost every case the reaction of the plant as a whole, or of its organs, was the primary object of research. This has also been largely the point of view of the experimental morphologist in dealing with animals.

Dehnecke ('80) has observed that, in cells of the starch-sheath of *Impatiens*, the large starch-grains, or clusters of them, would fall to the lower end of the cell by virtue of their own weight. This fact may be very readily verified by observing, with the microscope, cells of the starch-sheath of the plant in question held in a vertical position.

[Annals of Botany, Vol. XIII. No. LI. September, 1899.]

Dehnecke states also that, when the plant is revolved slowly in a horizontal position upon the klinostat, the starch-grains will fall from one edge of the cell to the other, and may thus be made to glide round within the cell in a plane parallel to the transverse axis.

It is evident, *a priori*, that the heavier inclusions of the protoplasm may be made to fall toward any side of the cell by means of centrifugal force. It was the purpose of the writer to determine, at least in a general way, what parts of the living substance and its inclusions could be displaced within the cell by means of a centrifugal force several hundred-fold greater than that of gravity acting for certain definite but usually short periods of time, and to see what effect such displacement might have upon the individual cell.

The results in many cases were surprising, and may prove to be of considerable theoretical importance, so that a somewhat detailed discussion will, no doubt, be of interest to the botanist, and especially to the experimental cytologist, whether his object of investigation be the plant or animal cell.

Various Algae, leaves of Mosses, of *Elodea* and *Vallisneria*, together with trichomes and seedlings of several Phanerogams, furnished material for investigation. Of the Algae those most easily procurable at the time were selected, such as *Cladophora*, *Spirogyra*, *Oedogonium*, *Vaucheria*, *Mesocarpus*, *Chara* and *Nitella*. Trichomes of *Urtica*, *Momordica*, *Cucurbita*, *Primula*, with many others ; stamen-hairs of *Tradescantia*, leaves of *Funaria* and *Elodea*, and sections from leaves of *Vallisneria*, together with seedlings of *Zea Mays*, *Vicia Faba*, *Ricinus*, *Phaseolus*, &c., were also found suitable for experimentation.

A centrifugal force varying from 1700 to 1930 times that of gravity was generated by the use of an ordinary milk-centrifuge driven by a gas-motor.

The objects selected for study were placed in strong glass cylinders, which were securely packed in the drum of the centrifugal machine. The drum permitted of a slight variation in the length of the radius, so that by moving the cylinders toward or from the centre a considerable difference

in intensity of the centrifugal force could be obtained without varying the speed of the motor[1].

Cladophora.

As *Cladophora* proved to be a suitable object for these experiments, and since the results in many respects were strikingly interesting and instructive, a detailed account of the behaviour of this plant may serve as an introduction to what follows, and also to give some idea of the phenomena discussed.

Fresh growing specimens, with tolerably large cells, were always selected for study.

Pieces of filaments about 2 or 2½ centimetres long were fastened upon a slide under a cover-glass by means of gypsum. One end of the filaments only is enclosed in an edge of the cast so made that this edge projects a short distance under one side of the cover-glass and overlapping the latter to the same extent. By means of sealing-wax, small grains of sand were first glued to the opposite corners of that edge of the cover-glass enclosed in the cast: in this way the cover-glass is held firmly and steadily in the same position. A larger grain of sand is similarly glued to the slide over which the cast is made in order to prevent any movement of the latter from side to side, and a small rubber-band holds cast and slide securely together. The preparations thus constructed are packed securely in the cylinders with cotton, and enough water is added to cover the specimens.

This method of preparation permits direct observation immediately upon removing the specimens from the centrifugal machine, and at any subsequent time.

[1] The centrifugal force may be calculated according to the following formulae:

$$\frac{4\pi R \text{ (in metres)}}{gt^2}, \quad \frac{4\pi^2}{g} = 4{\cdot}024, \text{ a constant.}$$

$$4{\cdot}024 \times \frac{R}{t^2} = \text{no. of } g \text{ (gravity).}$$

R = radius expressed in metres.

t = time in seconds of one revolution of the drum.

In order to observe the behaviour of the cells from time to time after the application of centrifugal force, the preparations were kept in a moist chamber; and, when the plants operated with were Algae, one end of the slide was allowed to dip into the water. The water was renewed every day or two to prevent the accumulation of bacteria and other foreign organisms. From time to time a small quantity of gypsum was thrown into the water of the moist chamber or small aquarium to prevent a too rapid dissolution of the gypsum casts, and as it was found that the Algae thrived better in the dilute gypsum-solution than in the tap-water alone.

For the most part the centrifugal force was allowed to act parallel to the longitudinal axis of the cell; but also frequently, for the sake of comparison, in an oblique and transverse direction.

We shall direct our attention first to the effect of centrifugal force acting longitudinally.

Specimens of *Cladophora* were subjected for about one hour and a half to a centrifugal force varying from 1700 to 1800 *g*.

The time necessary to bring about the desired displacement when the force remains constant varied with the size of the cell and the nature of the contents.

In cells of *Cladophora*, with the proportional dimensions of Figs. 1 and 2, immediate observation after centrifugal action showed that almost the entire contents were crowded into a dense mass at the end of the cell. Apart from an occasional chloroplast and sometimes a nucleus, nothing of the living substance or its inclusions remains undisturbed except the ectoplasm or *hautschicht* and the plasmic lamellae which penetrate the cavity of the cell and divide it into a number of irregular polygonal chambers varying much in size (Figs. 1 and 2).

Seen with the low power of the microscope, each cell appears perfectly colourless with a dense dark-green mass at one end. In all probability the lamellae always remain stationary or nearly so, and the chloroplasts and nuclei fall

through them, just as a small glass bead may be made to fall through a column of soap-foam.

As the lamellae are laid bare by means of the centrifugal force, thus enabling an unobstructed view of the interior of the cell, a word further as regards its structure may not be out of place here.

As is well known (Strasburger, '97), these lamellae may be distinguished in normal cells of *Cladophora* not too rich in chlorophyll. Upon them are usually distributed nuclei and chloroplasts. When the movable cell-contents are removed, the lamellae appear rigid, forming sharp angles with each other like so many cell-walls; in fact the cell looks as if it were filled with a thin-walled parenchymatous tissue. The lamellae shift their position little or not at all. On careful observation with high powers or oil-immersion, they are seen to be in a constant vibrating or quivering motion, and over their surface a thin layer of colourless cytoplasm is seen to move. It cannot be unreservedly stated that the lamellae quiver or vibrate constantly, but this movement was seen at each observation.

Immediately the preparation is taken from the centrifugal machine, the displaced contents begin to redistribute themselves, but, in *Cladophora* especially, very slowly, requiring often three weeks for a complete redistribution. During the first twenty-four hours the redistribution takes place more rapidly, after which it becomes slower and more uniform. The time of redistribution will vary with the size of the cell, being less in small narrow cells.

Fig. 1 was drawn twenty-four hours after the centrifugal action, when a portion of the chlorophyll and other inclusions had advanced somewhat into the colourless part of the cell. In the beginning all the movable contents occupied the space indicated by the densely shaded portion of the cell. As a rule the chloroplasts, and presumably the nuclei also, travel back toward the opposite end in a tolerably uniform layer, only a few isolated chloroplasts proceeding faster. The plastids and nuclei, together with the more coarsely granular

colourless cytoplasm, retreat partly along the ectoplasm and partly upon the lamellae.

It may be mentioned, however, that as soon as observation is possible after centrifugal action an active streaming of a thin layer of cytoplasm is to be seen upon the lamellae and along the *hautschicht.* This is true in all cells of the various plants observed at the time in question; the movement is, of course, toward the end of the cell from which the contents were made to fall.

Although, as previously mentioned, the retreating contents proceed in a tolerably uniform layer, yet this layer does not contain as many chloroplasts and nuclei as were originally distributed over the same area of the cell. Many of these bodies travel more slowly than others; for when the retreating layer or mass reaches the opposite end of the cell this end presents a light, pale-green colour which passes over gradually into the deep green of the other end into which the contents were thrown.

A noteworthy phenomenon presents itself in cells that were undergoing division at the time of centrifugal action. If the ring-shaped membrane were narrow, the contents of one daughter-cell would be forced through into the opposite end of the other, just as if no cellulose-ring were present (Fig. 2). If, however, the forming transverse wall had proceeded to such an extent that the circular opening remaining was only one-third or one-half the diameter of the cell, a few chloroplasts were generally lodged against the new wall. This was often the case in *Spirogyra*, to be mentioned later, where often an end or fold of some chlorophyll-band caught against the ring-shaped membrane. When, on the contrary, the opening remaining in the membrane was smaller, in large cells less than one-half the diameter, the chlorophyll-grains were not forced through.

During redistribution of the displaced contents in such cells, as just mentioned, the retreating mass creeps back through the opening in the incomplete transverse membrane, and becomes equally distributed as under ordinary circumstances.

When the opening is equal to about one-half the transverse diameter of the cell or a little less, the progress of the retreating contents is greatly retarded, but finally a portion succeeds in passing through and distributing itself in that portion of the cell.

Transverse walls which were being formed at the time of centrifugal action were never completed. Several instances were closely observed from day to day for three consecutive weeks, during which time the contents had become uniformly redistributed; but the cellulose-ring remained unchanged, except undergoing secondary thickening by the apposition of new layers in common with the entire wall of the cell.

This fact seems to indicate clearly that, when the relation existing between the transverse wall in process of formation and the nuclei and movable cytoplasm connected therewith is once destroyed, it is not restored again so as to complete the cell-wall. It was observed also that later some of these cells in question divided, and the division-wall was laid down near the old cellulose-ring. We shall return to this phase of the subject in a subsequent paragraph.

As already stated, a violent displacement of all movable contents in cells of *Cladophora* does not interfere materially with cell-growth or division, for a day or two after centrifugal action it was not at all uncommon to find cells dividing whose displaced contents had only fairly begun to redistribute themselves (Fig. 3). This fact may throw some light upon the relation of the amount of cytoplasm to cell-division. From Fig. 3 it will be seen that the resulting daughter-cells are of unequal size. The smaller presents a striking contrast in size and colour to the larger, which contains only a relatively small number of chloroplasts. Very often three or more such cells were to be seen in a piece of filament one and a half centimetres long. The entire process of division was followed in several cases, so that there can be no doubt as to the accuracy of the statement. So far as determined the number of nuclei was less in the longer cell (Fig. 3, *b*), but whether the quantity of cytoplasm is proportional could not be ascertained.

In the longer daughter-cell (*b*, Fig. 3), the chlorophyll-grains finally became uniformly distributed, when such cells from their pale-green colour form a striking contrast to the deep green of their neighbours, especially their sister-cells. So far as observation extended, subsequent growth in length seemed to be the same in both cells, i.e., the shorter did not increase in length more rapidly than the longer. In the larger cells, such as *b*, Fig. 3, with fewer chloroplasts, these bodies multiplied so that after a time the amount of chlorophyll in the cell was appreciably increased. For lack of time the ultimate fate of these cells could not be followed.

Centrifugal force acting transversely, i.e. at right angles to the longitudinal axis of the cell, caused the contents to collect into a band-shaped mass along the opposite side.

After transverse displacement, redistribution required a relatively short time, the distance to be traversed by the displaced constituents being much shorter.

Spirogyra.

Spirogyra, being in many respects a less hardy plant than *Cladophora*, is more susceptible to injury from violent centrifugal action. Specimens with tolerably large cells containing only one or two chlorophyll-bands were found suitable for experimentation. Subjected to the centrifugal action for the same length of time as *Cladophora*, a relatively large number of cells were badly injured, while many were killed outright. Consequently the time was shortened to periods of less than one hour. For the specimens operated with, three-fourths of an hour were sufficient to effect a complete longitudinal displacement of all movable contents of the cell. The cells to which the following paragraph refers contained but two chlorophyll-bands with abundant starch clustered about the pyrenoids. The specimens were fresh and actively growing with many of the cells in process of division.

Fig. 4 represents a cell drawn immediately the preparation

was removed from the centrifuge. It had been subjected for three-fourths of an hour to a force of 1820 *g*. All the movable contents were heaped up in the end of the cell. As soon as observation is possible after the cessation of centrifugal action (it required about five minutes to stop the machinery and remove the preparations), an active streaming movement toward the opposite end of the cell is seen in very finely granular cytoplasmic strands extending along the *hautschicht*. These strands generally anastomose. Not infrequently, and in very large cells especially, a cytoplasmic band is formed which encircles the cell in an exactly transverse direction. Movement within such a band follows the direction of the same. With the gradual redistribution of the cell-contents these bands, when present, progress uniformly in front of the chloroplasts.

About twenty or twenty-four hours after centrifugal action, a thicker layer of fine granular cytoplasm is distributed over the entire surface of the *hautschicht* (Fig. 5) i.e., the primordial utricle is now thicker. It is also to be seen (Fig. 5) that the chlorophyll-bands, together with the nucleus suspended by cytoplasmic strands which extend apparently from the pyrenoids, have begun to creep back to resume their position in the cell. It often happens, however, that the bands do not stretch out evenly, contiguous edges often adhering so as to give the bands a tangled appearance. This is generally true when a larger number of bands with steep turns are present in the cell. In such cases a longer time is necessary for an even and complete redistribution. Whenever only two or three bands are present, distribution is quite uniform and regular (Fig. 6).

In cells such as represented in Figs. 5 and 6, a complete redistribution may take place in seven or eight days, perhaps sooner, but often fifteen to eighteen, and even more, are necessary.

The rapidity with which redistribution takes place, as will be seen later, depends to some extent upon the temperature and perhaps illumination. In many instances when redis-

tribution was complete, there was nothing to indicate in any way that either bands or nucleus had experienced any change in position in the cell. The nucleus did not always resume an exactly central position in the cell; sometimes it would not quite reach the centre, or it might pass a short distance beyond it.

As in *Cladophora*, cells sometimes divided before a complete redistribution of the contents was accomplished, when the daughter-cells varied greatly in length, being to each other as 1 : 3. In the shorter cell the chlorophyll-bands necessarily remained crowded closely together, giving the cell a dark-green colour; in the longer, the relatively shorter pieces of bands stretched themselves the entire length of the cell, making not more than one and a half transverse turns. The nuclei always took up a central position in their respective cells.

Centrifugal force acting transversely upon the cells of *Spirogyra* often seriously affected their vitality, in fact killing many outright. As a rule a longer time was found necessary to effect a complete transverse displacement of the chlorophyll-bands. In large cells whose several bands wound at a sharp angle, these are usually broken at the side of the cell from which they are removed. In passing over to the opposite or 'lower side[1]' of the cell, one free end of each severed portion moves along in the primordial utricle upon one side parallel to the direction of centrifugal force, and the other upon the other side. Everything seemed to indicate that the ends arising from the severing of the bands did not move through the vacuole. In rather long and slender cells, however, with only few bands making long turns, the bands are not severed, and it seems that the displaced portion passes directly through the central cavity or vacuole.

Whenever the bands were broken at several points, the cell was so injured that death resulted soon after centrifugal action. It often happened that only a partial displacement

[1] By 'lower side' or 'lower end' is meant that against which or into which the contents are accumulated by the centrifugal force.

of the chlorophyll-bands was brought about, which consisted in a mere bending away of the displaced parts from the cell-wall. Under such circumstances recovery took place in one night. A redistribution of transversely displaced contents is accomplished in a relatively short time, especially in slender cells with few chlorophyll-bands which have not suffered appreciable injury.

As in *Cladophora*, the most interesting phase of the subject presented itself in the behaviour of those cells undergoing division at the time of the experiment. Material exhibiting dividing cells was obtained by placing the vessels containing fresh growing specimens on ice over night, and removing them the following morning to the temperature of the room (about 20° C.). During a larger part of the day dividing cells may be found without difficulty in material thus treated.

In dividing cells, the contents of one were forced through the circular opening in the transverse wall in process of formation provided that this opening was not less than one third or two-fifths the diameter of the cell. An end of a chlorophyll-band not infrequently caught against the edge of the partly formed membrane, when that end or portion remained in the otherwise perfectly colourless daughter-cell. In any case the bands on redistributing themselves began to creep back through the opening into the colourless cell. In *Spirogyra*, however, the redistribution of the cell-contents was never effected so regularly when a partly formed cell-wall interposed, and it could not be determined definitely whether the displaced daughter-nucleus returned to its cell. In *Mesocarpus*, where distribution is easier on account of the single straight chlorophyll-band, it was seen that the nucleus passed back with the chlorophyll-band into the colourless cell. In all such cases, division had not progressed far enough to sever the chloroplast. As in *Cladophora*, neither in *Spirogyra* nor in *Mesocarpus* was the transverse wall ever completed after its original connexion with the nuclei had been disturbed.

So far as known, cell-division in *Cladophora* stands in no

direct connexion with nuclear division, while in *Spirogyra* and *Mesocarpus* both processes take place at the same time, although the transverse walls seem to be laid down in the same way in each plant; that is, the membrane begins at the *hautschicht* and is developed inwards. The more intimate relation existing between the nuclei and these types of cell-wall formation, which may be designated as the *Cladophora* and *Spirogyra* types respectively, is virtually unknown notwithstanding the fact that both plants have become classical as objects of investigation.

Gerasimoff ('97) has shown that, when dividing cells of *Spirogyra* and *Zygnema* are exposed to a temperature below 0° C., the process of division is inhibited, and one of the daughter-nuclei may pass over into the other cell, thereby giving rise to an enucleated cell-chamber and one with two nuclei. He found also that similar results could be brought about by means of certain anaesthetics.

A similar behaviour was observed in the course of my own studies upon *Spirogyra*. As previously stated, the plants were kept on ice over night, but the temperature never fell below 0° C. As a rule, the temperature of the water containing the plants when the vessels were removed from the refrigerator on the following morning was 3° or 5° C.

In several cases observed, one of the nuclei moved back into the enucleated cell in four or five days after the experiment, but the ring-shaped transverse wall never developed further. Plasmic cords extended through the opening in the wall from one cell to the other. Other cases were noticed where both nuclei remained in the same cell-cavity, when one of them, and apparently that which had left its cell, became partly cut off from the rest of the cell by an irregularly-shaped membrane. For lack of time, a further study of this phase of the subject was impossible.

As regards the type of cell-wall formation displayed in the vegetative division of cells of *Cladophora*, *Spirogyra*, *Zygnema* and *Mesocarpus*, it may be safely stated that, when the relation existing between a cell-wall in process of formation and the

nuclei concerned therewith is violently disturbed, the completion of the cell-wall as such does not take place.

Stamen-Hairs of Tradescantia.

The behaviour of cells of the stamen-hairs of *Tradescantia* in response to violent centrifugal force proved to be of much comparative interest. These cells lend themselves readily to this as well as to many other sorts of experimentation.

By means of gypsum, young unopened buds were fastened in short pieces of glass-tubing just large enough in diameter to admit them. The tubes were then packed in the cylinders previously mentioned. After centrifugal action, the buds were removed from the tubes and kept upon wet filter-paper in a moist chamber where they remained fresh for two or more days, during which time the cells of the stamen-hairs divided as usual. For immediate observation after centrifugal action, a bud is opened and the stamen transferred to a slide. Fastening individual stamens or even sections through the bud upon the slide proved not only difficult and troublesome but also more or less injurious to the cells.

With buds arranged in this way, the line of centrifugal force was coincident with the longitudinal axis of the majority of the hairs, while many others lay at various angles to the same, making possible a displacement in all directions in the same bud.

We shall direct our attention first to cells still in the embryonic condition, i. e. capable of division. In all the younger cells of the hair, the nucleus, together with the movable cytoplasm and its inclusions, was forced into a dense mass in the lower end of the cell, while the vacuole or vacuoles were compelled to occupy the opposite end or half of the cell. In a short time after the cessation of centrifugal force a normal redistribution was effected.

In any stage of karyokinesis between that of the spindle- and the telo-phase, the figure is often forced into an oblique position in the cell, and the arrangement of the chromosomes

somewhat distorted. A distortion of the figure is, however, not the rule in *Tradescantia*, but slight distortions may be often present which are invisible in the living cell.

In Fig. 7 is shown a cell with dividing nucleus, in which the straight or slightly curved rod-shaped chromosomes have arrived at the poles. Each set of chromosomes is inclined slightly to the longitudinal axis of the spindle. Judging from this figure, it seems that the spindle-fibres extending from pole to pole offer some resistance to the weight of the chromosomes of the one daughter-nucleus which tend to fall into the end of the cell upon those of the other daughter-nucleus. In no case observed were the chromosomes at one pole thrown over upon those at the other. The chromosomes (Fig. 7) did not orient themselves again so that their long axes might coincide with that of the cell, but formed at once the daughter-spirem by the union of their respective adjacent ends. It must not be forgotten, however, that only a few of the finer details of the karyokinetic figure can be seen in the living plant-cell even in the most favourable cases. The spindle-fibres in the cell shown in Fig. 7 could be seen only faintly, the displaced cytoplasm in which the karyokinetic figure lay rendering the view less clear than under ordinary conditions, so that a more accurate knowledge of detail must await investigation by the indirect method. The division-wall resulting from this division (Fig. 7) was only slightly oblique. Its formation, which was observed continually, took place in about the same time as in cells not exposed to centrifugal action.

Fig. 9 *a–g* will serve to illustrate the process of cell-division immediately following a transverse displacement of the contents. The protoplasm capable of being moved by the centrifugal force was merely collected along one side of the cell, thus suffering displacement through a relatively short distance. When first observed the nucleus was in the anaphase (Fig. 9 *a*). The cell-plate is soon laid down within the connecting fibres and in contact with the cell-wall upon one side (Fig. 9 *b*). The connecting fibres gradually bulge out at the equator, forming the familiar barrel-shaped system. The bending out

is, of course, more pronounced on the side next to the vacuole (Fig. 9, *c* to *g*). The cytoplasmic strands running from the plasmic mass to the *hautschicht* change position, while additional ones appear. The growth of the cell-plate (Mottier '97) keeps pace with the diametrical increase of the system of connecting fibres at the equator, so that when the latter reaches the opposite wall a transverse membrane is formed and cell-division is complete. The increase in diameter of the system of connecting fibres is accompanied by a shortening of its length, so that when the cell-plate is formed the daughter-nuclei come to lie close to it. In a short time now the entire contents of each cell assume their normal orientation. The processes embraced by *a* and *f*, Fig. 9, required forty minutes ; between *f* and *g*, eight minutes.

A division of the cell often takes place before the nucleus regains its more central position, displacement having been longitudinal. The result of such a division is shown in Fig. 8, in which the daughter-cells are of unequal size. This figure was drawn twenty-four hours after displacement. In this case it is not known whether or not the cell was in division at the time of centrifugal action ; but other instances were observed in which both nucleus and cell divided while the former lay in the end of the cell. It is doubtful whether nuclear or cell-division can continue during the action of centrifugal force as great as that used, for the phenomena observed immediately afterwards seemed to indicate clearly that the karyokinetic process is inhibited. No special effort was made to determine whether the process is accelerated or retarded as a result of centrifugal action. The time required for the completion of that part of the process illustrated in Fig. 9 was nearly the same as for corresponding phases under ordinary conditions. The difference, if there be any, is certainly small, whether on the side of acceleration or retardation.

These observations seem to indicate further that, where the cell-wall is laid down through the direct instrumentality of the kinoplasmic connecting fibres, its position in the cell is

determined by that of the nuclei, while the proportional distribution of cytoplasm plays a secondary and minor part as a factor in regulating the division or size of the resulting daughter-cells.

The results of centrifugal action upon older cells of the stamen-hairs, i. e., those no longer capable of dividing, and whose rôle is purely vegetative in character, proved to be interesting, though of much less theoretical importance. A relatively longer time was necessary to effect the desired displacement than in those still in the embryonic condition. The following remarks apply more particularly to cells in which the cell-sap had become only slightly or not at all coloured. In mature cells the coloured cell-sap and the nature of the cell-wall interfered with a distinct view of the living contents.

In cells where a displacement of a larger part of the contents was effected, the nucleus, much cytoplasm, and all the larger inclusions, were massed in the lower end of the cell, the vacuoles occupying the remaining and larger part of the cell-cavity. The cytoplasmic strands which penetrate the cavity of the cell, or at least some of them, remained in position. As soon as observation was possible, an active streaming towards the lower end of the cell was seen in the strands, showing that the displaced cytoplasm had begun to redistribute itself rapidly. The larger inclusions also began to move, and in a short time they were normally distributed in the cytoplasm. The nucleus is often slow to move from its centrifugal position. Sometimes it may regain a position midway between the ends of the cell, shifting the same from time to time as under ordinary circumstances; then again it may remain almost stationary in the displaced position.

Other Trichomes.

Observations upon various other trichomes, although furnishing data of much less interest than those of *Tradescantia*, may not nevertheless be wholly without significance. Trichomes

of a large number of Phanerogamic species from widely different families were used, with results varying only in minor details. Those of *Urtica*, *Momordica* and *Cucurbita*, which seemed more favourable for this study, merit some special mention.

Sections of the leaf or petiole bearing hairs of suitable age were fastened upon the slide under a cover-glass in the same way as the algal filaments. Hairs of *Urtica* and *Momordica* are quite hardy, the cells living often for a week or ten days after subjection to a centrifugal force of 1820*g* for one and a half to two hours; though of course there are always cells that seem to be seriously injured or killed outright either by handling or by the centrifugal action.

The cell-contents of hairs of *Momordica* possess about the same orientation as in *Tradescantia*, except perhaps that a greater number of plasmic strands traverse the cell-cavity or vacuole.

Centrifugal action lasting for one hour and a half displaces nucleus, chloroplasts, and much of the cytoplasm with all conspicuous inclusions, massing them in the lower end of the cell. As in *Tradescantia*, a number of the larger plasmic strands persist. The streaming movement of the cytoplasm may not have been entirely stopped by the action of the centrifugal force, for it was seen immediately the preparation was taken from the centrifugal machine, which required from five to seven minutes. It is possible, however, that the movement may begin again within that time.

The contents of the cells redistribute themselves in a relatively short time, usually less than twenty-four hours. It sometimes happens that a portion of the cell-contents consisting of dead or inert particles will remain in the lower end of the cell separate, and perhaps entirely cut off, from the actively circulating cytoplasm.

In the long pointed hairs of *Urtica*, when the contents are forced into the attenuated end of the cell, the nucleus especially, which seems wedged in between the walls, finds more difficulty in extricating itself. At the end of centrifugal

action the displaced cytoplasm, including the nucleus, apparently fills the narrow tapering cavity for about one-half the length of the hair, except the small knob-like termination which still contains, in many cases at least, a vacuole. Although the tapering end seemed quite densely filled with cytoplasm and the nucleus was compressed into the form of an ellipse, yet a constant movement was kept up in the cytoplasm, passing into the point of the hair on one side of the nucleus and returning on the other. A rotating stream passed through the narrow neck into the knob on one side and returned along the other. In the broad basal portion of the cell in question, the rotation passed over gradually into the so-called streaming or circulating movement.

Twenty-four hours after centrifugal action, the cytoplasm was in a large part normally distributed. The nucleus in some cases remained for several days in the displaced position; in others it wandered back into the broad basal part of the cell, its usual position. After a week the cells of the hairs in question were perfectly healthy, the cytoplasm exhibiting an active movement. In a few cases the same trichomes of *Momordica* and *Urtica* were subjected a second time to centrifugal action, with similar results.

In many trichomes a much longer duration of the centrifugal action is necessary to displace the living contents as described, especially where the primordial utricle is very thin and fewer heavy inclusions are present. *Primula chinensis* possesses this sort of trichome. Those operated with were taken from the petiole of young leaves. The cells contain a few pale and scattered chloroplasts, and a relatively small amount of cytoplasm with few of the more conspicuous granules. The delicate plasmic strands traverse the cell chiefly in a longitudinal direction. After five hours' exposure to a centrifugal force of 1820 g, the nucleus, chloroplasts, and some of the finely granular cytoplasm, were found accumulated in the lower ends of the cells. On the next day all was normally distributed.

Hairs of *Cucurbita* obtained from seedlings were found to be

more susceptible to injury than those of the other plants. Many seemed to have been killed outright while others died soon after centrifugal action.

Funaria.

Small, fresh specimens of *Funaria* were fastened upon a slide in the usual way. Individual leaves to be observed in detail after centrifugal action were then carefully removed from the plant and mounted in water. When such leaves were returned to the moist chamber to be kept for subsequent observation, the cover-glass was removed to allow free access of air. The behaviour of the cell-contents in leaves that had been removed from the plant was about the same as in those which were not detached.

The character of displacement varied in different parts of the leaf. In the longer and larger cells near the base, displacement was more easily brought about than in the smaller isodiametric ones of the terminal third or fourth of the leaf. Sometimes the contents in basal cells experienced a total displacement, while in those at the apex of the same leaf no perceptible change in the position of chloroplast or any other inclusion could be detected. Such leaves then presented a complete transition from cells in which all the contents capable of being displaced were made to fall into the lower end of the cell, to those in which no change in the position of any chloroplast was apparent.

The reason for this is unknown to the writer, unless it be due to the density or firmness of that part of the primordial utricle in which the chloroplasts are imbedded. According to all appearances the chloroplasts were about the same size with the same quantity of starch enclosed in each. If their specific gravity be less than that of those in the basal cells, no method of settling the question suggested itself at the time.

An average cell with displaced contents is shown in Fig. 10. It appears that the contents fill about one-half

of the cell-cavity; but the side next to the vacuole is probably more concave than it seems, in which event the displaced mass would present the form of a cup. Delicate strands are frequently seen running along the *hautschicht*, from which ramifications may penetrate the cell-lumen. Displacement takes place in plasmolyzed cells very much as in normal ones (Fig. 11). This fact would seem to indicate that the density of the cell-sap in itself does not affect in any appreciable way the free movement of bodies held in the cytoplasm. Cells may, of course, be plasmolyzed to such a degree that all the chloroplasts are crowded together, when little space is left for displacement and when the contracted primordial utricle holds all inclusions firmly in a fixed position.

In order to see what effect, if any, a difference in temperature and illumination might have upon the redistribution of the cell-contents, one set of plants on being removed from the centrifuge was placed in a cold house at a temperature of 2° C., while another was kept in the laboratory at a temperature varying from 16° to 20° C. Of those in the laboratory, one portion remained in diffused light, another was kept in darkness, and a third within a cylindrical screen of heavy white paper beneath an incandescent electric lamp. In cells exposed to the diffused light of the laboratory room at 20° C., redistribution was about completed in four hours and a half. The chloroplasts were generally arranged along those cell-walls which are perpendicular to the surface of the leaf. In those kept in darkness at the same temperature (20° C.), redistribution was accomplished in the same time, the orientation of the chloroplasts being the same. Redistribution in the cells exposed to the electric light required about the same time, but here the chloroplasts were more uniformly distributed upon the entire inner surface of the cell. Different intensities of illumination, therefore, so far as observation extended, played no important part as a factor in the redistribution.

Temperature, on the contrary, seemed to have much to do with the movement of displaced portions of the living substance

and its inclusions. In cells exposed to a temperature of 2° C in the cold house, twelve to twenty hours were often required for a complete redistribution. The time varied, of course, in cells of different parts of the same leaf. In the smaller ones near the tip, where displacement is less easily brought about, recovery takes place more quickly under whatever conditions may prevail in which protoplasmic activity is possible.

Vallisneria.

Longitudinal sections of leaves were used for experimentation. *Vallisneria* proved to be a less favourable object for this study than the plants already mentioned. Even after a longer duration of centrifugal action, only a part of the chlorophyll was thrown into the lower end of the cell. On immediate examination after two hours of centrifugal action, a very decided movement was to be seen in many cells, both chloroplasts and starch-grains being carried along in the cytoplasmic stream. Many chloroplasts, were, however, collected into larger and smaller clusters. Some of these clusters were thrown into the end of the cell, where they remained for a time, while others lay stationary along the sides of the cell. In a short time the chloroplasts became more evenly distributed, those aggregated into clusters separating from one another. It seemed that in these cells the movement of the protoplasm was not brought to a standstill by the centrifugal action. Soon after the preparations had been removed from the centrifuge, the protoplasm began to rotate in many cells in which no movement was perceived at first, and it is reasonable to suppose that the motion had been inhibited in such cells. Even in many cells in which no protoplasmic movement was noticeable when the experiment was begun, the chloroplasts were displaced in part only.

Since it seemed that the movement of the protoplasm interfered somewhat with the displacement of the cell-contents, an attempt was made to determine, if possible, in what measure the movement hindered the dislocation of the chloro-

plasts and the inclusions. Consequently the movement was first inhibited by placing the sections, properly fastened upon a slide, in water containing from 3 to 5% ether. When all movement had ceased, the preparations were transferred to water containing just enough ether to prevent a return of the movement, in which they remained during centrifugal action. (It may be added here that when the movement had been stopped for several hours, the sections lying in ether-water all the while, it began again a few minutes after removal to fresh water.) In cells with the movement thus inhibited, more of the chlorophyll was made to fall into the lower end of the cell, but displacement was much less complete than was expected.

From this experiment it seems that there are other factors more potent in preventing a displacement of the cell-contents than the movement of the cytoplasm.

Chara and Nitella.

Chara and *Nitella* lend themselves much less readily to these experiments than the other plants investigated. As a rule the centrifugal force necessary to displace the chloroplasts which lie in the stationary part of the cytoplasm proved fatal. The cells are either killed outright, or retain their vitality for a short time only.

In order to bring about a complete displacement in certain cells only, it was necessary to continue the centrifugal action for five or six hours. It made no difference whether the rotation of the cytoplasm was first inhibited by ether or not. In *Chara* the results were different in different parts of the plant. In the uncorticated cells near the ends of the so-called leaves, displacement of the chlorophyll was effected in different degrees. (Only terminal portions of good thrifty shoots about one centimetre and a half long were used.) Frequently much was removed from a tolerably broad strip on both sides of the neutral zone; then again this thinning out of the chloro-

plasts is less localized, being generally more uniform throughout the cell.

In several instances rather striking phenomena presented themselves in uncorticated leaf-cells. In these, upon which the centrifugal force acted obliquely, almost the entire mass of chlorophyll was thrown into one corner of the cell, the remaining portion becoming thereby transparent. On the following morning, the specimens having been removed from the centrifuge at 6 p.m. of the day preceding, the mass of displaced contents remained in exactly the same position, apparently quite dead, while the colourless cytoplasm kept up a lively rotation. The living protoplasm of the cell had thus entirely separated itself from the displaced mass of chloroplasts and other granules. In the rotating protoplasm only a few chlorophyll-grains were present.

Other and similar cells of the same leaf were plasmolyzed and dead, while still others remained almost unchanged, i. e. no displacement was perceptible in them.

The cells of the whorl of undeveloped leaves closely enveloping the growing end of the shoot were virtually unchanged, no displacement having taken place except in the pointed distal cells which were killed.

Generally much of the contents was displaced in cells of the cortex. Here the chloroplasts and other bodies showed a tendency to distribute themselves, and sometimes this was in a large measure effected. Many cells died, however, as a result of the centrifugal force.

Displacement of the chlorophyll in the cortical cells often permitted to a large extent an unobstructed view of the interior of the large internodal cells of the leaves. In these cells, the chloroplasts and large colourless inclusions of the protoplasm were often thrown into the ends of the cells, filling them apparently for about one-fourth their entire length. In all such cases observed the displaced mass was dead on the following morning, and in many cells all other cell-contents as well.

In a few instances the colourless mass of protoplasm became

segregated into two or more rounded portions, displaying a rapid rotating movement within. This segregation of the living contents is similar to certain phenomena of disorganization brought about by other external stimuli such as the action of electrical currents, &c. (Klemm '95).

Again, whenever the displacement in the internodal cells was transverse instead of longitudinal, the displaced contents lay in a stationary band-shaped mass extending the entire length of the cell, while the colourless protoplasm enclosing the central vacuole kept up a constant rotation.

In both *Chara* and *Nitella* the centrifugal force almost always inhibited the movement of the protoplasm, which returned immediately the action ceased unless death ensued.

In *Nitella* displacement was less difficult. In every cell of the part of the plant used, the chlorophyll with the nuclei and other inclusions were crowded into a dense mass in the lower end of the cell; but this sort of treatment proved too severe for *Nitella*, for every cell died before the chlorophyll began to distribute itself. If the centrifugal force were allowed to act only long enough to produce a partial displacement, the cells seemed uninjured, and the chloroplasts were soon redistributed.

Vaucheria.

From what has been said in the preceding pages in regard to the several plants mentioned, we know, *a priori*, about what to expect from a similar treatment of a majority of all plants.

In *Vaucheria* the presence of oil-bodies adds a new factor to our problem, and it is in this respect that this plant offers anything additional to what has thus far been considered. Centrifugal action of about one hour and a half was sufficient to drive nearly all or a vast majority of the chloroplasts into the ends of the filaments. (Pieces varying in length from one-half to one centimetre were used.) Those chloroplasts which remained behind seemed to be smaller and

probably lighter than those displaced, or they may have been situated in a firmer part of the primordial utricle. Only a relatively small number of oil-globules are carried along with the chloroplasts ; usually they lie scattered in the more proximal portion of the filament left colourless by the removal of the chlorophyll. They are, of course, most numerous just back of the chlorophyll. Consequently, the end of the filament presents a dark-green colour, which shades over gradually into the nearly colourless part from which the chlorophyll has been removed.

Certain large oil-globules, in which are included one or more chlorophyll-grains, are carried along with the displaced mass. These larger oil-bodies doubtless arise from a fusion of smaller ones and the chloroplasts lying in contact with them.

It sometimes happens that several such globules, together with other inclusions of the protoplasm, collect at certain points in the filament and form a sort of plug, which completely prevents further movement at these places, where the contents will then accumulate as in the end of the filament. In several instances it was observed that those parts of the filament which were clogged in this way became injured and were cut off from the main portions by transverse walls, just as when any part is injured by crushing or otherwise.

Twenty-four hours after displacement, considerable progress in the redistribution of the contents is noticeable, and in a few days there is nothing to indicate that any disturbance had taken place in those filaments which received no injury through clogging or otherwise. The oil-masses with their included chloroplasts redistribute themselves more slowly. Many of them soon disappear, probably by breaking up, thereby liberating the chloroplasts, when the oil doubtless takes part in the processes of metabolism ; others, however, persist for a longer time.

A redistribution of the chloroplasts throughout the entire portion of the filament left nearly colourless by the displace-

ment did not always take place, especially in longer pieces in which recovery was less rapid. The pieces of filaments almost invariably grew in length, thereby creating more space and a movement of the contents in the direction of growth. When such was the case, the proximal end of the filament remaining colourless would die after a time, and be cut off from the rest by a transverse membrane as in normal vegetative propagation.

It sometimes occurred that the ends of the filaments became thick and club-shaped, apparently as a result of the pressure exerted by the displaced contents. Further growth at such club-shaped ends manifested itself in a prolongation possessing the normal diameter of the filament, which sprang usually a little to one side of the middle point of the end.

Further experiments were made regarding the behaviour of oil-globules and bodies containing this substance in which leaves of certain Jungermanniaceae and root-tips of *Ricinus* were used.

In cells of the leaves of the Liverworts, some of the bodies containing oil were displaced along with the chloroplasts, but not so readily as the latter, while many others remained stationary. It will be remembered, however, that these bodies do not represent pure fatty oil but contain more or less proteid, which makes them heavier than pure oil.

Root-tips of *Ricinus*, having been subjected to the centrifugal force, were immediately fixed in a mixture of chromic-osmic-acetic acid, imbedded in paraffin, and sectioned. The oil-globules are blackened by the osmic acid, so that they may be readily seen after the sections have been carefully stained and mounted in balsam. The proper combination of these acids must be used, or else the blackening may disappear, owing to oxidation caused by the action of the chromic acid.

Here the results were about what would be expected. The oil-bodies, being lighter, were completely separated from the other constituents of the cell. The nucleus, small starch-grains, and the bulk of the cytoplasm were accumulated in the

distal end of the cell; while oil and the vacuole, whenever the latter was present, occupied the proximal end. The oil formed a dense layer along the proximal transverse wall, and when present in sufficient quantities it filled the space between the vacuole and the corners of the cell.

The Nucleus.

To the writer, at least, one of the most interesting phases of the subject was that pertaining to the behaviour of the nucleus in root-tips of certain Phanerogams. Tips of the primary roots of *Zea Mays*, *Vicia Faba*, *Allium Cepa*, and *Phaseolus vulgaris* were used. Of these, *Zea* and *Phaseolus* proved to be most satisfactory.

The method of procedure was as follows. The seeds were allowed to germinate in moist sawdust, and when the primary root had reached a length of two or three centimetres the whole seedling of *Zea* was fastened by means of gypsum in a piece of glass tubing which had been widened out funnel-wise at one end. The seedlings were placed in the funnel so that the root passed freely into the tube while the body of the seed remained in the widened part.

A thin batter of gypsum was now poured into the funnel so as to surround the seed but not to cover it entirely. The root was loosely wrapped with shreds of cotton close to the grain to prevent the gypsum batter flowing into the tube of the funnel. Fastened in this way, both root and shoot were left perfectly free, while the body of the seed was held secure and immovable so that not the slightest injury was received by the plant from the excessive weight of the seed during centrifugal action.

On account of the large size of the seed of *Vicia*, the whole seedling was not used. Root-tips about one centimetre long were cut off and inserted into tubes just large enough to admit them freely. The end of the root was then pushed a short distance into the gypsum batter which had been introduced into the end of the tube. The tip of the root thus rests in its own

mould, and the pressure due to the increased weight is so evenly distributed that not even the cells of the cap itself sustain the least injury, except it be the outermost layer. The tubes and funnels are now packed into the cylinders previously mentioned, when all is ready for operation.

In order to observe the condition of the nucleus and the other cell-constituents just at the close of centrifugal action, tips of the roots were put at once into the fixing fluid (chromic-osmic-acetic acid). In order to observe the behaviour of cells at certain intervals subsequent to the action of centrifugal force, specimens of *Zea* remaining in the tubes were replaced in the sawdust or kept in a moist chamber. Of course, all tips cut from the plant previously to centrifugal action were fixed immediately on their removal from the centrifuge. The pieces of tissue were imbedded and sectioned. The sections were stained on the slide with safranin, gentian-violet, and orange G. By the method of preparing the tissue just outlined, the exact condition of all the protoplasmic constituents of the cell at the time of fixing is revealed in successful preparations with striking clearness.

As regards the cell-contents as a whole, we find here results similar to those already described; but, concerning the nucleus alone, phenomena are met with which merit special attention.

In certain cells of *Zea*, *Vicia*, and *Phaseolus*, not only do we find the contents of the nucleus displaced in its cavity as in the case of the cell, but the nucleolus may often be forced out through the nuclear membrane into the cytoplasm, when nucleus and nucleolus will be found lying some distance apart with all connexion between them severed. This shows quite clearly that the nucleolus is relatively much heavier than the other constituents of the nucleus.

Only one instance is known to the writer of any recorded observation upon this phase of the subject, namely that of Herrick ('95), referred to by Davenport[1] ('97) in his Experimental Morphology. As stated by Davenport, Herrick found

[1] Herrick's paper was not accessible to me.

that 'when the ovary of a lobster is killed, the nucleoli of all the nuclei are found in contact with that part of the nuclear membrane which was the lowest at the moment of killing' (l. c., Fig. 22), and that 'the weight of the nucleolus is relatively so great as sometimes to cause a depression in the part of the nuclear membrane upon which it rests' (l. c., Fig. 23).

Strong centrifugal force reveals a series of phenomena relative to the specific gravity of the nucleolus, to which we shall now direct our attention.

In the smaller and densely filled embryonic cells of the root-tip, very little change is to be noticed; but farther back, where the cells have elongated somewhat, a marked difference is at once apparent. In those nuclei in which a displacement is plainly perceptible, the nucleolus lies in contact with that part of the membrane toward which the centrifugal force has been directed. (As in the case of the cell, this part will be spoken of as the lower side.) Not only the nucleolus, but much of the nuclear reticulum is displaced along with the former (Fig. 12). In cases like this the nucleolus, together with a portion of the threadwork, causes a decided depression in the nuclear membrane. To what extent the reticulum is pulled along by the heavy nucleolus cannot be stated with certainty.

From the displaced mass of reticulum there extend back to various points in the nuclear membrane many linin-strands (Fig. 12). That part of the nuclear membrane directly opposite the displaced mass to which these linin-threads extend is depressed or sunk in. In a great many nuclei presenting a similar orientation of their contents, a similar indentation is present. It is not, therefore, due to shrinkage, and the fact indicates strongly that the linin-thread at certain points is pretty firmly attached to the nuclear membrane.

The nucleolus is often only heavy enough to cause a slight depression at the lower side as in Fig. 12, but frequently this depression is augmented to a protuberance or beak equalling

one-third to one-half the diameter of the nucleus before its membrane is ruptured and the nucleolus escapes into the cytoplasm. Sometimes the beak is drawn out into a long slender neck (Fig. 13). Such a condition is met with chiefly in the rapidly elongating cells of the central cylinder, but it may also happen in similar cells of the cortex. As a rule the nuclear membrane is ruptured sooner, so that only a short beak is formed. In the same section of a root-tip of *Zea*, *Phaseolus*, and others, every conceivable transition between Figs. 12 and 13 may be seen in the plerome-cylinder.

It is, however, only when the cytoplasm offers a considerable resistance to the movement of the nucleus that the nucleolus can be made to fall through the membrane of the former. If the nucleus is able to move more freely within the cytoplasm, as is generally the case except in elongating cells of the plerome, it readily sinks to the lower end of the cell, resting against the end wall, when an expulsion of the nucleolus is impossible. In the rapidly elongating cells of the plerome the nucleoli seem to be larger and heavier than those in the adjacent region of the periblem.

The linin-reticulum, if containing only a relatively small quantity of chromatin, as in Fig. 13, will experience little or no displacement. As a rule, the reticulum or nuclear thread is displaced in a certain degree along with the nucleolus even in embryonic cells. The nucleolus is generally accompanied, when displaced, by the colourless area or sphere in which it often lies in the resting nucleus, a fact so noticeable in *Zea*. Sometimes, when the nucleolus is forced out of the nucleus as in Fig. 13, the former does not come into contact on all sides with the cytoplasm, but may lie within a colourless space.

Since the colourless fluid surrounding the nucleolus does not retain any stain, it cannot be said with certainty that it is carried along with the latter. The facts seem to indicate, moreover, that the specific gravity of this colourless sphere is greater than that of the remaining part of the nuclear sap, and that it may be of a more viscid nature. In *Allium*, and

perhaps *Vicia*, the colourless area immediately surrounding the nucleolus is less marked than in *Zea*. It is certainly not an artefact due to shrinkage.

The foregoing remarks pertain strictly to the nucleus in the so-called state of rest. A few statements may now be made concerning the same in process of division.

In the stage of the hollow spirem of the anaphase, the chromatin-thread in vegetative nuclei runs in tolerably regular turns along the nuclear membrane with an occasional turn or loop projecting into or traversing the nuclear cavity. As the nucleus here considered does not increase much in size above that of the resting-stage, the chromatin-spirem seems tolerably rigid, especially when we remember that in all probability it is fastened at certain points to the nuclear membrane by delicate threads of linin or of cytoplasm. Nevertheless, it often happens that even in smaller nuclei of embryonic cells a number of turns of the spirem fall toward the lower side of the nucleus.

During later stages of karyokinesis the elements of the mitotic figure are variously affected. The chromosomes when arranged in the equatorial plate are often less regularly oriented. The entire spindle, as in *Tradescantia*, falls to the bottom of the cell; and, if the normal position were oblique, it would not infrequently be bent and sometimes forced to lie transversely in the cell. No very striking effects were observed during later phases of mitosis, save some irregularity in the position of the chromosomes. The resulting daughter-cells were often of unequal size, as in the case of the stamen hairs of *Tradescantia*.

Seedlings of *Zea*, as already stated, were allowed to grow after the action of centrifugal force in order to observe the condition of the nucleus at subsequent intervals. It required only a short time to recover from the shock, for at the end of twenty-four hours the rate of growth in the primary root differed but little from that of control-specimens, being sometimes accelerated, sometimes retarded. Only few comparisons were made along this line as it was not my purpose to study

the rate of growth as influenced by the action of centrifugal force.

Root-tips were fixed at subsequent intervals of six, eight, twenty, and twenty-four hours. In six or eight hours the displaced contents had become in a measure redistributed except in the long narrow cells of the plerome, and at the end of twenty or twenty-four hours no signs of a displacement were evident. But, if the nucleolus had been forced out of its nucleus, these two bodies were found lying separately in the cytoplasm, sometimes at considerable distances from each other. The nucleolus did not re-enter the nucleus even when a connexion remained between them as in Fig. 13. The enucleolated nuclei were often irregular in shape and not infrequently showed signs of disorganization.

All attempts to eject the nucleolus in cells of the various trichomes, &c., proved futile. Either the resistance offered by the cytoplasm to the movement of the nucleus was too feeble, or the nucleolus was too light to admit of its ejection by means of the centrifugal force employed. Negative results were also obtained with trichomes from the leaves of *Pinguicula*, whose nuclei contain large crystals. Neither crystals nor nucleoli were ejected.

It was thought that sharp-pointed raphides of such plants as *Agave mexicana* might be made to pierce the cellulose-membrane. In *Agave mexicana* and others the raphides occur in cells containing mucilage, and, although the entire bundle is thrown into the end of the cell so that the sharp ends of the raphides touch the cell-membrane, yet in no case could they be seen to penetrate the wall in the least. After the operation the raphides moved back to their former position in the cell.

Concluding Remarks.

One of the great tasks of the physiologist, and perhaps that which overshadows all others in theoretical importance, is to know what cells can do under any and all conditions

of life, and to understand the relation of these activities of the cell to each other and to the surrounding conditions. It does not matter whether these activities be of the so-called normal or abnormal kind; for who is prepared to say what is normal and what abnormal, and where the one leaves off and the other begins?

In this investigation centrifugal force was regarded largely as a stimulus, and the purpose constantly kept in view was to know what responses the living substance of the various cells would make to such a stimulus.

One of the most surprising phenomena, to the writer at least, is the great amount of what seemed to be severe treatment which many cells are able to endure. In *Cladophora* and various other Algae, where almost every visible trace of the living substance save the *hautschicht* (and the plasmic lamella in *Cladophora*) is crowded into a small compact mass in one end of the cell, and where three weeks or even a longer time is necessary to effect a redistribution of these contents similar to that existing under ordinary circumstances, not the slightest pathological phenomenon could be perceived in the vast majority of cases. Aside from the power of mere endurance in this respect, the factors that underlie the slow or rapid redistribution are still far from a satisfactory elucidation.

Of far greater interest, to the cytologist at least, are probably the principles underlying the various phenomena revealed in the types of cell-division which occur in *Cladophora* and *Spirogyra*. In the former not only does the formation of transverse cell-walls not stand in any direct connexion with nuclear division, but, so far as known, it is independent of the position or distribution of the nuclei. On the other hand, it is not improbable that further investigation will show that some direct relation does exist between the transverse septum in process of formation and the orientation of the nuclei. The process of the formation of a cell-wall in the cell-division of *Spirogyra* is in a measure transitional between the *Cladophora* type and that in which the cell-plate is laid

down through the instrumentality of the connecting fibres. The peculiar cellulose-ring formed preparatory to cell-division in *Oedogonium* may be brought into the same category with *Spirogyra* and *Cladophora*, bearing perhaps a closer resemblance to the type of the former.

It is not easy to understand why in *Cladophora* the membrane once begun as a cellulose-ring is never brought to completion after a displacement of the cell-contents, without assuming that some very close relation exists between the nuclei and the origin of the transverse membrane, and that this relation having been once disturbed is never re-established. Since centrifugation never plasmolyzed the cells in question, the ectoplasm or *hautschicht* remained always in contact with the cellulose-ring, so that no mechanical injury could arise from that source. There are therefore strong grounds for the conclusion that in the *Cladophora* and *Spirogyra* types of cell-division a close relation exists between the nucleus or nuclei and the forming transverse membrane, and that, when such relation is once disturbed through a displacement of the nuclei in the cell by centrifugal force, it is never fully restored and the protoplast is incapable of completing the membrane.

It might be of interest in this connexion to know whether spiral or other secondary thickenings would develop further, if, at the beginning of the process, the contents of those cells were displaced in a similar manner.

Again, such cases as that illustrated in Fig. 3 present phenomena for which the writer is, as yet, unable to find a satisfactory explanation. It would seem at first that the size of the resulting cells might be proportional to the quantity of cytoplasm, and that that factor is the determining principle. Cell *a*, Fig. 3, contains more chlorophyll and probably a greater number of nuclei than cell *b*, but whether the quantity of living substance apart from nuclei and chloroplasts is proportional to the sizes of the respective cells I am unable to say. It is more probable that the position of the division-wall is determined here also by

the nuclei, and that the rôle of the cytoplasm is largely secondary.

The results obtained in root-tips of *Zea*, *Allium*, *Vicia*, and *Phaseolus* have thrown more light upon the specific gravity of various cell-constituents, and especially those of the nucleus. There is no doubt that the nucleolus is relatively a very heavy body, and that its specific gravity is greater in the nuclei of cells destined to a greater constructive activity. Cells of the plerome-cylinder, which eventually develop into the various thick-walled elements, demand a much greater constructive activity of their protoplasts than the smaller thin-walled cells of the cortex. The nucleus, which is beyond any doubt connected with the secretion of cell-wall substances, must do more work where thick-walled elements are produced and consequently it must have more food. From this fact and from what is known of the behaviour of the nucleolus during karyokinesis (Mottier, '97), the statement seems justified that the nucleolus represents so much food-material that may be drawn upon by the nucleus whenever necessity demands.

During the comparatively short time at my disposal, it was possible to do but little more than to give a general survey of a part of the field, covering a tolerably wide range among plants, and to indicate lines along which fruitful results might be expected. It was not possible under the circumstances even to make an exhaustive and quantitative study of a single phase of the various problems touched upon.

In an investigation of this kind nothing is more strikingly evident than the necessity of combining the best indirect methods of the histologist and cytologist with those of the physiologist.

It is hoped that some fact or suggestion contained in the foregoing may arouse a sympathetic interest in others having at their disposal the necessary mechanical apparatus for the prosecution of such and similar studies; for there is no doubt but that careful and painstaking investigation here will yield fruitful results.

This work was carried out in the Botanical Institute at Leipzig, and I desire, in conclusion, to express publicly my indebtedness to Professor Pfeffer for his great kindness and constant advice throughout the entire work.

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EXPLANATION OF FIGURES IN PLATE XVIII.

Illustrating Professor Mottier's paper on the Effect of Centrifugal Force upon the Cell.

Fig. 1. *Cladophora.* A cell drawn eighteen hours after the action of a centrifugal force of 1930 *g* for one hour and a half. The displaced contents have begun to distribute themselves; in the colourless part the plasmic lamellae are to be seen.

Figs. 2, 3. *Cladophora.* Both figures drawn two days after centrifugal action. In Fig. 2 cell-division had begun before the application of centrifugal force; the cellulose-ring, appearing in optical section as two inward projections on either side, never developed further as a transverse wall. In Fig. 3 a division of the cell has just taken place.

Figs. 4–6. *Spirogyra.* Centrifugal force, 1820 *g*, duration three quarters of an hour. Fig. 4 was drawn immediately after centrifugal action at 5 p.m.; the two chlorophyll-bands, nucleus, and all cytoplasm capable of being displaced lie in a compact mass in the lower end of the cell. Relatively large starch-grains form a rosette about each pyrenoid.

Fig. 5. A cell larger than in Fig. 4, drawn at 9 a.m. the following morning; the primordial utricle is thicker, due to a redistribution of cytoplasm over the inner cell-surface. The bands with the nucleus have made some progress toward redistribution. Fig. 6 was drawn at a still later period.

Figs. 7–9. Cells from the stamen-hairs of *Tradescantia virginica.* Centrifugal force about 1820 *g*, duration one hour.

Fig. 7. Karyokinetic figure at the close of the anaphase; each set of daughter-chromosomes lies slightly inclined to the longitudinal axis of the cell. The beginning of the cell-plate is faintly visible; the vacuole occupies the upper end of the cell.

Fig. 8. Drawn twenty-two hours after centrifugal action; cell-division took place while the nucleus lay in the displaced position, thus giving rise to two daughter-cells of greatly unequal size. The fact that the diameter of the nucleus of the smaller cell is almost as great as the length of the cell itself, seems to indicate that the cell was in process of division at the time of centrifugal action.

Fig. 9 *a–g* (semi-diagrammatic) represents a part of the process of cell-division in a cell in which the dividing nucleus and surrounding cytoplasm were displaced transversely. Observation began immediately after centrifugal action; *a* to *f* represents forty minutes, *f* to *g* eight minutes.

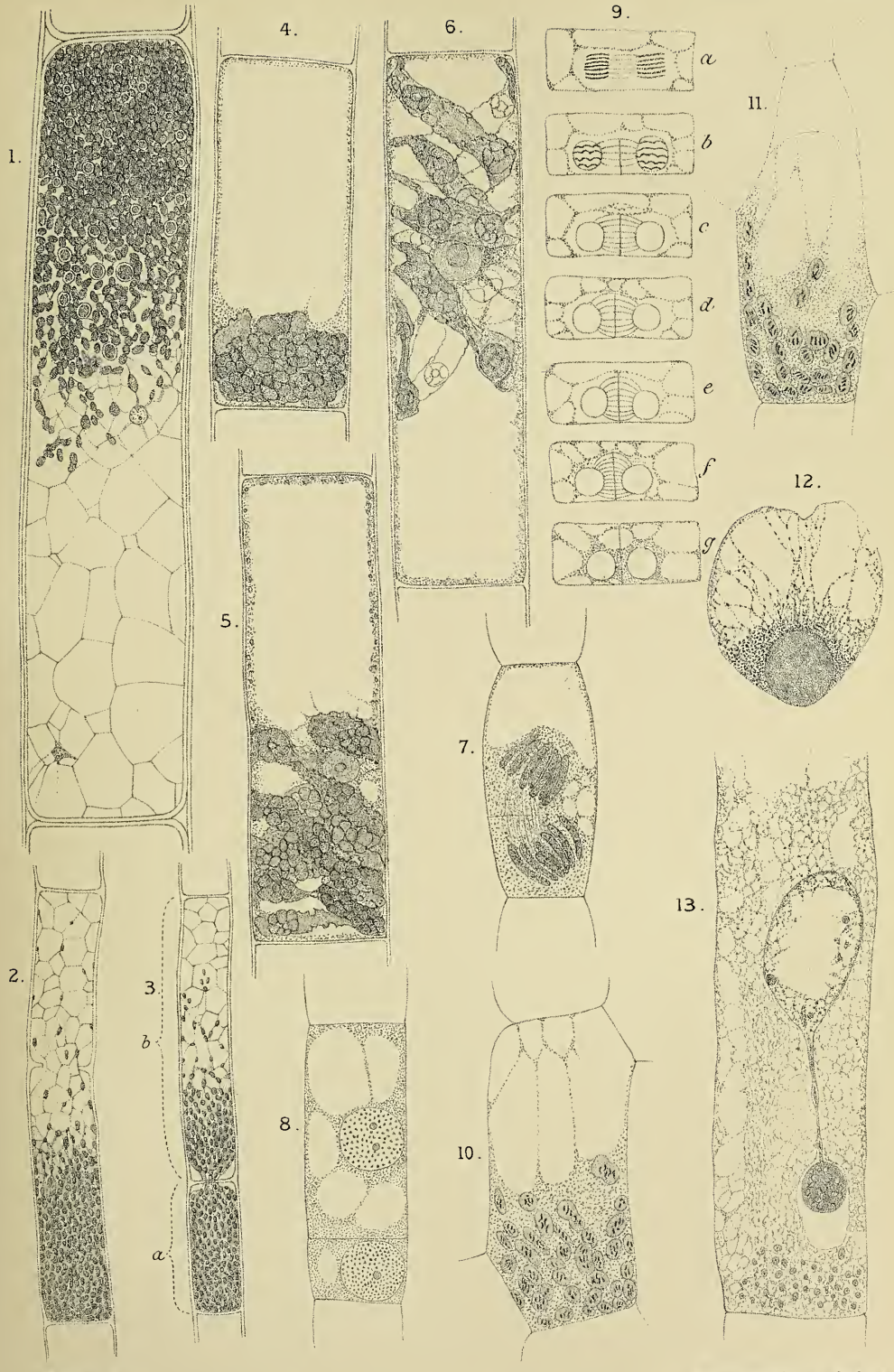
Figs. 10, 11. Cells of the leaf of *Funaria.* Centrifugal force 1700 to 1820 *g*, duration one hour and a half.

Fig. 10. A cell from basal half of leaf showing displaced contents.

Fig. 11. Displacement in a cell which had been first plasmolyzed.

Fig. 12. Nucleus from a cell of the plerome of a root-tip of *Allium.* From the displaced mass of nuclear thread, strands extend to various points in the nuclear membrane, especially to the sunken-in part opposite. The nucleolus was on the point of breaking through the nuclear membrane.

Fig. 13. Part of a long cell from the plerome of a root of *Zea Mays.* The heavy nucleolus has drawn a part of the nuclear membrane out into a long neck. Starch grains and other inclusions lie in the lower end of the cell.



Mottier del. University Press, Oxford.

MOTTIER. — CENTRIFUGAL FORCE.

A Fat-Destroying Fungus.

BY

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With Plate XIX.

AMONG some specimens of germinating Coco-nuts brought from Ceylon in January, 1898, by Mr. H. H. W. Pearson, one was found half full of a thick white flock of the mycelium of a Fungus growing from the basal end of the nut. On keeping the nut moist under a bell-jar, the mycelium increased considerably, and soon reduced the endosperm to a slimy, brownish-grey pulp which gave off a pleasant ethereal odour something like that of amyl butyrate. It seemed probable that one of the effects of the action of the Fungus was to destroy the coco-nut oil contained in the endosperm, so I began an investigation into the biology of the Fungus in the hope of obtaining some results in connexion with the destruction of fats. A detailed examination of the original nut could not be made owing to its being soon over-run by a *Coremium*-form of *Penicillium*, and later by *Eurotium*.

The Fungus was, however, easily obtained in pure culture from sowings of its conidia in plates made up with 1 per cent.

[Annals of Botany, Vol. XIII. No. LI. September, 1899.]

asparagin, 1 per cent. cane-sugar and gelatine, or with yeast-extract and gelatine. White patches of mycelium appeared in a few days, and the jelly surrounding them turned an olive-green colour. The mycelium was then lifted out with a sterilized needle and transferred to sterilized blocks of coco-nut endosperm, kept moist in plugged test-tubes by means of wads of cotton-wool saturated with water. The sterilization was effected by boiling in a steam-sterilizer for an hour on three successive days. Attempts were made to obtain pure cultures without this heating, and consequent change of the endosperm-contents, by sawing off a piece of the shell and a thin layer of endosperm, singeing the cut surface and cutting out blocks of endosperm with a sterilized cork-cutter, but without success. On infecting these blocks the Fungus grew more rapidly than on those sterilized by boiling, but a crop of *Penicillium* invariably grew at the same time.

A series of cultures were also made on sterilized Brazil-nuts. The Fungus grew equally well on these, and gave throughout the same results as obtained with cultures on coco-nut endosperm.

In a week's time the greater part of the infected block was covered with a coarse snow-like mass of mycelium, whether the soft endosperm or the hard brown testa was infected. This mycelium increased so rapidly that in a few weeks it half filled the tubes. During its growth large quantities of water were secreted. After about a month or six weeks the growth of the mycelium appeared to have ceased, except that at irregular intervals of time close spherical tufts of mycelium, from a quarter to half an inch in diameter, were formed. Meanwhile the shape of the blocks of coco-nut endosperm and of the Brazil-nuts altered considerably, owing to the breaking down of the cells of the interior into a pulp, which gradually disappeared, leaving only a thin contorted shell. The pleasant ethereal odour already mentioned was very noticeable in the tubes.

For anatomical investigation the cultures were taken up at weekly or fortnightly intervals, placed in Rath's solution for

three or four hours, washed for twenty-four hours in running water, and gradually dehydrated in alcohol.

Before my investigations had gone very far, it became evident that the Fungus in question was one of the Hypocreales [1], probably belonging to the group of the Nectrieae; but on this latter point I cannot at present speak with certainty. The evidence for this is given in detail below.

The conidia are elliptical in shape, with thin, smooth walls (Fig. 1 *a*); each contains a single nucleus, one or more highly refringent bodies, and on staining with an alcoholic extract of alkanet-root the presence of small quantities of oil can be detected. Single conidia placed in hanging drops of nutrient gelatine began to germinate in twenty-four hours at a temperature of 16° C. The mycelium grew rapidly, and the refringent bodies passed out into it, and in a short time showed signs of diminution in size. The first septum appeared when the mycelium was about six times as long as the spore. The first-formed cells could always be distinguished from those formed later, even in an old mycelium, on account of their being shorter, more swollen, and full of vacuoles. Each has, as a rule, one well-marked nucleus. The mycelium branches vigorously in all directions, and after about six days many of its hyphae turn abruptly from their original course in order to grow alongside other hyphae, rarely fusing with them however (Fig. 1 *b*). In this way strands, containing a dozen or more hyphae, are formed which give the masses of mycelium a characteristic coarse appearance. After a time the ends of these strands fray out, and the hyphae forming them abstrict conidia terminally and singly (Fig. 1 *b*). The formation of these conidia takes place at a great pace; in one case eight were formed in sixteen hours from the apex of a hypha.

On staining the mycelium with Delafield's haematoxylin at this stage, it appeared to be crowded with nuclei, there being often as many as twenty or thirty in a single young segment (Fig. 2). In the older parts of the mycelium, however,

[1] See Lindau, in Engler's Pflanzenfamilien, I, 1, p. 343 et seq.

they were few in number, usually one, but sometimes two, in each segment. At the points of branching a large single one, often surrounded by much smaller ones, was almost invariably to be found (Fig. 2 *b*), while the ends of hyphae which had finished abstricting conidia rarely contained any.

The variability of their size and their number did not seem to agree with the supposition that they were nuclei, so cultures were specially grown to determine, if possible, their nature. For this purpose conidia were sown in flasks of sterilized coco-nut milk, and when a healthy mycelium was visible to the naked eye it was lifted out and fixed in the usual manner in Rath's solution. The nucleus-like bodies were then found in great abundance. They stained deeply with haematoxylin, fuchsin, methyl-green, and with safranin-gentian-violet orange G. They gave no reactions when tested for glycogen and oil. On treating the mycelium with a solution of gastric juice at a temperature of 25° C. for twelve hours, and again staining, they were found to have disappeared, while the true nuclei could still be easily distinguished. From this fact and from their action towards staining agents it seems clear that the bodies are proteid in constitution. Too much importance must not, however, be assigned to these micro-chemical reactions. The bodies have been observed in many Fungi and described as nuclei. One of the most careful accounts of them is given in Guégen's study of the biology of *Penicillium glaucum* [1], and he hesitates in calling them true nuclei. In this case, however, their size and their difference in reaction when treated with gastric juice distinguish them from nuclei, and as they pass out into the conidia they may possibly serve as reserve stores for use during germination.

Here and there the nuclei showed signs of division by karyokinesis, but not definitely enough to make out any details. The most noticeable feature about them was the frequency with which they were found in close connexion with the vacuoles. In most cases they were to be found on

[1] Guégen, Bull. de la Soc. Myc., t. xv, p. 23; for literature also.

the margin of a vacuole; while sometimes, where a nucleus appeared to have just divided, the daughter-nuclei were separated by a large vacuole (Fig. 3).

In hanging-drop and plate cultures, chains of chlamydospores were developed from the mycelium which produced microconidia, especially as the gelatine began to dry. The chlamydospores are spherical, or slightly elliptical, in shape, with thick brown walls, and contain many of the proteid reserve bodies already mentioned (Fig. 4). On germination they gave rise to a mycelium which again produced microconidia.

These were the only forms of spores produced in the gelatine-cultures, but macroconidia were formed in the cultures on coco-nut endosperm and Brazil-nuts. Like the microconidia, they are abstricted terminally and singly from the hyphae. At first the young macroconidia cannot be distinguished from microconidia, but they soon become sickle-shaped; and when they have reached their full size they divide by transverse walls, so that ultimately each is composed of three or four cells each with its own nucleus (Fig. 5). Like the microconidia, they contain considerable quantities of oil.

Germination may occur from any one, or from all three or four cells. The mycelium formed is similar to that formed by the microconidia and the chlamydospores; but it is characterized by having many of its hyphae united into a network by the fusion of short lateral processes, very like those put out by conjugating *Spirogyra* (Fig. 6).

In cultures four weeks old the mycelium in the endosperm had given rise to a stroma formed from strands of hyphae running parallel with one another, and united on the surface of the blocks by branches running at right angles (Fig. 7). These branches interlace to such an extent that the mode of development is soon lost sight of, and the stroma appears to be composed of hyphae running in every direction. When mature it is chocolate-brown in colour and fleshy in texture. Its thickness is from one to two mms.

Even in this early stage rudimentary perithecia can be

distinguished in the stroma itself, and later they are formed in great abundance on its surface, where they stand out in warty brown masses two or more mms. high. They have been produced continually for the last nine months. Unfortunately they have proved barren in every case. They consist merely of a flask-shaped shell of thickened dark-brown hyphae, woven into a very close layer, enclosing a plexus of colourless hyphae often of considerable size (Fig. 8). Keeping the cultures at a temperature of 20° C. and 25° C. for a month, and altering the hygrometric conditions by partially drying with calcium chloride, has had no effect, and after making many attempts to force them to produce ascospores I have had to abandon the problem. Owing to this the further identification of the Fungus has to be postponed.

Yet another form of reproduction was met with, but once only, namely, a pycnidium with pycnidiospores. This occurred in a stroma on a culture four weeks old. The pycnidium was flask-shaped, with a narrow ostiole. Its walls were composed of smaller, less thickened hyphae, which were not so closely woven as those of the walls of the perithecia. The spore-abstricting hyphae were branched and directed to the ostiole. Under these circumstances the germination of the pycnidiospores could not be observed.

As the perithecia or ascocarps are open at the apex, the Fungus evidently belongs to the Pyrenomycetes, which may be subdivided into three groups, the Hypocreaceae, the Sphaeriaceae, and the Dothideaceae, the texture of the stroma and the position of the perithecia serving as distinguishing characteristics. The perithecia of the Sphaeriaceae are, as a rule, embedded in the stroma, and have hard, dark-coloured walls, while those of the Dothideaceae are so embedded that they have no distinct wall of their own. The Hypocreaceae, on the other hand, have soft, coloured perithecia, usually placed in groups on a fleshy stroma[1]. We may class the Fungus in question then among the Hypocreaceae, a group which contains several parasitic genera, some species

[1] Brefeld, Unters. aus d. Gesammtgeb. d. Mykol., Heft x, p. 162.

of which are only known at present in their conidial stages (e. g. *Fusarium*). For its further identification we require to know the colour of the ascospores, and of this we have, as yet, no evidence. If they are dark-coloured it belongs to the Melanosporeae ; if hyaline, yellow or red, to the Nectrieae[1].

In this connexion we may notice that Brefeld has shown that various species of *Nectria* reproduce by forms very similar to those already described. *Nectria coccinea* (Pers.) may serve as one example[2]. It forms microconidia and macroconidia, similar to, and in the same manner as, those of the Fungus described, while chlamydospores and pycnoconidia occur in *N. sinopica* (Fries) and other species.

Sections through blocks of coco-nut endosperm infected a fortnight previously show that the mycelium has penetrated the tissues considerably, and that in a very characteristic way. In longitudinal sections the tissue appears to be broken up into alternating bands of a white and grey colour (Fig. 9). The white bands consist of unattacked tissue, the grey of attacked tissue. In transverse section the attacked portion appears as grey patches surrounded by the unattacked white portion.

On staining with Delafield's haematoxylin, the mycelium can easily be distinguished in the cells, especially if the sections are previously treated with chloroform to remove the large quantities of oil they contain. Each cell of the attacked portion is then seen to be crowded with the luxuriantly growing mycelium, which here and there forms dense knots owing to its having turned on itself on reaching the transverse wall of the cell (Fig. 10). The growth in a longitudinal direction is singularly constant for the first three weeks ; then, however, numerous branches grow out at right angles and pierce the longitudinal cell-walls, sometimes in as many as twenty places in a single wall. In this way the first-formed bands of hyphae are united and a dense meshwork of mycelium is formed in the tissue.

[1] Lindau, l. c.

[2] Brefeld, l. c., p. 173, and Taf. iv, Figs. 23 and 24.

The piercing of the walls is evidently brought about by the secretion of a cellulose-dissolving enzyme, for they swell strongly, and the hyphae push their way directly through the gelatinized walls without showing any signs of constriction. The hyphae, on reaching the walls to be pierced, either swell directly into a bulbous ending, or give out two or three short branches, which then dilate and apply themselves to the wall. These endings each contain a large nucleus which stains deeply with haematoxylin, numerous small vacuoles, and oil-drops (Fig. 11). It is probable that they secrete the cellulose-enzyme, which presumably gelatinizes the cell-walls, just as the haptera of the Lily-disease *Botrytis* do[1]. The walls are pierced and dissolved to such an extent that in cultures six weeks old it is difficult to find a trace of the endosperm cell-walls, the whole mass being reduced to a slimy pulp, through which the mycelium runs in all directions. The hard brown testa undergoes little change, and that mostly mechanical, owing to the mycelium breaking through it and carrying out fragments into the stroma. No action on its thickened cell-walls could be detected. Meanwhile some very obvious changes have been brought about in the oil-contents of the endosperm; for, instead of large masses of fat being present in the attacked cells, the quantity has become exceedingly small, being in many cases only represented by a small quantity of a fine emulsion.

This is especially well seen in longitudinal sections of cultures two weeks old stained for six hours in an alcoholic extract of alkanet-root. They appear to the naked eye to be composed of alternating bands of deep and pale red tissue (Fig. 9). Under the microscope the unattacked cells are found to be full of the deeply stained oil, while in the attacked cells the oil is chiefly to be found in the mycelium. So abundant is it there that it is an easy matter to trace the course of the hyphae through the cell-walls by means of the dark red drops.

In cultures in which the stroma has been formed, the dis-

[1] Marshall Ward, Ann. of Bot., vol. ii, p. 339.

tribution of the oil is even more interesting. Sections stained in the same way, or, better still, stained with osmic acid by allowing the culture-blocks to remain in Rath's solution for twelve hours, show a plentiful supply of oil in the mycelium within the endosperm, and in the lower portions of the stroma. The upper portion of the stroma, however, rarely shows a trace of oil, though drops can be distinguished in the conidia standing away from it. The absorption of oil by the mycelium and its disappearance in the stroma points to its utilization as a food-stuff, and, as oils are indiffusible substances, to its conversion into diffusible bodies by ferment-action.

So far little has been done to establish the existence of fat-splitting enzymes, though they must be of very general occurrence in the vegetable kingdom, judging from the number of seeds grown solely for the purpose of extracting the oil they contain. Sachs[1] appears to have been the first to observe that the oil gradually disappears on the germination of oil-containing seeds, just as starch does in the case of farinaceous seeds. From this he was able to argue that oil was a reserve-substance in many cases. Fleury[2] went a step further, and from an examination of germinating seeds of Almond, Castor-oil, and Rape was able to show that a fatty non-volatile acid was formed at the same time. These results were confirmed by Müntz[3] in 1871, who suggested that the decomposition was brought about by means of an enzyme which split the fat into a free fatty acid and glycerine. He was, however, unable to detect the glycerine. This view was generally accepted, but it was not until 1889 that the enzyme was isolated by Green[4] from germinating seeds of *Ricinus*. He succeeded in obtaining extracts of the seeds with which an emulsion of castor-oil was decomposed into a fatty acid and glycerine. The glycerine could not be detected

[1] Sachs, Bot. Zeit., 1859, p. 178. Physiology, Eng. ed. p. 347.
[2] Fleury, Ann. de chimie, Sér. 4, t. 4, p. 38, 1865.
[3] Müntz, ibid., t. 22, 1871.
[4] Green, Proc. Roy. Soc., vol. xlviii, p. 370.

in the young plant, on account of its having undergone further changes, probably into sugars.

There is a great probability that this enzyme occurs as frequently in Fungi as in Flowering-plants. Thus fats are found in considerable quantities in many forms of spores, e. g. in the oospores and zoospores of the Chytridiaceae and Saprolegniaceae, in uredospores, &c., where they are without doubt a reserve store for the germinating spores to draw upon. Many Fungi contain large quantities of fat in the mycelium; *Lactarius deliciosus*, e. g., contains 5·86 per cent., while those typical reserve-stores, the sclerotia, as a rule contain very considerable quantities, amounting in the case of *Claviceps purpurea* to 35 per cent.[1]

In many cases a large fat-content is abnormal, and due, as shown by Loew and Naegeli[2], to fatty degeneration, but this can hardly apply to the cases mentioned.

Then again we are acquainted with Fungi which grow exclusively on fat-containing bodies, such as *Empusa*[3] and *Cordyceps* spp. on animal remains, and *Cyclonium oleaginum*[4] (Cast) and *Inzengaea asterosperma*[5] (Borzi) on olive fruits. Others, such as *Penicillium* and *Eurotium* spp., though not necessarily restricted to growing on oil-containing substances, may often be found doing so. One frequently finds them, for instance, growing in the layer of sweet-oil placed over bottled fruits to prevent decomposition. These Moulds also attack cotton, rape, and other cakes made from the waste left after crushing oleaginous seeds for the oil they contain. Ritthausen and Baumann[6] have shown that a great loss of oil occurs when cakes are attacked in this way; e. g. two samples of rape-seed cake containing 10·53 per cent. and 8·5 per cent. of oil contained after two years only 1·98 per cent. and 1·87 per cent. respectively when overrun by these Fungi.

[1] Zopf, Die Pilze, p. 138.

[2] Naegeli, Unters. über niedere Pilze, 1882. Zopf, ibid.

[3] Brefeld.

[4] Brizi, Bot. Centrbl., Bd. lxii, p. 81. (Abstract.)

[5] Borzi, Bot. Centrbl., Bd. xxiv, p. 14. (Abstract.)

[6] Ritthausen and Baumann, Versuchs-Stat., 1896, Bd. xlvii, p. 389. Reitmar, Versuchs-Stat., 1887, Bd. xxxviii, p. 373.

Penicillium is of especial interest in this connexion, for it has been shown to contain, among other enzymes, a lipase, which was successfully extracted by Gérard[1], and shown to be capable of splitting monobutyrine into butyric acid and glycerine.

Under these circumstances there can be little doubt that the disappearance of the oil from rape-seed cake, &c., is due to the Moulds being able to utilize it as a food-material.

Among the Bacteria several forms are known which are capable of splitting fats. An example of this is afforded by the *Bacillus fluorescens non liquefaciens* of Krueger[2], isolated from butter which had become 'cheesy.' Several pathogenic forms, such as *Bacillus typhi abdominalis*, *B. pyocyaneus*, and *Vibrio cholerae asiaticae*, have also been shown by von Sommaruga[3] to possess this power.

In order to obtain the mycelium in quantity to test for the presence of this supposed fat-splitting enzyme, large cultures were grown in flasks of sterilized coco-nut milk kept at a temperature of 20° C. In from eight to ten days the surface of the liquid was covered with a thick growth of mycelium and the liquid itself had become acid to litmus paper. In one series of flasks the milk was coloured with neutral litmus and infected with the conidia of the Fungus on Nov. 2. On Nov. 7 the purple colour had changed to a bright red, and a small quantity of mycelium was visible floating on the surface. The growth of the mycelium had therefore given rise to the formation of a free acid. Whether this was from the fat or from other constituents, such as sugar, remained to be proved.

The mass of mycelium was then taken out of the flasks, washed rapidly in distilled water, and ground to a thin paste with kieselguhr and water in a Bacterium-mill, or with clean sand and water in mortar. On filtering this paste under

[1] Gérard, Bull. d. la Soc. Myc. d. France, t xiii, p. 182.

[2] Centrbl. f. Bakt. vii, 1890, No. 14–16, p. 87.

[3] Zeitschr. f. Hygiene, Bd. xviii, 1894, p. 441. Lafar, Technical Mycology, Engl. ed., p. 199.

pressure through several thicknesses of filter-paper, a faintly brown opalescent fluid was obtained which gave a slight acid reaction. It showed no signs of oiliness. The extract was then neutralized with sodium carbonate and mixed with 2 per cent. potassium cyanide solution, to inhibit the action of the Bacteria which had entered during the process of extraction, and then placed in quantities of about 5 c.c. in sterile plugged tubes for further experiments.

In the first place thick sections of coco-nut endosperm were placed in three tubes containing the fresh extract, and in three containing the extract previously boiled and cooled to serve as a control. The tubes were kept at a temperature of 24° C. for a day, and the sections then taken out and stained over night in alkanet-extract.

In the control experiment the sections showed no signs of change; but in the sections from the tubes of fresh extract the oil had either completely disappeared or was reduced to a fine emulsion.

A further series of tubes were then prepared as before; but this time, instead of sections of endosperm, cover-slips coated with a thin layer of coco-nut oil were used. This substance is of special value in testing for lipases, as it is a pure fat free from acid, and contains, according to the analysis of Fresenius, 99·979 per cent. of fat[1]. The layer soon became liquid and formed an emulsion which floated on the surface of the extract and gradually diminished in quantity. Testing with litmus-paper showed that the liquid was acid, and on neutralizing with sodium carbonate solution the fatty acid formed floated to the surface[2]. The liquid had the same pleasant ethereal odour as the original nut.

To another series of tubes of this extract a 2 per cent.

[1] Kew Bulletin, 1890, Art. clxxiii, p. 230.

[2] This acid was not investigated further. I may note here that the literature on the question of the constitution of coco-nut oil is confused owing to the unfortunate habit of spelling coco-nut 'cocoa-nut.' As pointed out by Balfour (Ann. Bot., vol. i, p. 184), this is a mistake. Thus in Watt's Dict. of Chemistry, vol. ii, p. 231, we find 'cocoa-nut-oil or cocoa-butter,' cocoa-butter being the product of *Theobroma Cacao!*

mixture of monobutyrine and water was added[1]. Twelve hours later the mixture smelt strongly of butyric acid and gave an acid reaction with litmus.

These experiments leave little doubt that a fat-splitting enzyme is present in the Fungus.

Like other enzymes, it proved to be precipitated by the addition of an excess of absolute alcohol, as a flocculent precipitate. On drying this precipitate over calcium chloride, a whitish-grey powder was obtained which was readily soluble in cold water. A solution of the enzyme obtained in this way gave the same results, when tested with coco-nut oil and monobutyrine, as the original extract.

The course of action of the enzyme is first to emulsify the fat, and then to split it into a fatty acid and glycerine, which substance, as has often been shown, can be utilized by plants as a food-material and converted into sugars. Whether the fatty acid can be utilized in a similar way is doubtful, for the coco-nut milk becomes more and more acid as the mycelium increases. The presence of oil in the mycelium itself offers the same difficulty as the presence of oil in the cotyledons of germinating *Ricinus* did to Sachs. Whether the oil can pass directly through the walls, as Sachs supposed was the case, or whether it is re-formed from the products of its decomposition, cannot at present be determined[2]. Certainly the fatty acid cannot diffuse as a soap, as is the case with the fatty acids formed by the steapsin of the pancreatic juice, for the reaction of the infected coco-nut endosperm and milk is invariably acid.

The disappearance of the oil from the endosperm when attacked by this Fungus suggests the interesting question whether the coco-nut embryo on germination can utilize the oil in a similar manner by the formation of a lipase.

[1] Gérard, l. c.
[2] Cf. Green, l. c.

Botanical Laboratory, Cambridge,
May, 1899.

EXPLANATION OF FIGURES IN PLATE XIX.

Illustrating Mr. Biffen's paper on a Fat-Destroying Fungus.

Fig. 1. (*a*). Microconidia germinating.
(*b*). A strand fraying out into conidia-bearing hyphae.
Fig. 2. (*a*). Apex of a hypha which has not yet produced conidia.
(*b*). A hypha branching, showing the 'proteid reserves.'
Fig. 3. Daughter-nuclei separated by a vacuole.
Fig. 4. A chain of chlamydospores, from the hanging-drop-culture from which Fig. 1 was drawn fifteen days previously.
Fig. 5. (*a*). Young macroconidia on hyphae standing away from a stroma.
(*b*). Stages in development.
(*c*). Stages in germination after twenty-four hours at 16° C. in yeast-extract and gelatine, and after forty-eight hours.
Fig. 6. A portion of the mycelium formed by 5 *c*. seven days later.
Fig. 7. The development of the stroma from a culture on coco-nut endosperm, three weeks old.
Fig. 8. Median longitudinal section through one of the sterile perithecia, showing the parenchymatous wall and the plexus of hyphae within it.
Fig. 9. Section through a block of coco-nut endosperm, showing the alternating bands of infected and uninfected tissue. The infected tissue is shaded to represent the grey colour of the original. The stroma is beginning to form on the upper surface. The dark line on the lower surface represents the brown testa.
Fig. 10. Longitudinal section through a fortnight-old culture on coco-nut endosperm to show the distribution of the mycelium. On the right of the figure the growth at right angles to the original direction has commenced.
Fig. 11. The branched and swollen hyphal endings about to grow through gelatinized transverse walls.

Fig. 1.

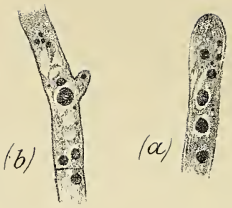


Fig. 2.

Fig. 3.

Fig. 6.

Fig. 7.

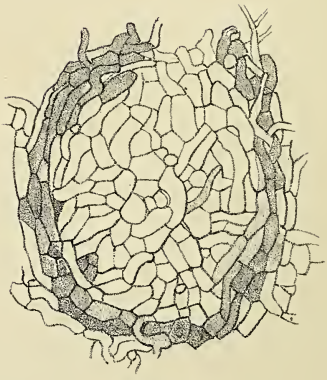


Fig. 8.

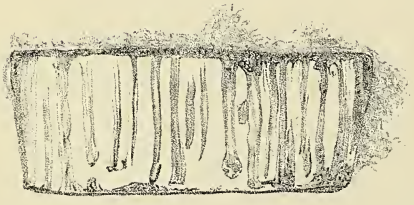


Fig. 9.

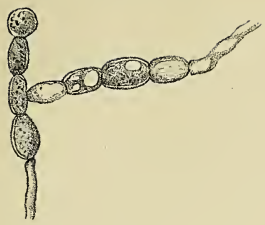


Fig. 4.

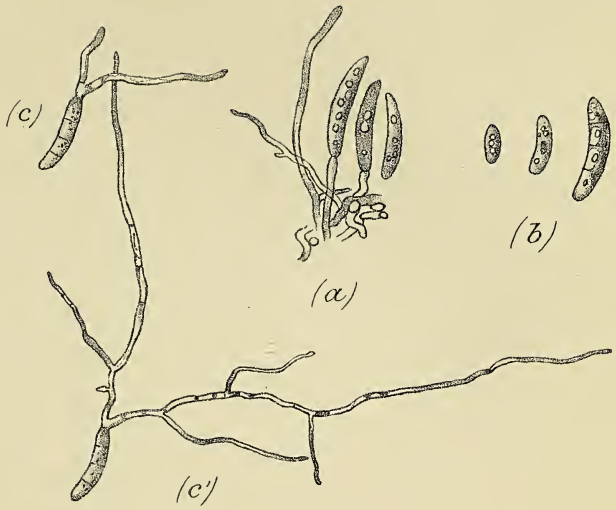


Fig. 5.

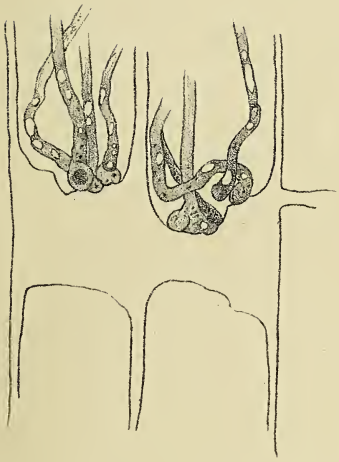


Fig. 11.

Fig. 10.

University Press, Oxford.

On some points in the Anatomy of the Ophioglosseae[1].

BY

L. A. BOODLE, F.L.S.

With Plate XX.

THE present paper is the result of an examination of *Ophioglossum vulgatum*, *Botrychium Lunaria*, &c., which was undertaken in the first instance for comparison with 'seedling' plants of *Botrychium virginianum*[2]. It seemed probable that such a comparison might throw some light on the meaning of the monarch roots of *Ophioglossum*, or on some other peculiarity in one or other of the two genera.

Some further investigation of the development of the root-stele in *Ophioglossum vulgatum* also appeared desirable, to determine whether the xylem or phloem showed any trace of diarch structure.

The accounts previously given of the structure, development, &c., of these plants will be referred to later on, after the present observations have been described. Those relating

[1] From the Jodrell Laboratory, Royal Gardens, Kew.

[2] Material of prothalli and seedling-plants of this species was kindly presented to Kew by Mr. E. C. Jeffrey of Toronto University.

[Annals of Botany, Vol. XIII. No. LI. September, 1899.]

to the structure of the root will be taken first. Then indications of secondary thickening in the root and stem of *Ophioglossum* will be described, and the concluding part will contain a summary of previous work on the points dealt with.

1. Structure of the Root.

The roots of *Ophioglossum vulgatum* run horizontally in the soil, so one may conveniently speak of the upper and lower sides of the root. The xylem-mass of the mature stele is roughly semicircular in outline[1] and occupies the lower half of the stele, the upper half being occupied by the phloem and conjunctive parenchyma (see Fig. 9).

The differentiation of the xylem from the procambial strand, as seen near the apex of the root, begins with the formation of a single tracheide on the lower side. It is shown at *px* in Fig. 1, where it is placed rather to one side of the middle point of the arc of cells, which afterwards forms the lower limit of the primary xylem. This is the usual position of the first protoxylem-element, though it is sometimes found at the middle point of this arc.

Comparison with sections further from the apex showed that in this root the second tracheide is developed in the position of the cell marked *a* in Fig. 1, and that the third tracheide is formed between the first and second but nearer to *a*. The second tracheide is sometimes placed as given for this root, but it may occur immediately adjoining the first, or in other positions.

To generalize from several series of sections cut through root-tips, one may say that the differentiation of the primary xylem always begins with the formation of a single tracheide at the periphery of the procambial strand, and then other tracheides are formed, in the same peripheral arc, but in no definite order, until it is complete, when the further develop-

[1] The shape of the xylem mass is variable.

ment takes place in a centripetal direction (i. e. towards the phloem). The xylem is therefore monarch.

For comparison with the transverse sections, a root-tip was prepared in the following way:—Some of the cortex was sliced off on two opposite sides; the remaining part, which contained the stele intact, was then boiled in potash, washed, and mounted in strong glycerine. The stele was thus rendered transparent. It is represented in Fig. 2, where *px* is the first protoxylem element. Two of the tracheides are shown more highly magnified in Fig. 3, where it is seen that the thickenings in these early protoxylem-elements are annular, with an approach to reticulate thickening in places.

The first protoxylem-element is marked *px* at about the same point in Figs. 2 and 3.

To return to the transverse section; the phloem is seen at the upper limit of the procambial strand. It consists of an arc of sieve-tubes (*s.t.* Fig. 1), (which are in contact with the endodermis, *e*), together with a certain amount of the parenchyma lying between the sieve-tubes and the xylem. The walls of the sieve-tubes are, at this stage, already thickened, and stain strongly with haematoxylin; the paucity of contents also serves to distinguish these elements. A few sieve-tubes are still undifferentiated, viz. the inner ones opposite the middle region of the arc. Thus, when differentiation is complete, the row of sieve-tubes is usually one element thick at the ends, and often two elements thick in the middle. When mature the sieve-tubes show the characteristic granules in contact with the walls.

A section at an earlier stage, before the differentiation of any xylem, showed the arc of sieve-tubes easily recognizable, and with one or two sieve-tubes near each end slightly thicker-walled than the rest. This suggests the presence of two protophloems. Their position, determined by comparison with younger stages, is shown at *pp.* in Fig. 1.

It will be seen from Fig. 9 that in the mature root there is a considerable zone of parenchyma between the sieve-tubes (*ph*) and the xylem. The outer part of this parenchyma,

about two or three cells thick, bordering on the sieve-tubes, is differentiated and should be regarded as belonging to the phloem. In roots collected in December, this zone of cells differed from the parenchyma below it in having very little or no starch, and in the dense granular proteid contents of many of the cells. The rest of the parenchyma may be called conjunctive [1].

The roots of several other species of *Ophioglossum* are also monarch. Prantl [2] gives monarch roots as a character common to the whole section *Euophioglossum*, which includes twenty-seven species in his monograph [3]. Poirault [4], however, states that this does not hold good, and that *O. ellipticum* is an exception. The two remaining species in Prantl's classification, are *O. pendulum* and *O. palmatum*. The former he describes as having triarch and tetrarch roots, and the latter diarch. *O. pendulum* has sometimes diarch roots, and an interesting fact (for comparison with *O. vulgatum*, &c.) is that monarch structure may occur as a modification in these roots. This was seen in the basal region of a rootlet [5]. The diarch rootlet is shown in transverse section in Fig. 5, and its monarch base in Fig. 4, which represents the stele of this rootlet where it passes through the cortex of the parent root. A comparison of Figs. 4 and 5 makes it pretty clear that the xylem-group in Fig. 4 represents the right-hand one in Fig. 5, and that the

[1] In Fig. 9 the distinctions between sieve-tubes and parenchyma are not clearly represented. The rather large elements, one to two cells thick, touching the endodermis, and marked *ph*, are sieve-tubes. The next two or three layers, including some rather small cells, are phloem-parenchyma; the rest of the tissue down to the xylem is conjunctive parenchyma (*p*).

[2] Prantl, Beiträge zur Systematik der Ophioglosseen, in Jahrb. d. k. bot. Gartens zu Berlin, Bd. iii, p. 297.

[3] Many of these rank as synonyms in Hooker and Baker's Synopsis Filicum, where only six species are given under the section *Euophioglossum*.

[4] Poirault, Sur l'*Ophioglossum vulgatum*, Journ. de Botanique, t. vi, p. 71.

[5] This was clearly a true rootlet, not a case of dichotomy of the root as described by Rostowzew and Poirault for *O. vulgatum*. It is curious that dichotomous and monopodial branching should occur in roots of the same genus. Possibly the dichotomy may be restricted to the monarch roots; cf. Van Tieghem (Symétrie, &c., p. 108). In speaking of the root of *O. vulgatum* he says: 'Si elle vient à se diviser, nous savons à l'avance que ce sera par dichotomie.'

left-hand group of the diarch plate of Fig. 5 is absent in the part shown in Fig. 4, which is consequently monarch [1].

Several series of sections were cut through the bases of diarch rootlets in *Botrychium Lunaria*. They also have a monarch region, as seen in Fig. 6. Here the phloem is interrupted opposite the protoxylem (*px*), but continuous at the other side of the stele. A little further out in the same rootlet the structure becomes diarch (Fig. 7)[2]. The two protoxylems are marked *px*, and the phloem is interrupted opposite both [3]. As arranged in the figures, the lower xylem-mass of Fig. 7 is the only one present in Fig. 6, while the upper one in Fig. 7 has just appeared, and is only represented by two tracheides. A comparison of these two sections is much more conclusive than the example of *Ophioglossum pendulum* figured.

It is interesting to notice that these sections (Figs. 6 and 7) closely resemble, in the distribution of their xylem, two roots of *Ophioglossum Bergianum* figured by Bower [4], and described by him as monarch and diarch respectively.

The above facts lead to the conclusion that the rootlet-base has in these cases probably become monarch by reduction from diarch structure; one xylem-group being abortive and the two phloems joined on the other side where the missing xylem-group would be. The monarch structure of the roots of *Ophioglossum vulgatum* may admit of a similar explanation, the reduction extending throughout the roots.

The possibility of these monarch structures being primitive must be acknowledged, but what evidence there is points rather to reduction. Thus, though the bases of diarch

[1] This cannot be said with absolute certainty until the development in this region has been observed. Unfortunately the material obtainable, both in this species and in *Botrychium Lunaria*, did not include any rootlet at the right stage for determining the matter developmentally.

[2] Figs. 6 and 7 both represent sections of the *free* rootlet near its base.

[3] A few of the sieve-tubes in these two sections are difficult to determine, and a little doubtful, but the sieve-tubes are at any rate approximately as shown.

[4] Bower, Studies in the Morphology of Spore-producing Members: II, Ophioglossaceae, Figs. 114 and 113.

rootlets in *Botrychium Lunaria* are monarch, the bases of the *roots* bearing them are diarch; and the base of the root is more likely to show primitive structure than that of the rootlet. For the roots of *Ophioglossum vulgatum*, the presence of two protophloems favours the hypothesis of reduction from diarch structure by suppression of one of the xylem-groups.

Van Tieghem and Bower have both studied the passage of the root-stele through the cortex of the stem. The former described the stele as twisting through 90° on its way out [1], while the latter was unable to observe this rotation [2].

Series of sections were made transverse to the root-stele on its way through the cortex of the stem, and these showed a distinct change in orientation. Starting from the outside, on entering the cortex the xylem is directed downwards and the phloem upwards; but in passing inwards a twisting takes place which amounts, at any rate in some cases, to nearly 90°, so that the xylem and phloem become lateral to an approximately vertical plane before fusing with the stem-bundle. This is illustrated in Fig. 8, where the sieve-tube on the right is about parallel with the axis of the stem, and thus shows the orientation of the xylem of the root. Perhaps the root-stele may sometimes pass in without any twisting, as described by Bower, but the twisting appeared to be the rule in the plants examined.

Holle [3], from sections and from an examination of the vascular skeleton, comes to the conclusion that each leaf-trace bundle, after passing down the stem for a certain distance and fusing with commissural bundles, bends out again as a root-bundle, there being one root to each leaf.

This would give a simple explanation of the monarch structure of the root. Its vascular bundle would then be a continuation of the leaf-trace bundle, which, after passing down the stem, bends outwards without any twisting, and with no change in structure, the monarch bundle being

[1] Van Tieghem, Traité, p. 1394. [2] Bower, l. c., p. 73.
[3] Holle, Bot. Zeit., 1875, p. 251.

practically identical with a collateral bundle. This relation between leaf-trace and root is not, however, without exception [1], and the structure seen in several sections has much more the appearance of a root-bundle abutting on a stem-bundle at or near the part forming the downward prolongation of the leaf-trace. It is certainly difficult to follow the tracheides of a leaf-trace down as far as the insertion of the corresponding root; but in one series of transverse sections the root-stele (as also happened in several other cases) was attached quite to the lateral edge of a stem-bundle, and here appeared to be connected with commissural elements rather than with leaf-trace tracheides.

On Holle's interpretation, the twisting of the bundle, described above, would also seem purposeless, and would amount to this:—the leaf-trace, instead of simply bending outwards without any twisting, when it would obtain the correct orientation for the root, bends sharply through about 90°, and then bends back again through the same angle in passing through the cortex.

Series of sections were also cut through the root-stele in the cortex of the stem of *Botrychium Lunaria*. When the orientation could be clearly made out, the xylem-plate, in the case of diarch roots, appeared to be horizontal [2] just before joining the stem-bundles; that is, there was a protoxylem-group on each side of the vertical plane. If the protoxylem on one side of the vertical plane were abortive, and the two phloem-masses were to fuse on the other side (a kind of

[1] Bower, l. c., p. 67. 'This regularity, however, does not hold for the lowest leaf of a shoot, nor is the regularity always maintained.' Holle (l. c., p. 314) also mentions that the first two leaves have no roots belonging to them. Rostowzew (Recherches sur l'*Ophioglossum vulgatum*, in Oversigt Videnskab. Selskabs, 1891 (No. 2, p. 72), states that very rarely there are two roots to a leaf; and Prantl (*Helminthostachys zeylanica*, u. ihre Beziehungen zu *Ophioglossum* u. *Botrychium*, Ber. deutsch. bot. Gesellschaft, i, 1883, p. 156) also calls in question the relation between leaves and roots as described by Holle.

[2] This agrees with Holle's observations on this and two other species of the genus, l. c., p. 268; and with those of Van Tieghem on *Botrychium Lunaria* (Symétrie de structure des plantes; Annales des Sciences Nat., Bot., v. sér., t. xiii, p. 105).

reduction, which takes place in rootlet-bases of *Botrychium Lunaria*), one would obtain the structure of the monarch root of *Ophioglossum vulgatum* and with the orientation that it has at its junction with the stem-bundle.

The first root of seedlings of *Botrychium virginianum* is of typical diarch or triarch structure [1], and this point may be regarded as slight evidence that the monarch structure in roots of *Ophioglossum* and rootlet-bases of *Botrychium* is a case of reduction. If monarch structure were primitive for the group, and retained in some species, one would expect to find it in the first root of the seedling in others.

If the monarch structure in *Ophioglossum vulgatum* is to be regarded as reduced from the diarch type, there should be some physiological peculiarity with which this reduction is connected.

The horizontal position of the roots is no doubt connected with the production of adventitious buds, the roots keeping at a convenient distance below the surface of the soil. Monarch structure, of the type and orientation found in *Ophioglossum vulgatum*, may have arisen from the diarch form as a means of favouring the development of numerous adventitious buds. Thus, to suppose a phylogenetic series, the *diarch* root would probably produce an adventitious bud about opposite one of the protoxylems. If the two phloem-groups now become approximated at this side of the stele (which we may call the upper), and abortive towards the other side, the development of the bud will be favoured, because most of the phloem will convey nourishment to the adventitious bud, and there will be only a little phloem towards the lower side of the stele to carry nourishment *direct* to the root-apex. Further modification in the same direction would result in the fusion of the two phloems on the upper side of the stele, where the adventitious bud is. This would lead to the abortion of the protoxylem on that side,

[1] Jeffrey, The Gametophyte of *Botrychium virginianum*; Transactions of the Canadian Institute, vol. v, 1896–7, p. 283. Specimens obtained from him showed diarch structure in the first root.

as its absorption from the cortex would be interfered with by the phloem outside it. Further, this would be compensated for by the spreading out of the other protoxylem into a long, peripheral arc, such as is found in this plant.

Adventitious buds occur on roots of *Ophioglossum pendulum* and other species. Prantl [1] has observed them in *O. lusitanicum*, *O. coriaceum*, *O. capense*, *O. Luersseni*, *O. ellipticum*, *O. japonicum*, *O. pedunculosum*, *O. reticulatum*. They may possibly occur in most of the species, but Welwitsch [2] notifies their absence in *O. fibrosum*.

The theory suggested above is purely tentative, owing to want of material and of sufficient data. It may be stated thus:—The monarch structure in *Ophioglossum* is an adaptation for favouring the growth of numerous adventitious buds on the roots, in the case of comparatively small and slow-growing species, where the supply of nourishment from the parent plant is limited. Owing to the monarch structure, the assimilated food has to reach the root-tip viâ the adventitious bud, so that the latter may be able to divert all that is necessary for its growth. To test this theory it would be necessary to observe whether many or few adventitious buds were produced in proportion to the size of the assimilating surface of the plant; this is not possible at present, but one or two cases may be mentioned.

O. pendulum produces adventitious buds, but has a large assimilating surface, more than ten times that of any species in the section *Euophioglossum*, judging from data of leaf-measurements given by Prantl. There is thus presumably plenty of material for producing buds in this species without putting any restriction on the further growth of the roots which form them. And this plant has diarch, triarch, and tetrarch roots.

O. palmatum is also a large-leaved species, having diarch roots.

O. fibrosum would be an exception, as, assuming the observa-

[1] Prantl, l. c., p. 308.

[2] Welwitsch, quoted by Prantl, l. c., p. 308.

tions of Welwitsch and Prantl [1] to be correct, it produces no adventitious buds but has monarch roots. Such cases might arise in the event of a species giving up the production of buds but retaining monarch structure.

O. ellipticum is another exception as it produces adventitious buds, but has not monarch roots, according to Poirault. However, one cannot really test the probability of the theory without knowing the number of buds produced in proportion to leaf-area, &c.

O. Bergianum has a small leaf-area, and produces adventitious buds [2], and has both diarch and monarch roots [3]. It would be interesting to know whether the *monarch* roots are those that produce the buds.

2. Secondary Thickening.

In sections of mature roots of *Ophioglossum vulgatum* one sometimes finds developing tracheides at the periphery of the xylem-mass. By staining transverse and longitudinal sections with methyl-green and eosin this is clearly shown. If the double staining is carried out so as to give the right balance to the two colours, the mature xylem is bright blue (or bluish green) and the phloem and parenchyma are pink; while any half-lignified tracheides present take up both stains and become purple, so that they are made very conspicuous [4].

Fig. 9 is a transverse section of a root in which are five developing tracheides (*t*). Fig. 10 is more highly magnified, and shows one developing tracheide (*t*) with its protoplasmic contents. Fig. 11 is a longitudinal section showing part of the xylem with one developing tracheide on the left, containing protoplasm and nucleus (*n*). In the preparation from

[1] Prantl, l. c.

[2] Poirault, Journal de Botanique, vi, p. 73.

[3] Bower, l. c.

[4] The stain used was watery solution of methyl-green, followed by alcoholic solution of eosin. It unfortunately fades.

which the drawing was made, this tracheide was well differentiated from the others by the staining of its walls.

These developing tracheides must, it seems, be regarded as secondary, as the addition of such elements takes place in roots several years old. Thus the part of the root shown in Fig. 9 was cut close to the attachment of an adventitious plant, on the side towards the parent plant; and, judging from the number of young leaves on the adventitious plant, this part of the root must have been four or five years old. The secondary tracheides are only formed in small numbers; and, as they are added one here and one there, there is no definite cambial layer, though a newly divided cell may occasionally be seen with its thin division-wall, as in a cell bordering on the xylem in Fig. 10.

The greatest addition of secondary elements was seen in the region of the root near the base of an adventitious plant. Now and then the outermost tracheides show radial arrangement; in many cases no doubt these elements are secondary, but their arrangement does not give a safe criterion, as occasionally the xylem may have a roughly radial disposition from the first.

The root-material examined was collected in June and December. As one would expect, no good cases of developing tracheides were found in the December material.

The stem also was examined for secondary thickening, with the result that it showed an exactly similar addition of tracheides, though this was more difficult to observe on account of the oblique course of the bundles. The tracheides here appeared to be added on the outer side of the wood only, while in the root they may be added on all sides. Fig. 13 shows part of a stem-bundle in which there are five developing tracheides on the outer side of the xylem. This bundle must have been differentiated four or five years; and developing tracheides were seen in one or two still older bundles.

Sections of the young stem show that the leaf-trace bundles arise as distinct procambial strands, and that the first-formed

xylem-elements are at the inner limit of the strands; that is, the bundles are endarch. This is an important character, because, as Professor Farmer informs me, the vascular ring in the rhizome of *Helminthostachys* is distinctly mesarch. An examination of some material of the mature rhizome of *Helminthostachys* [1] led me to the same conclusion.

There is a considerable amount of secondary thickening at the bases of many of the roots of *Botrychium Lunaria*, where they are immersed in the cortex of the stem. This sometimes makes it impossible to tell the position or number of protoxylem-groups, as one sees in the case of Fig. 14. For this reason some of the series of sections cut to determine the orientation of the root-stele at its junction with the stem-bundle were useless.

The well-known secondary thickening in the stem of *Botrychium Lunaria* makes it not surprising to find a trace of it in the stem of *Ophioglossum vulgatum*, but secondary thickening was hitherto unknown in the root of any recent Pteridophyte.

In the petioles of unfolded leaves of *Ophioglossum vulgatum* the vascular bundles sometimes show developing tracheides (as in Fig. 12), but it seems uncertain whether these should be regarded as secondary or not.

Conclusion.

Some of the works on *Ophioglossum* have been referred to above, but it remains to quote the results of some authors more in order and detail.

The main facts as to the structure and development of the root of *Ophioglossum vulgatum* were described and figured by Stenzel [2] as early as 1858. His Fig. 4 (Tab. 58) is a very good representation of the early stage of the stele, in which

[1] Kindly sent by Mr. A. C. Seward, F.R.S.

[2] Stenzel, Unters. über Bau u. Wachsthum der Farne, I. Stamm u. Wurzel v. Ophioglossum vulgatum, Nov. Act. Acad. Leopold.-Carolin. Nat. Cur., xxvi (1858).

the first two tracheides have been differentiated, and in this case they are close together (one cell apart). Later stages are also given; the production of the adventitious buds is described, and the conclusion arrived at that they are formed on true roots, not stolons.

Russow[1] gives a short description and a diagram of the root. He describes the root as monarch, the protophloem as being in contact with the endodermis, and as appearing some time before the protoxylem, and describes the formation of a peripheral arc of xylem, followed by centripetal development of the remainder; but his description and figure would give the impression that the first-formed protoxylem-element was practically at one end of the arc, and that the development proceeded regularly towards the other end of the arc. He also, curiously enough, states that the xylem occupies the upper half of the stele, and the phloem the lower.

Van Tieghem in his earlier work[2] states that the first vessels are formed at a single point in the circumference (thus making the xylem monarch), and describes the root-bundle as being inserted on the stem with its xylem downwards and phloem upwards. Thus he had not at that time discovered the rotation afterwards described by him. He goes on to show that the root of *Ophioglossum* corresponds in structure to half the root of *Botrychium*; and says that, to refer the structure in *Ophioglossum* to the diarch type of *Botrychium*, it is only necessary to suppose that a dichotomy (producing monarch branches such as he here claims for roots of *Botrychium*[3]) takes place in the cortex of the stem, and that the upper branch is constantly abortive. This would mean that he interpreted the xylem as truly monarch, a view which he abandoned afterwards; for in the Traité de Botanique[4] the xylem is described as diarch. It is curious that the

[1] Russow, Vergl. Unters., in Mém. de l'Acad. Imp. des Sciences de St.-Pétersbourg, vii. sér., t. xix, No. 1, 1872, p. 122, and Taf. xi, Fig. 31.

[2] Van Tieghem, Symétrie de structure des plantes, Ann. des Sci. Nat., Bot., v. sér., t. xiii, 1870–71, p. 107.

[3] l. c., p. 109.

[4] Second edition, 1891, p. 1394.

orientation as described by him comes out wrong, both on his earlier and later interpretations, when compared with *Botrychium*[1].

Holle's paper has been referred to above for the relation of roots to leaves. According to his account the lignification of the leaf-trace proceeds acropetally, and when it reaches the part opposite the young root, it then proceeds outwards into the root and upwards in the leaf-trace. This simultaneous lignification in the adjacent organs is important physiologically for the purpose of conduction to the young leaf; but, as mentioned above, the junction has not always the appearance of direct continuity. Holle[2] also states that, if one disregards the fusion of the leaf-traces of the lowest two leaves into the solid stele at the base of the adventitious plant, the trace of the first leaf is continuous with the bundle of the posterior part of the parent-root, while the trace of the second leaf is continuous with the bundle of the anterior part of the parent-root. This is probably roughly correct.

Van Tieghem's theory of the monarch root of *Ophioglossum vulgatum*, as given in his Traité de Botanique[3], has been quoted above, and is that it represents a diarch root with one phloem-group abortive.

Rostowzew[4] states that the stem of *Ophioglossum vulgatum* possesses secondary thickening of very short duration, but gives no details, reserving them for a more complete memoir. The later paper is written in Russian[5], and no detailed abstract appears to have been published, but there is an explanation of the figures in German. None of the illustrations, however, refer to secondary thickening. There is a figure[6] of a transverse section of a young root-stele showing

[1] Van Tieghem, Symétrie, &c., p. 105.
[2] l. c., p. 314.
[3] Van Tieghem, Traité, p. 1394.
[4] Rostowzew, l. c. (Oversigt), p. 72.
[5] Rostowzew, Beiträge zur Kenntniss der Ophioglosseen; 1. l' *Ophioglossum vulgatum*; Moscow, 1892.
[6] Plate II, Fig. 14.

the first two protoxylem-elements, which in this case are a considerable distance apart.

In his first paper he describes the stele of the root as being always monarch, though he states that he has sometimes seen protoxylem at two opposite points: but he adds, 'Thus the root in this case appears to be diarch, but it becomes afterwards monarch, because the lower part of the phloem does not develop.' Thus he apparently regards the xylem as representing a diarch plate, and one of the two phloem-groups as absent. The dichotomy of the root is described[1] by him, and the stele of the root is stated to become concentric before the branching, several phloem-elements appearing on the lower side of the xylem, and the two steles after the dichotomy being at first concentric.

The above description is not very definite as to the amount and distribution of the phloem, and is supplemented by the observations of Poirault[2], who says that the stele before division possesses several sieve-tubes on the lower side[3], but that these are scattered and not grouped as in the normal phloem-mass[4]. The other details of the dichotomy need not be referred to here, but this occurrence of scattered sieve-tubes on the lower side of the xylem is quoted because the same thing sometimes occurs in the mother-root near the base of the adventitious plant, on both sides of the latter. It may be explained as a local development of the part of the phloem which is normally abortive (viz. the lower parts of the two phloem-masses).

Poirault[5] describes another anomaly as occurring in roots of *Ophioglossum vulgatum*, namely, the occurrence of a second phloem-group, *similar* to the normal one, on the lower side of the xylem, these two phloem-groups being connected by a series of sieve-tubes round the ends of the xylem-mass.

[1] l. c. (earlier paper), p. 75.

[2] Poirault, Journal de Botanique, t. vi, 1892, p. 70.

[3] He writes 'upper side' by mistake.

[4] This agrees with a figure in Rostowzew's Russian memoir (Fig. 10) representing the young stele of a dichotomised root.

[5] Poirault, l. c., p. 70, and Comptes-rendus, 1891, p. 967.

This anomaly was traced by him for a distance of 30 cm. with no sign of dichotomy. This is referred to again in a later paper [1], and the conclusion arrived at that the anomaly is due to the root preparing for division, where the processes of the dichotomy proceed slowly, the first stage being very persistent, or that it is a case of abortive dichotomy.

The view given in the earlier paper [2], that it was a case of return to diarch structure, is given up, as it had been suggested by a supposed case of another species in which monarch roots (of the type of *O. vulgatum*) as well as diarch and triarch roots occurred. This was found to be due to the roots of two species being mixed in the material used.

In this case of *Ophioglossum vulgatum* with two phloem-masses, no definite conclusion can be drawn without a knowledge of the development of the xylem-mass.

Poirault states that after dichotomy the roots have only one phloem-group, and are not concentric, as described by Rostowzew.

The observations in Bower's paper [3] concerning the monarch structure in *Ophioglossum* have been quoted above. His conclusion, that the xylem is monarch, has been confirmed by the facts described in the present paper, but the latter do not tend to support the view of the primitive character of monarchy in *Ophioglossum* to which he inclines.

I am indebted for material of *Botrychium Lunaria* to Mr. J. Lloyd Williams of University College, Bangor. The rest of the material, with the exception of the young plants of *Botrychium virginianum* sent by Mr. Jeffrey, was obtained from the Royal Gardens, Kew.

I wish to express my thanks and obligation to Dr. D. H. Scott, F.R.S., under whose direction this research has been made, for his kind advice and criticism throughout the work.

[1] Poirault, Recherches sur les Cryptogames vasculaires, Ann. des Sci. Nat., vii. sér., t. 18, 1893, p. 144.

[2] Journal de Botanique, t. vi.

[3] Bower, l. c.

Summary.

1. The root of *Ophioglossum vulgatum* is monarch as regards its xylem, but the phloem has an indication of the presence of two protophloems.

2. Monarch structure also occurs at the base of diarch rootlets in *Ophioglossum pendulum* and *Botrychium Lunaria*.

3. The root-stele of *Ophioglossum vulgatum* has probably become monarch by reduction from diarch structure, viz. by the abortion of one of the *xylem*-groups and the fusion of the two phloem-groups.

4. A small amount of secondary thickening takes place in the root and stem of *Ophioglossum vulgatum*, xylem-elements only being added.

5. Secondary thickening also occurs in the root-bases of *Botrychium Lunaria*.

EXPLANATION OF FIGURES IN PLATE XX.

Illustrating Mr. Boodle's paper on the Ophioglosseae.

Abbreviations :—*e.*, endodermis ; *p.*, parenchyma ; *ph.*, phloem ; *s. t.*, sieve-tubes ; *px.*, protoxylem ; *t.*, developing tracheides.

Fig. 1. *Ophioglossum vulgatum.* Transverse section of young stele of root (near apex). Most of the sieve-tubes are differentiated, but only one tracheide (*px*). *a* is the position in which the second tracheide appeared in this root ; *pp*, position of the two protophloems. × 270.

Fig. 2. *Ophioglossum vulgatum.* Young stele of root in a transparent preparation ; slightly diagrammatic. The tracheides are of course not all in one plane, but are included in the drawing as if they were. × about 150.

Fig. 3. *Ophioglossum vulgatum.* Parts of first two elements of the protoxylem more highly magnified to show the thickenings more accurately. The first tracheide is marked *px* at about the same point as in Fig. 2. × 375.

Fig. 4. *Ophioglossum pendulum.* Transverse section of stele of rootlet within the cortex of the parent-root, showing monarch structure. × 135.

Fig. 5. *Ophioglossum pendulum.* Stele of same rootlet when free, showing diarch structure. × 130.

Fig. 6. *Botrychium Lunaria.* Base of rootlet showing monarch structure. × 275.

Fig. 7. *Botrychium Lunaria.* Same rootlet becoming diarch. × 275.

Fig. 8. *Ophioglossum vulgatum.* Stele of root in cortex of stem, showing change of orientation. × 88.

Fig. 9. *Ophioglossum vulgatum.* Transverse section of root, showing five partly lignified developing tracheides (*t*). × 130.

Fig. 10. *Ophioglossum vulgatum.* Part of transverse section of root more highly magnified, showing one developing tracheide with its protoplasmic contents. × 365.

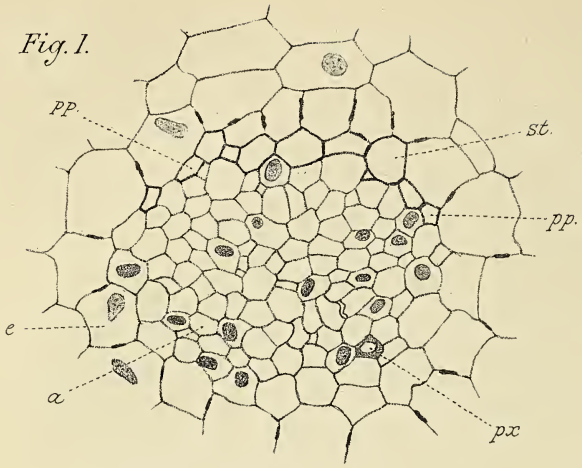
Fig. 11. *Ophioglossum vulgatum.* Longitudinal section of root, with nucleus (*n*) and protoplasm in the developing tracheide on the left. × 375.

Fig. 12. *Ophioglossum vulgatum.* Transverse section of one bundle of petiole. × 130.

Fig. 13. *Ophioglossum vulgatum.* Part of one vascular bundle of the stem cut transversely, with five developing tracheides. × 130.

Fig. 14. *Botrychium Lunaria.* Transverse section of stele of root-base in the cortex of the stem, showing secondary thickening. × 195.

Fig. 1.



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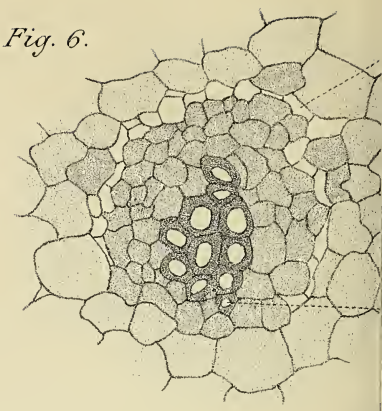

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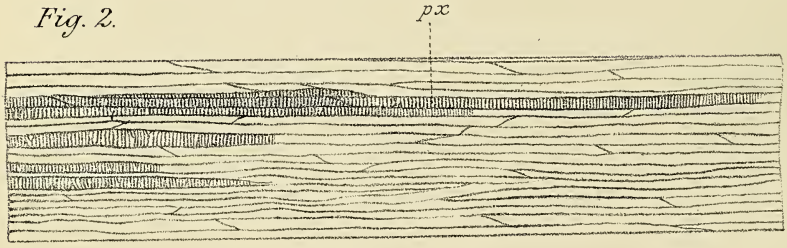

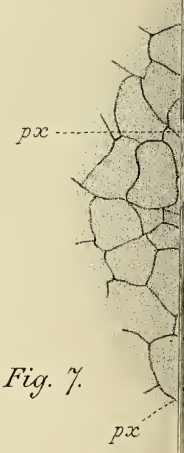

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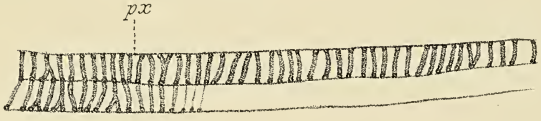

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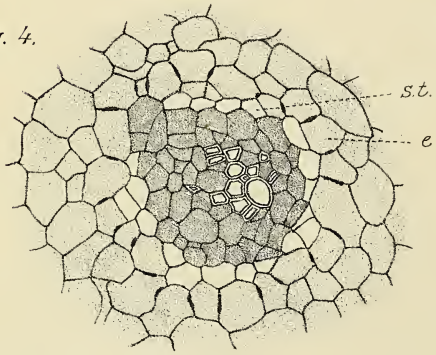

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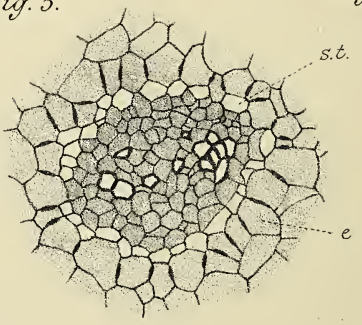

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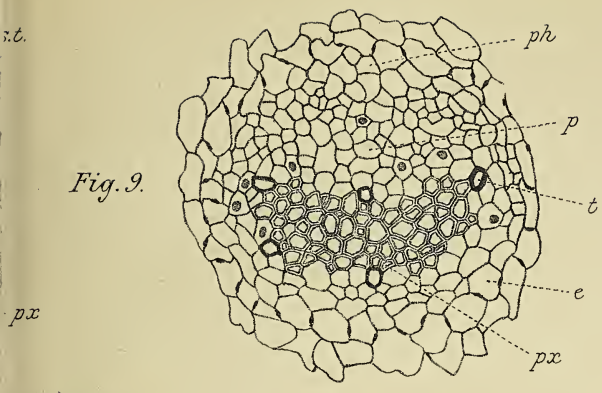

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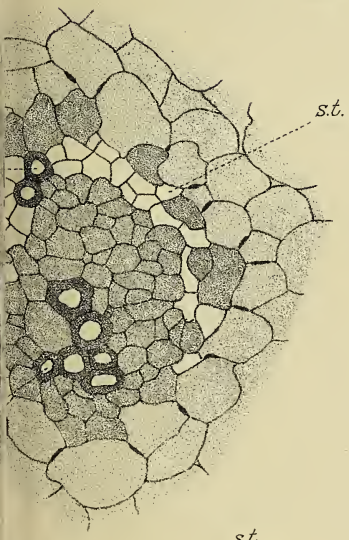

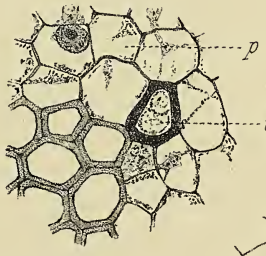

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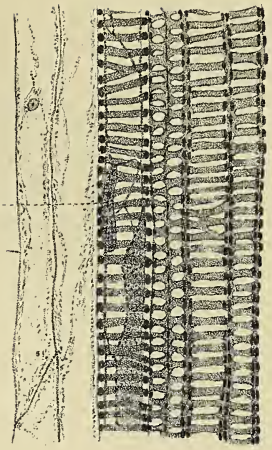

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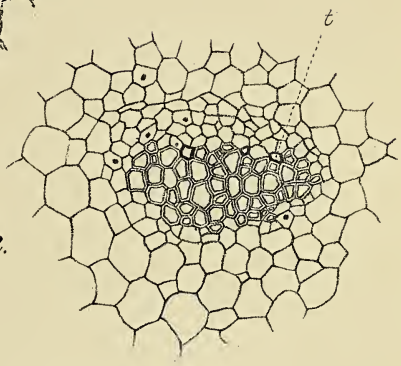

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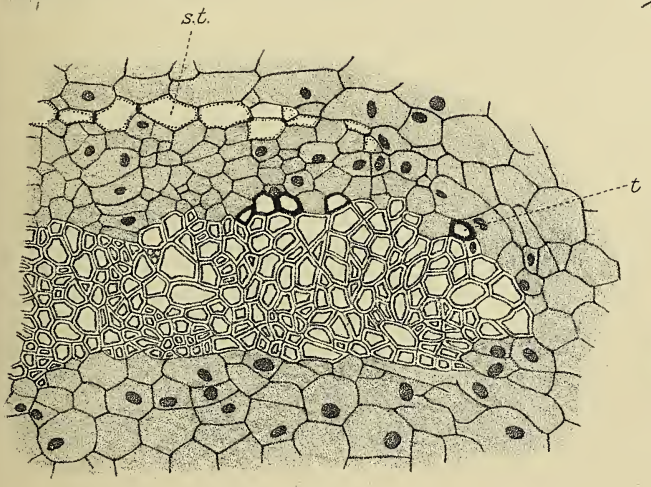

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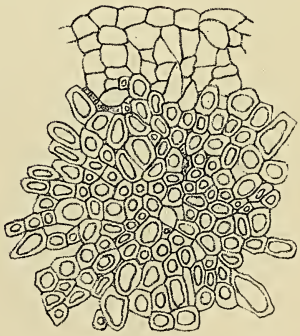

Fig. 14.

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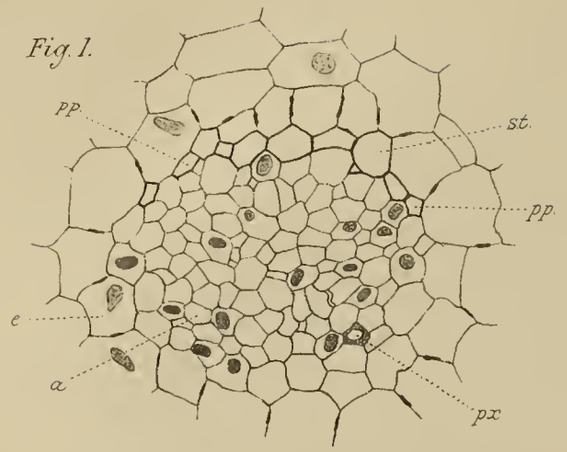
Fig. 1.



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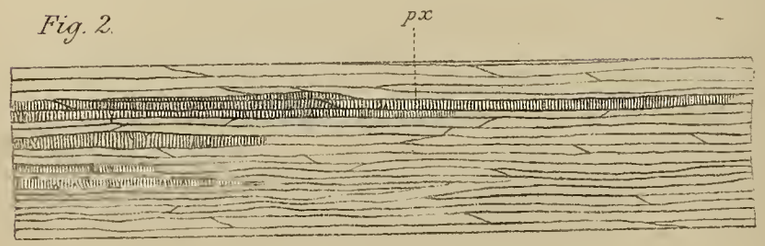

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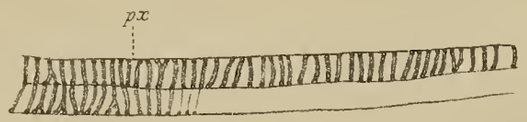

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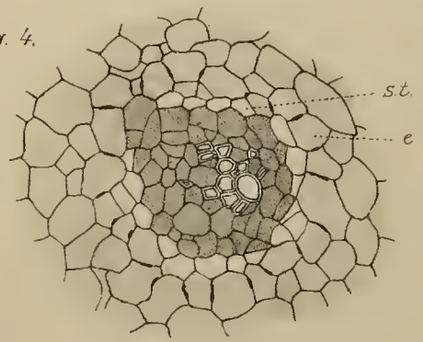

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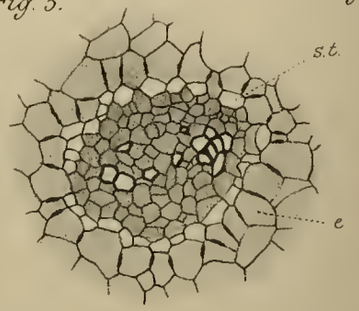

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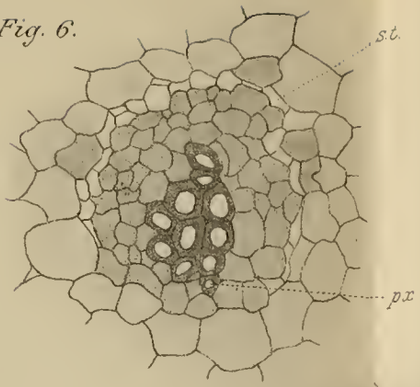

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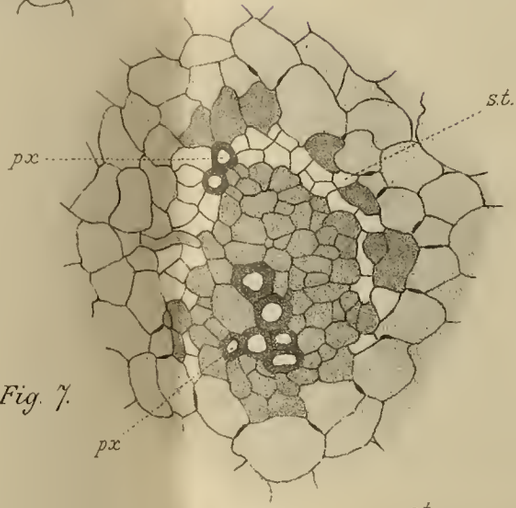

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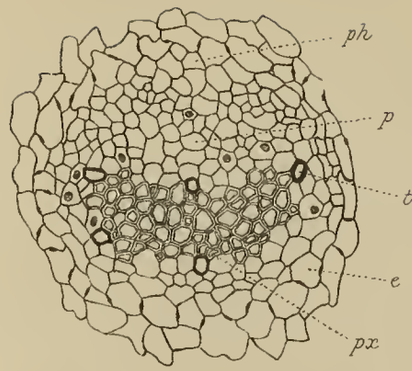

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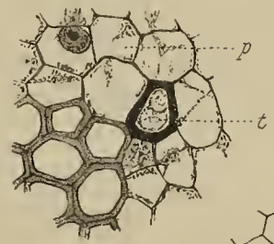

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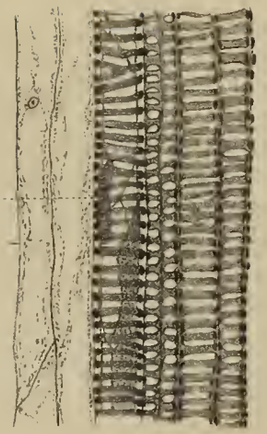

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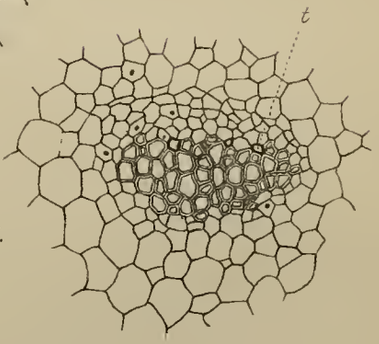

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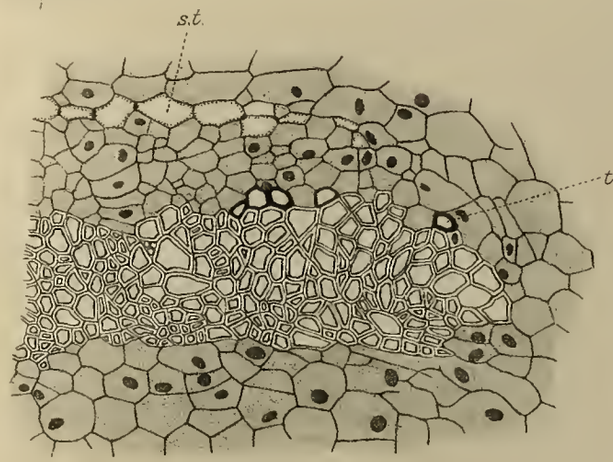

Fig. 14.

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BOODLE. — OPHIOGLOSSEAE.

University Press, Oxford.

On Biastrepsis in its Relation to Cultivation.

BY

HUGO DE VRIES,

Professor of Botany in the University of Amsterdam.

IN his very suggestive volume 'Materials for the Study of Variation, treated with especial regard to Discontinuity' (1894), Mr. W. Bateson says (p. 574), with regard to the relation of the study of variations to the great questions of the origin of species and the operation of natural selection, 'The only way in which we may hope to get at the truth is by the organization of systematic experiments in breeding, a class of research that calls perhaps for more patience and more resource than any other form of biological inquiry. Sooner or later such investigation will be undertaken, and then we shall begin to know.'

It is hardly necessary to point out that such experiments in breeding can be much more easily carried out with plants than with animals, especially when it is necessary to have a large number of individuals under observation. This is the case at present, since selection is the chief point at issue, so that the validity of the conclusions to be drawn depends mainly on the number of the individuals in each experiment. In the case of plants it is a simple matter to raise several hundred individuals, and to retain but a few as the parents of the succeeding generation; it is a much more complicated and

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costly affair to do this in the case of animals. Moreover, it is easy to cultivate plants under quite natural conditions, whilst the breeding of rats and mice, or moths or other insects, for experimental purposes, can only be carried on under conditions which are far from being natural, and which cannot be said to be favourable to the normal development of the animals. On these and other similar grounds, breeding experiments relating to inheritance and variation can be most satisfactorily instituted with plants.

For more than ten years I have been occupied with experiments of this nature. The object which I have had in view is to study the effect of selection under the most favourable conditions in producing breeds and varieties, on the one hand; and, on the other, the influence of various external conditions upon the production and further development of these variations.

In the course of such experiments the important distinction to be drawn between *individual* and *partial* variations becomes at once apparent. The former are deviations from the type which show themselves, though not necessarily in a uniform degree, in all the homologous members of the body of an individual, as, for instance, a variation in colour of the flowers or the fruits. The latter are manifested in but few members; thus only a single leaf or flower may exhibit some special peculiarity. I have also found that the partial variations are in a much higher degree dependent upon external conditions than are the individual variations; hence the former are more useful than the latter as a means of studying the effect of external conditions.

Amongst the partial variations, fasciations and twistings of the stem are especially serviceable for this purpose. These phenomena used to be regarded as accidental monstrosities, but I have shown by breeding experiments that they are hereditary[1]. They are typical examples of partial variation;

[1] Ueber die Erblichkeit der Zwangsdrehungen, Ber. d. deutsch. bot. Ges., 1889, vii; also, Monstruosités héréditaires offertes en échange aux Jardins Botaniques, Kruidkundig Jaarboek v. h. Genootschap Dodonaea, Gent, 1897, p. 62.—

on each sufficiently branched individual some of the branches are normal; the normal branches are in fact usually more numerous than the abnormal.

A few years ago I showed, in *Crepis biennis*, what results can be obtained by means of selection and cultivation in the case of fasciation[1]; and in an earlier work[2] I demonstrated that from a few isolated individuals of *Dipsacus sylvestris* with twisted stems a breed can be produced by selection, such that the abnormality recurs annually in a larger or smaller number of the plants raised from seed.

Such hereditary breeds or races afford the best material for comparative investigation of the conditions upon which the development of abnormalities depends. According to circumstances each successive generation is richer or poorer in so-called 'heirs'; that is, in individuals which manifest the monstrosity. If the breed is in itself poor in heirs, that is, if it shows, even after careful annual selection of the seed-producers, only a small percentage of monstrous individuals, it is one which is obviously not suitable as material for such an investigation: but if, on the contrary, under normal conditions,

[NOTE. The expression 'twisting of the stem,' used above in the text, is intended to render into English the term 'Zwangsdrehung,' applied to this abnormality by Al. Braun (Monatsber. d. k. Akad. in Berlin, 1854), for which there is no recognized English equivalent (see Masters, Vegetable Teratology); it might perhaps be well to adopt the term 'biastrepsis,' proposed by Schimper (Flora, 1854, p. 75). The addition of a brief description of this 'Zwangsdrehung' or 'biastrepsis,' in its characteristic form, may be of use to the reader. It occurs only in plants the shoots of which have opposite or whorled leaves; the phyllotaxis becomes spiral instead of verticillate, the successive leaves of the spiral being connected by their bases. The effect of this cohesion of the leaf-bases is to prevent the normal elongation of the internodes, which therefore become spirally twisted and often much dilated and otherwise monstrous in form. When, as is sometimes the case (e.g. *Dipsacus*), the stem is hollow, the pith-cavity is normally interrupted by diaphragms at the successive nodes; but in the twisted stems the pith-cavity is continuous, the diaphragms of the normal stem being represented by a rib projecting into the pith-cavity and following the course of the leaf-spiral.—ED.]

[1] Sur les courbes Galtoniennes des monstruosités; Bull. Scientif. de la France et de la Belgique (Giard), 1896, t. xxvii, p. 396.

[2] Monographie der Zwangsdrehungen, Pringsheim's Jahrb. für wiss. Bot. xxiii, 1891. See also, 'Eine Methode Zwangsdrehungen aufzusuchen,' Ber. d. deutsch. bot. Ges. xii, 1894; and 'Bijdragen tot de leer van de Klemdraai,' Kruidkundig Jaarboek, Dodonaea, iv, 1892, p. 145.

say one-third of the progeny show the variation, whilst the remainder are atavistic, it may be anticipated that changes in the external conditions will manifest their influence by causing fluctuations in this percentage.

On this principle are based the investigations which I purpose to describe in this paper, and which were almost exclusively carried out with my twisted breed of *Dipsacus sylvestris*, after this had attained in the fourth generation a degree of heredity exceeding 30 per cent.

The publication of the results which I have obtained during the last six or eight years has, furthermore, a practical object. I am convinced that the cultivation of this twisted breed, if generally adopted, would afford an easy means of investigation. Since I showed that monstrosities in plants are, as a rule, hereditary, and more particularly that twisted forms can be cultivated in Botanic Gardens as hereditary breeds, investigations have, as a matter of fact, been made in various places. But it has become apparent that the cultivation of such breeds is not so simple a matter as it appeared to be at first. Whilst some botanists have succeeded in raising from seeds obtained from me as striking and as numerous monstrous specimens as I myself, others have been less successful.

The cultivation of plants having twisted branches, fasciations, &c., makes greater demands on the gardener than that of normal plants of the same species. These monstrosities are, in the first place, only partial and not individual variations ; certain parts only of the body deviate from the type in the given direction. Some parts only show the abnormality, and in the case of twisting (biastrepsis) it has so far been observed to extend over a larger or smaller area of the main stem or of the branches.

Individual variations are obtained pure from pure seed ; the conditions of germination and of subsequent cultivation have no effect upon this. Hence their cultivation does not involve anything more than that of the species to which they belong. However, it often happens that the seed, obtained by exchange or by purchase, is not quite pure, and therefore

selection by weeding out may have to be exercised. Partial variations, on the other hand, depend almost as much upon the cultivation as upon the seed. The purest seed is no absolute guarantee for a satisfactory batch of seedlings, unless the treatment has been appropriate. Then again, in the more familiar breeds, rarely more than one-third of the individuals exactly reproduce the abnormality, the others reverting more or less completely to the type.

With regard now to the raising of plants with partial variations. The seed must, of course, be taken from those members of each generation which show the abnormality (twisting, fasciation, &c.) in the highest degree. The plants selected as seed-bearers must be isolated before flowering, either by digging up all the rest or by removing all their flower-buds. In their cultivation the following points must be regarded. In the first place the sowing must be made at the proper time, usually in April ; it is best to sow in pans under glass. The seedlings should be pricked out when they have developed the second leaf, and should either be planted out at once in their ultimate position, or be kept singly in pots for a few weeks (pots of 8–10 cm., or 3 in., are the best). Further, most monstrous plants require an open, sunny situation, plenty of space, and much manure. The attempt should not be made to grow the plants in a shady place ; sometimes it may be successful, but the result is very uncertain. The plants should, from the very beginning, be planted so far apart that they do not touch or overshadow each other at all, or at least not until they have grown so far that the monstrosity has made its appearance. The amount of manure required obviously depends upon the nature of the soil, but it can hardly be given in excess. I made use of dried cow-dung, so-called cattle-guano, and horn-meal (crushed and steamed horns and hoofs), and gave $\frac{1}{2}$–1 kilog. of this mixture to every square metre of soil. The seed-pans must not be manured at all, otherwise *Botrytis cinerea*, the great enemy of all special cultures, will be developed.

I propose to describe now a series of experiments, all of

which were made with my twisted breed of *Dipsacus sylvestris*. As I have already remarked, this breed, with proper treatment, gives a progeny 30–40 per cent. of which have well-marked twisting of the main stem, besides other individuals which show less marked twisting of the branches, and others again which have their leaves in whorls of three. If, however, the treatment is unsatisfactory as regards any one of the essential conditions mentioned above, this high percentage of monstrous forms is not obtained; there may, in fact, be none at all.

The scientific results of my experiments may be summed up by saying that *Biastrepsis, especially in the case of* Dipsacus sylvestris, *is in a high degree dependent upon the conditions which obtain during and subsequently to the germination of the seed. Conditions which favour the vigorous development of the plants promote also the size and the number of the portions of the main stem and of the branches which show the twisting.*

The practical result of my experiments is, I consider, the recognition of the fact that the cultivation of monstrosities should either be carried out strictly according to rule, or not be attempted at all. Without the requisite care the attempt only results in doubt being cast upon the goodness of the seed and the hereditary properties of the breed.

I treat of the subject under the following heads :—

A. The normal cultivation of the breed.
B. The influence of space.
C. The influence of the soil.
D. Summer-sowings.
E. Autumn-sowings in the open.
F. Autumn-sowings under glass.
G. Cultures in other Botanic Gardens.

A. The Normal Cultivation of the Breed.

In my monograph on the subject (1891), to which allusion has already been made, I have given an account of the morphology of biastrepsis in *Dipsacus sylvestris*, and of the

means by which I obtained the hereditary breed. I was then in a position to report upon the first four generations of this biennial; I now give the results of my observations on the subsequent generations.

My breed was derived from two individuals with a twisted main stem, which made their appearance among plants obtained from a sowing in 1884. Before they flowered, all the other individuals had been removed. From the seed of these two I obtained the *second generation* in 1886. At that time I was unaware of the special conditions essential to the successful cultivation of these plants, and, doubtless on this ground mainly, I found only two twisted individuals among about 1650 seedlings. Both of these flowered in isolation, and bore seed abundantly. From this seed the *third generation* was raised in 1888–9, consisting of about the same number of plants, among which were sixty-seven with twisted stems, that is, about 4 per cent. of the total number. The seed-bearing plants selected were flowered in isolation.

The seed for the *fourth generation* was sown, in part, in 1890, and gave about 10 per cent. of twisted individuals, which, owing to an accident, could not be used for the propagation of the breed. The remainder of the 1888–9 seed was therefore sown in 1891, and, with a better knowledge of the requisite conditions, I obtained 34 per cent. of twisted individuals, a percentage which has been approximately maintained, but not materially exceeded, in subsequent generations.

The improvement in the method of cultivation was essentially this, that more space was allowed to the young plants from the very beginning. In the two previous generations about fifty individuals were grown to the square metre; in this generation the number was reduced to about twenty-five by the repeated weeding-out, during the month of June, of all superfluous plants so soon as they began to touch one another.

The sowing on the beds was done in the middle of May, 1891. At the beginning of October I found that about half a dozen plants in every hundred had spiral phyllotaxis

within the dense rosette; and at the beginning of November I had rather more than half of the plants removed as being undoubtedly normal and atavistic. Towards the end of May, 1892, when the shoots were being vigorously pushed up, the plants were finally gone over and counted. Including the figures for the previous November, I obtained the following result for the whole sowing:—

Twisted stems	37 = 34%
Stems with $\frac{1}{3}$ phyllotaxis	12 = 11%
Normal (atavistic)	58 = 55%
Total	107

It is of importance to remark that the number of twisted individuals had increased, with the larger space, not merely relatively in percentage but absolutely per square metre. In the third generation there were fifty plants to the square metre of which 4 per cent. (1–7 per cent.), or about 1–4 plants, were twisted: in the fourth generation I had four square metres with thirty-seven twisted individuals, that is, about nine to the square metre.

I selected from this bed the seven best plants as seed-bearers, all of them having local biastrepsis in some of the branches, and I isolated them before flowering.

The *fifth generation*, 1893–4, gave less favourable results: it yielded only 20 per cent. of twisted main stems. In this case I had not, as previously done, sown the seed on the beds, but in pans standing in the greenhouse of my laboratory. This method has since proved itself to be the more convenient and certain, and it was adopted with both the succeeding generations.

The seed harvested in September, 1892, was sown in the middle of March, 1893. About the middle of April the best seedlings were transplanted singly into 10 cm. pots, containing well-manured loam; and about the middle of May they were planted out in the beds at about the same distance from each other as in the previous experiment (twenty-two plants to the square metre). In the next year all the shoots

shot up, and on counting them the following results were obtained:—

	A	*B*	%*A*	%*B*
Twisted main stem	5	2	20	10
Phyllotaxis $\frac{1}{3}$	1	1	4	5
Normal (atavistic)	19	17	76	85
Total	25	20		

A and *B* are two groups of plants grown from seeds specially collected from two of the 1892 seed-bearers.

The number of plants is obviously too small to admit of attaching much importance to the percentages obtained.

In the autumn of 1894 the seed of the four best plants, which had been isolated from the rest, was harvested.

The *sixth generation*, 1895–6, yielded a much more satisfactory result, viz. 42 per cent. of individuals with twisted main stems; a result which was due, in part at least, to the greater distance of the plants from each other, the other conditions of cultivation being as before. The seed of 1894 was sown about the middle of March, 1895, in pans kept in the greenhouse, the seedlings being transplanted into pots early in April, and planted out in the beds at the beginning of May. But there were only thirty-three plants to four square metres, that is about eight to the square metre. At the end of October I found that fourteen of the leaf-rosettes showed spiral phyllotaxis in the centre, seven showed $\frac{1}{3}$ phyllotaxis, and twelve were normally decussate; that is, 42 per cent. had spiral and 21 per cent. $\frac{1}{3}$ phyllotaxis, and 36 per cent. were decussate. In May, 1896, I confirmed this result, and then all but the spiral plants were weeded out. The six most strongly twisted individuals were selected as seed-bearers, and were isolated before flowering.

The *seventh generation*, 1897–8, was raised in much the same way. The seed of 1896 was sown in the greenhouse on May 5, 1897; the seedlings were potted off, and about the beginning of July they were planted out, seventy in all, in the beds, sixteen plants to the square metre.

At the end of May, 1898, I found that these consisted of—

Plants with twisted stems	32 = 46%
,, $\frac{1}{3}$ phyllotaxis	21 = 30%
,, normal decussate	17 = 24%
Total	70

The proportion of stems in which the twist was to the left or to the right respectively was maintained unaltered in this generation, there being usually about an equal number of the two kinds, as shown in the following table :—

3rd generation, 1889	29 right	27 left
4th ,, 1892	21 ,,	33 ,,
7th ,, 1898	14 ,,	17 ,,

The whole history of the breed may be summarized in a tabular form as follows :—

Generation.	Sowing.	No. of plants.	No. to square metre.	Percentage of twisted stems.
1. 1884–5	Bed	—	—	—
2. 1886–7	,,	1643	—	0·1
3. 1888–9	,,	1616	35	4
4. 1891–2	,, May 15	107	25	34
5. 1893–4	House, Mar. 17	45	22	10–20
6. 1895–6	,, Mar. 11	33	8	42
7. 1897–8	,, May 5	70	16	46

Whilst at first a large number of plants, crowded together, were used with imperfect success, in the later years fewer plants with plenty of space have afforded much more satisfactory results. This improvement is due in part to the more favourable cultural methods, in part to the continuous selection ; it is impossible in this case, as usually in other such cases, to discriminate between the effects due to these two causes respectively. It will, however, be shown in the next section that the better cultural methods were of considerable importance in bringing about the result.

B. The Influence of Space.

The condition most essential to the successful cultivation of these twisted plants is that each plant shall have sufficient room in which to develop freely ; the plants must neither

touch nor overshadow each other. This result is clearly indicated by the various cultures previously described.

It is instructive, in connexion with this point, to compare the plants growing on the borders of a bed with those growing in the middle. Whenever the space becomes insufficient, the twisted stems are mostly or even entirely confined to the plants growing on the borders: this was the case in the second and third generations, when there were about fifty plants to the square metre. On the other hand, the occurrence of spiral rosettes in the plants on the borders, and the absence of them from those in the middle of the bed, is one of the best indications whether or not the plants have sufficient space allotted to them.

In order to demonstrate in a simple manner the truth of the above statement, I instituted the following experiments.

In 1889 seeds, obtained from the second generation in 1887, were sown on two neighbouring beds and in the same manner. When, in June, the plants began to touch each other, they were not thinned out to an equal extent; on the one bed 300 plants were left, on the other 540. As each bed had an area of twelve square metres, there were in the one case twenty-five plants to the square metre, in the other forty-five. On examining the elongated shoots in May of the following year the results, which were widely different in the two cases, were—

Bed, 25 plants to square metre	6% twisted stems.
,, ,,	5% phyllotaxis $\frac{1}{3}$.
Bed, 45 plants to square metre	1% twisted stems.
,, ,,	1% phyllotaxis $\frac{1}{3}$.

I made a second experiment, in which the plants were still more crowded together. In the first summer I allowed 136 plants to grow on a bed of 2·1 square metres area, that is, sixty-five plants to the square metre. In order to eliminate the effect of the border-position, I surrounded the bed with a margin of plants placed about as closely as the others, but these marginal plants were not subsequently counted in with the others. When I examined the rosettes in the following

February, no trace of spiral arrangement could be detected. A control-experiment, with sixty-two plants from the same seed, but planted twelve to the square metre, gave 10 per cent. of individuals with twisted stems.

Hence lack of sufficient space can entirely prevent biastrepsis from making its appearance.

A similar result was obtained with regard to the exposure of the beds. Shade, whether of trees or other objects, is always prejudicial ; a good proportion of twisted stems can only be obtained when the plants are grown in an open situation fully exposed to the sun. Thus, in 1890, a sowing in the shade of a tree gave only 3 per cent., whilst another quite similar sowing, but with full exposure to the sun, gave 7 per cent. of plants with spiral phyllotaxis.

The time for sowing the seed, whether in the open or in pans in the greenhouse, varies from March to the beginning of May. The time and mode of sowing seems to be of little or no importance as compared with the requirement of sufficient space.

When sowing in the open, about 3–5 c.cm. of seed per square metre should be used, containing 200–300 seeds. Of these only a portion will germinate, and of these some are lost or are weeded out, so that eventually only about 20–25 plants result. On the other hand, seeds sown in pans kept in the greenhouse nearly all germinate ; the seedlings are very uniform, and can be potted off without any selection. The latter method therefore yields by far the more reliable figures of the two.

In weeding out the seedlings obtained by sowing in the open, either the most vigorous plants may be left, or no attention may be paid to this point ; but the result is materially influenced by the course pursued, for the weaker plants are much less likely than the vigorous to show twisting. They are plants which apparently have been badly nourished, either in the seed-stage whilst still on the parent, or during or after germination. I made an experiment on this point in 1888–9. In June, 1888, I planted two similar

beds, each of 12 square metres area; the one with 360 very vigorous plants, the other with 410 weakly plants, from seeds of the same parent. The weakly plants were small, and therefore required less space. When the plants shot up in the following year, the one bed gave 8 per cent., the other only 3 per cent. of twisted stems.

C. Influence of the Soil.

The richer the soil, and the more vigorous the plants, the better is the prospect for a high proportion of twisted stems.

In the year 1891, in addition to the culture on good loose soil (see p. 401), I had a control-experiment on poor hard ground. The good soil was manured with 2 kilog. of guano and dried cow-dung per square metre; the poor soil received only one-eighth of a kilog. of guano per square metre; the treatment in other respects was identical. The result was—

On the good manured soil	34% twisted stems
On the poor soil	14% „ „

In very poor soil the proportion of twisted stems may sink to nothing. I made an experiment of this kind in 1894 with a bed which consisted, to a depth of half a metre, of nothing but sand. For seed I used two samples, gathered in 1891 and 1893, which gave, in control-experiments, 10 per cent. and 25–30 per cent. of individuals with spiral phyllotaxis; half a bed (4 square metres) was sown with each of the samples. The seeds were covered with garden-soil in order to ensure germination. The two half-beds gave respectively 94 and 124 plants, that is 24 and 31 per square metre, which, as they were for the most part small, only touched each other here and there. The plants were examined in May, 1895, and it was found that, without exception, the phyllotaxis was decussate.

This is a convenient opportunity to direct attention to a circumstance which has hitherto been insufficiently recog-

nized in comparative experiments with plants: I refer to the inequality of external conditions as regards the individual seedlings growing on one and the same bed. This inequality is much greater than might be supposed, especially during germination. It is a familiar fact that the seeds of a sowing do not all germinate simultaneously; and those which germinate on a sunny day in moist soil are at a great advantage as compared with those which germinate in dry soil or on a dull day. The unevenness of the surface of the soil leads to some spots being dry and others relatively moist, and germination is so much expedited in the latter that the seedlings are often found growing in scattered groups. The mixture of the soil with the manure is not uniform, even when the greatest care is taken; and this leads to an unequal distribution of moisture in the soil, especially when fresh farmyard manure is used. Then again, injury by birds or insects gives rise to great differences among the seedlings. All these various conditions have as their result that the young plants, after germination is over, soon show striking differences in development. According to the goodness or badness of the weather in the course of the summer, these differences either become more marked or they tend to disappear. And then, if the plants are crowded so as to touch each other, the existing differences become accentuated, the more vigorous developing rapidly at the expense of the weaker.

Much more in the same strain might be added: but what has been said suffices to prove that the individual differences between plants growing on the same bed are mainly caused by the inequality of the conditions under which they have been developed. If now the most vigorous and best-developed individuals be selected as seed-bearers, it is almost certain that they are those which have been the most highly nourished throughout their lives. Selection in this case means the selection of the best-nourished.

It would appear to be quite permissible to extend this conclusion to the case of biastrepsis. During the ripening of the seeds, during their germination, and then during their

subsequent development, the individuals of the same sowing of the same seed are exposed to very different conditions of life, although every effort may have been made to secure uniformity in this respect. And it is these differences of external conditions which determine which and how many of the seedlings shall develop twisted stems, the seedlings belonging, of course, to the proper hereditary breed.

The plants with the most strikingly twisted stems which are selected as the seed-bearers are thus generally those which have been the most highly nourished. And as this mode of selection is pursued in successive generations, so the best-nourished plants have for many years had the best-nourished individuals as their ancestors. Thus the influence of nutrition accumulates as the generations succeed each other.

I may add that I have made similar observations in the case of other plants and of other types of monstrosities.

D. Summer-Sowings.

The sowings associated with the observations which have been already given were made between the beginning of March and the middle of May, and the particular time of the sowing was without influence upon the percentage of twisted stems. Under proper treatment the plants come on so vigorously in the course of the summer that whatever differences may have originally existed gradually disappear, so that they cannot be detected in the winter or in the second summer after the sowing has taken place.

Dipsacus sylvestris torsus is strictly biennial. In spite of careful observation, I have been unable to discover any annual individuals. I obtained seeds of *D. sylvestris* from various Botanic Gardens and made large sowings, but in no case did an annual form occur. This is the more remarkable since most biennials (such as *Beta*, *Daucus*, *Oenothera*) produce numerous individuals which 'bolt,' from which an annual

breed can easily be raised. Under these circumstances the question arises, What would be the effect of sowing the seed, not in the spring, but in summer or in autumn? and I have endeavoured to find an answer to this question by the experiments of which the following is an account.

Seeds were sown in the summers of 1892 and 1893, at the beginning of June, at the end of July, and in the middle of August. The seed used belonged to the fourth or fifth generation of my breed which had already attained 34 per cent. of twisted main stems. The chief result was that the plants obtained by summer-sowing regularly developed their shoots in the following year; but, with the exception of a single individual of the June sowing, the stems were not twisted. The details of the various cultures are as follows:—

Sowing of June 1, 1893. The seed was that of 1892, from a crop which contained 34 per cent. of twisted stems; it was sown in rows on the bed. The seedlings came up regularly; and up to October superfluous plants were weeded out so soon as they began to touch one another. About twenty plants per square metre remained, so that the condition of space was very favourable (see p. 404); there were 179 plants in 9 square metres. In May, 1894, all the rosettes shot up without a single exception. As soon as the phyllotaxis of the shoot could be made out, the plants were examined. One stem was found to be characteristically twisted, another had three-leaved whorls, whilst all the others had the normal decussate phyllotaxis and showed no twisting of the stem.

All the plants, with the exception of the twisted individual, were dug up. This plant clearly showed itself, in the course of the summer, to be less vigorous than the twisted individuals of a normal culture, and it flowered later. Its stem was twisted right up to the inflorescence.

Sowing of July 28, 1892. Seeds harvested in 1891 were sown on a bed having an area of 4 square metres, and the soil was kept moist by means of a lawn-sprinkler. The seeds germinated rapidly, and before the autumn had formed vigorous rosettes of radical leaves. By the end of the follow-

ing May all the seedlings, with the exception of a few weakly ones, had thrown up their shoots. When the shoots had attained a length of 50–75 cm., and the terminal capitulum could just be seen, it was found that all the plants were quite normal, having decussate phyllotaxis ; the only observable abnormality was one divided leaf. There were 131 plants with elongated shoots, and twenty-nine with only rosettes, 160 plants in all, on the 4 square metres, so that the proportion (forty) per square metre was high ; but this number did not appear to be excessive, inasmuch as the plants were relatively small and did not touch each other more than would vigorous plants at a greater distance from each other. After the counting was done, I allowed five of the best specimens to remain ; they developed strong stems over 2 metres high, which were not noticeably less vigorous than ordinary normal plants of the breed.

In a control-experiment made with the same seed sown in the spring of 1892, I obtained twenty-two individuals out of sixty, that is, almost 37 per cent., having twisted stems.

Sowing of August 14, 1893. The seed used was obtained from two plants of the 1892 crop, and was the same as that used in the June sowing of this year. They quickly germinated in the bed, and by the autumn had formed relatively small rosettes, so small, in fact, that sixty plants per square metre could well be left. Notwithstanding this, they nearly all (235) threw up their shoots in the following spring ; but here and there, where germination had been tardy, some plants, twenty in all, remained as rosettes, that is, about 8 per cent. The stems were weak, of only about half the normal thickness, and their leaves were decussate up to the inflorescence ; there was no sign of twisting of the stem.

Summarizing these results of summer-sowing, we find that, given adequate space and suitable treatment, there is an almost entire absence of twisted stems. The experiments included 179, 131, and 235 plants, giving a total of 545 shoots, of which only one was twisted and one had three-leaved whorls, both of these exceptional plants belonging to

the June sowing. In contrast with this, the spring-sowings gave 34–37 per cent. of twisted stems.

I conclude, therefore, that late sowing does not allow the plant sufficient time in which to attain normal vigour in the rosette-stage. Hence, when the shoots are thrown up, they are too weakly to develop spiral phyllotaxis. This result accords fully with those obtained in my observations on the effects of too limited space and bad soil.

E. Autumn-Sowings in the Open.

I began an experiment on September 11, 1891, which may be regarded as a control to the summer-sowings. The seedlings which are produced in a sowing so late in the season do not throw up their shoots in the following year, but remain in the rosette-stage, the plants growing vigorously until the following autumn. They do not throw up their shoots until the third year. The lateness of the sowing has, therefore, the effect, not of weakening, but of materially invigorating the plants.

At the beginning of the first winter the young plants had only two or three pairs of leaves, which were not much more than 3 cm. in length. It is known that the throwing up of the shoot of biennials in general is the result of stimulation, and that this stimulation is given by the winter. Indeed, when Rape is sown early, this effect may be produced by the late night-frosts. But if the plants are too young they appear not to be susceptible to this stimulation. This is a phenomenon which has been too much overlooked by physiologists.

In the middle of September, 1892, just one year after sowing, I examined the plants. There were twenty-three of them in a small bed of 2 square metres area. Not one of them had thrown up a shoot; but ten of them (44 per cent.) showed spiral phyllotaxis in the heart of the rosette, and four had three-leaved whorls.

Hence it appears that the effect of the prolongation of the rosette-stage is rather to increase than to diminish the proportion of abnormal plants. Moreover, this experiment leaves no doubt as to the correctness of the interpretation which I have placed upon the results of summer-sowing.

F. Autumn-Sowings in the Greenhouse.

The object of these experiments was so to accelerate, by cultivation in a greenhouse under favourable conditions of temperature, illumination, &c., the germination of the seeds and the subsequent growth of the seedlings during the autumn and the winter, that the plants should be in a position to throw up their shoots in the following summer. In order to maintain the earth in the pots, day and night, as nearly as possible at the temperature (22° C.) which I found to be most suitable, I made use of a large shallow water-bath, 10 cm. in thickness, which occupied a closed space in the small greenhouse attached to my laboratory, and was distant only 20–25 cm. from the glass above it, being inclined so as to be about parallel to it. The pots were 10 cm. in diameter; each either contained a single plant from the beginning, or two plants, the weaker of which was removed so soon as they began to touch each other. By a control-experiment it was ascertained that it would not suffice to heat the water-bath only during the day-time; plants treated in this way threw up no shoots in the following summer. Continuous heating was required, and this was carried on from the middle of September, when the sowing took place, until the middle of November, at which time the seedlings had five or six pairs of leaves, the leaves being about 14 cm. in length.

The experiments were begun on September 17, 1892, and on September 15, 1893, the seed used being in each case that which had just before been harvested. With the success of the experiments the whole life-cycle of these biennial plants was brought within the limits of a single year; and it would

eventually become possible thus artificially to grow them as annuals, and perhaps in time to establish an annual variety by selection.

Sowing of September 17, 1892. The seed was harvested and sown on the same day: it was obtained from a plant the seeds of which gave 20 per cent. of twisted stems when cultivated in the ordinary way. Germination and the early stages of growth were quickly and satisfactorily gone through. The water-bath was heated until the middle of November; subsequently only the greenhouse was heated. At the end of January, 1893, the plants were put out into a cold frame, where they proceeded to form new leaves, those which had been formed in the greenhouse now dying off. In the middle of March they were planted out, and after the middle of April they were no longer protected by glass.

At the beginning of June there were nineteen plants with vigorous shoots 50–75 cm. in height, and twenty-two rosettes. At the end of June the nineteen shoots were nearly two metres in height, and were as vigorous as average plants of my breed. The examination of these shoots showed (1) that eight of the plants had normal decussate phyllotaxis; (2) that there were seven plants which, although their phyllotaxis was decussate, had each a four-leaved whorl owing to the suppression of an internode; (3) that there were four plants with slight and local twisting in the shoots which were otherwise straight. One of these showed well-marked twisting in one of the lateral branches. Hence only four plants out of nineteen (about 20 per cent.) showed slight and local twisting of the stem; that is, just the same proportion as that of typically twisted stems occurring in a control-experiment[1]. These four plants flowered in isolation and ripened seed before September 15, 1893; that is, within a year from the time of sowing.

[1] It should be remarked that in the control-experiments only the entirely, or almost entirely, twisted stems are counted: no attempt was made to ascertain whether or not slight local twisting of the stem would develop in the other plants, for most of these were dug up before they had completed their growth.

The effect of the method of culture described above is that, in these artificially annual plants, whilst twisting of the stem is not altogether prevented, it is reduced to a minimum.

Sowing of September 15, 1893. The seed of one of the previously described artificially annual plants was immediately sown, and germination took place under the same conditions as in the previous year. The seed-bearing plant was the one which showed twisting in one of its lateral branches. At the end of January the plants were brought out of the greenhouse and were kept for a time under glass. Two weakly plants remained in the rosette-stage, but the others, thirty-five in number, threw up shoots in May: the shoots were vigorous, of about the same height and thickness, and were better and more uniformly developed than were those of the preceding generation. The examination of them on June 18 gave the following results :—

Nine normal decussate shoots ;

Ten decussate shoots, each having one four-leaved whorl ;

Three shoots with two four-leaved whorls ;

One shoot with three four-leaved whorls ;

One shoot in which the leaves of one of the pairs were separated.

Eleven shoots with slight local twisting (30 per cent.).

The result is thus the same as that obtained with the preceding generation. I was unable to allow these plants to flower, for fear of interfering with the normal cultivation of the breed.

Sowing of September 3, 1894. Professor G. Le Monnier of Nancy, who has for years cultivated my breed of *Dipsacus* on a larger scale than I have myself been able to do, was good enough to send me some freshly-gathered seed of twisted individuals early in September, 1894. In the more southerly climate of Nancy the seed had ripened a fortnight earlier than with me ; consequently I was able to repeat the experimental sowings of the two previous years with this new and favourable factor. The plants grew more rapidly than those of previous years on the heated water-bath, and by the middle

of November had formed about twice as many leaves. They were kept warm until the middle of December, when they were taken out and put under glass. By the end of May they had all (thirty-six) thrown up their shoots, but these shoots were decussate right up to the inflorescence.

In a control-experiment made with the same seed, but without artificial heat, the plants were potted off soon after germination and were kept under glass in the garden: twenty-nine of them threw up stems in the following year, which, as was to be expected, were decussate; nine plants remained in the rosette-stage.

The conclusion to be drawn from all these experiments is that whilst it is possible to contract into one year the life-cycle of the biennial *Dipsacus sylvestris torsus,* by sowing the seed immediately it is ripe and by hurrying on germination and the early stages of growth, this takes place at the cost of the biastrepsis, which is either altogether wanting or is manifested in only a relatively slight degree.

G. Cultures in other Botanic Gardens.

When, in the summer of 1889, the third generation of my breed began to show a distinct increase in the percentage of twisted individuals, I distributed seed of it for the first time; and I did so at the friendly request of Professor J. Urban of the Botanic Garden at Berlin. So successful was the cultivation of the breed there, that in the published catalogue of the seeds gathered in 1891 there are enumerated no less than five varieties of these plants with twisted stems or with three-leaved whorls. Since then I have distributed seed in increasing quantity, and the cultivation of the breed has been successfully carried out in various Botanic Gardens, although in some cases difficulties have been encountered. The plant requires not only a good deal of space, open exposure, and loose well-manured soil, but special attention in addition, which is often begrudged since the species grows wild and is to be met with

even in overgrown localities and on bad soil. Carefully raised garden-plants are, however, much more sensitive than wild ones, and with this the tendency to biastrepsis is intimately connected. For instance, the cultivated plants are often killed by frost in damp winters with us, or only the main flowering shoot is killed. In the latter case lateral shoots are thrown up in the following summer which usually show but little twisting; but the plants may be used as seed-bearers. The plants should therefore be protected during the winter, if there is no snow, with straw or leaves. A single dead leaf placed over the crown of the rosette suffices to protect, if only it is kept in position, and does no harm however long it remains.

The most detailed report which I have received regarding the cultivation of this breed is that of Professor Le Monnier, the Director of the Botanic Garden in Nancy, who most kindly co-operated in some of the experiments already described. Since 1892 he has annually raised several hundred plants of my breed, and cultivated them under the most favourable conditions. In November of that year he had 490 plants in the rosette-stage, of which 20–30 per cent. showed spiral phyllotaxis, and 60–65 per cent. had three-leaved whorls, so that there were very few atavistic individuals. The proportion of twisted stems agrees with my own observations (see p. 404); but the number of plants with three-leaved whorls far exceeds anything that I have, even now, obtained in Amsterdam. Moreover, the spiral phyllotaxis appeared earlier in Nancy than in Amsterdam: it was detected there in many rosettes as early as July; but with me, even in early sown plants, it could never be seen before August, and, in the case of April or May sowings, not before September.

Professor Le Monnier had also the goodness to repeat at Nancy the experiment of sowing the seed immediately after it is ripe. This was done at the beginning of September, 1894: the seedlings grew in the open, without any artificial heat, more rapidly and vigorously than they did at the same time in Amsterdam when all possible care was lavished upon them (see p. 415). Nevertheless in the following year they threw up

shoots which showed no trace of twisting or of three-leaved whorls. At Nancy, as at Amsterdam, the production of seed within a year could be induced, but at the expense of biastrepsis.

The seed, gathered at Nancy, sown in 1897, gave in the following summer only seven twisted stems, and ten with three-leaved whorls, out of about 100 shoots. This remarkable state of affairs must probably be ascribed, as Professor Le Monnier suggests, to the pollination of the twisted seed-bearing plants of 1896 by pollen brought by insects from other plants of *Dipsacus* growing at a distance. This experience is the more important since I have myself observed that an interval of 100 metres is often insufficient to prevent the crossing of two varieties of the same species if in flower at the same time.

Conclusions.

1. The seed of *Dipsacus sylvestris torsus* yields, under proper cultivation, a progeny of which about one-third have twisted stems. This proportion was first attained in the fourth generation, and since then it has rather increased than diminished on the whole (see p. 404).

In addition, there occur plants with three-leaved whorls, with divided leaves, or with local twisting of the lateral branches, and occasionally the other anomalies which I have described in my monograph [1].

2. The development of biastrepsis, that is, the transition from decussate to spiral phyllotaxis, depends not only upon the hereditary properties of the individual latent in the seed, but also in a high degree upon the external conditions under which the individual develops.

3. The more favourable the conditions of life, and con-

[1] In addition to the references given on pp. 396 and 397, I may mention the following papers which I have published on this subject: Ber. d. deutsch. bot. Ges., xii, 1894; and Kruidkundig Jaarboek van het Genootschap Dodonaea in Gent, iii, 1891, and iv, 1892.

sequently the more vigorous the growth of the plants, the richer is the progeny obtained from any given seed in individuals with twisted stems, and the more marked is the twisting in the individuals.

4. The most important condition is that the plants shall have plenty of space for their growth : they must not overshadow each other, and they should touch each other as little as possible. There ought never to be more than 20–25 plants to the square metre ; and even then the plants come into contact with each other in the autumn : it would be better not to have more than 10–15 plants per square metre.

When too closely planted, the number of plants with twisted stems per square metre is less than that obtained with more remote planting ; so that the greater number of individuals is not an advantage but a disadvantage. In the case of close planting, the twisted individuals are confined, either exclusively or for the most part, to the border of the bed.

5. The time of sowing is of importance, for this determines the length of life of the plant up to the time when the shoot is thrown up : the longer this period, the conditions being favourable, the greater is the prospect for biastreptic individuals.

Sowings in the summer or in the early autumn yield seedlings which throw up shoots in the following year, which shoots show little or no tendency towards biastrepsis. On the other hand, good results are obtained from autumn-sowings, yielding plants which do not throw up their shoots until the next summer but one, and have therefore a longer rosette-stage : the proportion of biastreptic plants is in this case rather larger, if anything, than in the case of ordinary spring-sowings.

With regard to the spring-sowings, it does not appear to be a matter of great importance whether the seed be sown in March, in April, or early in May ; or whether the seed be sown directly in the beds, or in pans in the greenhouse, the seedlings being subsequently planted out. For various reasons

I have for some years adopted the latter method; it is more convenient, and it is more certain, especially when the spring is dry.

6. Good, loose soil, well manured with nitrogenous matter, is an important essential. On unmanured sandy soil it is impossible to raise, even from the best seed, any twisted individuals; if the soil is hard or unfertile, the percentage of such individuals diminishes.

7. It is possible to contract the life-history of *Dipsacus sylvestris torsus* into the limits of one year, if the seed be sown immediately it is ripe and the conditions be favourable. By this means an additional generation can be obtained each year; and it might perhaps be possible, by selection, to produce an annual twisted breed. However, so far as experiment goes at present, it appears that the annual character is developed at the expense of the biastrepsis; for in such plants there is little or no twisting of the stem.

8. The statement that, with a given hereditary tendency, a monstrosity becomes more marked the more favourable the conditions of life, and therefore the more vigorous the growth, is true not only for the biastrepsis of *Dipsacus sylvestris*, but is established for the most various plants and different monstrosities by the observations which I have made during the last ten years [1].

[1] Vide, Ueber die Abhängigkeit der Fasciation vom Alter bei zweijährigen Pflanzen, Botan. Centralblatt, Bd. lxxvii, 1899; and, Sur la culture des fasciations des espèces annuelles et bisannuelles, Revue générale de Botanique, Tom. xi, 1899, p. 136.

I am always ready to supply seed of *Dipsacus sylvestris torsus*, even in considerable quantity. I can generally supply specimens of twisted stems.

On the Structure and Affinities of Helminthostachys zeylanica.

BY

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AND

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With Plates XXI—XXIII.

HELMINTHOSTACHYS ZEYLANICA, Hook., the sole representative of an interesting genus of Ophioglossaceous Ferns, is somewhat widely distributed in the Eastern tropics, occurring as it does both in Asia and in Australia. Our own material was collected by us in the low country in Western Ceylon, where the plant is locally abundant, and we have also to thank Professor F. W. Oliver and Mr. A. C. Seward for their kindness in supplementing our stock.

Helminthostachys has a creeping and markedly dorsiventral rhizome which bears the branched leaves characteristic of the plant in two rows on its upper surface, whilst the roots spring from its flanks and under surface. The roots, as was pointed out by Prantl[1], do not stand in any definite relation to the leaves either in number or in position, and in this respect

[1] Prantl, Helminthostachys zeylanica in ihrer Bez. z. Ophioglossum u. Botrychium, Ber. d. deutschen Bot. Gesellsch., 1883.

[Annals of Botany, Vol. XIII. No. LI. September, 1899.]

Helminthostachys approximates more to *Botrychium* than to *Ophioglossum*. It is true that Holle[1] believed that he had discovered in both these genera, a regular relationship between the roots and leaves; but though this perhaps may hold for *Ophioglossum*, it has been already questioned by others for *Botrychium*, and indeed Holle's own figure does not give much support to his views. Thus *Botrychium*, in this respect, would occupy a position intermediate between *Ophioglossum* and *Helminthostachys*. The roots are, at any rate in the latter Fern, more numerous than the leaves, and are fleshy brittle structures like those so characteristic of the other Ophioglossaceae. They branch freely and monopodially, but the branches in the great majority of cases are either short-lived or rudimentary, and their vestiges form mere warty swellings on the surface of the parent root, which thus appears, on superficial examination, to be unbranched. In this branching of the roots another indication of affinity with *Botrychium* may perhaps be traced.

As in the family generally, root-hairs are absent, although in one or two instances slight outgrowths of the external cells seemed to indicate their possible formation under more favourable conditions. In any case, however, the amount of root-surface available for them is very limited owing to the early cuticularisation of the subjacent cortical layer and the consequent exfoliation of the outermost cells. The roots persist for a considerable number of years, and their bulky cortex serves without doubt to increase the storage-room for the starch which is contained so abundantly by the plant.

No adventitious buds were seen on any roots, although when collecting the plants, these were specially looked for; it would appear therefore that this form of vegetative reproduction which is not uncommon in the other members of the family is absent from, or at least rare in, *Helminthostachys*.

The creeping rhizome forms a normally unbranched structure, and persists for a great number of years, as is shown

[1] Holle, Ueb. Bau u. Entwickl. d. Vegetationsorgane d. Ophioglosseen, Bot. Zeit., 1875.

by the number of leaf-scars on the dorsal surface, each one probably representing an annual period of growth. In one instance, however, two fertile leaves were seen on the same rhizome, one immediately behind the other, thus proving that in *Helminthostachys* there may be, sometimes at any rate, more than one leaf produced in one season's growth.

The rhizome is somewhat compressed dorsiventrally and is fleshy; as it gets older the growing end becomes successively thicker until a certain diameter is reached, after which a fairly constant thickness is maintained. Naturally, different individuals vary somewhat in their degree of development, but about 1 cm. may be taken as the average diameter of a well-developed specimen, though many stems fall short of this.

Although normal branching does not occur, new branches occasionally arise as adventitious buds upon the older parts of a rhizome, and always, so far as we have seen, at a considerable distance from the apex. Indeed it is most common to find the buds connected with portions of stem which are extremely short, and from which the cortical tissues have almost disappeared, indicating perhaps that it is only on detached fragments that the buds arise at all. In fact it was hoped that the small fragments of the parent stem attached to the buds might prove to be tuberous prothallia, but this was not the case. It is of interest to note that the young leaves formed on these adventitious buds are of a simpler form than those found on mature plants.

The leaves arise, as has already been stated, in two rows on the upper surface of the rhizome. The successive leaves are thus inclined first to the right and then to the left of the median line. Each leaf divides, at the upper end of the petiole, into three sterile main branches, which themselves commonly branch still further; and in the case of the fertile leaves, a fourth—sporangiferous—branch springs from the adaxial side of the petiole at the same level, or slightly below it, at which the sterile branches diverge. The leaves are sheathed at their bases by remarkable stipular structures which are comparable with those occurring in other Ophioglossaceae, and the still

unfolded leaves may be easily detected as swellings on the rhizome in front of the one actually unfolded. They are perfectly ensheathed in the stipular structure just alluded to, and the actual arrangement strikingly recalls that met with in *Botrychium*, and, though to a less extent, that in *Ophioglossum* also.

It will be remembered that in *Ophioglossum* there is a funnel-shaped appendage in front of each leaf, which somewhat closely resembles an ochrea, and that this ensheathes all the younger leaves (which repeat the same arrangement) and also the stem-apex. The ochrea in *Ophioglossum* is formed by a ring-like upgrowth of the stem in front of the leaf; this may be regarded as representing a foliar structure the insertion of which completely encloses the stem, as may be seen, for example, in the leaf of the Onion. In *Botrychium*, however, the sheath is somewhat simpler, and more obviously confined to the leaf. Holle[1], who investigated *Botrychium Lunaria* as well as *Ophioglossum*, gives a good account and figures. Our own observations, which are illustrated by the diagram (Pl. XXI. Fig. 3), confirm him in all important respects.

In *Botrychium Lunaria* the leaf-rudiment, as soon as it is differentiated from the apical meristem, grows much faster on one side than it does in length, and thus a sort of hood or flap is produced which extends over the apex, its edges fitting into a corresponding depression situated on the opposite side of the stem. The base of the leaf, though broad, does not surround the stem, and the chamber which is formed by the projecting flap communicates with the exterior through the narrow slit left between the free edge of the hood and the depression into which it fits. The foliar part of the leaf is developed from the back of the leaf-rudiment taken as a whole, and therefore the flap comes to lie as a sort of adaxial appendage of it. These relations are very difficult to make out in the older leaves, as the changes produced by growth and extension of the tissues tend to obscure them. Thus the blade-portion of the older, but still enfolded, leaves

[1] Holle, loc. cit.

are found to lie in a cavity in the petiole of an older one. Ultimately, when a leaf unfolds, it ruptures the sheath provided for it by the base and petiole of the older leaf.

The same arrangement in all essentials is met with in *Helminthostachys*, and the chief points of difference are such as are readily referable to its dorsiventral habit as opposed to the radial structure characteristic of *Botrychium*. Each leaf as it arises becomes very early differentiated into a basal portion and an upper part, at first represented by the blade and sporangial regions, the petiole only becoming visible as an intercalated structure at a much later epoch. A broad flap grows out from the adaxial face of the leaf-base, and it has a broad insertion on the stem also, there being direct continuity of the stem- and flap-tissues on the edges near the leaf, and thus the stipule comes to partially envelop the stem, leaving however a slit-like orifice into its cavity at the apical end, exactly as in the case with *Botrychium*. The apical edge of the flap grows completely over the stem-apex and meets the tissues of the ventral face of the rhizome on the other side of the growing-point. This is facilitated by the fact that the apex is sunk in a depression and thus is arched over by older tissues on all sides. The Fig. 4 on Pl. XXI clearly explains these relationships of position.

As a still younger leaf arises, it also produces a similar outgrowth under the flap belonging to the next older leaf; hence the apex is enclosed in a series of hoods, separated from each other by narrow air-spaces. One result of this arrangement is that of ensuring protection for the young leaves and the apex of the rhizome from desiccation, whilst at the same time the growing tissues are in direct communication, though only by means of tortuous passages, with the external air. The risk of drought is further lessened not only by the fact that the apertures left between the series of flaps and the stem-tissues are all on the lower side, *i.e.* next the soil, but also by the presence of mucilage-forming hairs which spring from the ends of the flaps and grow into the chambers. These hairs are similar to those found in *Botry-*

chium and especially in *Ophioglossum*, but they are perhaps rather less abundant in *Helminthostachys* than in the two other genera.

As the leaves approach maturity they press on their covering flaps, and finally rupture them ; hence when expanded, the base of the petiole is ensheathed by the torn edges of the ruptured stipule belonging to an older leaf. The stipules are then very clearly seen to be continuous with the stem tissues down the flanks of the rhizome.

The Vascular Skeleton.

The rhizome is traversed by a single axile stele, in the upper face of which narrow triangular foliar gaps occur at intervals, one in front of each leaf-trace ; but in the remaining portion the vascular cylinder forms a ring enclosing a central core of parenchyma in the older stems. It is, however, almost solid in the youngest rhizomes. In general character the stele recalls that of *Botrychium* to which it approximates more in character than to that of *Ophioglossum*, with the possible exception of *O. Bergianum*, some specimens of which, to judge from one of Bower's figures [1] of a transverse section of the stem, resemble our stem as seen in transverse section ; but this aspect of the matter will be considered by-and-by.

In accordance with the position of the leaves on the rhizome, the leaf-traces arise right and left of the middle line on the upper surface of the stele. The trace to each leaf is single, at least at its origin, and it runs for some little distance in the cortex, only diverging from the main stele at a very narrow angle before it obviously branches, and these smaller strands turn sharply upwards into the petiole. We say *obviously branches*, because, as will be seen later, the trace, though apparently single, in reality consists already of at least two separate strands which can be readily distinguished from each other.

In the character of its leaf-trace, *Helminthostachys* more

[1] Bower, Studies in the Morphology of Spore-producing Members : II. Ophioglossaceae. London, 1896.

closely approaches the Ophioglosseae than any of the other groups of Ferns; and although it in some respects resembles some of the Lycopods, especially certain Selaginellas, it differs from them inasmuch as the gap in front of the trace is a foliar and not a ramular one as in all those Selaginellas concerning which we have been able to obtain information [1]. For a short interval behind the place of exit of each leaf-trace, the gap of the preceding one has closed up, and hence a section of the stele in this region exhibits the form of a complete ring of vascular tissue enclosing a central strand of parenchyma.

When the foliar stele has traversed the cortex of the rhizome and passes up towards the base of the leaf, it divides rapidly into about seven or eight (sometimes however fewer) bundles by a series of dichotomous branchings. These run up into the petiole of the leaf and form the ring of bundles which are situated near its periphery. There is some anastomosis between them as they continue their course through the leaf-stalk up to the level where the leaf branches. Just below this spot a very complex series of anastomoses accompanied by a redistribution of the bundles occurs which results in a tolerably regular vascular supply being given off to each branch of the leaf. There are three of these branches in a sterile, and an additional adaxial sporangifervus one in a fertile, leaf. Each midrib of the three vegetative leaf-branches receives typically four bundles, of which the two outer are the stronger. These soon break up again, and it appeared useless to attempt to trace them further. The fertile leaf segment also receives four (or five) bundles, but these are clearly separated from the strands of the barren segments at some little distance from the point of branching, though at this spot there are again cross-connexions visible between the various bundles. The transverse sections figured in Pl. XXI. Figs. 7, 8, 8 a, will serve to convey a general idea of the distribution of the vascular strands in this region.

[1] See Harvey Gibson, Contributions towards a knowledge of the Anatomy of the Genus Selaginella, Annals of Botany, vol. viii.

As regards the steles which are given off to the roots, one fact becomes immediately prominent, viz. that there is no regular connexion between them and the leaf-traces. They spring from the lower part of the flanks or from the ventral surface of the stele, and never arise in connexion with either the leaf-traces or the margin of the foliar gaps. Their irregular distribution is equalled by the lack of uniformity in their number; sometimes two or even three root-strands are found to be given off between a single pair of leaves.

This irregularity was pointed out by Prantl[1], who rightly, as we think, criticized Holle's attempt to show that a definite relation could be made out in *Botrychium*. At least we have not succeeded in confirming Holle's account, and moreover his own figures do not seem to lend much support to his contention.

The rhizome-stele itself can be followed up to the apex of the stem beyond the youngest leaves and roots, and hence it is cauline in nature, and differs in this respect from what is stated by Holle to be true for other Ophioglosseae. There can, however, be no doubt as to the condition of matters in *Helminthostachys*, for the dorsiventral structure obviates some of the difficulties which meet one in dealing with radial stems. The vascular tissue can be traced on both the upper and the lower surface (from which leaves are absent) quite up to the apical meristem, and, as will be seen later, the actual differentiation of tissue begins to set in just behind the apex.

Histology.

The Rhizome. A transverse section of the creeping stem shows an ill-defined dark-coloured peripheral band composed of a few cell-layers in thickness which serves to protect the inner tissues. On the upper surface, and starting in connexion with the bases of the cast-off leaves, the cells grow out somewhat and undergo periclinal divisions resulting in the formation of an irregular layer resembling cork. But this formation

[1] Prantl, loc. cit.

does not extend round the rhizome, and in this respect it differs from the Ophioglosseae as described by Holle, but the difference is probably to be ascribed, as are other features already alluded to, to the dorsiventral character of *Helminthostachys*, the spots where cork-formation originate not being, in this Fern, distributed at intervals around the stem but confined to one longitudinal strip of its surface.

Beneath the external protective layers, there is to be seen a very well-developed cortical parenchyma, the cells of which are gorged with starch, at least at the season at which our plants were gathered. The cells are rounded in form, and thus necessarily leave intercellular spaces between each other. Their walls recall those of other Ophioglosseae in their pale colour, in their well-developed pits, and in their somewhat gelatinous appearance; though in respect of this last character they are far less striking than are the cortical walls of *e.g. Botrychium Lunaria.*

The pits are remarkable, forming as they do not merely simple depressions in the walls, but rather being massed together in areas like the pores of a sieve-plate. Indeed they may be fairly termed pitted areolae, and they do not differ essentially from the actual sieve-plates of the sieve-tubes themselves in this plant. The areolae are of various forms, circular or oval, or they may be less regular in contour. They contain also a varying number of depressions or pits which are separated from each other by bars or ribs of thickening which thus divide up each areola into its thickened and unthickened portions. Of course the pits on either side of the middle lamella of two adjacent cells correspond, and we have in a few favourable cases been able to demonstrate fine protoplasmic continuity through the pit-membranes.

The cortex of this plant offers a fine example of the occurrence in the intercellular spaces of the substance identified by Russow, Terletzki, and others, as protoplasm, but determined by Mangin to consist of pectose-compounds.

The cortical parenchyma is limited internally by the outer endodermis, which is extremely well marked in *Helmintho-*

stachys. It cannot, however, be referred to a definite cell-band which can in any way be identified as 'the innermost layer of the cortex,' for the characteristic markings sometimes extend over two bands of cells, and at other times they extend also to cells which obviously appertain to still more external cortical layers. Thus, in this plant at any rate the endodermis, whilst retaining its physiological importance, lacks the definiteness which alone can give it that degree of morphological importance which is ascribed to it by many investigators. The cells composing the endodermis are strongly cuticularised over their radial and often over part of their tangential walls, a fact which is abundantly demonstrated after treatment with strong sulphuric acid. The pitting of the walls is also very characteristic, and gives a peculiar striped appearance to the cells.

Within the endodermis a somewhat broad layer of parenchymatous cells which are somewhat elongated in form can be readily seen, and this forms the pericycle of the stele. Immediately within this band the sieve-tubes are disposed. They form a very easily recognizable layer, both on account of their form and especially by reason of their thickened cellulose walls. In respect of their curious walls they resemble other Ophioglosseae as described by Poirault[1]; and while rather exaggerating the peculiarities observed by him for *Ophioglossum* and *Botrychium*, they plainly belong to the same type. When seen in longitudinal section they are found to consist of elements perhaps about eight or ten times as long as their diameter, and of a somewhat irregular form. The reason for this latter peculiarity will appear subsequently. Their walls, are, as has been said, very thick, and this is due to the swelling of the middle layers of the walls in which pectic substances are abundantly present. The sieve-areas are freely distributed over the lateral walls, and they occur, though not so frequently, on the end walls also, where two elements are in contact. The sieve-areas are very well marked

[1] Poirault, Recherches anatomiques sur les Cryptogames Vasculaires, Ann. Sci. Nat. (Bot.), sér. VII, t. XVIII.

as depressions in the swollen walls, and they are divided up into still smaller areas or pits, much as in the case of the pits in the cortical parenchyma alluded to above. Continuity of the protoplasmic contents of two contiguous sieve-tubes can be fairly easily demonstrated, and it is then seen that the membranes of the individual pits (which collectively make up a plate) are pierced by very fine holes through which the protoplasmic strands pass across. When the sieve-tubes are isolated, by macerating the tissues, they are seen to possess a firm internal membrane which is continuous with that forming the pits, and the swelling is proved to be due to a substance which dissolves away in the Schultze's fluid employed to separate the cells. The pits then appear of course as protrusions of the cell-wall (see Fig. 20, Pl. XXIII) since the intervening substance has been cleared away in solution.

In many instances it seemed almost certain that there was a sieve-connexion, not only between the sieve-tubes themselves, but also between the tubes and the parenchyma of the phloem. If our observations on this point are correct it would seem that the parenchyma in this Fern can function as companion-cells, as has been stated by some investigators for other Ferns. But there is no evidence here of the (possibly) functional companion-cells having been derived together with the sieve-tube, from a common mother-cell, and therefore the arrangement is one of physiological interest only. The sieve-tubes are differentiated and easily recognizable immediately behind the apical meristem; they are separated by a rather broad band of parenchyma from the xylem of the stele.

The wood of this stem presents several points of interest. In the first place the protoxylem-elements are not developed on the inner side of the ring, as in the other Ophioglosseae, nor at its outer side as in those Selaginellas[1] which, *e.g. S. laevigata, var. Lyallii*, bear some resemblance to *Helminthostachys* in respect of their steles. They are differentiated

[1] Harvey Gibson, loc. cit.

in a zone occupying a position about midway between the interior and the periphery of the xylem, although they are not very regular in their exact locality. The xylem then belongs to the Mesarch category. This is of especial interest considering the weight which has been attached to this peculiarity in certain groups of plants in which it characteristically occurs. But it is clear that, as with almost any other single character, it is possible to overstrain its importance, and as a matter of fact the relative position of the protoxylem is seen to vary in some cases within quite narrow limits. Thus in the Ophioglosseae so far as at present known, mesarch strands are confined to *Helminthostachys*, the rest of the species investigated possessing strictly endarch xylem. We have ourselves studied *Ophioglossum vulgatum*, not only in material prepared by ourselves but also in other excellent serial sections kindly lent us by Mr. Boodle, and we find that the protoxylem is invariably endarch in development. The same arrangement also occurs, apparently exclusively, in *Botrychium Lunaria* [1] as well as in the other species of which the anatomy is known.

Again, in the Lycopodineae, the bundles are typically exarch, the protoxylem being placed peripherally, but Harvey Gibson [2] has shown that in *Selaginella spinosa* the protoxylem is centrally situated. And again, as Bower [3] has pointed out, the xylem in the peduncle of *Phylloglossum* is mesarch, just as we find it to be in *Helminthostachys*. The case of *Phylloglossum* is interesting, since it closely resembles in the general outline of its xylem that of the Fern now under discussion, and at the same time a similarly formed xylem-strand is met with in some specimens of *Ophioglossum Bergianum* [4]; in the last named plant, however, the xylem is endarch as in the rest of the genus. Our own observations confirm this. And, to quote *Selaginella* once more, the species, *S. laevigata, var. Lyallii*, which bears superficially a close resemblance to *Helminthostachys* in the structure of its vascular

[1] See Russow, Vergl. Unters. d. Leitbündel-Kryptogamen.
[2] Loc. cit. [3] Bower, loc. cit. [4] Loc. cit.

strand, differs entirely from it in the fact that the xylem is exarch as in the majority of the other species of *Selaginella*. Again, the fact need not be lost sight of that mesarchy seems to have prevailed extensively in those fossil-genera which have been grouped together under the name of Cycadofilices; and moreover it is tolerably characteristic of many Leptosporangiate Ferns of the present day[1]. The occurrence, however, of the same mesarch character in *Helminthostachys*, on the one hand and in *Phylloglossum*, which is almost generally admitted to be a primitive Lycopod, on the other, may possibly indicate something more than a mere coincidence. Amongst the Ophioglosseae, *Helminthostachys* may be regarded as possessing some claims to be regarded as a representative of an old stock: for quite apart from the wide geographical distribution of the monotypic genus, it is, in respect of the stipular character of its leaves, in reality simpler than *Ophioglossum*, and perhaps even than *Botrychium* also. It is however not very easy to estimate the weight to be attached to this feature when the dorsiventral habit of the plant is borne in mind.

The metaxylem consists of tracheids with characteristic bordered pits of an oval or even almost circular form, but other elements also occur in which the pits assimilate to the more scalariform type met with in other members of the family. The Ophioglosseae show amongst themselves some considerable deviation from the more regular scalariform type met with in the majority of Ferns, but so far as we are aware, *Helminthostachys* goes considerably beyond them in this character; and it may be worth while recalling the fact that, judging from the fossil remains, it resembles in this respect many of the extinct Vascular Cryptogams, *e.g.* some of the Cycadofilices. Of course no argument is here drawn from this, either as to a possible relationship of the groups just mentioned to each other, or to *Helminthostachys* itself, but it seems worth while recalling the evident fact that this

[1] Mr. Seward has kindly drawn our attention to the fact that *Lygodium*, amongst the true Ferns, possesses exarch xylem in the stem.

form of tracheid marking was a wide-spread one amongst these ancient types of Vascular Cryptogams, whereas in another alliance, that of the Calamites, &c., it is conspicuous by its absence.

The forms of the tracheids themselves are often highly irregular, recalling those figured by Bucherer in the rhizome of *Dioscorea*[1]. Some of them may branch or fork, others exhibit nodular outgrowths. A few of the shapes commonly to be met with are sketched in Fig. 17 (Pl. XXII). The reason for the remarkable outlines thus exhibited seems to be most easily sought in the fact that the individual tracheids continue to grow long after the stem has ceased to elongate, and hence a considerable amount of sliding growth obtains. As will presently be seen, the differentiation af the procambial strand proceeds with unusual slowness, and consequently the last-formed tracheids have to accommodate themselves to the possibilities of room as best they may. One result of all this is that many of them pursue a very tortuous path ; and that hence, in a transverse section of the rhizome it is possible to meet with elements which have been cut almost longitudinally, and this is especially the case as one examines older parts of the stem. Sliding growth is doubtless facilitated by the apparently gelatinous or pectosic character of the middle lamella. The tracheids are grouped together in bundles, each mass being separated by parenchymatous tissue, which in some instances can be traced as a continuous band extending from the cortex to the axile strand of parenchyma lying within the xylem ring.

Passing inwards from the xylem, it is seen that there is no phloem on the inner surface of the wood, and this is also a feature which *Helminthostachys* shares in common with other Ophioglossaceae. Even when the continuity of the vascular ring is interrupted by a foliar gap, the phloem stops short abruptly at its margins. A remarkable feature in this Fern is the occurrence of an internal endodermis which often abuts directly on the xylem-elements, though it is sometimes

[1] Bibliotheca Botanica, vol. iii.

separated from them by one or more layers of parenchyma. It is apparently connected with the foliar gaps, for in a few instances the tissue in question was traced as a direct continuation of the outer endodermis, being connected with it over the margin of the gap. This direct connexion can, however, by no means always be made out, even when the gap is a wide one, the outer ring ending at the margins of the gap or merely bending out over the leaf-trace, but not dipping into the interior of the stele. In any case, however, the distribution of the inner ring is irregular in most places; often the characteristic markings can only be observed on groups of cells which are separated from the next part of the band by normal parenchyma from which the cuticularisation is absent. It is however very constantly to be met with inside the stele at a spot where a vascular strand to a root is emitted. In fact the inner endodermis may be roughly compared to a sort of irregular net-work on the inner side of the xylem. Its existence is of special interest, as Poirault[1] mentions and figures an inner endodermis for the *young* stem of *Botrychium*, but states that it becomes indistinguishable in the larger stems of older plants. In *Helminthostachys*, however, precisely the reverse would appear to obtain; for whilst it is extremely obvious in all the older rhizomes which we examined it is certainly not so in the young stems; although a slight indication of radial thickening was seen here and there it did not answer to the tests of either stains or treatment with sulphuric acid. On the whole it seems clear that its occurrence is secondary; and as already stated, is connected with the opening of the strand where the leaf-traces originate, just as was ascertained by Leclerc du Sablon[2] for *Osmunda* and *Pteris*, save that in these plants the case is still further complicated by the presence of internal phloem, and that in *Helminthostachys* the endodermis does not, as apparently in them, line the inner part of the stele as a continuous sheet of tissue.

[1] Poirault, loc. cit.

[2] Leclerc du Sablon, Recherches anat. sur la formation de la tige des fougères. Ann. Sci. Nat. (Bot.), sér. VII, t. XI.

In the stems of the youngest plants which we were able to obtain it was seen that the axile strand of parenchyma which we may term the pith, is but scantily developed, xylem-elements extending into the centre. But nevertheless it is obvious (see Fig. 23, Pl. XXIII) that a pith is beginning to form by the increased differentiation of parenchyma at the expense of the tracheidal tissue. It can hardly be doubted that in still younger plants the xylem would form a more or less solid axile core in the stele just as is the case with other Ferns.

As regards the question of the existence of secondary thickening in the stem of *Helminthostachys*, we have come to the conclusion that it is quite absent. But owing to the slow differentiation of the procambial strand, already alluded to, it is easy to mistake the late differentiation of tissues which are really primary for a secondary formation. In spite of careful search we did not find any satisfactory evidence for anything like a secondary cambial division and subsequent new formation of fresh tissues, such as goes on in the stem of species of *Botrychium*, in which secondary tissue-production is a characteristic feature. There appears to be a slight cambial activity in *Ophioglossum*[1], but it would seem to be quite wanting, at least normally, in *Helminthostachys*.

Following the tissues of the rhizome to the apex, the vascular strand is seen to terminate immediately behind the somewhat sharply marked meristem. The actual apex is sunk in a depression formed by the over-arching of the youngest leaves on the dorsal and lateral edges, whilst on the ventral edge the tissues of the stem itself contribute to the same end. The cells which occupy the apical depression are all elongated at right angles to the surface, and it is extremely difficult to identify any one cell as the parent of the whole. But we succeeded in more than one instance in recognizing one cell as the probable common ancestor of the rest, though even in the most favourable cases it is rather difficult to be quite certain. The character of the protoplasm and nucleus affords a valuable clue, whilst the argument drawn from the

[1] Boodle, Anatomy of Ophioglosseae, p. 386 of the present number.

arrangement of the cells themselves is not so conclusive as is commonly the case. This latter circumstance is doubtless to be attributed to the slowness with which the new meristem-tissues are formed, coupled perhaps with the relative rapidity with which the tissues are differentiated; consequently one might perhaps expect to find that the slowly developing meristem would be affected by these conditions, and show some variation in its disposition which would depend in a measure on the time which had elapsed since the last segmentation. And indeed from whatever cause, we find this to be a fact. The apical cell was recognized with probable certainty in one case, when its form was seen to be triangular; in another, as Rostowzew appears to have found for *Ophioglossum*[1], it seemed to have the form of a truncated prism. But these less usual forms are not very difficult to reconcile with the more common type of a tetrahedral cell, when the long protracted growth of the segment-cells and the resulting disturbance of the space relations are borne in mind.

Following the development of the tissues, the vascular region is early distinguished both from the pith and the cortex, owing to the numerous tangential divisions and the somewhat more regular arrangement of its cells, which are smaller than those of the adjacent tissues. The phloem is differentiated very early, and shows the characteristically swollen walls between the numerous sieve areas almost up to the apex of the strand. The lignified elements of the xylem are slow in differentiating. A number of isolated protoxylem-groups, or even single elements, are dotted about in a manner which at once betrays the mesarch character of the strand as a whole; but the formation of the full complement of tracheids is only effected at a considerable distance behind the apex. But these are all present *ab initio* as rudimentary structures; there is no evidence of any new ones arising as the result of a secondary cambial activity.

[1] Rostowzew, Ophioglosseae, Moscow, 1892 (Russian). Figs. 8 and 10 in the text.

The Foliar Bundles.

The leaf-trace-strand as it leaves the stele is seen to be enfolded, though sometimes only on its outer and lateral faces, by an endodermis which is derived in the first instance from, and is in any case directly continuous with, that of the stele itself. As the trace bends out the stele is disconnected on one side first, and the foliar strand hinges on the still unbroken side in a somewhat characteristic fashion. It recalls that of *Gleichenia hecistophylla* as figured by Poirault [1], although the resemblance between the two cases does not extend to points of detail.

After the trace has ceased to be connected with the parent stele, the endodermis is usually found to completely surround it. The strand soon betrays evidence that it consists of two main bundles. At first the protoxylem is in an almost central position conformably with the position which it occupied when the trace still formed a sector of the stele; but the parenchyma soon becomes more markedly aggregated on the inner side of the central part of the trace, and the protoxylem is then grouped in two main portions just outside this. Finally this parenchyma, which at first looks like a rather irregular pith, separates the xylem on the side directed towards the stele of the stem, and at the same time a similar division is effected on the opposite side; and thus, by a gradual series of transitions, the trace comes to consist of two main collateral bundles with their protoxylem on the inner side of their chief mass of wood [2]. However, it may be observed that traces of the original mesarch structure can often still be detected (and this is true of bundles here and there in the petiole of the leaf itself), owing to the formation of a very few elements which arise on the inner side of the protoxylem.

[1] Poirault, loc. cit., p. 173.

[2] It is of interest to note in this connexion that the leaf-traces of *Botrychium virginianum* belong, according to Jeffrey (Mem. of the Bost. Soc. of Nat. Hist., vol. 5, p. 160), to the concentric type.

Still the general endarch tendency in the leaf-bundles is very obvious.

The xylem and phloem of the two bundles, which are still united into one strand, are seen to become still more divided up by parenchyma which runs, roughly speaking, in the radial direction across them, and thus paves the way for the sudden branching of the strand into its petiolar bundles just under the insertion of the leaf. This division occurs whilst the trace is still in the cortex, and the separating bundles diverge and pass sharply up into the petiole, where they are arranged round its periphery. A certain amount of anastomosis between the bundles in the petiole occurs, especially, as already stated, below the place where the leaf branches.

Each bundle possesses phloem on its outer face, and parenchyma on the inner side of the xylem. This parenchyma belongs to the bundle and may easily be mistaken for phloem, but a careful study has convinced us that these bundles are not concentric but truly collateral. The protoxylem consists of a narrow band of elements on the inner side of the xylem, and they are often seen to border on a band of peculiar parenchyma similar to that which Russow [1] termed 'Lücken-parenchymstreifen.'

The cells of this tissue are large and thin-walled. The transverse walls are clearly seen in longitudinal section, and the general impression which the observer acquires is that he is dealing with some kind of a gland.

We endeavoured to ascertain whether there was any obvious difference between the proportion of phloem in the bundles distributed to the fertile and barren leaf-branches respectively; but we are unable to detect any, although on *a priori* grounds it appeared not improbable that, having regard to the abundant sporangia, and consequent drain on plastic materials, such a difference might be found.

As regards the venation of the blade of the leaf in *Helminthostachys,* it conforms in a general way with the type

[1] Russow, loc. cit., p. 104.

characteristic of *Neuropteris*. This is in striking contrast with the venation of *Ophioglossum*, in which, as is well known, the venation is remarkably reticulate. On the other hand, it strongly resembles that in *Botrychium*, which also belongs to the same type. Each leaf-lobe in *Helminthostachys* is traversed throughout its length by a stout midrib from which are given off on either side, a number of lateral bundles which fork once, or sometimes twice, and then run out in an approximately parallel fashion to the margin of the leaf. In *Botrychium*, however, the course of these bundles is not quite so markedly parallel, a fact which may perhaps be correlated with the more trapezoid form of the pinna. The fact that it also recalls the appearance of the venation of *Angiopteris*, may be urged as a ground for not attaching too great importance to this similarity in the venation of *Helminthostachys* and *Botrychium*, nor should we have dwelt on it at all had it not been supported by many other and, as we think, weightier characters.

The minute structure of the leaf-blade is sufficiently illustrated by the Fig. 18 on Pl. XXIII. The cells of the upper and lower epidermis both contain chlorophyll, and the stomata are confined to, or are at any rate far more abundant on the lower than on the upper surface. Each bundle is enclosed in a sheath of elongated parenchymatous cells on to which the assimilating tissues converge.

We did not attempt to follow the development of the sporangia, as this has been recently done by Bower in his monograph on the Ophioglosseae, and our own observations do not contribute any new facts to his excellent account.

The Roots.

The roots originate from the stem at a very short distance behind the apex, and their vascular tissues are united to those of the rhizome by a somewhat broad insertion. The pith of the root is in connexion with that of the stele of the stem, but the communication is not a very direct one, the

xylem-parenchyma, which is dilated somewhat in this region, serving as the link between them.

In transverse section the roots are seen to be most commonly hexarch, though different roots vary in this respect from tetrarch (rare) to heptarch. In respect of the number of its xylem- and phloem-groups in the root, *Helminthostachys* stands somewhat isolated from the rest of the Ophioglosseae, in which the number is much smaller; whilst on the other hand it approximates more to the Lycopodiaceous type, many of the plants in this group possessing complex root-strands. The Marattiaceae, which are likewise noted for the numerous bundles in their roots, may also be kept in mind, although, as will have already been rendered evident, in nearly all essential features there exists no real similarity between them and *Helminthostachys*. There is a very well-developed central pith, and the sieve-tubes in the phloem are easily recognized. Outside the vascular elements there is a sudden transition to a layer of large-celled pericycle, which in its turn is enclosed in a tolerably distinct endodermis.

The bulky cortex consists of rounded parenchymatous cells with prominent intercellular spaces between them. The cells of the outer cortex are more closely united, and in passing to the peripheral layers the intercellular spaces altogether disappear. The outer wall of the peripheral band of the cortex is thickened and of a brown colour, and is distinctly suberised.

In longitudinal section the apex of the root beneath the root-cap is found to be occupied by a single apical cell of triangular form, from which segments are cut off parallel to the four sides in the ordinary way. The segments however cannot be recognized individually save when very young. In this respect, as in so many others, the behaviour of the other Ophioglosseae is once more recalled.

It very often happens that the apical cell is destroyed, either owing to the attack of some organism, or to a degeneration which owes its existence to causes inherent in the root itself. A very complex arrangement then frequently

results, since the youngest segment-cells often begin to proliferate and to fill up the gap left by the dying cell (see Fig. 16). Almost every root available for study was thus affected; and if the process has gone on to any great extent it produces an apex of exceedingly singular structure, in which apparently several apical cells may be present. Indeed at one time we were tempted to compare the root of this plant with that of *Angiopteris*[1] rather than with that of *Botrychium*, which, when in a healthy condition, it much more closely resembles.

The root, as already remarked, branches freely, but we were unable, owing to the absence of suitable stages in our material, to ascertain whether the branches originate from the endodermis or from the pericycle.

Summary.

1. *Helminthostachys* is dorsiventral; the roots which spring from the flanks and lower side of the rhizome bear no definite relation to the leaves which arise from its upper surface.

2. The leaves are provided with stipular appendages which enclose the youngest leaves and pass over the apex of the stem, where they fit into a corresponding depression in the tissues. They more closely resemble the leaf-appendages of *Botrychium* than those of *Ophioglossum*.

3. The rhizome possesses a single stele. The vascular elements in all save the youngest stems enclose an axile pith.

4. There is an inner irregular and an outer well-developed and regular endodermis.

5. The xylem is mesarch in character and differentiates very slowly from the procambial strand.

6. There is no secondary formation of vascular tissue.

7. Adventitious buds may be formed on the stem.

8. The leaf-traces are single at their origin, and divide to the full number of petiolar bundles in the cortex of the rhizome. The bundles are collateral.

[1] Farmer, On the Embryogeny of *Angiopteris evecta*. Ann. of Bot., vol. vi.

9. The apex of both stem and root is provided with an apical cell, but the sequence of segmentation very early becomes unrecognizable.

10. The vascular strand of the root varies from tetrarch to heptarch.

11. The root branches monopodially: but as a rule the lateral roots are abortive, or at any rate do not commonly persist.

In reviewing the observations contained in this paper, with the object of attempting to determine the nearest affinities of *Helminthostachys*, we find that they by no means point in one direction only. This is partly due to the difficulty of establishing a criterion of value which will enable the weight of any given facts to be duly estimated; but it is also partly due to the fact that the plant itself has leanings towards different groups of plants.

Without going so far as to regard *Helminthostachys* as constituting in any way a link between the Ophioglosseae and the Lycopodineae, it can hardly be disputed that whilst in most important respects it is clearly nearly related to the former, it nevertheless in some of its salient peculiarities recalls the generalized type of Lycopodinous structure.

Thus in the character of the stele, whilst it resembles in a measure that of both *Botrychium* and *Ophioglossum Bergianum*, it cannot be doubted that the resemblance with that of *Phylloglossum* is more obvious still, in spite of the absence from it of intercellular spaces in the xylem which occur in the last-named plant. Again, the structure of the root-stele recalls that of some Lycopods far more than it does those of either of the other genera of the Ophioglosseae. But on the other hand, the evidence for intimate and real relationship with the Ophioglosseae is of a much stronger order, as might from the general habit of the plant have been anticipated. In the consistency, texture, and appearance of the stem and roots (apart from the dorsiventrality of the former), it irresistibly recalls *Botrychium* and *Ophioglossum*. Again, in the mode of formation of the stipules it strongly resembles

the former genus, whilst in respect of its foliar bundles it shows more affinity with the latter, especially with *O. pendulum.*

Again, whilst it differs from both in respect of the complexity of its sporangia, it merely forms in this respect a member of a series which can be constructed with *Ophioglossum* as its starting-point, and passing on through *Botrychium.*

It is a matter for regret that material for the study of the prothallium was altogether lacking ; it would be of the greatest interest to know whether the antherozoids are multiciliate (as is probably the case) like those of the Ferns, or whether they exhibit a leaning to the biciliate type characteristic of the Lycopods.

It would seem, however, that a near relation with the true Ferns, whether Eusporangiate or Leptosporangiate, is very doubtful. The stipulate character which might suggest Marattiaceous lines will not bear close examination, as the plants are in reality very different from each other in almost all important respects. Rather it would appear that, like some of the surviving species of *Ophioglossum* and *Botrychium*, *Helminthostachys zeylanica* represents a very old form ; and that, whilst retaining some of the ancient and more generalized characters which also recur sporadically in some members of the Lycopodineae, it has developed along lines sufficiently parallel with the other members of the Ophioglossaceae to justify its being united to this family : nevertheless it has also acquired a strong individuality of its own which tends to obscure almost any single link of relationship on which we may definitely concentrate our attention.

EXPLANATION OF FIGURES IN PLATES XXI–XXIII.

Illustrating Messrs. Farmer and Freeman's paper on *Helminthostachys.*

Fig. 1. Naked-eye drawing of rhizome: *F.* flap, *R.* root, *L.* leaf, *P.* petiole, *L.S.* leaf-scar.

Fig. 2. Adventitiously formed plant. (We owe this drawing to the kindness of Mr. T. G. Hill.)

Fig. 3. Apex of the stem of *Botrychium.*

Fig. 4. ,, ,, *Helminthostachys: a.* stem-apex, *l.* leaf, l_1 *and* l_2 older leaves with their flaps, f_1 and f_2; f_3 the flaps of a still older leaf.

Fig. 5. Vascular skeleton: *L.* leaf-trace, *R.* root-strand, *F.g.* foliar gap.

Fig. 6. Shows the rhizome-stele giving off leaf-trace (*L.T.*) breaking up higher into its petiolar bundles: *R.* root-trace.

Figs. 7, 8, 8*a*. Sections through the petiole just at branching: *S.b.* sterile branches, *F.b.* sporogenous branch, *W.* wing of petiole.

Fig. 9. Median section through stem-apex: *L.* leaf, *F.* flap of older leaf, *S.* lower part of stem, *a.* apical cell.

Fig. 10. Apex of another rhizome: *a.* apical cell, *H.* hairs.

Fig. 11. Transverse section of stele and part of cortex: *O.E.* outer endoderm is, *I.E.* inner endodermis, *S.T.* sieve-tubes.

Fig. 12. Young part of stele just differentiating: *P.X.* protoxylem.

Fig. 13. Longitudinal section of stele: *C.* cortical parenchyma with pits on the walls, *E.* endodermis, *S.T.* sieve-tubes.

Fig. 14. Stele of rhizome, diagrammatic: *R.* root-strands, *L.T.* leaf-trace, *O.E.* outer endodermis, *I.E.* inner endodermis, a discontinuous band.

Fig. 15. Transverse section through leaf-trace, showing the separation of the strand into two halves.

Fig. 16. Root-apex with damaged apical cell. Segments bulging out.

Fig. 17. Abnormal forms of tracheids.

Fig. 18. Transverse section of a leaf-blade.

Fig. 19. Spiral element (double spirals) } after maceration.

Fig. 19. Pitted tracheid (*b*) }

Fig. 20. *a.b.* sieve-tubes macerated.

Photographs.

Fig. 21. Transverse section of rhizome, root-trace to left, foliar gap above.

Fig. 22. Transverse section of rhizome after closing of foliar gap.

Fig. 23. Transverse section of small rhizome showing wood-elements extending to centre of stele.

Fig. 24. Longitudinal apex of stem: *a.* apical cell.

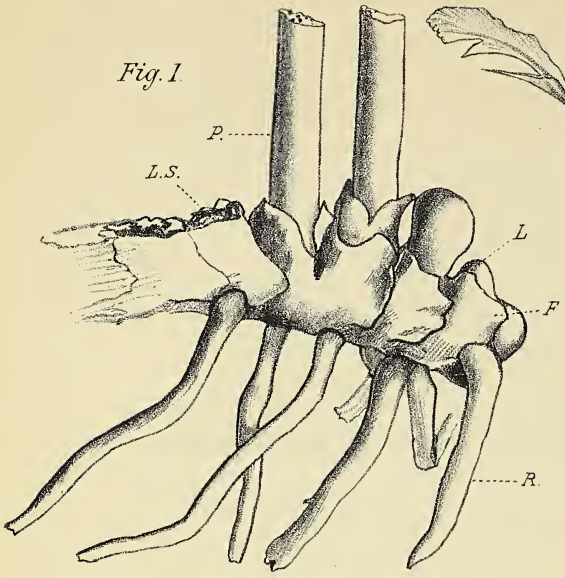
Fig. 25. Longitudinal section of sieve-tubes, showing sieve-pits.

Fig. 26. Transverse section of petiole-bundle.

Fig. 27. Longitudinal section of root-apex: *a.* apical cell.

Fig. 28. Transverse section of root.

Fig. 1.



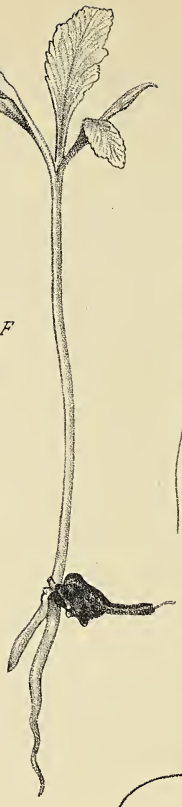


Fig. 2.

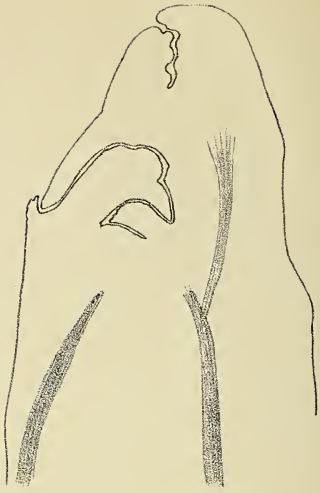


Fig. 3.

Fig. 7.

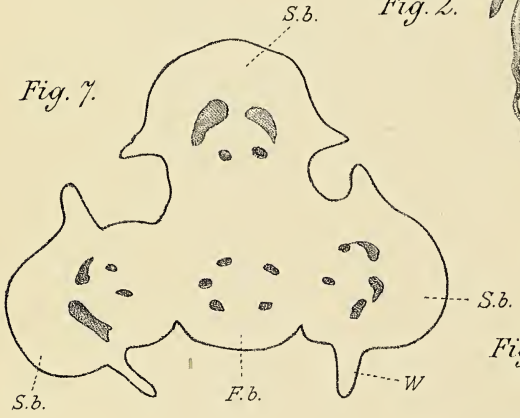
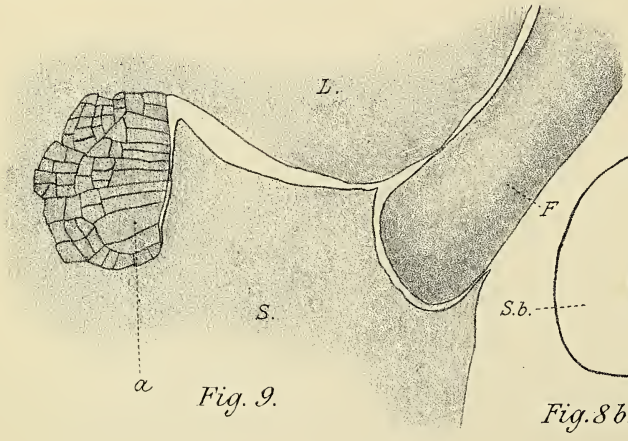


Fig. 8a.



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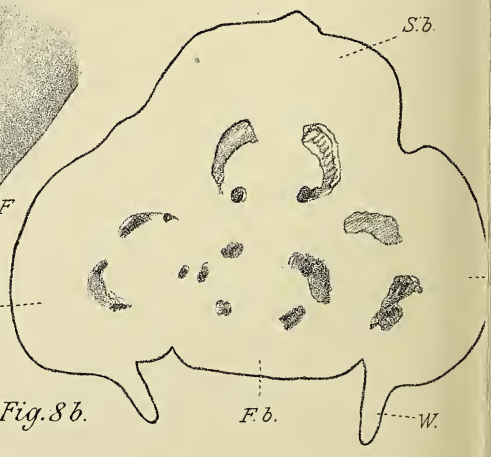

Fig. 8b.

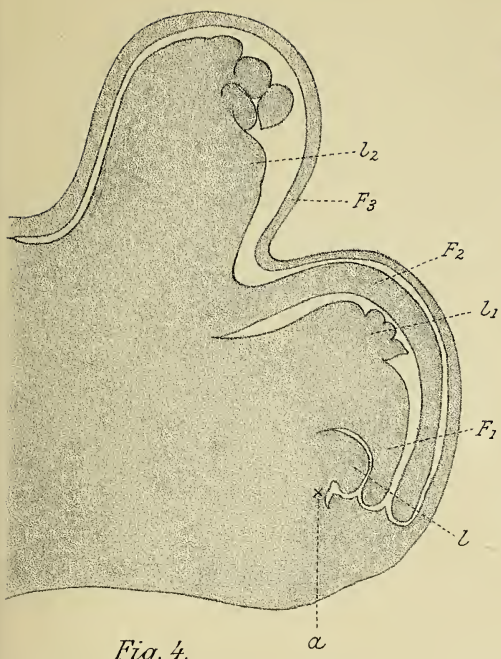

Fig. 4.

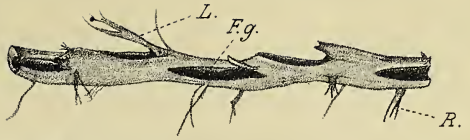

Fig. 5.

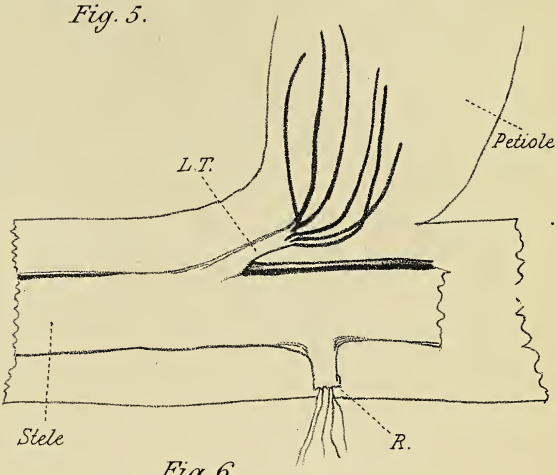

Fig. 6.

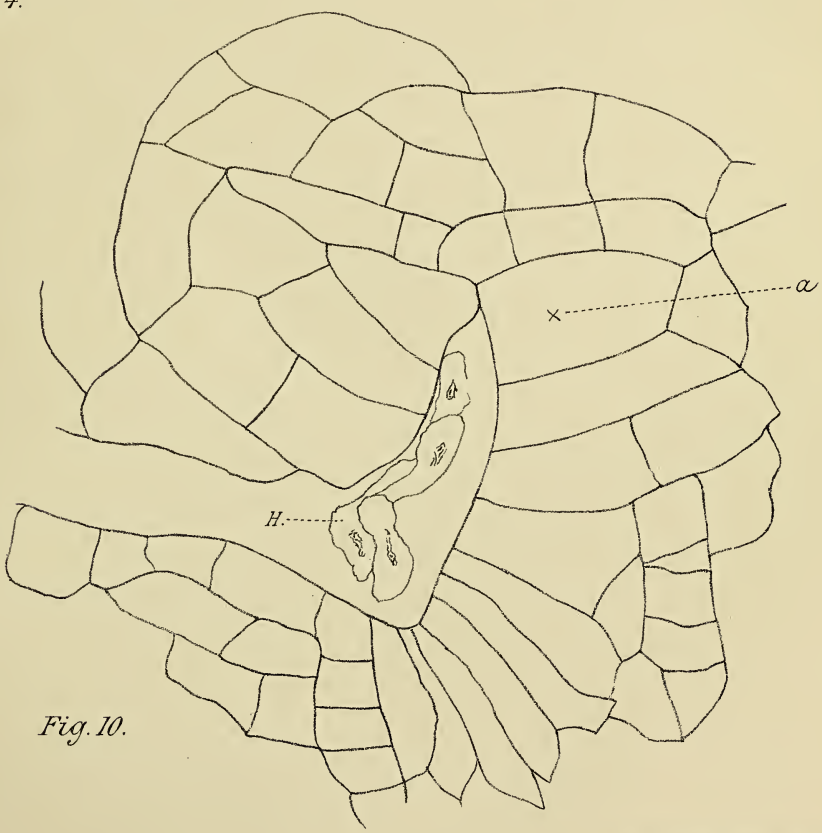

Fig. 10.

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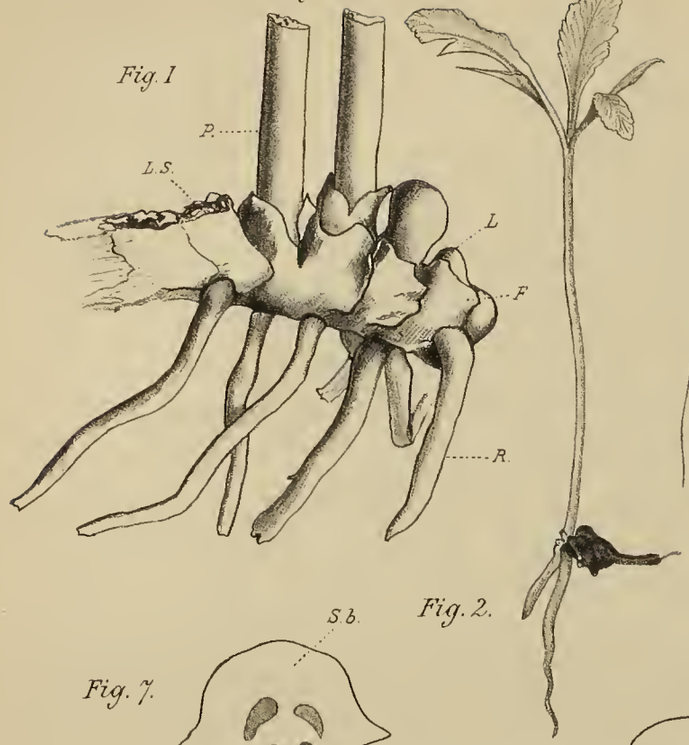

Fig. 1

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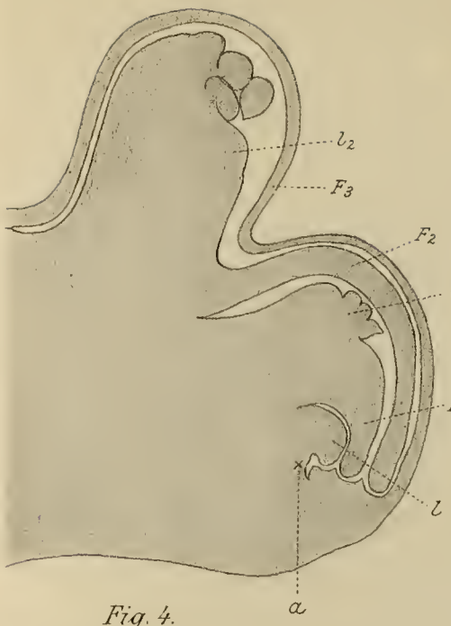

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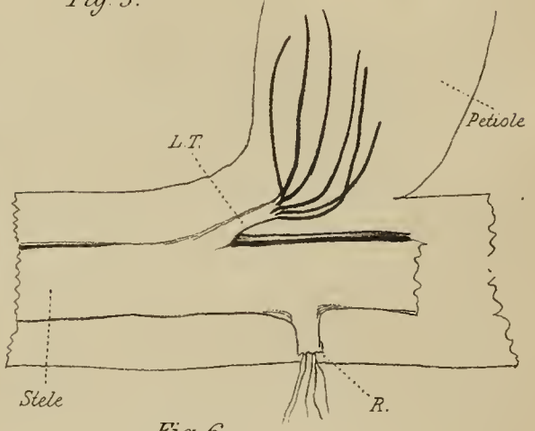

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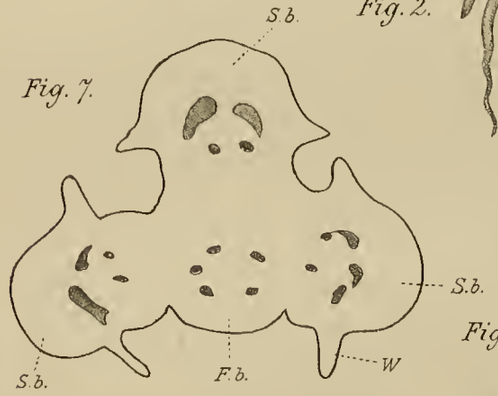

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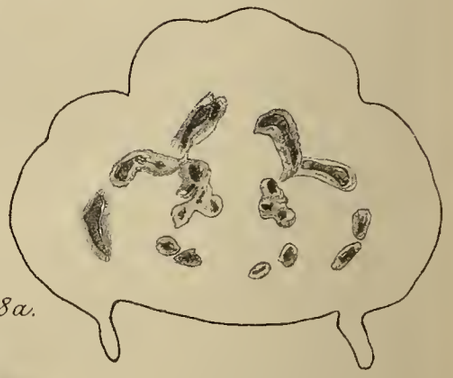
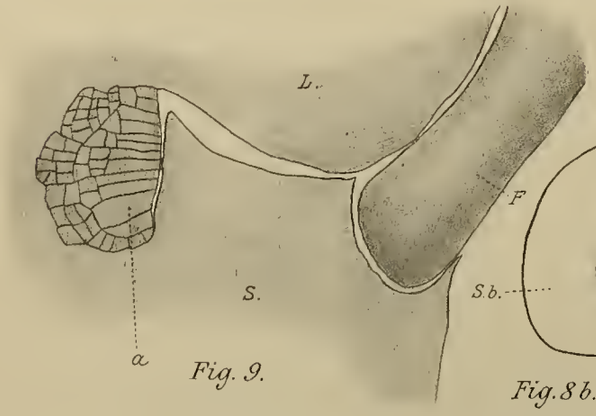


Fig. 8a.



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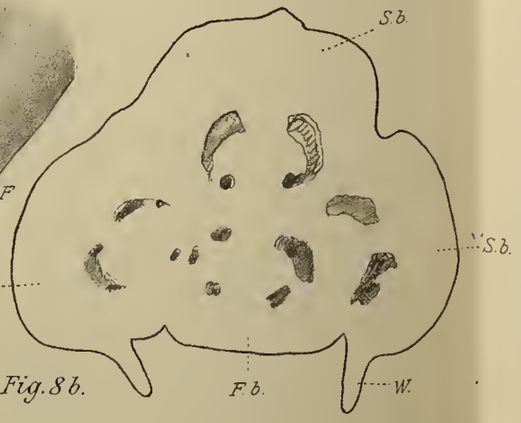

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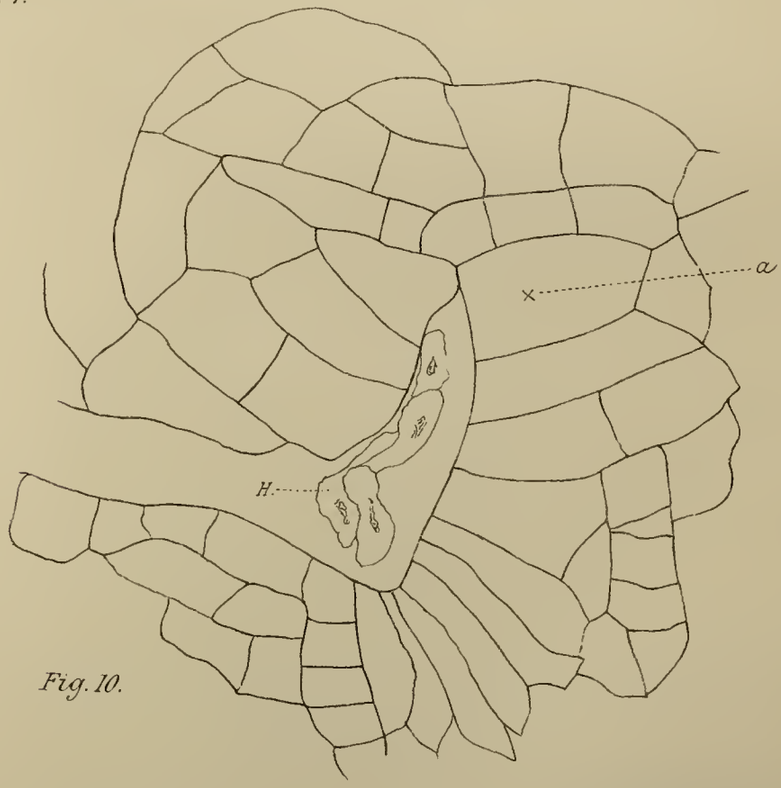

Fig. 10.

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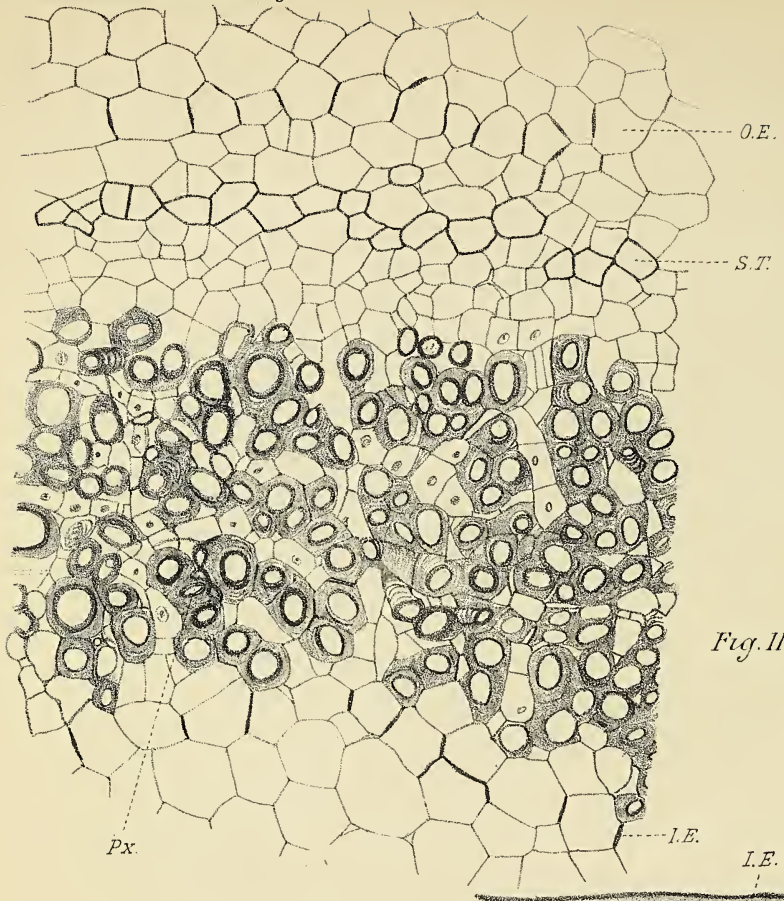

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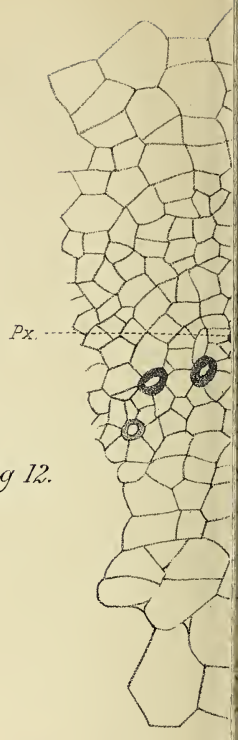

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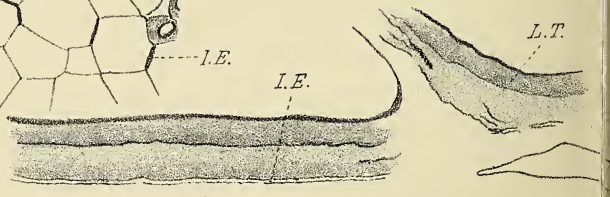
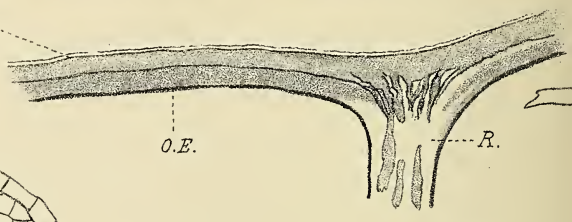


Fig. 14.



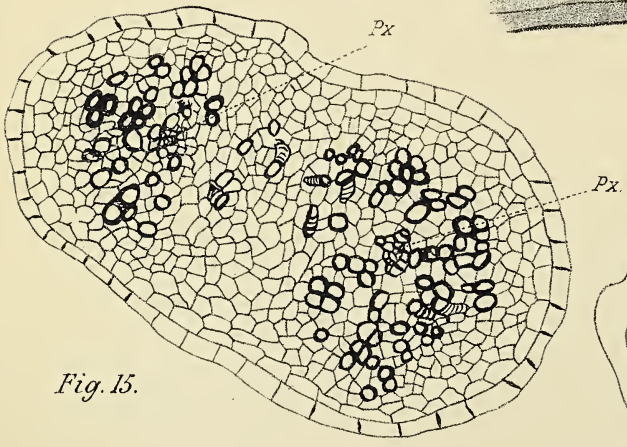


Fig. 15.

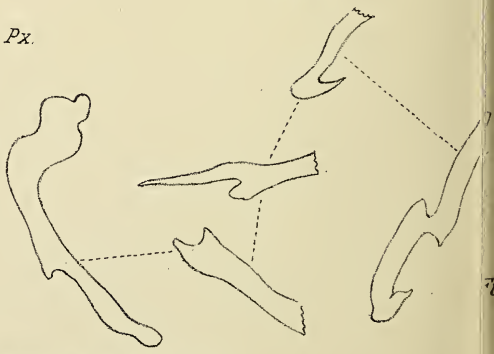
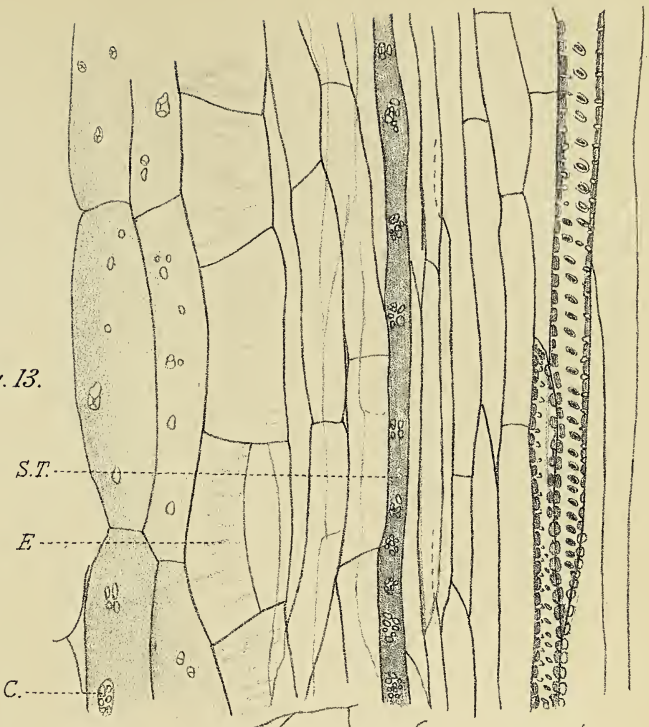


Fig. 13.



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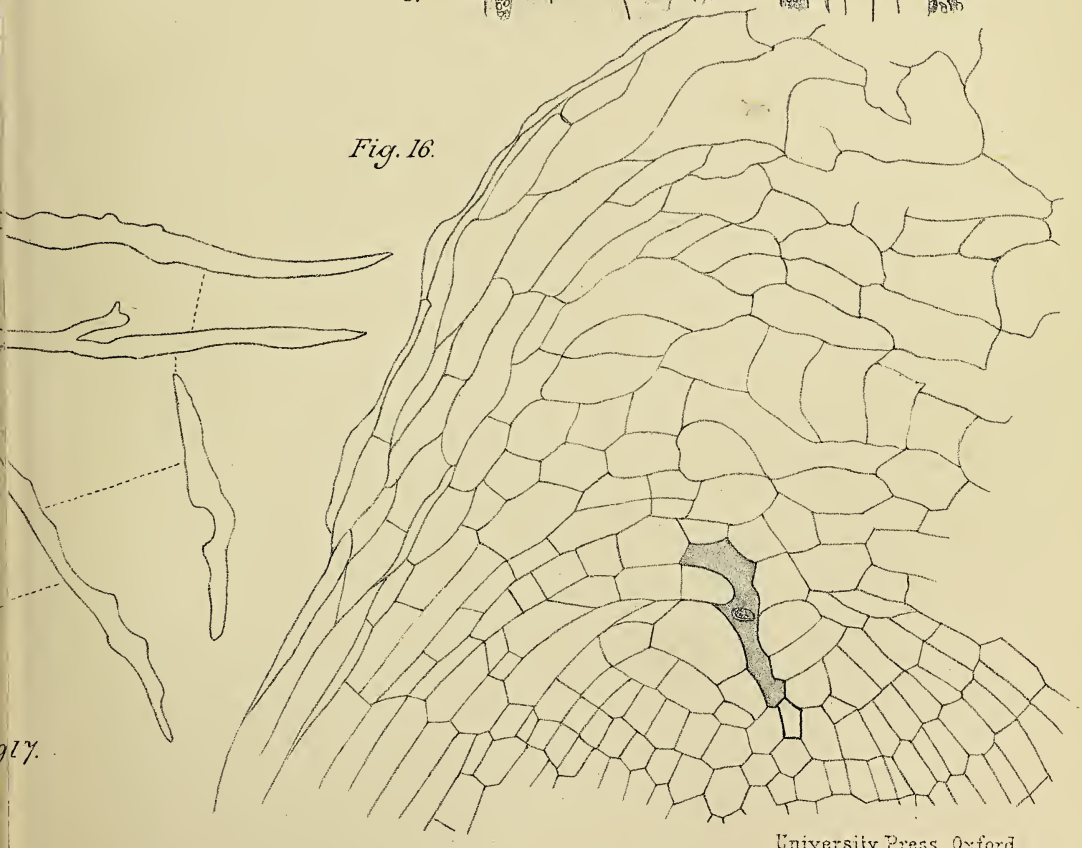

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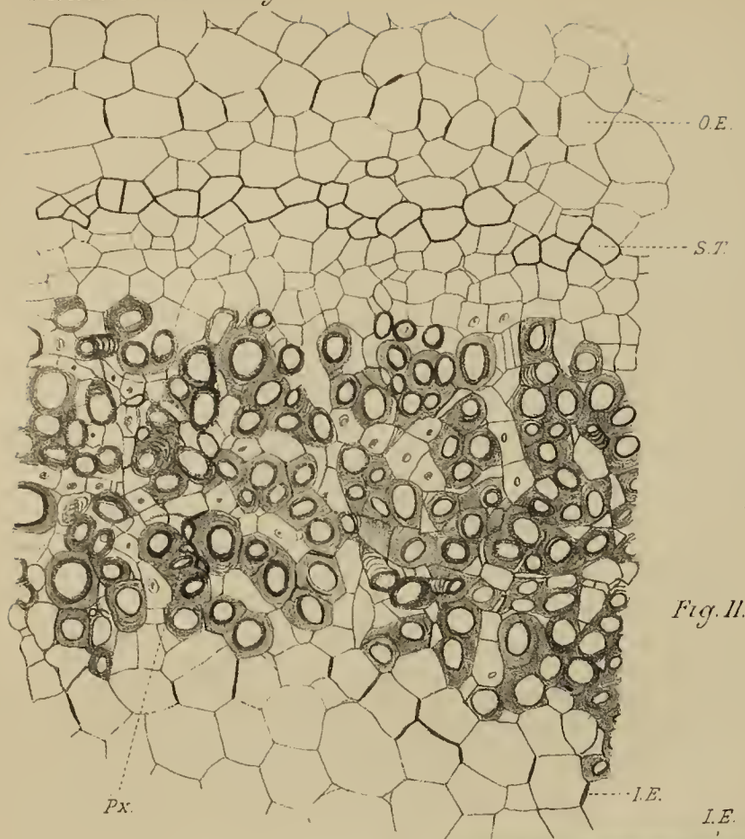

Fig. 11.

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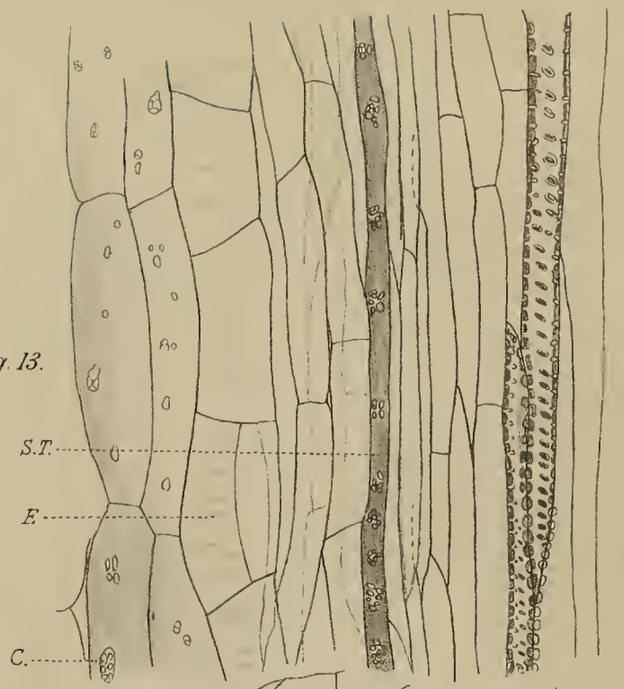


Fig. 13.

Fig. 14.

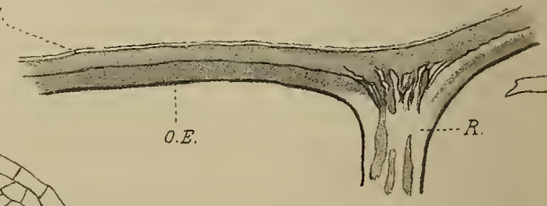


Fig. 15.

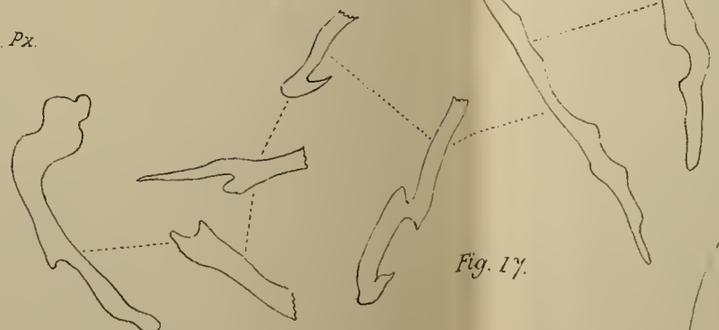
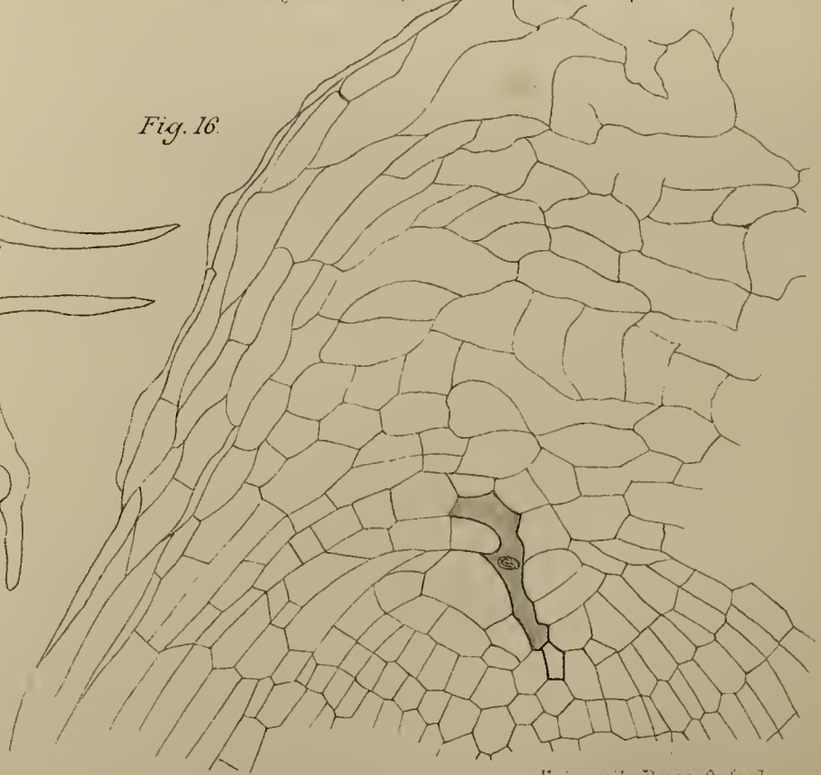


Fig. 17.

Fig. 16.



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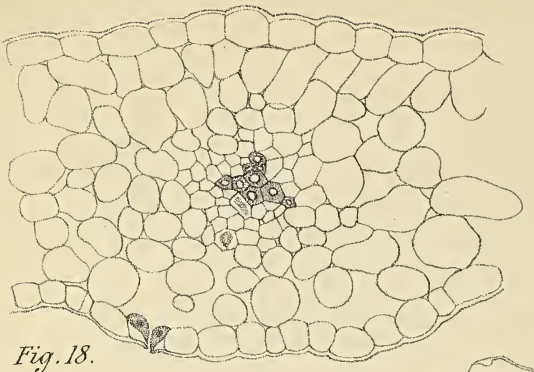

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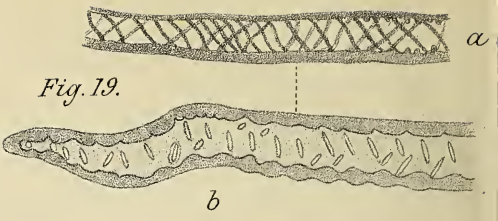


Fig. 19.

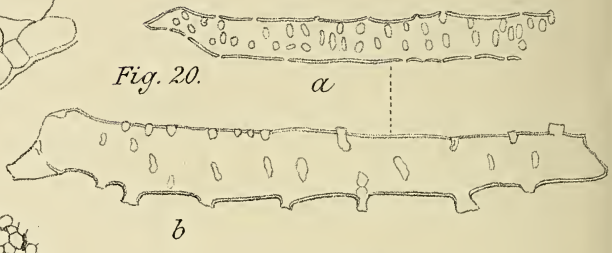
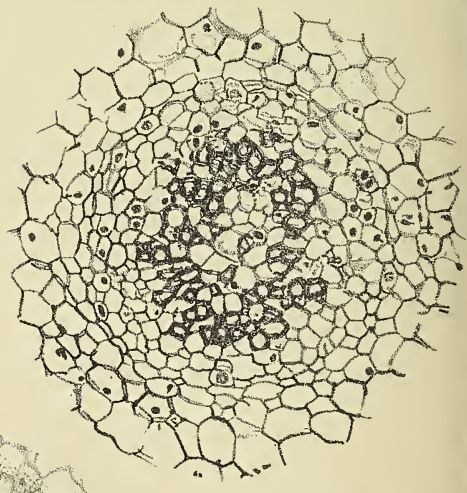


Fig. 20.

Fig. 21.



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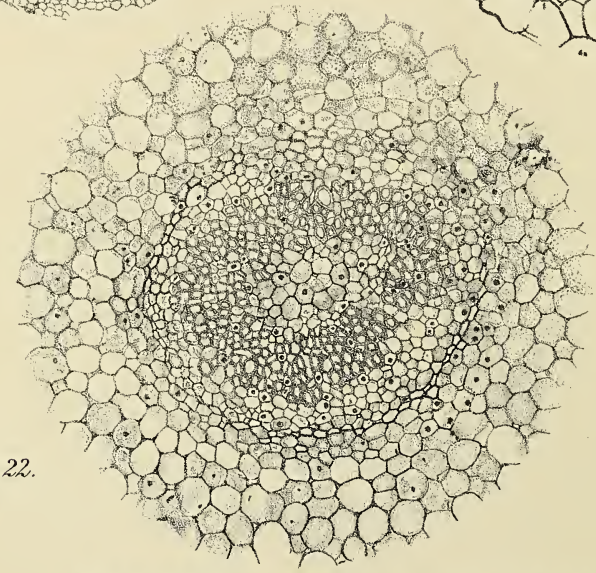


Fig. 22.

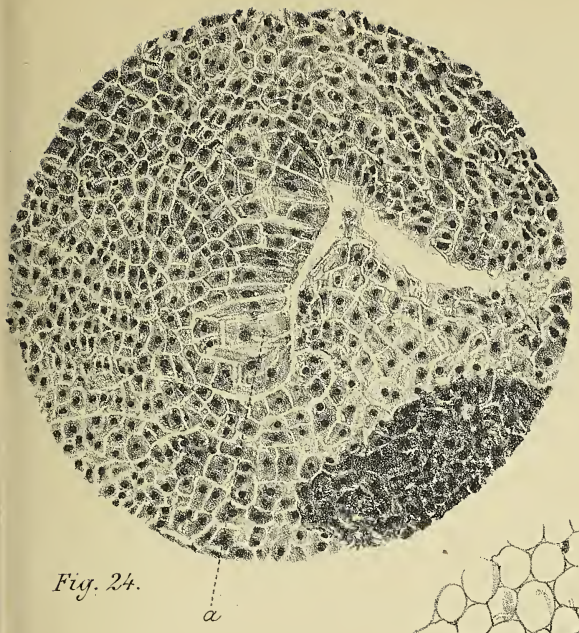


Fig. 24.

Fig. 25.

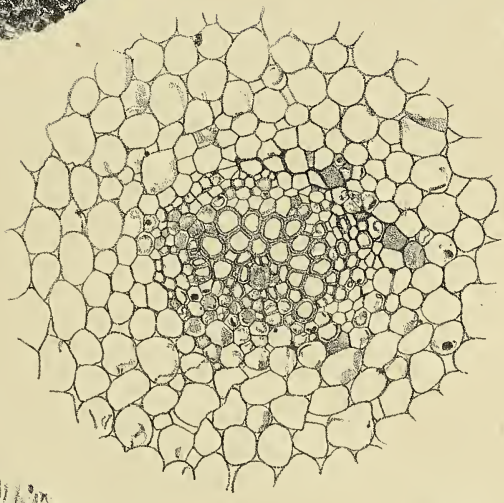
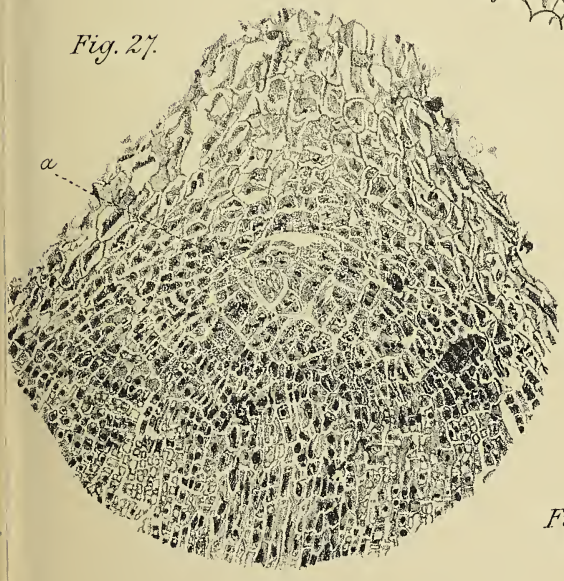


Fig. 26.

Fig. 27.



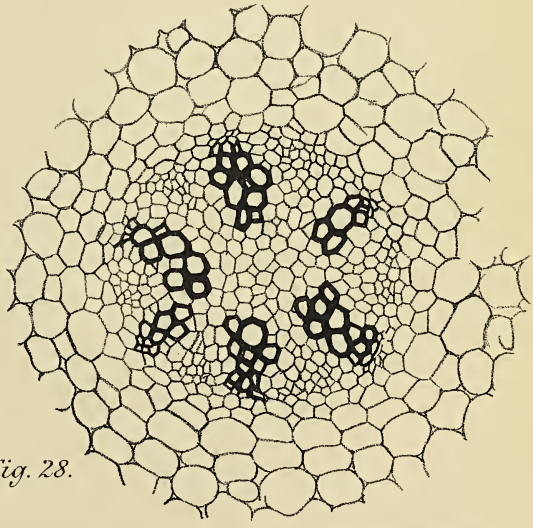


Fig. 28.

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The Alleged Fertilization in the Saprolegnieae.

BY

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THE question of the 'sexual' reproduction of the Saprolegnieae is one of the oldest still debated in Botany. Pringsheim's first results were contested by Cornu, who, however, was himself led astray by too close an approximation to the Peronosporeae; De Bary then took the matter up, and showed from observations on the living Fungus that, despite the formation of male organs, similar in the two groups, those of the Saprolegnieae were functionless. Pringsheim then approached the matter afresh; he alleged new evidence in favour of a true sexual process. This was refuted by De Bary, Marshall Ward, and Zopf from different standpoints. Modern cytolological methods enabled me to complete the work of De Bary by means of new facts and new interpretations; and the late Professor Humphrey confirmed my work. Professor Trow has recently attacked the problem anew with the aid of the method of sections, and has published a revival of Pringsheim's views, on grounds which appear to merit a full examination. This is, indeed, the more imperative, as my refutation of his paper of '95 in a short note [1] has remained practically unanswered, even in his recent paper.

[1] Annals of Botany, vol. x, 1896.

[Annals of Botany, Vol. XIII. No. LI. September, 1899.]

The 'sexual' reproduction in the Saprolegnieae is briefly this:—The hyphae swell into rounded enlargements, which become partitioned off; these are the 'oangia.' The protoplasm in these oangia aggregates into rounded masses, the oospores, which, instead of escaping like the zoospores, round off, become invested with a thick laminated cell-wall within the oangium, and remain for a long time in a resting condition. This is complicated by the fact that in most, but not all the forms, smaller hyphae, which we may call 'male' hyphae, grow into contact with the oangia; and the dilated end of the male hypha is shut off as an 'antheridium' by a septum closely attached to the oangium. On the loss of turgor by the oangium, the antheridium emits tubes which grow from the antheridium into the cavity, and press closely against some or all of the young oospores, without contracting any permanent adhesion to them, as may be clearly seen when the laminated wall is formed round the oospores. On comparison with allied forms the tubes appear clearly identical with the fertilizing tubes of the Peronosporeae, and may receive the same name.

We have first to consider the appearances in the maturing oangium. In this a central vacuole is established, if not already present at the moment of closure and septation; and vacuoles appear at fairly regular intervals in the peripheral protoplasm, which disappear by opening into the central cavity, as I showed, so that the layer of plasma is gradually and steadily thinned down. At the commencement it is multinucleate; and as the plasma shrinks the nuclei are brought closer to one another; in *Achlya* they may be seen approximated in files; and their total relative number now steadily decreases. At the same time they show changes of form and structure; many are seen to become ovoid, instead of spherical or lenticular. Again, at first the staining material of the single central nuclein-mass is in the form of a fairly large central body suspended by threads of linin; but as the process goes on its size diminishes and several are seen in each nucleus; in the oval nuclei we

may frequently find these staining masses aggregated in two distinct groups with linin-strands traversing the centre of the nucleus. The nuclear wall is less distinct from the cytoplasm, and the nuclein bodies stain less deeply. Here comes the first point of difference between Trow and myself; I cannot interpret these facts as other than the fusion of nuclei, and the multiple staining bodies as representing the originally separate nuclein-masses of the nuclei that have fused. When there are two groups of granules, each would thus represent the granules of an already complex nucleus at an early, incomplete stage of fusion with a similar one. Trow regards these figures as indicating indirect nuclear division, identifies the several steps with those of ordinary mitosis, and concludes that these are the ordinary gamete-specializing divisions. The reduction in number which goes on steadily from beginning to end of these changes, he ascribes to the digestion of most of the unfortunate nuclei in the cytoplasm.

The reduction in the amount of staining material in a nucleus is, however, not an unexampled phenomenon in the passage from a vegetative to a reproductive cell; the germinal vesicle of the ovarian egg of Vertebrata is a notable case in point. The issue between us is essentially this:—Trow believes that the nuclei divide, and that the greater part of the daughter-nuclei thus formed are bodily digested in the cytoplasm: whilst I infer from my observations that the nuclei fuse two and two at the same time that they shrink in size, while the amount of staining material is simultaneously reduced; and in this way the number of nuclei is so reduced that finally there is but one nucleus left in each oospore. The reduction in staining properties may be regarded as due to a digestive process; but according to my views the digestion would be entirely intra-nuclear. I found that in *Achlya* this reduction is seldom complete at the moment of the resolution into oospores, so that at first each is frequently binucleate; and Humphrey confirmed this: in *Saprolegnia*, on the contrary, each oospore is usually uninucleate at its very beginning. However, I have noted that there are occasional exceptions:—

'In a few cases, however, I have seen two nuclei of unequal size in the young oospore; and from this I might have inferred a sexual fertilization, had not the oangium in these very cases happened to be free from all signs of antheridial branches, let alone fertilizing tubes ('95, p. 694, Pl. XXIX, Figs. 25.)' This observation seems to have been inexplicable to Professor Trow: in his '95 paper he wrote (p. 638), 'the fact that Hartog did not find binucleate oospores in *Saprolegnia* may be traced most probably to an examination of apogamous forms like *S. Thureti*.' But it was just in an apogamous oangium that I figured such a binucleate oospore, and not in cases where the antheridia and fertilizing tubes were well developed. This fact I recalled in my criticism ('96): 'I have indeed found and figured a young oospore of *Saprolegnia* with two nuclei, as is so frequently the case in *Achlya*; but in this case there was no antheridium present whatever, while many hundreds of oangia [sc., of *Saprolegnia*] with attached antheridia and contained antheridial tubes did not supply a second case of a binucleate oospore in this genus' (p. 99). In his '99 paper Trow cites the passage in the *oratio obliqua*, and in such a context as to suggest that it applied to *Achlya*. He goes on to say (p. 167): 'I have never been able to absolutely prove the absence of an antheridium from an oogonium except by the method of following the development throughout on separate hyphae. In the case of oogonia taken haphazard, I have great doubts as to the possibility of demonstrating the complete absence of an antheridium by Hartog's method.' There cannot be the least opening for any doubt in examining carefully prepared specimens *in toto* (whether merely floated out, or carefully spread out with needles) as to the presence or absence of antheridial branches, antheridia, or fertilizing tubes. My specimens were laid before, I think, two meetings of the British Association, and were accessible to any one interested to go over them with me, as Mr. Wager did; and I do not admit that Professor Trow has any right to assume either wilful negligence or incapacity on my part, in a matter

of such great importance, merely because my observations contradict the conclusions which he drew from his own.

His account is as follows: 'In *Saprolegnia mixta*, the young oospores have frequently a second nucleus near the point of contact of a fertilizing tube. No opening can be seen in the tube, and it would in any case probably be very difficult to prove its presence even were it there. But a permanent opening does not appear to be necessary for fertilization to take place in such cases. No one, for example, has been able to determine a permanent opening at the end of the pollen-tube of the Angiosperms, where fertilization undoubtedly takes place' (95, p. 635). True, but in Angiosperms (1) the pollen-tube becomes permanently attached to the embryo-sac, as is the case in other siphogamous fertilization; (2) the pollen-tube loses its turgescence. Neither of these is the case in the Saprolegnieae; as De Bary, Marshall Ward, Humphrey, and I have seen, the fertilizing tube may grow up to the naked oospore, and even dent it, but it then glides off from its surface and grows past it without losing turgescence. Trow's conclusions appear then to be based, so far as *Saprolegnia* is concerned, on forms in which the final fusion of the last two nuclei left in the oospore-origin is habitually delayed to the same extent that I found, exceptionally, in a form free from male organs.

In *Achlya*, he writes in '99 (p. 159), 'large numbers of sections were examined to try and elucidate the mode of fertilization, but only one section, that reproduced in Fig. 45, appeared to be capable of throwing any light on the actual process. In this case it was possible to trace the fertilization-tube without a break into an egg which was already surrounded with a thin membrane.' The figure as it stands appears, at first sight, to be conclusive. But there are points about it that make me hesitate and finally reject its evidence. The cell-membrane is represented on the oosphere and the antheridium at the surface of the oangial wall, but the tube itself is represented as a grey homogeneous mass, with neither plasmic network, granules, nor nuclei, and

without any trace of a cell-wall, on its free course to the oospore; this is inexplicable. Now Professor Trow has already told us: 'In the preparations which I have made and used for further study, I have never seen a loose or misplaced section, and have never been at a loss to locate any section of a series. It is fortunate that it is possible to attain certainty in this direction, for there can be no doubt that the oogonia and oospores are too thick and dense for clear pictures of their contents to be obtainable by any other method than one which involves sectioning' (p. 151). But he has omitted to figure the upper and lower sections of the very set to which this solitary case of penetration belongs; he has not noted or explained the absence of the cell-wall from the free part of the fertilizing tube, and the absence of the beautiful cytoplasmic network which is so obvious even in the specimens prepared *in toto*, and which *ex hypothesi* should be so much more clearly seen in sections. For the present, then, it is at least permissible to regard the preparation as unsatisfactory, and to explain the case as one in which the fertilizing tube has passed over the surface of the oospore without penetrating it. Indeed, Professor Trow's observations on the living specimen confirm those of the supporters of the apogamy of *Achlya*: 'Neither in this case nor in that of many others examined, have I been able to trace the entry of the fertilization-tube into the egg; it seems to grow past it, and on the side furthest removed from observation, possibly influenced thereto by the illumination from below' (p. 142). Thus, like the rest of us, he has found that the fertilizing tube passes on, leaving no trace of its futile attempts to perform the ancestral functions. He assumes, however, that a gameto-nucleus is transmitted to the oospore by the unseen, temporary penetration of the oosphere; and gives no hint as to what becomes of the part of the tube that he wishes us to believe has penetrated into the egg. There is no trace of it on the wall of the oospore at any time, even in his own sections. The tube bears no branch, reveals no scar; one can therefore only infer that it must be withdrawn after it has

done its work, like the male organ of a Metazoan[1]. 'I have satisfied myself, however,' he writes, 'of the presence of two nuclei in the egg *at all times* in this stage, one peripheral and the other central, and the peripheral one always close to the point of attachment on a fertilization-tube' (p. 159). Professor Trow showed his exquisite drawings at the Botanical Section at Bristol; they conveyed clearly to me that this 'peripheral nucleus' was in no way different to other collections of granules—microsomes—lying more or less peripherally in the egg, and his plates are equally convincing: such pseudo-nuclei he has figured in his Fig. 45 (upper oospore) above the entrance of the dotted line. In Fig. 46, the lowest oospore contains two of these in the angle between the two dotted lines to the south of that noted as the 'male nucleus'; and in the right-hand oospore at the north-west of the 'male nucleus,' and separated from it by two vacuoles, is another of these. On the other hand, in Fig. 44 the left-hand egg seems to show two nearly central true nuclei, of which he has only recognized one. The explanation I am inclined to give is that the stain he used, gentian-violet and eosin, does not sufficiently differentiate nuclei and microsomes, and can give no certain results.

I wrote in '95 (p. 698–700): 'The apogamy of Saprolegnieae does not imply a complete absence of processes comparable with fertilization. The essence of ordinary fertilization consists in the union of two cells, cytoplasm to cytoplasm, nucleus to nucleus (and in most cases, archoplasm to archoplasm—referring of course to the views of Fol and Guignard, which have since been somewhat shaken). In this way is formed a new complex cytoplasm, nucleus, &c., which, as such, have never been associated before. In Saprolegnieae the nucleus of the oospore is formed by the fusion of many nuclei, which possibly wandered from different parts of the plant, and which

[1] This is a revival of Pringsheim's erroneous observations and conclusions; De Bary I think compared the passage of the gametoplasm, leaving no trace of exit from the fertilizing tube or entrance into the oospore in the cell-walls of either, to the passage of spiritual mediums like the renowned Mrs. Guppy through brick-walls and closed doors and windows.

are, in any case, essentially different from all those formed by repeated nuclear fission in the previous life-history of the plant. . . . Obviously such endogamous nuclear unions cannot occur in plants with distinct walls partitioning them into distinct cells. But we do find parallel cases in other apocytial plants. In the Dasycladeae the nuclei fuse several together in a gametangium, and round the single fusion-nuclei the protoplasm aggregates to form uninuclear [isogamous] gametes which pair with those of other plants. In *Derbesia*, zoospores with fusion-nuclei formed in precisely the same way have apparently lost the faculty of pairing, and develop directly. . . . In Uredineae the teleutospore is bicellular; in each cell the nucleus divides; and then in the cell the two sister-nuclei fuse again, so that each of the two resting-spores of which the teleutospore is formed has a fusion-nucleus. And in Ustilagineae each spore is primitively binucleate; but becomes uninucleate by the fusion of the nuclei. . . . I refer also to the formation of the large "basidial nucleus" of the Basidiomycetes by the fusion of two or more; from this, by two bipartitions, are formed the nuclei for the basidiospores. In most animals the ovarian egg divides at maturity into a brood of four, three of which are functionless and are termed polar bodies; while the fourth is the functional oosphere which is usually fertilized by the male cell, the spermatozoon. In several cases of so-called parthenogenesis the second cell-division remains incomplete : the cell-nucleus divides as if to give rise to the nuclei of a polar body and an oosphere respectively; but then the former nucleus moves back and fuses, playing the part of a male [I should rather have written 'the same part as the sperm-nucleus'] with its sister, just as we have seen in the Uredineae.' These facts I quoted especially from Boveri and Hertwig in '91; but since then Brauer of Heidelberg confirmed them in the fullest way for *Artemia*; and Wilson's 'Cell in Development and Inheritance' (where they are quoted with figures) should be familiar to every one who, like Professor Trow, proposes to deal with questions of cytology.

To these examples we may add the fusion of the sister-nucleus of the oosphere with one of the basal group in the embryo-sac of Phanerogams when it is in an apocytial condition, and the fusion in the apocytial Heliozoan *Actinosphaerium* of the nuclei, several together, into a much reduced number, as observed by Brauer; and as an intermediate case the formation of the gameto-nucleus of the oosphere in *Sphaeroplea*, which, according to Rauwenhoff's description, resembles the process as 'seen in the formation of the oospheres of *Saprolegnia*' ('91, p. 19). All of these (save *Actinosphaerium* and *Artemia*) were cited in my '95 paper, or in the '91 'Problems of Reproduction,' to which I referred, and which contains the full statement of my views on these subjects. Yet Professor Trow writes, without taking any account of these facts—'The question of wholesale nuclear fusions is one which we expect to see proved conclusively by actual demonstration. It is so improbable in itself, at any stage in the life-history of a plant, that we may be pardoned, in the absence of such demonstration, for unbounded scepticism as to its occurrence. . . . But Hartog's theory, even as a theory, has an unsound foundation. Consider what it means. The nuclei in question, surrounded by their protoplasmic masses—energids, let us say—are female gametes, and very highly developed ones too' ('99, p. 165). Yet I think there can hardly be greater general unanimity on any matter connected with the history of reproduction, than as to the view that regards the conjugating nuclei as *equivalent* nuclei, and free as far as possible from the taint of binary sexual differentiation which is an adaptation of the cytoplasm only. A point on which Weismann, O. Hertwig, and Maupas are agreed is surely worth taking into account.

He proceeds: 'The theory, then, is that of the multiple endogamous union of highly developed female gametes in plants where very dissimilar male gametes are well known. Imagine a botanist broaching as a theory the multiple union of *Fucus* eggs to form a zygote, and the universal incredulity with which it would be justly met, and we have some idea of

the great improbability of Hartog's theory.' Professor Trow is singularly unfortunate in his illustration. Between such widely different groups as the Metazoa, and the Phaeophyceae, it was hardly to be expected that homologies could be found; yet, thanks to his own teacher, Oltmanns, we know that the abortive oospheres of the Fucaceae are absolutely comparable with the polar bodies of the Metazoa.

Professor Trow's careless treatment of facts and records shows everywhere save in his figures. Thus whilst he figures the well-known cellulin-corpuscles of Pringsheim (Fig. 42), he not only fails to recognize them, but volunteers an absolutely impossible explanation of their nature; 'occasionally, as represented in the figure, we have in the large central vacuole what we may regard as skeletons of fat-globules; these skeletons are very delicate spherical shells, almost invisible in some preparations, but generally well seen in those stained with haematoxylin (p. 157) . . . The oil-globules are represented by the cavities in which they lay, and occasionally, in the case of those lying either partly or wholly in the vacuole, by the skeletons we have already described' (p. 158). In '95 I noted that the cellulin-granules become almost invisible in balsam, and what Professor Trow calls shells are certainly (as he figures one in Fig. 43) solid spheres of this substance; that it stains with haematoxylin, was, so far as I remember, one reason for my rejecting that pigment. He throws doubt (p. 166) on the validity of my preparations, for representing in my Figs. 21, 22, oospores of *Achlya*, the outer part occupied by dense protoplasm, 'the central part appears in the form of a big vacuole, the nucleus being suspended in the middle by fine strands of protoplasm'; yet in both these a thin continuous layer of protoplasm is figured surrounding the nucleus. I am far from being a good draughtsman, and my sketches were unfortunately redrawn instead of being printed in their inartistic condition. But in Fig. 25, that of the spore of *Saprolegnia* with two unequal nuclei not yet fused, the larger nucleus especially has a dense investment of cytoplasm, continuous with the reticulum.

Professor Trow says that the fixing solution was 'a saturated aqueous solution of mercuric chloride, and it was applied hot as advised by Hartog and Humphrey' (p. 150). Now my methods have been repeatedly published; in the report of the Congrès de Botanique, of '89, printed in the Bulletin de la Société de Botanique de France, vol. 26 (translated in the Journal of the Royal Microscopical Society); and in the fullest possible detail in the memoir of '95; nowhere do I speak of applying hot sublimate, for the results of the cold solution were good enough; Humphrey however warmed his fixative, and an ambiguous sentence of his possibly explains Professor Trow's error.

I have termed the central deeply staining mass in the nucleus a 'nuclein-mass'; my figures show that I saw it as very frequently containing deeply staining granules embedded in linin; Humphrey calls it a chromatin-mass, evidently in the same sense. Professor Trow is not justified in writing 'we cannot then regard it either as a nucleolus as has been done by Dangeard, or as chromatin or a chromosome as has been done by Humphrey, Hartog, and myself' (p. 152). For this implies that the synonymous terms used by Humphrey and me are also equivalent to his own use of 'chromosome,' than which nothing can be more inexact. I indeed described this nuclein-mass as resolving itself into four chromosomes in division!

Professor Trow writes, 'had Hartog been able to follow the division of the nucleus in the oogonium (as he did in the antheridium)' (p. 164). Here again is an inaccuracy: I expressly state that 'the nuclei multiply by division, *as shown by the small size and large number of those found in the "fertilizing tubes"*' ('95, p. 694). I wish I had been able to 'follow the divisions.' The curious point is that it is a repetition of an error in his '95 paper, to which I expressly called attention in my criticism of '96 (p. 98, note). All these are, singly, small matters; but collectively they count in judging the value of the author's observations and inferences.

Professor Trow's terminology is rather perplexing at whiles.

The sentence, 'The clear material through which the threads pass we may regard as nuclear sap or nucleo-hyaloplasma, the threads themselves as linin-threads' (p. 152), conveys no clear meaning to my mind as to what he understands by nucleo-hyaloplasma; most botanists would apply the term to the linin-threads themselves minus the microsomes. Again, 'It is difficult to determine the substance which fills these true vacuoles, whether it be hyaloplasm or cell-sap' (p. 154). His conception of hyaloplasma is certainly an unfamiliar one.

With regard to the closing discussion in Professor Trow's paper on nuclear reduction and alternation of generations, I have nothing to say here. A complete and recent statement of my views is to be found in two papers 'The Fundamental Principles of Heredity,' and 'Nuclear Reduction' in 'Natural Science' for Oct.–Nov. '97, and July '98, both translated in the Biologisches Centralblatt for 1898. These contain a comparison of the essential relations of gametogeny to other brood-cell formation in Metazoa, Metaphytes, and Protista. A short abstract of my views contributed by me to the discussion on Alternation of generations at the Bristol meeting of the British Association, has appeared in the Annals of Botany. It is, I presume, because these are all so accessible, that no reference is made to them in Professor Trow's contribution to the subject.

In conclusion, I have at least to thank Professor Trow for having given me the occasion to go back again to my old specimens, and to find that the lapse of years has in no way damaged their goodness or their legibility, while his exquisite drawings have again convinced me that sections can, in this group at least, demonstrate nothing more than good mounts *in toto*. A little more care in reading his own specimens, as in reading the published observations of others, would have saved me the painful task of criticizing unfavourably the outcome of a difficult and laborious research.

List of Authorities referred to.

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NOTES.

THE PLURILOCULAR SPORANGIA OF PETROSPONGIUM BERKELEYI.—While collecting Algae in July last year at Murlough Bay and the Giant's Causeway in the County Antrim, Mr. E. M. Holmes, with whom I enjoyed the privilege of collecting, kindly pointed out to me some specimens of *Petrospongium Berkeleyi*, Naeg., growing on the rocks very near low water-mark at Murlough, a little to the east of Miss Clarke's cottage; later I found it at the Giant's Causeway, but nowhere was it abundant.

The late Professor Harvey, writing of this plant (Phycologia Britannica, 1846–51, Pl. CLXXVI), says, 'On the west coast of Ireland it is plentiful in several places, and probably is pretty generally distributed along our shores, being overlooked on account of its being often nearly the colour of the rock on which it grows, and resembling in its fleshy appearance and feel the collapsed body of the common *Actinia*.' It belongs to the division of Brown Algae or Phaeophyceae, which are usually considered to multiply asexually by means of zoospores produced in large numbers in unilocular sporangia; these zoospores show a well-marked red eye-spot, two laterally fixed cilia, and a chromatophore. A sexual method of reproduction also obtains among many genera, consisting in the conjugation in pairs of isomorphous planogametes with the formation of zygotes. To this division, the Phaeosporeae, belong the well-known Tangles or Oarweeds of our coasts, together with a host of more inconspicuous and delicate forms differing very much from one another in habit and mode of growth.

Although each species is supposed theoretically to have both asexual unilocular sporangia and sexual plurilocular sporangia or gametangia, each cell of the latter rarely giving rise to more than one zoospore or gamete, there is a large number of plants in which only one kind of reproductive organ has as yet been observed. It was with a view of looking for the plurilocular sporangia in *Petrospongium*, which hitherto

have not been observed, that I collected a quantity of material for microscopic investigation, preserving it for this purpose in 3 per cent. formol.

A few weeks ago, when examining a series of sections prepared from this material, after being hardened in alcohol, stained in bulk in Delafield's haematoxylin, and embedded in paraffin in the usual way, I found a small number of plurilocular sporangia taking the place of a number of peripheral filaments, in close proximity to unilocular sporangia, both occurring on the same plants. At first I was in doubt as to their real character. In one section the plurilocular

Unilocular and plurilocular sporangia.

sporangia were observed to arise more or less at right angles from a basal filament running nearly parallel to the surface of the thallus, recalling the characters of a parasitic *Ectocarpus* or *Streblonema* growing attached to its host-plant; on more careful observation, however, I convinced myself of the direct transformation of the peripheral filaments giving rise to these sporangia, which were much fewer in number than the unilocular sporangia. They varied as regards shape and size, measuring from ·0459 mm. to ·1134 mm. long, the average breadth being ·0108 mm.

An interesting point observed in the sporangia is the difference in size between the loculi of the same sporangium; and since each loculus usually contains but a single zoospore, there is a corresponding

inequality of size among the zoospores, and one is tempted to look for a physiological distinction between them.

The unilocular sporangia arise from the cells at the base of the cortical filaments, and do not ever seem to arise from the apices of the latter. The cortical filaments divide in an irregularly dichotomous manner, and in a few cases the apical cell of a filament becomes enlarged and divides so as to form two tiers of four cells each; this may represent a stage in the development of a plurilocular sporangium, although no intermediate stages were observed.

Petrospongium is closely allied to *Leathesia*, differing from it in form, consistency, and colour, and being at all stages in its life-history solid and fleshy; it would also seem to be nearly allied to *Castagnea* on account of the similarity between their reproductive organs, and also to *Mesogloia*. If we assume that it is not impossible that *Mesogloia* may give rise to plurilocular sporangia by a modification of some of its peripheral filaments, there is no very clear distinction between the three genera except one of habit and form, and these differences may be the result of a different mode of life.

Since the researches of Thuret in 1851, very little attention was given to the behaviour of the zoospores of these sporangia until, in 1881, Berthold demonstrated at Naples the fusion of two zoospores from the plurilocular sporangia of *Ectocarpus siliculosus*, thus proving that they were true gametes which produced zygotes after fusion. The gametes showed a physiological distinction during the process of conjugation; one of them became fixed and lost its cilia, while the other remained motile, so that we may speak of the former gamete as female and the latter as male.

Quite recently the accuracy of these statements has been doubted by Oltmanns, who attributed them to erroneous observation; however, Sauvageau, and more recently Kuckuck, have succeeded in repeating Berthold's observations, although Sauvageau says that conjugation takes place between the gametes much less frequently than Berthold found to be the case, a difference which he considers as depending partly on locality and time of year [1].

Kuckuck's experiments with the same plant were unsuccessful: but in the autumn of 1897 he made experiments with the zoospores from the plurilocular sporangia of *Scytosiphon lomentarius*, and in

[1] Oltmanns has since withdrawn his objections. Flora, 1899.

many instances immediate conjugation was observed between the gametes. He further found that the zoospores which failed to conjugate immediately after being set free from the sporangium, very soon entered on the resting condition, and in this state were incapable of being fertilized.

This fact leads us to inquire if, when the zoospores from the plurilocular sporangia fail to conjugate—assuming for the moment that in all cases they are true gametes—they perish; or are they capable of germinating parthenogenetically and giving rise to healthy plants? Sauvageau has been enabled to settle this question, and to prove that in *Ectocarpus virescens*, at any rate, these zoospores are not obligatory gametes, in the sense that they must conjugate or else perish; for after escaping from the plurilocular sporangia they move about for some time, and then become fixed, and later on germinate 'with the greatest ease and regularity.'

The researches of Berthold, Sauvageau, and Kuckuck, would seem to place beyond all doubt the true sexual nature of the conjugating gametes of *Ectocarpus siliculosus* and *Scytosiphon lomentarius*; but, in other cases, not yet fully examined, it is impossible to say definitely whether we have to deal with asexual zoospores, or with zoospores possessing a potential sexuality, on account of the great variability of their behaviour after they are set free from the sporangium.

Having regard to the great similarity between the antherozoids of *Ectocarpus siliculosus* and those of *Fucus*, and to the resemblance between the modes of the sexual process in these plants, there seems to be no reason why the Phaeophyceae should be divided into the two series, Phaeosporae and Phaeogamae.

My best thanks are due to Professor Johnson, who suggested to me some time ago to examine material of *Petrospongium* for plurilocular sporangia, *P. Berkeleyi* being found plentifully at low water at some points on the Irish coast.

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SOME REMARKABLE MARINE MONOCOTYLEDONS IN JAPAN.—A discovery of unusual interest to Japanese botanists was made in the Luchuan Islands. On my botanical tour in the Yaye-yama Archipelago, situated close to Formosa, I found a remarkable

marine Monocotyledon, growing abundantly in the shallow sea between the islands of Irumuti and Uchibanari. Many specimens bearing ripe fruit at the apex of the spirally-wound peduncle, were secured. These proved on examination to be *Enhalus acoroides*, Steud. (*E. Koenigii*, Rich.), a peculiar genus of Hydrocharideae, found in Malayan seas, and extending to Africa in the west, the Pacific Ocean in the east, and to Australia in the south. The linear dark-green leaves of this plant, which attain the size of more than 60 cm. by 1–1½ cm., afford sufficient food to the Dugongs (*Halicorne Dugong*), which frequent the shallow sea of the Luchuan Islands. Some of the specimens of *Enhalus* in my collection have the leaves partly bitten off by the herbivorous mammal, the skulls and teeth of which I brought home for identification, and the flesh of which I tasted and found delicious. The Luchuan name of *Enhalus* is 'Sūsanuha.'

In the same sea, growing together with *Enhalus*, I also found *Halophila ovata*, Gaudich. (*H. ovalis*, Hook. f.), in abundance. This plant is now known to extend to the Pacific coasts of the principal island (Hondō) of Japan; and I collected it myself in 1896 at a depth of between eight and eighteen fathoms in the Bay of Kagoshima, in Kiusiu in Southern Japan.

There is a specimen, collected by me in the sea of Miyako-jima, of another marine Monocotyledon, which, I think, might possibly be referred to *Thalassia stipulacea*, Koen.

TOKUTARO ITO, Tokyo.

RHIZOPHOREAE IN JAPAN.—Having paid attention to this subject for the last six years, I think it well to put on record three species of Mangroves now ascertained to be indigenous to Japan. They are *Kandelia Rheedii*, Wight et Arn., *Bruguiera gymnorrhiza*, Lam., and *Rhizophora mucronata*, Lam. In the island of Kiusiu in Southern Japan, only the first of these species is to be found; in Amami Ōshima, we have the first two species; while, in Uchinā (Okinawa or Great Luchu) and in the Yayeyama Archipelago, all the three species are to be found together in luxuriance. Thus the northern limit of Mangroves in Japan might be attributed to the coast of Kiiré in the Bay of Kagoshima in Kiusiu, and extending through Amami Ōshima and Uchinā to the Yayeyama Archipelago, where I found them as flourishing as those on the Malayan coasts.

The Mangrove-forests of Satsuma, being situated in 31° 20′ N. lat., might be considered at least as the northern limit of Rhizophoreae in Asia. I may here state in passing, that the specimen of Mangrove collected by Döderlein in Kagoshima and determined by Professor Engler (Bot. Jahrbücher, vi, 1885, p. 63) as '*Rhizophora mucronata*' should be *Kandelia Rheedii*, on the grounds that the former does not extend beyond the Luchu proper in the north, and that in the Kagoshima Prefecture the only species to be found there is the latter species.

TOKUTARO ITO, Tokyo.

FLOATING-APPARATUS OF THE LEAVES OF PISTIA STRATIOTES, L.—This curious aquatic Aroid, commonly growing in ponds, ditches, and other stagnant waters of tropical and subtropical countries, has fleshy, cuneate or obovate leaves arranged in a rosette. These leaves are provided on the underside with a large swollen body, about the size of a pigeon's egg. In *Trapa natans*, L., the swimming-organ is developed on the long petiole; but in the case of *Pistia Stratiotes*, L., the leaves being sessile, the part a little above the base becomes swollen into an obovate form, and serves as a floating-apparatus. On making a longitudinal section of the leaves, this organ is found to be composed of spongy parenchymatous tissue, containing air. The upper and lower surfaces of the leaves are densely covered with minute depressed hairs, which protect the leaves from being wetted. By these means, the leaves of *Pistia Stratiotes*, L., are enabled to maintain their position above the surface of the water, the upper side is kept dry, and thus the transpiration and respiration are constantly carried on. The above observations were made on the plant growing luxuriantly in a pond in Shui in the Lūchū Islands.

TOKUTARO ITO, Tokyo.

Cell-Division in Sporangia and Asci.

BY

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With Plates XXIV–XXVI.

THE most thoroughly studied case of spore-formation in sporangia is that of *Saprolegnia* and *Achlya*. Here the slenderness and transparency of the objects made accurate observation of the processes taking place in the dividing protoplasm possible, without the employment of any very refined technique. The behaviour of the nuclei has not been sufficiently investigated in this case, but the observations of Humphrey[1], Trow[2], and others leave little doubt as to the main facts. The sporangium, when cut off from the parent hypha, is multinucleate, and the ultimately formed zoospores are probably uninucleate. In the light of Belajeff's[3] observations on the formation of the cilia in antherozoids, it would be extremely interesting to determine

[1] Saprolegniaceae of the U.S., Trans. Am. Phil. Soc. Vol. xvii, 1892–3.

[2] The Karyology of Saprolegnia, Ann. of Bot. ix, 1895, p. 609.

[3] Über die Cilienbildner in den spermatogenen Zellen, Ber. d. D. Bot. Gesellschaft. Bd. xvi, p. 140, 1898.

[Annals of Botany, Vol. XIII. No. LII. December, 1899.]

the relations of the nuclei to the formation of the cilia. As to the actual method of cell-division whereby the protoplasm of the sporangium is cut up into the swarm-spores, the earlier observers seem to have been too much influenced by theories of cell-formation in general. A brief summary of the views of Nägeli, Unger, Schleiden, Meyen and others, is given by Pringsheim[1] in the introduction to his account of the development of *Achlya prolifera.* Büsgen[2] also reviews briefly the views of the older authors as to the nature of the spore-formation and its bearing on cell theories.

Unger held that the sporangium was formed simply by the building of the basal septum, and was an example of simple meristematic cell-formation.

Nägeli interpreted the same phenomena of sporangium-formation as indicating the building of a complete new cell in the end of the hypha with a new wall just inside the sporangium wall. It was for him a case of 'wandständige Zellbildung,' and was used to still further support the doctrine that all new cells arise in old ones by free cell-formation. Nägeli further believed, in agreement with the views of Schleiden, that the individualizing of the spores was due to preceding formation of cytoblasts (nuclei), centres around which the cell is differentiated. According to Pringsheim's own account of the process in *Achlya prolifera* (*Saprolegnia ferax*, Ktz.) the septum which cuts off the sporangium is a double membrane running up on the sporangium wall and down on the wall of the hypha for a short distance, thus forming in optical section three-cornered intercellular spaces. The septum becomes convex downward. A lumen now appears in the sporangium due to influx of water (areola of Unger).

The spores appear first as prominences pushing into the sporangium-lumen. No boundary line about them is visible.

[1] Die Entwicklungsgeschichte der Achlya prolifera, Nov. Act. Acad. Caes. Leop. Carol. Nat. Cur., vol. xxiii, p. 1, 1850.

[2] Die Entwicklung der Phycomycetensporangien. Jahrbücher für Wiss. Bot., Bd. xiii, 1882.

Their surface consists of granules just like those in the mass of the protoplasm. A boundary line appears when they separate from each other and from the sporangial wall. They are thus completely bounded off from each other before a cellulose cell-wall appears, though this is formed shortly after their separation.

The protoplasm cleaves simultaneously, and not by successive bipartitions breaking into larger and then smaller portions. There is no deposition of slime between the plasma masses during the process of division. The septum now becomes convex upwards, due to the pressure of the protoplasm below.

No cytoblast (nucleus) is present in the spores. They are bounded by a cellulose wall, and beneath this the protoplasm is covered by a primordial utricle. The oospores are formed in the same fashion as the swarmers. The primordial utricle of the sporangium and oogonium is used up in spore-formation, but cannot be considered as dividing to form the entire new primordial utricle for each spore. Pringsheim recognizes that Nägeli's distinction of 'wandständige Zellbildung' and free cell-formation is dependent entirely on the degree to which the sporangium is filled with protoplasm.

Büsgen[1] investigated the sporangia of the Phycomycetes after Strasburger's description of the cell-plate in the Phanerogams, and is evidently much influenced in his interpretation by Strasburger's work. He describes the cleavage as occurring simultaneously by means of cell-plates. These cell-plates which are formed at first break down, and are later reconstituted for the definitive separation of the spores.

This period of disappearance of the cell-plate, as we shall see later, doubtless corresponds to the period of swelling when the spores are so closely pressed together that their boundaries become indistinct. The whole process is supposed to be analogous to full cell-formation, as described by Strasburger for *Eranthis hiemalis*.

[1] l. c.

Berthold[1] describes the cleavage essentially as follows, basing his description chiefly on a study of the oogonia, and assuming that the processes in oogonium and sporangium are identical. The first step consists in differentiating a dense granular wall-layer around a central vacuole out of the, at first, more homogeneous protoplasmic contents of the oogonium. This layer of protoplasm then, at a certain definite number of centres, begins to form rounded protrusions into the central vacuole, the protoplasmic layer between these eminences becoming as a consequence thinner. These centres of aggregation become constantly larger, pushing out more and more into the central vacuole. The whole protoplasmic mass also contracts so as to be set free from the oogonial wall. Finally the different masses become entirely separated and round themselves up, though threads of protoplasm may connect them for a time after they have become well individualized.

These balls of protoplasm now swell so as to become pressed together and flattened against each other, but ultimately round themselves up again and ripen into the mature oospores.

From another point of view, the cleavage, according to Berthold, might be interpreted as proceeding from the central vacuole outward by means of very broad furrows which finally cut from the vacuole to and through the plasma membrane. No furrows from the surface inward are found. Berthold sums up the process for sporangium and oogonium under three heads: first, the separation of the contents of the sporangium into a wall-layer and a central vacuole; second, the loosing of this wall-layer from the cell-wall, and its division into a number of distinct heaps which finally separate entirely; third, the forming of these masses into swarm-spores or eggs.

Berthold's object throughout is the explanation of the mechanics of the process. He regards the position of the spores as determined by a number of new centres of attraction

[1] Studien über Protoplasma-Mechanik, p. 308: Leipzig, 1886.

toward which the protoplasm of the wall-layer is drawn together.

He notes very clearly a preliminary contraction followed by swelling, and a second final contraction of the spore masses.

Berthold[1] regards the process of cleavage in *Saprolegnia* as derived from free cell-formation and as representing a higher stage of development than the latter, since, in this case, the spores are able to separate out of the parent plasm and maintain their independence without the sacrifice of materials involved in the formation of periplasm. This view involves of course a reversal of the process as conceived by Brefeld, who holds that the ascus has been derived from the sporangium. The sporangium, according to Berthold's view, has been developed from types which form periplasm rather than the reverse. The ascus being in the latter group represents the more primitive type. It is to be noted that, while insisting definitely on the derivation of the sporangium as seen in *Saprolegnia* from some periplasm-producing type, Berthold does not go into the question of establishing relationships between the groups of fungi on this basis, his interest in the matter being wholly from the mechanical physiological standpoint. Berthold's doctrine of the stratification of the cell-body, with polarity determined by the position of the nucleus, influences throughout his interpretation of the spore-formation in the sporangium. Also the position of the developing oosphere, he thinks, is determined by the appearance of certain new and characteristic relations in the symmetry of the wall-layer of the oogonium.

The differentiation of the sporangium-contents into a central vacuole and a peripheral protoplasmic layer is perhaps an example of stratification in a cell. The position of the forming spores is ultimately determined, as we shall see, by the position of the nuclei. Whether the distribution of the nuclei is influenced by diffusion streams, I shall discuss more fully later on.

[1] l. c. pp. 313–4.

Rothert[1] gives a very full account of the formation of the basal septum of the sporangium. He finds that the dense protoplasm at the apex of the sporangiophore excretes a hyaline plasma in the region where the septum is to be formed. This hyaline mass is at first in the form of a ring which gradually widens and cuts off the sporangium. It is sharply bounded below and passes gradually into the dense plasma above. It may be thicker than the breadth of the sporangium at that point. A few seconds suffices for the process of building this zone. The lower part of the hyaline zone condenses next to a cross-wall without sharp contours at first. Cellulin granules are abundant in this region, and are dissolved in the hyaloplasm and used to form the wall.

Pringsheim[2] had already pointed out a similar participation of cellulin bodies in the formation of cell-walls.

In sporangia which are full of protoplasm, Rothert finds that the hyaline disk may be built simultaneously across the entire diameter of the hypha. Cleavage occurs in sporangia with a central vacuole by clefts which extend from the vacuole outward. They are not formed by a gradual progressive splitting, but simultaneously in their entire depth. These clefts become connected to form a continuous net. They contain cell-sap and not slime, and divide the protoplasm into polygonal areas of equal size. The net appears simultaneously throughout the sporangium. The plasma is not first divided into larger and then progressively smaller portions. At first numerous protoplasmic connexions are present between the spores, but these soon disappear for the most part.

In full sporangia the process is the same, except that clefts appear in the midst of the protoplasm and are enclosed by it on all sides. In sporangia with little protoplasm aggregation occurs at certain points as in the case of the oogonia. The polygonal masses which are to form the spores now begin to contract and round up, but are still connected next the

[1] Die Entwicklung der Sporangien bei den Saprolegnieen. Beitr. zur Biologie der Pflanzen, Bd. v, p. 291, 1892.

[2] Ber. d. D. Bot. Gesellsch., Bd. i, p. 303, 1883.

sporangium-wall, as can be shown by plasmolyzing the whole contents of the sporangium. After this stage of greatest contraction, twenty-five minutes after the full differentiation of the spore rudiments, swelling begins. The central 'lumen' disappears, vacuoles appear in the spore masses, and they become polygonal by mutual pressure but do not fuse. It was this swollen condition which led Büsgen to suppose that the spores, after separating, fused once more into a homogeneous mass. Cleavage is now completed and the whole sporangium shrinks, due to the escape of cell-sap. The stages of contraction and rounding up of the spores and subsequent swelling followed by another contraction in ripening as described by Rothert seem to be of general occurrence, as will be seen from my own observations described later.

Humphrey, in the paper already referred to, confirms most of Rothert's observations as to cleavage in the sporangium. He also describes the cutting off of the sporangium by a basal transverse wall. The process of wall-formation is preceded by the formation of a disk of hyaloplasm which arises by the withdrawal of the microsomes from the originally granular protoplasm of the region just below the forming sporangium. A comparison of his description with that of Oltmanns, relating to the cutting off of the oogonium of *Vaucheria*, leads to the surmise that Humphrey may have mistaken a flattened lens-shaped vacuole for a disk of hyaloplasm.

Pringsheim[1] first observed and figured the process of progressive cleavage from the surface inward in the sporangia of *Pythium entophytum*. In this plant, according to the author, the protoplasm escapes from the sporangium before the spores are formed, but remains attached to the end of the sporangium as a spherical mass enclosed in a very delicate membrane. Division begins by furrows on the surface of this mass, which advance towards its centre and finally separate it into a mass of swarm-spores in a fashion very much like that described below for *Synchitrium*. As already noted, however, he takes

[1] Beitr. zur Morph. u. Phys. der Algen, ii. Die Saprolegnieen. Ges. Abhandl., Bd. ii, p. 63.

quite a different view of the process as seen in the sporangia of *Achlya* and *Saprolegnia.*

The sporangia of the Mucorineae have been much less studied than those of the Saprolegniaceae on account of their extreme density and opaqueness. Only very fragmentary and unsatisfactory references to the cleavage are to be found in the older literature. All of these older observations were made on the living material, in which the differences of refractive index of the various parts of the protoplasm are so slight as to preclude the possibility of recognizing with certainty the parts played by vacuoles, nuclei, plasma-membrane, &c. The accounts of spore-formation in these sporangia given in the standard text-books [1] are extremely inadequate and most of the details given are inaccurate, as will be seen later. The relatively large and opaque sporangia of *Mucor Pilobolus*, *Sporodinia* and *Synchitrium* can only be satisfactorily studied by means of microtome sections, and this explains the worthlessness of the earlier observations.

Corda [2] describes and figures the sporangial development in *Ascophora Mucedo* [3]. Prior to spore-formation, the plasma-mass is filled with transparent vesicles. In the lower part of the sporangium the columella is differentiated out, and at the same time in the upper part ill-defined and isolated cells begin to appear. These cells multiply and finally fill the entire sporangium. These cells are the spores. They are arranged in radial rows and probably connected in chains. Büsgen [4] gives a very brief account of the cleavage in *Mucor*, based on a study of small living sporangia. He considers that the cleavage is accomplished here as in *Saprolegnia* by the formation of granular cell-plates, and notes that the surface of the sporangium is first marked off by the cleavage lines into larger areas, which are progressively cut up until the size of the spores is reached. This progressive division has been regarded as including the idea of successive bipartitions of

[1] De Bary's Morph., Physiol. u. Biol. d. Pilze u. s. w., pp. 74-5.
[2] Icones Fungorum, Bd. ii, p. 19, 1838.
[3] *A. Mucedo*, same as *Rhizopus nigricans*, Ehrbg.
[4] l. c. pp. 278–9.

the spore-plasm, though this is by no means involved in the description given by Büsgen.

Recently Maurice Léger[1] has undertaken a very extended investigation of the cytology of a large series of Mucorineae. Léger points out the difficulties in technique which beset the investigation of the Mucorineae, and a careful reading of his paper is sufficient to convince one that the technical methods which he has employed are inadequate for overcoming these difficulties. A real insight into the cleavage phenomena was impossible from the preparations he was able to obtain. Léger himself is aware (p. 3–4) that there are very considerable gaps in his account.

Léger used alcohol for fixing, and cut sections in celloidin. In some cases he relied on results obtained from preparations of crushed sporangia. In my experience alcohol is a very unsatisfactory fixing agent for these materials, and not to be compared with those of Flemming, Merkel and Wilson.

To his reliance on alcohol for fixing is probably due Léger's inability to distinguish nucleolus and chromatin. He describes the nucleus as spherical, provided with a central nucleolus, which stains strongly, and a membrane; between the nucleolus and the nuclear membrane is a colourless zone which takes no stain. It is probable, judging from its relative size in his figures, that Léger's nucleolus is a fused mass made up of nucleolus and chromatin together. Léger is convinced that the nuclei regularly divide by direct division, and that karyokinesis is only met with in the germinating spore. He does not tell us just how thick his sections cut in celloidin were. The difficulty of obtaining very thin sections in this medium may well have been responsible in part for his poor nuclear figures.

Léger studied spore-formation in a very considerable series of forms from the genera *Sporodinia*, *Mucor*, *Rhizopus*, *Chaetocladium*, *Thamnidium*, *Pilobolus*, *Pilaira*, *Mortierella*, *Syncephalis*, and *Piptocephalis*.

In *Sporodinia* the young sporangium is filled with homo-

[1] Léger, Rech. histol. sur les Mucorinées, 1897.

geneous protoplasm in which the nuclei multiply rapidly by direct division. He notes nothing in the cutting off of the columella, except that the plasma is divided into an outer denser sporogenous part, and an inner less dense part with fewer nuclei about which the columella-wall is laid down. For the formation of the spores Léger quotes Van Tieghem's account, with the correction that the nuclei are continuously present during the process. He accepts the doctrine that the cleavage of the spore-plasm is due to a differentiation into a granular portion, which condenses itself into polyhedric masses which gradually round themselves up, and a hyaline intersporal plasma which fills the spaces between the polyhedric masses and forms a continuous layer beneath the sporangium-wall. The spores are formed by a process of free formation, and enclose themselves in a cellulose-wall by secretion from their surfaces. The masses are separated simultaneously, and Léger figures the separating zones of intersporal substance as being plates of equal thickness throughout. It is hard to see how he could have failed to observe the furrows which cut into the protoplasm gradually from the surface, and which can be observed readily on living material with an ordinary lens.

Léger studied the sporangia of *Mucor Mucedo*, and the remaining forms with large sporanges, at the stage of spore-formation, chiefly by means of crushed specimens and optical sections, and concludes that the process is the same as in *Sporodinia*, a differentiation of the plasma into polyhedric spore-bodies and intersporal substance which separates them.

In *Rhizopus nigricans* he notes a stage where the polyhedric masses are formed before the intersporal substance appears, and says he has not observed this elsewhere. In *Mucor racemosus* the plasma cleaves into sporogenous polyhedric bodies containing three to seven nuclei. In small sporangia with few spores, each contains a larger number of nuclei than do the more numerous spores in larger sporangia. The remaining forms investigated show nothing especially characteristic in their method of spore-formation.

In the case of *Mortierella candelabrum* Van Tieghem's and Monnier's views as to the appearance and disappearance of nuclei are opposed by Léger. Three to eight nuclei are present in the spores, and the conditions in this respect are the same as in other genera.

For *Syncephalis* the author accepts Van Tieghem's and Monnier's view that the spores are formed inside sporangia whose walls are absorbed after spore-formation. The nuclei divide directly at the base of the sporangium. Each spore contains from four to five nuclei or rarely three to six. The spore rows are held together after solution of the sporangial-wall by intersporal protoplasm.

In *Piptocephalis* the sporangium-rods divide into two spores by differentiation of intersporal plasm. The sporangial walls disappear as in *Syncephalis*. Léger distinguishes three types of spores in the Mucorineae: sporangiospores, chlamydospores and zygospores. In germination stages neither nucleolus nor nuclear membrane is present; the nuclei are granular chromatin-like bodies. They divide once while still in the spore by indirect division. Rarely this division takes place in the germinating tube, but more than one indirect division is never observed. With the beginning of the independent nutrition of the mycelium the nuclei begin to divide directly.

In all these cases Léger considers cleavage as the direct formation of the polyhedric sporogenous bodies which have the same number of nuclei as the ripe spores.

Strasburger[1] gives a brief account of the spore-formation in *Mucor*. He considers that the cleavage occurs here as in *Saprolegnia* by means of cell-plates. The entire mass of protoplasm is used for forming spores, and there is no epiplasm. He considers that the 'Zwischensubstanz' of Brefeld and others is derived from the cell-plates.

Dangeard[2] has studied the sporangium-formation in *Synchitrium Taraxaci*. He finds that the single primitive nucleus of the fungus-body divides by direct division to form the

[1] Zellbildung und Zelltheilung, 3rd Ed.

[2] Dangeard, Rech. hist. sur les Champignons, Le Botaniste, ii. sér. pp. 77-86.

numerous nuclei which appear prior to sporangium-formation. In assuming the existence of direct division here he confirms the observations of Rosen [1], who finds that the nuclei of *S. Taraxaci* regularly divide directly, and not by karyokinesis. Dangeard, to be sure, thinks karyokinesis occurs in some cases, but his figures give little evidence that he had really seen mitotic figures. Dangeard describes the division of the primitive cell to form sporangia as a process of simultaneous fragmentation, whereby the multinucleated mass is at once cut into a larger or smaller number of polyhedric multinucleated portions. These are the sporangia. They become enclosed in a membrane, round themselves up, and later the zoospores are formed equalling in number the nuclei in the sporangium.

Dangeard notes merely this simultaneous fragmentation of the protoplasmic mass. The portions are separated from each other by a layer of colourless protoplasmic liquid.

Very recently C. M. L. Popta [2] has studied spore-formation in several of the so-called group of the Hemiasci, with special reference to the comparison of the phenomena in these forms with those in the ascus and in the sporangia of the lower Fungi. According to Popta, the process of delimiting the spores in *Ascoidea rubescens* is initiated by the appearance of abundant vacuoles in the protoplasm. These vacuoles are separated from each other by numerous protoplasmic plates and threads. This reminds us of the condition in *Pilobolus* as I have described it further on, but according to the author these angular vacuoles, instead of cutting through the plasma-plates and fusing edge to edge to divide the plasma into spore-masses, gradually become rounded up and disappear. The protoplasm now becomes charged with oil drops and granules. Later, hyaline non-granular protoplasm begins to gather round the nuclei, and these non-granular plasma-masses become delimited by membranes and, ultimately, uninucleated spores. Almost at once the single spore-nucleus

[1] Cohn's Beitr. zur Biol. d. Pfl., Bd. vi.

[2] Beitrag zur Kenntniss der Hemiasci, Flora, Bd. lxxxvi. pp. 1–46, 1899.

divides into at least four daughter nuclei. Gradually the granules between the spores are absorbed, and the latter are separated only by an oil-like *Zwischensubstanz*. The spores are then pressed out by the growth upward of a new sporangium from below. These processes were studied by the author on living material and on sporangia stained and studied *in toto*, and I can but believe that the process would show more resemblance to that in *Pilobolus* if studied on abundance of material in all stages and prepared in serial sections. The appearance of the angular vacuoles may well be a stage in the cleavage process, and their disappearance later may be due to the swelling of the already separated spores, whereby they are so closely pressed together that their boundary lines are invisible except in thin and differentially stained sections. It is to be remembered that Büsgen similarly was convinced by a study of the living material that the spores of the Saprolegnieae are formed completely only to immediately fuse once more into a homogeneous mass which is later cut up a second time into the definitive spore-bodies. It is doubtless the case in *Saprolegnia* that this apparent fusion after the spores have been once cut out is merely due to the swelling, whereby they become so closely pressed together that the plasma-membranes bounding each are indistinguishable without special preparation. If it could be assumed that the formation of angular vacuoles brings about the separation of the protoplasm into spores in *Ascoidea* the process would agree with what I have described below for a number of sporangia. If, however, the definitive cleavage is brought about by aggregation of hyaline plasma about the nuclei as centres, these hyaline masses becoming in some unknown fashion surrounded by a wall, the process is so far unique, and its homologies must be sought in some forms other than the asci or sporangia which have yet been studied.

In *Protomyces Bellidis*, according to Popta, the contents of the sporangium become differentiated into a wall-layer and a central vacuole. The wall-layer is divided then simul-

taneously into a single palisade layer of rod-like spores. This spore layer is bounded inside and out by a plasma-membrane (Hautschicht). Later the spores migrate from their first position and collect in a ball at the apex of the sporangium. No intersporal protoplasm is to be observed, but an outer and inner boundary layer of protoplasm about the spore-mass is present. In *P. macrosporus*, instead of a single layer three layers of spores are formed at once from the wall-layer of protoplasm in the sporangium. The spores here also migrate to form a ball at the apex of the sporangium. This spherical mass of spores is surrounded by a space containing cell-sap and numerous radially arranged protoplasmic threads and plates. These spores contain from four to seven nuclei. The protoplasm outside the spore-mass is also multinucleated. In this last point can perhaps be noted something similar to the multinucleated periplasm in the oogonia of *Cystopus*. Popta describes the cleavage in these cases as simultaneous rather than progressive, which would suggest division by cell-plates rather than by superficial furrows.

My own observations concern the phenomena of cleavage and spore-formation in certain sporangia of the lower Fungi, which perhaps may be taken as typical at least of the groups to which they belong, and further an account for purposes of comparison of the process of free cell-formation in the asci of *Lachnea scutellata*.

I shall describe the process in species from three genera of the lower Fungi, *Synchitrium*, *Pilobolus*, and *Sporodinia*. The sporangium of *Synchitrium* may represent a type of the cyst-like reproductive bodies of the one-celled fungal organisms, while *Pilobolus* and *Sporodinia* are types of the vegetative reproductive bodies of the Phycomycetes. Whether these sporangia are homologous with the zoosporangia of the Algae is perhaps still an open question, as we shall see later. In all three cases spore-formation consists in the cleavage of a multinucleated mass of protoplasm and the formation of numerous smaller reproductive bodies.

Synchitrium.

In *Synchitrium decipiens*, parasitic on the hog peanut, the swarm-spore, after penetrating an epidermal cell, develops the orange-coloured sorus in the hypertrophied host-cell. The so-called initial cell contains a single nucleus until it has reached practically its full development. It is then a mass of protoplasm visible to the naked eye (Fig. 1), and its nucleus has a diameter several times as great as that of the nuclei of the adjacent cells of the host-plant. This nucleus now divides with great rapidity till some hundreds of much smaller daughter nuclei have been produced, which are still, however, much larger than the nuclei of most Fungi. These daughter nuclei lie irregularly distributed in the initial cell which has thus passed from the single nucleated to the multinucleated condition. It is a question whether this process is to be regarded as a part of reproduction rather than the formation of a multinucleated vegetative thallus.

Since nuclear division does not begin until the initial cell has reached nearly its full size, I am inclined to regard the division as the beginning of spore-formation and the multinucleated cell as a sporangium, the uninucleated cell being the typical vegetative body of the *Synchitrium* plant.

Cleavage of the protoplasm of the sporangium now begins. This does not take place by repeated bipartitions nor by simultaneous precipitation of cell-walls about each nucleus. The process is rather analogous to that in the dividing protoplasm of the germinal disk of the chick, or still more nearly like that in those insect eggs where a series of nuclear divisions precedes protoplasmic segmentation [1]. The cleavage is progressive from the surface of the initial cell inwards, and divides the mass into successively smaller portions (Fig. 2). The actual division of the protoplasm is accomplished by furrows formed on the surface, and growing deeper and deeper in a more or less exactly radial direction. These grooves are in reality so narrow as to appear as plates, which grow wider

[1] Hertwig, Die Zelle und die Gewebe, p. 187.

by additions along their inner margins till they intersect, and thus divide the protoplasm into irregular blocks or sometimes pyramids with their bases in the surface of the initial cell. (See Figs. 1, 2, 3, which are median sections through an initial cell in which cleavage has just begun.) Only at the very periphery the separation of the cut surfaces of the protoplasm to form a shallow notch, as it appears in section, reveals the true nature of the process as a pushing in of the free surface to form a deep though extremely narrow constriction.

In many cases at first there is no separation of the newly-formed surfaces; they remain closely appressed, up to the periphery of the cell. The groove appears in section, merely as a single line which the Zeiss apochromatic lens 140 ap. fails to resolve into two closely appressed surfaces (Fig. 3). The position of the line is further emphasized by the arrangement of the vacuoles, which are pushed aside and form in section two more or less regular rows in the plane of the newly-formed surfaces on each side of the furrow (Fig. 3). Such a line might be taken for a cell-plate which subsequently splits to form the boundaries of the protoplasmic segments or which is metamorphosed into the cellulose walls of the spores. That this line, however, in reality represents from the start two closely appressed surfaces is abundantly shown in many cases. Shrinkage in volume of the protoplasm goes on during the early stages of cleavage. This shrinkage is probably associated with the throwing off of water which has been seen to accompany sporangium-formation in many Zygomycetes. As a result of this shrinkage we frequently find that the cleavage of the protoplasmic mass is followed at once by the separation of the newly-formed surfaces, so that open furrows appear. This is regularly the case in *Pilobolus*, as we shall see later. There can be no doubt that the processes in forming the broad open furrows and the narrow cleavage lines are identical. In one case the surfaces have remained so closely appressed as to be indistinguishable, while in the other they have been pulled apart at once. The two conditions can hardly be regarded as distinct stages

in cleavage, since in many cases the grooves open as fast as they are formed, while in others they remain closed till a much later period (Fig. 4). The separation of the surfaces thus formed need not proceed from the periphery inward. Frequently furrows are found closed at the periphery and widely open deeper in the mass of the cell. The formation of the cleavage furrows is the essential process; whether the surfaces thus formed separate earlier or later is a matter of secondary significance. That these cleavage lines are not long meridional furrows dividing the initial cell symmetrically, as is the case in the sea-urchin's egg for example, is clearly seen from surface sections of cells in the first stages of segmentation, as shown in Fig. 5. We see here that the cleavage surfaces intersect each other at very varying angles, so as to mark off the surface of the cell by an irregular network of grooves in which the meshes are of very irregular shape and of unequal dimensions. The grooves seem to appear first, at least in some cases, on one hemisphere of the cell, and to spread gradually outward over the other. In other cases cleavage seems to begin almost simultaneously over the whole surface of the cell. In the first set of cases I endeavoured to determine whether cleavage began on the superficial or deeper surface of the cell, as it lies in the epidermis of the host, but I could discover no regularity in this respect.

The cleavage is progressive from the surface inward, the furrows deepening in general in a radial direction. Still they may be curved, and are inclined to each other at very varying angles and frequently form intersections at points near the surface of the cell, thus cutting off superficial blocks of protoplasm of varying shapes and sizes. Sometimes this tendency to cut off a superficial layer of segments is very marked (Fig. 2), so that we have a central solid mass or cell of protoplasm surrounded by a layer of superficial cells; in other cases the furrows grow radially inward without intersecting till near the centre, thus forming narrow cones and pyramids with their bases outward. As the process of cleavage goes

on the contraction of the protoplasm becomes much more marked, the furrows open widely and the masses of protoplasm tend to round themselves up.

The cleavage-segments produced in this way are seen to be unequal and as a rule multinucleate. The cleavage-furrows have so far shown no tendency to orient themselves with reference to the nuclei. But as the process advances, and the protoplasm is cut into smaller and smaller masses, we find the nuclei more evenly distributed, and it is seen that none of these portions are left without nuclei. In the end the result is always the separation of the protoplasm into uninucleate masses (Fig. 6). The process which at first seemed to be independent of the nuclei is seen finally to be directed solely from the standpoint of their distribution. The cleavage, as has been already noted, is attended by a very pronounced shrinkage of the protoplasm. This began while the segments were still multinucleate, and by the time cleavage is complete the segments are so reduced as probably not to occupy more than one-third of the volume of the primordial cell. An exact estimate of this reduction is very difficult. Rothert [1] estimates the shrinkage of a *Saprolegnia* sporangium at a corresponding stage at thirteen per cent. It is to be noted that the cell does not entirely fill the membrane at these stages, sections always showing a considerable open space on one side or the other (Figs. 4, 5). This indicates that loss of water and volume begins prior to cleavage. To be sure, this reduction prior to cleavage may be artificial and due to fixation, though the structure and appearance of the protoplasm give no evidence of this. The cavity around the protoplasmic mass prior to cleavage must be filled with water or at least by solutions which are not precipitated in fixation nor stained by ordinary reagents, but at the end of cleavage the uninucleated segments are floating or, better, imbedded in a liquid which contains an abundance of dense oily droplets, which blacken with osmic acid and take up stains (especially orange G.) with considerable avidity. There is generally a layer of

[1] l. c., p. 315.

segments around the inside surface of the sporangium-wall. The remaining segments are not evenly distributed, but are gathered in clumps which stick together and press upon each other even to the extent of giving the normally spherical segments a polygonal outline. Frequently there is one large central clump of segments, and between this and the surface layer appears the emulsion-like oily liquid mentioned. I have spoken of this liquid as thrown off by the protoplasm by a process of drying, connected with ripening. Why it should contain oily matter is not apparent. It perhaps should be mentioned that it is not at all protoplasmic in its apparent nature, and cannot in any sense be compared with the epiplasm of the ascus or the periplasm of the oogonium of the Peronosporeae. It is apparent already that the method of spore-formation by constriction from the surface, as we find it in *Synchitrium*, precludes the possibility of a true periplasm in these forms. With the stage shown in Fig. 6 the process of cleavage is completed, and the uninucleated plasma masses so produced may be taken as typical vegetative spores homologous with those of *Saprolegnia*, and the process as representing typical asexual reproduction. Still their formation marks the completion of only the first stage of development of the reproductive bodies of *Synchitrium decipiens*. This first stage has been a process of cleavage, by which the multinucleated secondarily developed vegetative body of the fungus has passed back for purposes of reproduction to the primitive uninucleated condition. The bodies so formed may be called protospores. We now find a second series of changes initiated in the sporangium, sharply distinguished from the cleavage processes, and which I think we shall see are to be considered as initiatory stages in the germination of the bodies formed by cleavage. The first step in this second stage of intrasporangial development consists in a growth or swelling of the protospores. This proceeds until the primordial cell-wall is completely filled once more with its protoplasmic contents, as it was in earlier stages prior to cleavage. The segments as a result of this growth become pressed together, and take on

as a consequence a sharply angled polygonal outline in cross sections. The appearance of the sections at this stage is characteristically different from that in the last. The segments seem to be delimited and separated from each other by rather broad granular plates, and if one had not observed the preceding stages it would be easy to believe that these figures indicated a stage in cleavage where the protoplasm was being cut up simultaneously by granular plates into uninucleated spores. The granular plates are very conspicuous in stained sections (Fig. 7), and careful observation shows that, in the case of every cell, cleavage is complete rather than in progress at this stage, the granular plates being really double, and composed of the closely appressed membranes of the swelling protospores. Still I am inclined to believe that the appearance of this stage in unsectioned and unstained material of *Saprolegnia* such as Büsgen studied was what led him to advance the doctrine of a fusion of the once separated spores, followed later by a second more definitive cleavage by granular cell-plates. As a result of this growth in volume the liquid in which the primary segments floated disappears. Whether it is taken up again as food by the spores from which it has been extruded or simply is forced out of the sporangium, or whether both of these processes take place, is not easy to determine. The spore-mass at this stage fills the sporangium. The protoplasm of the protospores is evenly vacuolar and not apparently more or less dense than in the cleavage stages.

The next further step in development consists in a rapid multiplication of the nuclei by division. The divisions do not proceed as simultaneously as in the case of the earlier nuclear multiplication prior to cleavage, and we find spores containing 1, 2 or 4 nuclei in the same section (Fig. 7). The apparent irregularity is due in part to the fact that the spore appears in several sections. The process of division continues however till each segment contains from eight to twelve or possibly more nuclei. There is no evidence to be gained that the different spores finally contain exactly the same number of nuclei nor that each nucleus is removed by the same number

of generations from the original single nucleus of the protospore. It seems rather that the number of divisions in each spore depends on individual conditions of nutrition, &c., the same which determine the slight variability in size of the ripe spores. This is to be noted especially as showing that we have in the divisions of this spore-nucleus nothing directly comparable to the determinate number of divisions passed through by the ascus-nucleus to form the eight ascospore-nuclei.

This period of nuclear multiplication is followed by the third stage, in which the spores ripen preparatory to being set free from the sporangium for distribution. In this stage they become for the first time enclosed in a true cell-wall. Through the preceding stages they have been mere naked bits of protoplasm bounded only by their plasma-membranes. Fig. 8 shows the spores when the wall first appears as a very delicate membrane which stains strongly with orange G. At first it is only to be detected in cases where slight plasmolysis has occurred in fixation, so that plasma-membrane and spore-wall are somewhat separated. Later it becomes much thicker and shows a double contour. With the formation of the spore-wall the protoplasm of its body undergoes contraction once more, and the spores which were closely pressed together and polygonal in outline during the period of nuclear division draw apart from each other and become more rounded in outline (Fig. 8). With these changes the spores are finally ripened and ready for dissemination. The spore germinates as a sporange. The stages of germination consist in the breaking up of the multinucleated spore into a number of uninucleate swarm-spores. This may occur after a resting period in the case of *S. decipiens*. In *S. Taraxaci*, as is well known, the spores germinate to form swarm-spores at once. This formation of swarm-spores must be considered as a continuation of the stage of nuclear division in the spores which was interrupted by the ripening period. The whole may be roughly characterized as an embryonic stage in the life of the fungus.

The changes which take place in the process of cleavage of the protoplasm into the swarm-spores I have not as yet been able to study. The functional analogy between the nuclear divisions and growth of the protospore which takes place in the mother sporangium and the development of the egg in the tissues of the parent in the higher plants is sufficient to justify the distinction of an embryonic stage in the life-history of the fungus.

In *Synchitrium Taraxaci* this whole process of cleavage is abbreviated by the omission of the protospore stage. The multinucleated segments formed by the first cleavage-furrows round up, their nuclei become still more numerous by division, and they are then set free at once and germinate as sporangia. The life-history of the fungus is thus much shorter, and it is able to spread with great rapidity when conditions are favourable. As is known, provision is made in this species for its existence during unfavourable periods by the formation of the so-called resting spores. The relation between the cleavage processes in *S. decipiens* and *S. Taraxaci* is the same as that between the segmentation in *Pilobolus* and in *Sporodinia*, as we shall see later.

The question arises whether the protospore or the swarm-spore is the homologue of the gamete in the higher plants, but the resemblances are too remote, and the probability of any genetic relationship between these particular lower forms and the higher plants is too slight, to justify any speculation on this point. If we now summarize the life-history of the fungus we can roughly, and for purposes of comparison with other forms, distinguish five stages.

1. The multiplication of the nuclei by which the uninucleated vegetative body becomes multinucleated as a preparation for the formation of a corresponding number of reproductive bodies.

2. The progressive cleavage from the surface inward of the multinucleated mass into uninucleated masses or protospores.

These two processes constitute the original and fundamental elements in spore formation.

3. Embryonic development, in which the spores increase in size and become multinucleated.

4. Ripening, in which a protective wall is formed about the spore and the protoplasm passes into a denser resting condition with abundant inclusions of reserve food-materials.

5. Germination, which is a mere continuation of embryonic development by which a sporangium produces a number of embryos (swarm-spores).

A mechanical explanation of the cleavage I have just described is by no means easy. The formation of superficial constriction-furrows cutting into the protoplasm is analogous to what is seen in the division of the animal cell especially, as noted above, to the phenomena of cleavage of certain insect eggs. Heidenhain [1] and later Kostanecki [2] have attempted to refer the constriction which divides the animal cell to the contraction of fibres arranged about a centrosome. Such sets of fibres are certainly not concerned in the division of the *Synchitrium* sporangium. The gradual cleavage, first into large multinucleated segments and only later into uninucleated spores, which are formed in *S. Taraxaci*, could not well be referred to any such simple systems of contractile fibres as the above-named authors discuss.

The decrease in volume of the protoplasm during the process might suggest that the cleavage is connected with the loss of water, and the formation of superficial furrows might be compared to the cracking of the surface of a drying mass of a colloidal substance. Such an explanation however in itself alone is entirely inadequate to explain the definiteness and certainty with which the final result of the cleavage is attained. The nuclei are seen to be the centres of the ultimately formed segments, which would by no means necessarily be the case as a result of simple cleavage by drying. We might indeed assume that the loss of water was least in the neighbourhood of the nuclei, which would meet the demands of the case without however simplifying the

[1] Archiv für Mikr. Anat. 1894, p. 423.

[2] Ibid. 1897, p. 651.

matter. The nuclei are certainly the centres about which the cleavage planes ultimately orient themselves, and it seems necessary to assume that some influence has passed outward from them and directed the whole process of the cleavage.

Strasburger [1], in connexion with Noll's [2] doctrine that the protoplasmic membrane is the direct controlling organ in the growth of the coenocytic Algae, has pointed out that the nuclei may be the real controlling centres working through the protoplasmic membrane, even in resting cells, by means of impulses transmitted through the kinoplasm. My own observations on spore-formation in the ascus point to the assumption that the plasma-membrane is itself kinoplasmic in its origin.

If in *Synchitrium* the nuclei are centres for the formation of kinoplasm, and it proceeds outward from them by diffusion in all directions till it reaches the plasma-membrane, this will be correspondingly increased in thickness, and if the mass be decreasing in volume by loss of water and tending to split up like a mass of drying starch, the membrane might perhaps press into the furrows thus formed, so as to become the surface layer of the forming segments. The first furrows will show no specific orientation with reference to the nuclei, but as the process continues the nuclei forming the centres for building up and distributing this membrane-material will by reason of this very fact tend to occupy the centres of cleavage-segments, until ultimately each nucleus will have furnished sufficient material for enclosing itself in an independent membrane. The evidence in favour of the explanation of cleavage just suggested is by no means adequate; still it will perhaps serve to bring out more sharply the elements of the problem to be solved.

Pilobolus.

The method of spore-formation in the Zygomycetes is of especial interest, since we have here one of the most

[1] Über die Wirkungssphäre der Kerne und die Zellgrösse. Hist. Beitr. v, p. 981, 1893.

[2] Die Wirkungsweise von Schwerkraft u. Licht auf die Gestaltung der Pflanze. Nat. Rundschau, Bd. iii, 1888.

widely distributed, commonest, and in some cases such as *Pilobolus* one of the most highly perfected methods of asexual reproduction and spore distribution known among the Fungi.

Brefeld [1] has given very full accounts of the external anatomy of *Pilobolus* and has also described the behaviour of very numerous forms under cultivation with varying conditions of temperature, &c. He has not especially investigated the phenomena of spore-formation. He attempts however to maintain and strengthen the doctrine that the mucilaginous jelly in which the spores of many Mucorineae are imbedded is identical with the epiplasm of the ascus.

The study of these forms of the Zygomycetes, apart from their interest as illustrating the mechanics of protoplasmic division in coenocytes, has gained a certain further importance since, as is known, the doctrine has been advanced by Brefeld that they illustrate in their vegetative sporangia the ancestral types in the development of the ascus which is, according to this view, only a sporangium which has become definite in its size, form, and in the number of spores which it produces. Evidence for or against this doctrine can perhaps be obtained by an exact and complete study of the processes in the two structures, and I hope to contribute something to this end in the following account.

I have investigated especially the phenomena in the sporangium of *Pilobolus*, and have used the common species *P. crystallinus*, from which all the figures illustrating spore-formation are made. I have, however, confirmed the principal points in the process by studies on *P. oedipus* and *P. microsporus*. *Pilobolus* is very readily cultivated in the manner described by Brefeld. The fungus always appears on horse-dung left for a few days under a bell jar, but to get an abundance of material at the proper stage of development it is better to resort to the simple culture methods described by Brefeld. These consist simply in infecting a mass of dung,

[1] Botanische Unters. üb. Schimmelpilze, 1. Heft, 1872.

partially sterilized by repeated exposure to the temperature of boiling water, with spores from a culture which has appeared spontaneously. The sporangia can be caught on a clean slide as they are thrown off, and the spores are sufficiently free from bacteria when simply rubbed in a drop of water to use in infecting the partially sterilized dung. The culture can conveniently be made in a crystallizing dish of any size. In seven or eight days the sporangiophores begin to appear. Successive crops of sporangia are matured for several days, and thus abundant opportunity is given to secure any desired amount of material at any stage in development. The sporangiophores stand in dense turfs, completely covering the surface of the substratum. Not the least favourable feature of this fungus for cytological study is the exact regularity with which the different stages succeed each other through the day and night. The well-known yellow bulb-like swellings of the mycelium from which the sporangiophores arise appear in abundance on the dung in the early afternoon. This is a favourable period for studying nuclear division in this fungus. The vegetative nuclei in these bulbs divide rapidly to form the numerous nuclei which are afterwards cut off in the sporangium to form the spores. A little later the sporangiophores bud out from the bulblets and grow toward the light. Late in the afternoon the ends of the sporangiophores begin to swell to form the sporanges. The columella wall is formed about midnight. Segmentation occurs between one and four o'clock a.m. Embryonic development of the spores goes on from four to seven, and the ripe sporanges will be thrown off from nine till twelve a.m. The whole process is repeated at approximately the same daily periods for three or four days, when the culture is exhausted. The above periods of course vary somewhat with differences in temperature, moisture, &c., and the development of different sporangia is also not exactly parallel. Still, material fixed at the periods mentioned has always shown in my experience the majority of the sporangia in the stages noted. The general facts as to the development of *Pilobolus* have been very well worked out

by Cohn[1], and the whole process, as noted above, has been described and figured by Brefeld, so I need only mention the more especial points bearing on spore-formation. The mycelium of *Pilobolus* is one-celled and multinucleate, and when the sporangiophores are to be formed, the protoplasm collects at certain points in the mycelium, producing barrel-shaped swellings. These swollen portions are cut off by both peripheral and proximal walls. The division of the protoplasm is accomplished by simple constriction-furrows, and in the bulb so formed, as noted above, a rapid multiplication of nuclei takes place[2]. This dividing period probably corresponds to the dividing period in *Synchitrium*, whereby the one nucleated cell becomes multinucleated. The protoplasm of *Pilobolus* is already multinucleate, but preparatory to reproduction these vegetative nuclei multiply rapidly, apparently simply as a preparation, for the production of a sufficient number of spores. The protoplasm in the bulbs becomes much more densely packed with nuclei than that in the adjacent vegetative portions of the mycelium. This period of division is completed before the budding out of the sporangiophores. I have never observed nuclear division going on in the growing tip of the sporangiophore nor in the young sporangium before the cutting off of the columella. The growth of the sporangiophore is apparently largely accomplished by the flowing out of the material stored up in the bulb. As the sporangium becomes larger the bulb is more and more emptied of its protoplasmic contents, though a layer sufficient for maintaining the turgor of the central vacuole is always maintained, and in this layer also numerous nuclei are present. The sporangiophore grows in length, and is bright yellow throughout its upper portion, due to the presence of yellowish, perhaps fatty, granules in the protoplasm. At first it is slightly narrowed at the tip, but after

[1] "Die Entwicklungsgeschichte des Pilobolus crystallinus." Nova Acta Acad. Caes. Leop. Carol. Nat. Cur., vol. xviii, pt. 1.

[2] A detailed description of these and some other nuclear divisions in the fungi I expect to give in the near future in another connexion.

reaching a height of one to several millimetres the tip becomes rounded and swells rapidly to form the young sporangium (Fig. 9). No especial aggregation of the nuclei at the apex is to be noted at any time during this growth. They are rather evenly distributed throughout the sporangiophore-tube, and are present also in the hyaline plasma at the very tip. The growth is largely apical, rather than by stretching and deposition of new layers of cellulose on the inner surface of the stalk already formed. Well-fixed specimens show the paths of the streaming protoplasm in the sporangiophore very distinctly; each streaming thread is seen to have marked a path for itself through the protoplasmic structure. It is bounded by continuous delicate films, quite distinct from the spongy structure of the adjacent plasma. Whether these streams flow upward only cannot be determined from fixed material. That these delicate threads are really the paths of streaming plasma-currents is evident from the further fact that after the sporangium is full grown and the columella has been formed, all trace of the threads disappears from the protoplasm of the sporangiophore. The paths of the protoplasmic streams in the young sporangium are very well shown in sections such as are given in Figs. 10, 11.

As the currents passing up in the stalk reach the enlargement of the sporangium the streams seem to widen and thicken, indicating a checked velocity in the suddenly enlarged channel. This gives us the thickened ridge about the upper edge of the stalk shown in Fig. 2. From the upper edge of this ridge the currents spread into what in section is seen to be a fan-shaped series, distributing themselves to the surface of the sporangium from its apex through about 90°, thus building up the thickened layer which is to constitute the spore-plasma. In the lower half of the sporangium there is indicated a slight backward flow toward the mouth of the sporangiophore, thus filling the space gradually around the ridge which is built up around the mouth of the sporangiophore. The whole process of the growth of the sporangiophore and the inflow of protoplasm into the young sporangium

is seen to be analogous to the process in the growth of a pseudopod on an *Amoeba*, and the same distinction of central outflowing currents and slight peripheral backward currents can be observed. Noll has already pointed out that the growth of a coenocytic alga like *Caulerpa* is to be compared to the creeping of an *Amoeba*, and the comparison is equally striking here.

The central portion of the young sporangium is largely filled with cell-sap, only a few strands of protoplasm cutting through it. Outside this central area the protoplasm is spongy and thready, becoming denser toward the periphery. The outer layer is dense and quite homogeneous from the start, and is slightly thinner at the apex of the sporangium. The appearances in these young sporangia are suggestive with reference to the question as to the gross structure of protoplasm. In Figure 11 for example the plasma is not vacuolated in the ordinary sense, though an immense amount of cell-sap is present. This sap is not contained in more or less rounded cavities like the vacuoles as commonly described. The protoplasm forms rather a spongy framework in whose meshes is the cell-sap, The whole mass of sap occupies the interstices of the plasma-net, and is not divided off in isolated vacuoles. The sap is a *continuum*, in which the plasma-strands float as a net or framework. Later, after the upward flow is complete, the plasma passes into a truly vacuolated condition, when the cell-sap is contained in rounded cavities whose outlines are determined by surface tension. A similar transition from a foamy vacuolated structure to a spongy structure will be described later in the account of the germination of the spores. The whole sporangium continues to grow in size for a considerable period (5 p.m. until about midnight), and its wall in the apical region becomes thickened and dark coloured. The layer of spore-plasma becomes thicker by the increase in density of the spongy plasma on its inner surface. The circular ridge becomes higher, and to the outside, between it and the spore plasma, an open spongy mass is maintained which already indicates the outline of the

columella wall. The radiating strands from the upper edge of the ridge are shorter as the distance from the edge of the ridge to the dense spore-plasma is less. By midnight the layer of spore-plasma reaches its definitive thickness. The dome-shaped columella cavity is still a large cell-sap cavity, and the inner surface of the dense spore-plasma passes gradually into this by means of a spongy layer which is rapidly becoming typically vacuolar as the streaming ceases. In the outer portion we can now roughly distinguish three parts in the protoplasm of the sporangium ; the outer dense spore-plasma, passing into a spongy layer on its inner surface, and the central cavity of the columella. In the outer part of the spongy layer the split between the spore-plasma and the columella-plasma is to be formed. The first indication of this cleavage is seen in the gradual appearance of a layer of vacuoles larger than the rest, and lying in the curved surface which marks the outline of the columella. The vacuoles become flattened in their radial axes parallel to the surface of the sporangium, and form thus disk-like openings which tend to fuse at their edges. At the same time a circular cleft is seen to start from the edge of the sporangiophore opening just outside the protoplasmic ridge above noted, and to develop upward, cutting into the vacuoles so that they become connected into a continuous furrow (Fig. 12). Whether this furrow is continued upward to enclose the whole dome-shaped columella, or whether the vacuoles in the upper portion fuse edge to edge before the cleft reaches them, is difficult to determine. The process is a progressive one, the cleavage being complete in certain portions sooner than in others, and at a very late period strands of protoplasm are seen connecting the spore-plasma with that in the columella. It is not impossible that many of the apparently disk-shaped vacuoles are sections of curved openings which burrow through the plasma from below upwards. Frequently vacuoles which are distinct in one plane are seen, by focusing up or down, to be connected. There can be little doubt however that a considerable part of the cleavage of the columella is accomplished

by flattening and lateral fusion of originally ellipsoidal or spherical vacuoles, that is, the cleavage is not entirely by a furrow from the plasma-membrane at the mouth of the sporangiophore, but is at least in part a process of separation by excretion of liquid into vacuoles and their fusion side by side *in situ*. These vacuoles are not situated on the extreme boundary of the protoplasm adjacent to the large central vacuole, but are placed where the dense spore-plasma first becomes characteristically spongy. At the base of the sporangium indeed they cut through plasma as dense as the densest spore-plasma of the sporangium. Why the cell-wall of the columella could not be deposited on the surface of the central vacuole as well as on the surface of the small vacuoles, and thus enclose all the protoplasm in the sporangium, is an interesting question. The necessity is evident that the cleavage should proceed through a tolerably dense plasma, and this is perhaps due to the need of two protoplasmic surfaces in contact, in order to form a cell-wall. It would seem otherwise quite natural that the spore-plasma should simply bound itself off by a wall against the central vacuole of the columella, in which case only the plasma at the mouth of the sporangiophore would need to be cut through, and the cleavage process would be apparently simplified.

The cleft separating the spore-plasma from that of the columella is quite wide and irregular at first. Later, the turgor of the two separated plasmic masses causes them to be firmly pressed together. This whole process of the delimitation of the spore-plasma is accomplished without the formation of a cell-wall, and consists simply in the establishment of new protoplasmic membranes. The cell-wall is deposited later, in the cleft between the two membranes.

With the cutting off of the columella, the so-called collar begins to form on the basal wall of the sporangium, just outside the mouth of the sporangiophore. It appears first as a thin layer of homogeneous material which, with Flemming's triple stain, becomes faintly blue in colour (Figs. 14 and 15). After the columella-wall is laid down it increases rapidly in

thickness and finally extends up the base of the columella. It is thickest at the angle which the columella makes with the sporangial wall and thins out toward the edges in both directions. The substance of which it is composed is the same in appearance and staining reactions with the intersporal substance (Fig. 24) which appears later, and which Brefeld has attempted to homologize with the epiplasm of an ascus. It is plainly to be seen here that this collar-substance is not protoplasmic, and is an excretion deposited outside the plasma-membrane bounding the spore-plasma. It is an excreted material, and designed for a special function in distributing the sporangia as Brefeld has pointed out; but its perfectly homogeneous structure and its position outside the plasma-membrane of the spore-plasma prior to spore-formation show that it is in no way comparable to the protoplasm left behind in the ascus in the process of free cell-formation as it takes place there.

It is to be noted that the columella-wall has the same dome-shaped outline when it is first formed as it has later, when the spores are ripe. It is not at first a flat transverse septum which is later pushed up into a dome as the spores ripen. Cleavage of the spore-plasma begins very shortly after the columella is complete. The protoplasm becomes somewhat vacuolar, and the nuclei are rather evenly distributed through its mass. Cleavage-furrows then appear around the base of the sporangium, cutting the surface of the protoplasm into irregular polygonal areas (Fig. 14). At the same time in the interior of the protoplasm, where hitherto only rounded or spherical vacuoles have been found, angular vacuoles, frequently three cornered in optical section, appear, and their edges cut outward through the protoplasm to meet similar cleavage-furrows from adjacent vacuoles (Fig. 14). The surface-furrows have also been growing deeper, and meet and become continuous with the edges of the vacuoles. The surfaces of the vacuoles from being convex outwardly have become concave from the pressure of the adjacent plasma-masses, and the latter appear as intercellular spaces between the proto-

plasmic cleavage-segments. The spore-plasma is thus roughly marked out into irregular blocks apparently without reference to their size or the number of nuclei they contain (Figs. 14–15). Further progress of this furrowing cuts the first-formed blocks into oblong rounded sausage-shaped masses generally containing two to four nuclei in a row (Fig. 16). These oblong bodies are now divided transversely to form rounded or spherical masses each with one or few nuclei (Fig. 17). With this the primary cleavage phenomena are complete. The multinucleated spore-plasma has been cut up into units with one or few nuclei, corresponding to the protospores produced by the cleavage in the sporangium of *Synchitrium*.

But these are by no means the definitive spores of *Pilobolus*. A further period of growth and cleavage is to be passed through before the spores with protective walls are formed ready for dissemination. These uninucleated masses are perhaps morphologically the equivalents of the *Saprolegnia* swarm-spores, but they have no cell-walls, and are but a transitory developmental stage in the *Pilobolus* sporangium. The succeeding periods are, however, at once distinguished from the cleavage stages so far described. The protospores enter on a period of growth and division, and since this growth occurs in connexion with the parent tissue and nourished by it, it may be fairly regarded as an embryonic stage. The first step in the further development of the protospores is a period of nuclear division, wherein the nuclei divide rapidly, so that the masses become once more multinucleated (Fig. 18–19). After the nuclei have thus divided the cell divides by constriction, the nuclei being separated into two groups in the halves so formed. The nuclei may then divide still further, their divisions being followed by further cell-divisions, but finally nuclear division ceases. Cell-division, however, continues until the masses are cut up into regularly oblong binucleate cells. A series of these final cell-divisions, whereby an octinucleate mass is cut up into binucleate spores, is shown in figures 20 to 23. After each cell-division the nuclei are separated into two equal groups in opposite halves of the

cell (Figs. 20–21). The equatorial plane between the groups becomes more hyaline, and constriction of the plasma-membrane gradually cuts the cell in two (Fig. 22). The primary cleavage is complete about four o'clock, and the period of embryonic growth and division lasts until about seven a.m. The binucleated spores are at first naked masses of protoplasm, but soon are covered by a wall. Their protoplasm passes into a resting vacuolar condition, and reserve food products appear as drops of oil. Frequently a large oil drop occupies the centre of the cell, and the nuclei lie at its ends. The spores in this ripened condition are difficult to stain, their walls being extremely resistent to fixing and staining reagents. The best figures were obtained by killing in Flemming's solution, and staining with methylene blue. By this method very sharply defined nuclear figures are brought out in different shades of blue. The nuclear sap is colourless, the nucleolus deep blue, and the chromatin lighter blue. The spores are killed, fixed to the slide with albumen-fixative, and exposed about five minutes to one-tenth of one per cent. methylene blue solution. Spores that have been imbedded and sectioned on the microtome are not as favourable for the above staining as those freshly killed and fixed without dehydration and imbedding in paraffine. After staining, the preparations are dehydrated, cleared in clove-oil, and enclosed in balsam. The ripe spores lie imbedded in a shining mass of intersporal substance which can be stained readily with gentian violet. It is similar in all respects to the material in the so-called collar, and is presumably an excretion of the protoplasm during the ripening of the spores. As was noted above, the method of spore-formation as it occurs here, by cleavage of the entire protoplasmic mass from the surface inward, precludes the possibility of any comparison between this intersporal substance and the epiplasm of the ascus.

Before passing to the germination of the spores we may note the second enlargement of the sporangiophore, just below the sporangium, which is to become the explosive vesicle for firing off the ripened sporangium. It appears first

late in the afternoon, and continues gradually to enlarge during the night (Fig. 10). It is lined by a protoplasmic layer no thicker nor more numerously supplied with nuclei than the remainder of the sporangium. The cell-wall of the narrow neck between this vesicle and the sporangium is very thin, indicating the line of fracture when the vesicle finally bursts. The central vacuole of the vesicle contains only cell-sap, the protoplasmic primordial utricle forming only a lining layer just inside the wall. The throwing off of the entire sporangia during the morning hours by the swelling of the collar and bursting of the vesicle beneath, and the wonderful heliotropic reactions they show in the direction of the discharge, have been fully described by Brefeld. During ripening the sporangial wall has become very much thickened and brittle over the top of the sporangium. It thins out rapidly, however, and is light-coloured beneath the collar, and offers less resistance to the swelling of the latter at the time of the explosion.

The germination of the spores in a decoction of horse-dung can be readily studied in all its phases by fixing in Flemming's solution, fastening them to the slide with albumen-fixative, and staining with Flemming's triple stain or Mayer's 'Carmalaun.' The spores swell tremendously before pushing out a germ-tube, and the two nuclei divide to form eight or more. 'Carmalaun' gives the best results for staining the swelling stages, but Flemming's stain serves better after the germ-tube is formed. Figs. 25, 26, and 27 show these germinating stages. The nuclei may be readily demonstrated at these stages, but the plasma is too dense and the wall too impenetrable to allow a full study of their division. The development of the living spores can be well watched in their earlier stages under an immersion lens, and Figs. 28 to 29 show the appearance of spores of an undetermined species of *Pilobolus* during germination in water. As soon as the spores swell the nuclei can be made out without staining. They undergo considerable amoeboid changes of form, and seem even to change position in the spore by this means. In this case

nuclear division does not precede the pushing out of the germ tube as it does in *P. crystallinus*. The gross structure of the plasma is at first typically vacuolar. The vacuoles are spherical or flattened against each other, and are entirely distinct, each maintaining its outline apparently by surface tension of the contained liquid. So soon, however, as the germ-tube is pushed out from one side the whole structure is transformed, the vacuoles become elongated and fuse, the protoplasm is gathered into strands, forming a loose spongy net in which streaming is begun at once, and continues as long as the cells can be kept under observation without suffering from lack of oxygen. This change of the structure of the protoplasm can be followed with the greatest ease and accuracy, and is entirely similar but in reverse sequence to what takes place in the sporangium just before the columella is formed. It is apparently a necessary change if streaming is to take place in the germinating spore, and is doubtless a result of the same influences that lead to those phenomena.

Figs. 30–32 show the appearance of living spores under the 2 mm. apochromatic lens when germinating in a decoction of horse-dung. The change from vacuolar to thready structure is shown just beginning in Fig. 32. The thicker germ-tubes produced when the spores are germinated in a nutrient solution instead of water have already been noted by Brefeld.

The processes of cutting off the sporangium and dividing the multinucleated spore-plasma into spores may be summarized as follows:—

1. Arrangement of a series of vacuoles in a dome-shaped, curved stratum on the inner boundary of the dense spore-plasma.

2. Flattening of these vacuoles and the formation of a cleft through them, beginning at the edge of the opening into the sporangiophore.

3. Smoothing of the new plasma-membranes thus formed, by turgor of spore-plasma and columella-plasma.

4. Formation of a columella-wall in the cleft between the new plasma-membranes and vacuolization of the spore-plasma.

5. Formation of surface-furrows which deepen and meet the vacuoles, and thus cut the plasma into irregular and finally oblong sausage-shaped multinucleated bodies, which are further divided to form one or few nucleated energides, the protospores.

6. Nuclear division followed by cell-division, constituting embryonic development of protospores into mature binucleated spores.

Sporodinia.

Sporodinia is a further type of the Zygomycetes, in which the asexual reproductive apparatus is considerably modified. The fungus occurs in nature as a parasite on various Hymenomycetes, especially *Boleti*, and, as described by Brefeld, is very easily grown on bread cultures. It is a very favourable object for the study of spore-formation in the sporangia. The whole development and ripening of the spores is here passed through in a very brief space of time, one hour from the first swelling of the end of the sporangiophore sufficing for the development of the ripened spores. The spores are thin-walled, variable in size, short-lived, and by no means so resistent to unfavourable environmental conditions as those of *Mucor* and *Pilobolus*. They retain their capacity for germination only a week or two. Zygospores are produced much more abundantly by this fungus than by other members of the group, and serve the purpose of maintaining the existence of the fungus through a period of unfavourable conditions.

The sporangiophores are here dichotomously branched, and with the ripening of the spores become regularly septate.

The ends of the sporangiophore-branches swell to the form of flattened spheroids, whose transverse diameter is only about twice that of the extremely thick sporangiophores. The spore-plasma becomes aggregated in the form of a cap-shaped mass, thinning at its edges and lining the upper two-thirds of the spheroid. In sections the spore-plasma shows a regularly crescentic outline. The central and lower parts of the

sporangium are filled with a characteristically foamy protoplasm. Between this foamy plasma and the spore-plasma there is at first a very gradual transition (Fig. 33). Later, the difference in density is more sharply defined, the transition being sudden, on the lower surface of the dense spore-plasma, to a very open foamy structure. Just along this line of changed structure a series of larger vacuoles become arranged which gradually flatten in a curve, outlining the surface of the columella (Figs. 34, 35). It is perfectly evident that these are ordinary vacuoles which have grown larger and begun to flatten. At first it is not easy, in many cases, to say whether a particular vacuole is to take part in the formation of the series or not. As they grow larger they mark very sharply the boundary of the dense plasma. One wall of any one of these vacuoles will be dense plasma, while the opposite wall abuts on a number of smaller vacuoles. The nuclei[1] are somewhat more numerous in the spore-plasma, but not strikingly so. The mechanism of the flattening of these vacuoles is not easy to understand, except in so far as the denser resistent spore-plasma might be supposed to resist the action of surface-tension of the cell-sap on the side adjacent to it.

These vacuoles become more and more flattened and tend to fuse side by side (Fig. 35). Especially large vacuoles are to be seen at the edges of the crescent-shaped section of the spore-plasma. These come to lie closer and closer to the cell-wall at that point, and finally break through the plasma-membrane, thus bringing the cell-sap in direct contact with the sporangial wall through which it doubtless filters out and is evaporated. I have detected no cleavage-furrows cutting in to meet these vacuoles at the boundary of the sporangium, as was the case in *Pilobolus*. The remaining vacuoles in the series now fuse, thus separating spore-plasma and columella-plasma by a wide irregular cleft filled with cell-sap (Fig. 36). The turgor of

[1] In the figures given of *Sporodinia* the magnification is not sufficient to show the structure of the very minute nuclei clearly, and hence I have represented them merely as dots.

the two protoplasmic masses very soon brings the two membranes together and the contents of the sporangium are thus cut off from the sporangiophore by the new plasma-membranes. Between these a cell-wall is soon laid down. Cleavage of the spore-plasma begins at once by surface-furrows and clefts, which are rather wide and without the aid of vacuoles cut from below upward (Fig. 36) and, later, from both the upper and lower surface of the sporangium, thus dividing its contents into irregular polygonal blocks. There are two or three layers of these blocks in the apical thicker portion of a well-developed sporangium. At the edges of the spore-mass it is cut into only a single row of blocks (Fig. 37). These masses are very variable in size, and each contains a large but variable number of nuclei. They correspond morphologically to the blocks into which the spore-plasma of *Pilobolus* is first divided by the surface-furrows and vacuoles. They generally, however, contain many more nuclei than do the latter. With this the process of cleavage is complete in *Sporodinia.* A thin wall is built about each of the cleavage-masses which becomes at once a spore. The sporangial-wall is never thickened as in *Mucor* and *Pilobolus*, and bursts immediately for the escape of the spores. I have not detected by stains any intersporal substance, though the spores tend to adhere slightly when the sporangium is broken on a microscope slide. In nutrient liquids the spores round themselves up and put out a germ-tube very soon without any noticeable swelling. They contain from ten to fifty or so nuclei.

Sporodinia shows thus a very interesting abbreviation of the process of spore-formation as compared with *Pilobolus* or *Synchitrium decipiens.* It is seen, however, that the process in *Sporodinia* is related to that in *P. crystallinus* very much as the cleavage in *Synchitrium decipiens* is related to that in *S. Taraxaci*, except that in the latter the spore germinates as a sporangium. In *Sporodinia* and in *Synchitrium Taraxaci* the process stops with what is the initial stage of the process in the other two forms, and

it is plain that the ripe spores in the two types are not exactly homologous structures. Such variations in closely related forms are rather surprising, but in each case the abbreviated cleavage process is evidently an adaptation to meet the demand for a more rapid method of multiplication in the species in which it appears. Investigation will doubtless lead to the discovery of still further modifications of the process. It may be noted that the absence of winter spores in *Synchitrium decipiens*, as compared with *S. Taraxaci* where they are present, may be correlated with the longer process of cleavage and embryonic development in *S. decipiens*. It is hardly worth while to propose changes in nomenclature to distinguish the different forms of spores already described until a larger number of forms have been thoroughly studied. It is plain, however, that the term sporangiospore, as used by Léger, includes several structures quite distinct as to the method of their formation. The use of the term protospore for the ultimate product of cleavage in such forms as *Synchitrium decipiens*, *Pilobolus* and probably *Saprolegnia*, is certainly conducive to clearness. The ripened product of the sporangium, whether the cleavage process is more or less complete, and whether embryonic development is present or absent, may still be loosely designated as a spore or sporangiospore.

Cell-division also occurs in *Sporodinia* in the cutting off of the gamete-cells from the suspensors in sexual reproduction. This is an interesting case, since the mass of protoplasm to be cut in two is nearly as great in diameter as in the sporangium when the columella-wall is formed. There, as we have seen, the cleavage is accomplished by a row of vacuoles that flatten and fuse edge to edge. Here in the gamete-formation, however, the cleavage is accomplished entirely by a circular furrow proceeding from the surface to the centre, as in the cutting off of the bulb in *Pilobolus*. The nuclei are extremely numerous and are distributed in the protoplasm without any apparent relation to the plane of cleavage. The ingrowth is simply a deep narrow furrow and not the growth inward of a ring of fungus-cellulose. The cellulose-wall is formed

later. The process is exactly like that described by myself for the cutting off of the conidia in *Erysiphe*.

It is interesting that two such modifications in the method of cell-division should occur in the same plant. It is possibly evidence against the homology of the sporangium and gamete that the two are cut off so differently. It may be that the two methods are adapted to the difference in the form of the cell-wall to be developed in the two cases. Division by vacuoles may be better adapted to the formation of a dome-shaped cross-wall, while constriction suffices to form a plane cross-wall.

Lachnea scutellata.

I have already described the process of spore-formation in the ascus as represented in *Erysiphe communis*, and have given some figures indicating that the process in *Ascobolus* and *Peziza* is essentially the same as in *Erysiphe* [1]. The process is so unique in the method of cutting out the spore from the protoplasm of the ascus that I have continued the investigation of it in a number of other forms. *Lachnea scutellata* has proved especially favourable, from its abundance and the relatively large size of its nuclei, for investigating the phenomena in the ascus. And since, as I have already noted, the doctrine is widely accepted that the ascus is a more highly developed and specialized member of the same group of endogenous spore-producing cells as those I have been describing, it will be worth while to describe the process here once more as a basis for comparison, as well as to note minor differences from what has been already described for the asci of the above-mentioned forms.

L. scutellata is a very common cup-fungus growing on decaying wood in moist, shady places. The whole plant is bright red and the disk is bordered with long, stiff septate and pointed bristles. The material fixed in Flemming's solution is clear and transparent, neither so dense nor so

[1] Jahrbücher für wiss. Bot., Bd. xxx, p. 249.

much blackened as is the case with many Discomycetes. I will first describe some of the stages in the nuclear divisions in the ascus which I had failed to find or had been less successful in fixing and staining in the forms of cup-fungi already studied. In my earlier paper, for the sake of showing details on a larger scale, only the nuclei and the protoplasm immediately adjacent to them were figured. In the present case I have figured in each case the entire upper part of the ascus (from one-half to one-third) so as to show the appearance of the entire spore-bearing portion as a whole.

In the almost fully developed ascus of *Lachnea* there is a differentiation of the protoplasmic contents such as was described in part by De Bary and Strasburger. The lower two-thirds is filled by a very foamy vacuolated protoplasm which is convexly rounded off at its upper end against a denser granular plasma which fills most of the remainder of the ascus. The very apex of the ascus is filled by a cap of fine granular protoplasm, between which and the dense granular spore-plasma there is an oval foamy mass like that found in the lower portion of the ascus. Through the centre of this upper foamy area a vertical dense strand of protoplasm extends, at certain stages of development, from the under surface of the granular cap tapering downward to the upper surface of the spore-plasma. It is regularly present in the uninucleate stage of the fully developed ascus (Fig. 38), and again after the spores are fully delimited, and suggests the possibility of a fountain-like streaming of the protoplasm in the upper end of the ascus analogous to that described by Klebs[1] for the cells of some higher plants.

In my figures of the late diaster stages of the first division in *Peziza Stevensoniana* I was unable to make out a system of polar rays. In *Lachnea scutellata*, however, the asters attain a very noticeable development in this stage (Fig. 38). The rays from the upper nucleus extend almost to the apex of the ascus, and those from the lower one stretch far down into

[1] Form und Wesen der pflanzlichen Protoplasmabewegung, Biol. Centralblatt, Bd. i.

the spongy plasma, beyond the dense special spore-plasma. They are straight, unbranched and fine through their whole length. Some are much longer than others, and these longer rays, as they pass between the vacuoles of the spongy plasma, modify the shape of the latter noticeably. The vacuoles are pinched into a slightly ovoidal shape with flattened sides, the narrowed end being directed toward the centre of the aster, indicating that the system of rays is of some rigidity as compared with the other structures in the protoplasm. This deformation of the vacuoles is especially apparent where the rays pass from the denser spore-plasma into the foamy protoplasm below it. The appearance is given here as if the dense plasma was carried out around the rays as a rapidly narrowing sheath for each, suggesting that the rays have pushed forward from the dense plasma into the foamy plasma as the daughter nuclei separated, and that the dense protoplasm adhered to them and was carried along a certain distance. The connecting fibres, the old nucleoli, and the dense chromatin-masses of the daughter nuclei appear here essentially as in *Peziza Stevensoniana*. Abundant fine red-staining granules are scattered throughout the protoplasm, analogous to the so-called extranuclear nucleoli of cells in the higher plants.

In the earlier stages of division of the ascus-nucleus of *Lachnea* I have found nothing to add to my previous accounts. Also the reconstruction of the daughter nuclei proceeds in *Lachnea* essentially as in *Peziza Stevensoniana*, except that the polar rays continue for some time after the diaster stage, and there is no appearance of a disk-like central body on the equatorial side of the daughter nucleus. Figures 37, 38, 39 show stages in the nuclear divisions that follow and give the appearance and distribution of the protoplasm in the upper third of the ascus during these stages.

Figure 39 shows the equatorial plate stage of the division of the first two daughter nuclei. It is seen here, as is frequently the case in *Lachnea*, that the long axis of the spindle does not lie in the long axis of the ascus, though it quite regularly takes that position in *Peziza Stevensoniana*. Fig. 40 shows a diaster

stage of the same division. Here also the old connecting fibres do not lie in the long axis of the spindle. The diasters here duplicate in all particulars the diaster of the first division, but on a smaller scale. In this and the following figures the tip of the ascus is bent in such fashion that the section is not median, and the apical cap of dense protoplasm shown in Fig. 38 does not appear. Fig. 41 shows three of the spindles of the third division lying transversely to the axis of the ascus and the fourth at some distance off and parallel to the ascus-wall. This displacement of the fourth nucleus at one end or the other of the series was observed several times, and indicates the independence of the individual nuclei of each other and of the general proportions of symmetry in the ascus. The ends of all these spindles in the equatorial plate stage are decidedly broad and blunt, and the central body in which they end is flat and disk-shaped, as in *Peziza Stevensoniana* and *Ascobolus*. It stains more densely than the rays or spindle fibres, but there is no indication that it is more than a denser mass of kinoplasm formed by the meeting of the spindle and ray fibres. Figure 42 shows five daughter nuclei of the octinucleate stage of development in the ascus. They are in a late (anaphase) stage. The chromatin is still aggregated near the central body. The nucleolus has not yet appeared. The vesicular nuclear membrane encloses a clear space into which at a later stage the chromatin will be distributed. The remaining three nuclei appear in the next section of the ascus, which is here split lengthwise. It is seen that the systems of rays which formed the polar asters in the last divisions are still present. No indication of a beak-like elongation is to be noted. The central body lies close on the membrane of the spherical nucleus. The nuclei in almost every case lie quite near the wall of the ascus, though this is not shown in the figure, since the figures have been somewhat displaced vertically in drawing in order to bring them all into one plane. The aster in general lies toward the plasma-membrane, the rays here quite generally ending in the latter. The whole effect is somewhat as if the nuclei were hung up on the plasma-membrane by the

aster-rays. I am inclined to think this may have significance as indicating a resemblance in constitution between rays and membrane. It might be taken to indicate a connexion of the rays with the cell-surface such as is assumed, by Heidenhain and others, to exist between the astral rays and cell-boundary in the animal cell. It might be further assumed then that the rays give an anchorage for the spindle poles as claimed by Van Beneden and others, and that the daughter nuclei are drawn up to the walls in the position indicated above by the contraction of these rays during the early anaphases. However, such a connexion of the rays to the membrane is plainly lacking in the first and second divisions and in the divisions in the ascus of *Erysiphe*, as I have pointed out in my earlier paper. The spore-plasma at this stage again shows rather sharp boundaries against the foamy plasma above and below it, a condition which is obscured during the second and third divisions by the appearance of a considerable number of vacuoles in it. These vacuoles perhaps represent the nuclear sap set free by the disappearance of the nuclear membranes during division. Ultimately, however, this sap is either absorbed or extruded into the foamy epiplasm, and at the period just before the beginning of the delimitation of the spores, the spore-plasma is quite dense and homogeneous. There is no indication at this stage of a tendency to aggregate in denser layers about the nucleus. Such aggregation as was described in my former paper [1] represents in reality a later stage after the plasma-membrane is established, although I was not able to demonstrate the membrane at this stage from the preparations I then had. In *Lachnea* it is perfectly clear that the spore-boundaries are in no way outlined at the stage shown in Fig. 43. There is nothing to indicate that the nuclei are centres of any particular limited areas of cytoplasm about them. The further reconstruction of the daughter nuclei proceeds in the fashion described for *Erysiphe*. In Fig. 43 we have a stage in which the chromatin has been distributed through the nucleus and a nucleolus has appeared. The

[1] Ber. d. D. Bot. Gesellsch., Bd. xiii, p. 67, 1895.

chromatin lies for the most part on the nuclear membrane and is still in lumps and masses rather than threads or granules. A beak-like prolongation of the nuclear membrane beneath the central body and aster has appeared, and the nucleus has come to lie a little further from the plasma-membrane of the ascus and may be inclined at almost any angle to it. It will be readily seen that the nuclei being distributed on the inner walls of the cylindrical ascus, with the axis of the nuclear figure placed in general radially to the plasma-membrane, the beaks and asters will appear in polar view, in the case of such nuclei as have the beak-like prolongation extending vertically upward or downward from the plane of the section. Of the six nuclei appearing in this figure this was the case with the second and third nuclei from below, which extended almost vertically downward from the upper surface of the ascus, and their centres and asters do not appear in the figure. In this section some irregular openings appear in the spore-plasma and frequently red-staining granules are associated with them, lying on their borders but never free in their interior. In such cases the appearance suggests that the openings are due to breakage of the protoplasmic framework, whose elements have then shrunken together in the form of dense granules on the margins of the rents. This suggests that these red-staining extranuclear nucleoli may be formed artificially by partial breaking down of the protoplasmic structure in fixation. Whether this is a general explanation of this very common appearance in the cells of higher plants, I have not been able to satisfy myself. The process of metamorphosis of the aster in cutting out the spore is essentially the same as in *Erysiphe*. The rays revolve on the central body, and the cone-shaped opening, triangular in optical section, which indicates the first motions of the aster-rays is well shown in the case of the lower nucleus in Fig. 43, and in the upper nucleus in Fig. 44. The asters being in contact with the plasma-membrane, the latter seems to be pulled in as the fibres fold over, and thus a depression is produced in the surface of the protoplasm of the ascus. At first the central

body is very close to the plasma-membrane, but as the process of folding over of the rays continues, the whole figure migrates somewhat toward the centre of the ascus, as is seen by comparing the upper two nuclei in Fig. 44. An examination of Figs. 44 and 45 shows that the lower nuclei of the series tend to be a little in advance of the upper nuclei in their development, though this is by no means a general rule. Fig. 44 shows stages in the cutting out of the spores from the early stages in the displacement of the aster-rays in (*a*) to the completion of the plasma-membrane (*f*). In *d* and *e* the enclosure of the spore is not quite complete, and it is seen that the last part to be enclosed need not necessarily be antipodal to the central body. The shape of the mass first enclosed is also seen to be quite variable. In Fig. 44 (*c*) the nuclear figure is seen to be at the side rather than at the end of an ellipsoidal spore-mass. Of the two spores (*e* and *f*) the latter lies somewhat higher in the section and overlaps *e*, though they are drawn as if lying in the same plane. Irregularities of shape and in the position of the nucleus in the forming spore are much more numerous in *Lachnea* than in *Erysiphe*, and are due no doubt to greater crowding in the formation of the eight spores before they become evenly distributed in the elongated, relatively narrow ascus, while in the broad oval asci of *Erysiphe* the two to three spores are more evenly distributed from the first. These irregularities, however, make still more clear the relatively independent activity of the aster-rays, for, however the nucleus may be placed, they succeed in enclosing in every instance practically equivalent volumes of the spore-plasma, as is seen by comparing the size of the spores after they are completely outlined. The orientation of the nuclear figures, though tending in earlier stages of spore-formation to be radial to the surface of the ascus, is extremely varied, as seen in Fig. 46. This figure shows also that, though the nuclei are in general rather evenly distributed in the spore-plasma, the process can be carried out just as perfectly when several nuclei are in close proximity to each other, as are the three, *b*, *c*, *d*, in this section. In some

cases the young spore is decidedly lobed as in *b*, Fig. 45. *d*, in the same section, shows the plasma-membrane of the young spore indented, as a result of some disturbance in fixation which also produced the vacuole-like space lying opposite the indentation. In Fig. 47 we have a section showing seven of the eight ascospores completely enclosed in plasma-membranes isolating them from the adjacent spore-plasm. They have come to lie now in a more nearly median row in the ascus, though *a* and *b* are still overlapping, and the eighth at the lower end of the series was almost wholly in the next section and was hence omitted from the drawing. The astral rays have disappeared, though in *a*, *b*, *c*, and *f*, the beak and central body have not yet been drawn back to the nucleus. The nuclei have increased considerably in diameter and the chromatin forms a regularly distributed network. The spores are flattened upon each other and maintain their individuality and rounded outlines, indicating the existence of surface tension in their plasma-membranes. No cell-wall is present as yet.

As I have pointed out in my former paper the existence of surface tension in the spores at this stage is most conclusive evidence for the view that the plasma-membrane is a specially differentiated layer. As we have seen, the protoplasm of the ascus is entirely homogeneous before the spores are cut out by the metamorphosis of the aster, and to prevent the plasma in the spore from mixing and becoming continuous with the epiplasm outside, the assumption of the differentiation of a membrane-layer is essential. It is not necessarily implied that this differentiation is chemical; it may consist perhaps in an increased density and absence of coarser granules. I am inclined however to consider a very decided differentiation as necessary, since it can hardly be seen how a merely denser, more homogeneous colloidal layer could fail to lose its identity and diffuse into a chemically similar compound on both of its surfaces. If it be assumed, as claimed by Rhumbler for *Amoeba*, that the ectosarc is formed from the endosarc as a result of contact with water, and that in the ascospore, as

a result of metabolic changes beginning at the nucleus, the spore-plasma becomes chemically altered and different from the adjoining epiplasm as soon as it is cut off by the fused rays, we might imagine that the interaction of the thus differentiated spore-plasma and epiplasm could be maintained and a bounding layer produced, as it is supposed to be formed by the action of water on the surface of the *Amoeba*. That such differences could be so soon produced, and that the epiplasm could produce on the spore-plasma the same effect which so foreign a substance as water produces on the surface of an *Amoeba*, seems difficult to imagine. Bütschli's theory of a permanently differentiated outer layer (oily in its nature) fits much better with the phenomena here. The independence of motion of the rays through the ascus-plasma, comparable, as I have already pointed out, only to the vibratile activity of cilia, also seems to demand the assumption of a very considerable difference between their composition and that of the medium in which they move. In later stages the spores enlarge somewhat, and come to lie in a single row in the axis of the ascus. In Fig. 48, owing to a sharp bend in the upper end of the ascus, the spore-row seems to come nearer the apex of the ascus than is really the case. Spore-walls are still lacking at this stage. The spores are less flattened on each other, and are beginning to take on the elliptical outline of the fully ripened condition. Central bodies, such as are quite conspicuous on the spore-nuclei of *Erysiphe* at this stage, are hardly ever to be discovered in *Lachnea*.

In the above description I have not attempted to redescribe all the points in the process of spore-formation which are identical with those already described for *Erysiphe*. I have rather aimed merely at presenting those minor points in which the process varies in *Lachnea* from the condition in the other forms described.

The main point of difference between *Lachnea* and *Peziza Stevensoniana* and *Ascobolus furfuraceus* in this process is that in *Lachnea* the anaphases in the last division forming the

eight nuclei in the ascus proceed as far as the formation of a nuclear membrane and the appearance of the nucleolus, before the metamorphosis of the aster into the plasma-membrane of the spore is completed, whereas in the other forms the delimitation of the spore is accomplished while its nucleus is still in the densely aggregated stage with no nuclear membrane yet formed. In this *Lachnea* more nearly resembles *Erysiphe*. It will be seen, however, that *Lachnea* really occupies in this respect an intermediate position between the other two Discomycetes mentioned and *Erysiphe*, since in reality the daughter nuclei are not completely developed in *Lachnea* at the time when the spores are completely delimited, but increase in size later, as is seen by comparing Figs. 43 and 46. Such differences as this are doubtless adaptations to particular growth-conditions in the different genera. As was suggested before, the habit of producing successive series of asci in brief periods when external conditions are favourable may have led to the modification of the process in the Discomycetes as compared with *Erysiphe*.

Summary.

If we compare now the methods of spore-formation in the ascus and in the sporangia studied, the differences in the two cases are at once apparent. In the ascus, as in the higher plants, the cutting out of the daughter cell from the mother cell is effected by the agency of the same fibrous kinoplasmic elements as were concerned in the division of the nucleus. In the higher plants the flat cell-plate is formed by the 'cone-principal' of the karyokinetic figure as named by Van Beneden, while in the ascus the daughter cell is cut out of the protoplasm of the mother cell by an ellipsoidal cell plate formed from the fibres of the antipodal cone. In this process the daughter cell is cut out of the interior of the protoplasm of the mother cell, so that it remains surrounded on all sides by the material of the mother cell. The daughter cells do not

contain all the protoplasm of the mother cell, a considerable mass remaining as the so-called epiplasm. This is typical free cell-formation, as I have pointed out before. In all the sporangia studied, the cleavage is from the surface of the protoplasm, or from the surface of vacuoles of the mother cell. The daughter cells are thus separated by cleavage-furrows, and the nature of the division from the surface inwards precludes the possibility of the formation of an epiplasm.

That cleavage-furrows may start either from the superficial surface of the mother cell or from the surface of vacuoles imbedded in its protoplasm, is evidence that the vacuolar membranes and the external plasma-membrane of the cell are closely related structures, as has already been maintained by De Vries and Pfeffer. In both ascus and sporangium the daughter cells first formed are naked bits of protoplasm bounded only by plasma-membranes and without cell-walls. In the sporangia studied, the cleavage does not form uninucleate cells at once, though this is probably the case in the sporangia of the Saprolegniaceae. In the large sporangia of *Synchitrium* and *Pilobolus* the cleavage is progressive, first dividing the mother cell into multinucleate masses, which are gradually split up into the uninucleate protospores. This progressive segmentation has no parallel in the asci, where from the start a single nucleus forms the centre for the formation of each daughter cell. This progressive cleavage is by no means a series of bipartitions of a multinucleate cell, such as was suggested by Büsgen's work on the sporangium of Mucor, but is entirely irregular, forming at the first segments of varied size which contain a varying number of nuclei. I know of no process which is analogous to it in this respect either in plant or animal cells. The growth of the protospores in *Synchitrium* and *Pilobolus* to form sporangia in the one case and by division the typically binucleated sporangiospores in the other, is perhaps analogous to the growth in certain asci whereby the typically one-celled uninucleate ascospore becomes several or many celled or in some cases multinucleated

while remaining one celled. My earlier studies of ascospores led me to the erroneous conclusion that the one-celled ascospore was regularly uninucleate. I have since found ascospores in which nuclear division had occurred without cell division, and such cases have also been pointed out by other investigators [1]. These cases are interesting because of the possibility of a reduction of the number of chromosomes at this stage, and should be investigated further as to the phenomena of nuclear division.

That in *Synchitrium* the embryonic growth in the sporangium produces sporangia, while in *Pilobolus* and in all asci with multicellular spores the germination is by a germ-tube from each spore-cell, may be, as has been suggested for other similar cases, entirely a matter of adaptation in the one case to germination in water, and in the other to germination in air or solid media. The aim of the process is always to produce a larger number of reproductive bodies, and thus to use up advantageously any excess of nutritive materials accumulated in the sporangium or ascus. In the Exoasci an analogous embryonic growth takes the form of yeast-like budding, and the number of conidia produced is plainly dependent on the amount of nutriment available in the ascus.

A possible comparison might be made between the uninucleate stage of *Synchitrium* and the uninucleate stage of the ascus. Each is followed by a multinucleated stage and cleavage to form uninucleate spores, but the comparison is an artificial one. The divisions in *Synchitrium*, though they lead directly to spore-formation, are probably morphologically equivalent to the nuclear divisions that take place in the mycelium and bulb of *Pilobolus*. At any rate, some possible connecting types should be found to give the comparison any value.

As the mechanism which effects the division of the mother cell into daughter cells, we have in the ascus the kinoplasmic fibres, and in the sporangium constriction-furrows from the

[1] Dittrich, Zur Entwicklungsgeschichte der Helvellineen, Beiträge zur Biol. der Pflanze, 1899.

plasma-membrane of the surface or of vacuoles. This appears to be a very fundamental distinction. It may, however, be taken as indicating possibly a further resemblance between the plasma-membrane and the fibres of the karyokinetic figure.

If we consider now the bearing of the observations presented, on the doctrine that the ascus is a more highly developed and specialized modification of the sporangium of the Zygomycetes, it is plain that the very different methods of cleavage in the two cases are opposed to the assumption of any close relationship between them. In fact, it seems rather difficult to imagine any intermediate stages which could connect the process of cleavage by surface-furrows, as seen in the sporangium, with the free cell-formation of the ascus. It must be noted too that Popta's work on *Ascoidea* and *Protomyces*, which Brefeld considers intermediate forms between the lower Fungi and the Ascomycetes, has failed in any way to bridge this gap. Until some transitional forms have been found between these widely separated methods of cell-division, their evidence must be considered as decidedly against the assumption of any morphological relationship between the sporangium and the ascus. The method of division in the sporangium by surface-furrows is easily connected with other cases of division by constriction, such as are seen in the cell-division of *Cladophora* or in the formation of the conidia in the Erysipheae as I have described it, but the free cell-formation in the ascus seems as yet entirely unique, and its occurrence in widely separated forms of the Ascomycetes is justification for considering it as perhaps the most important and specific feature by which to distinguish the ascus from other spore-producing cells. The presence of epiplasm has always been considered one of the most distinctive features of the ascus, and those who have contended for the relationship of the sporangium and ascus have been much concerned to discover a similarity between the epiplasm and the intersporal slime in the sporangium. It is, however, sufficiently apparent that these two

substances are entirely distinct in their origin and consistency. The presence of epiplasm is a necessary result of the peculiar method of cutting out the daughter cells in the ascus, and together with this, may be taken as a criterion for distinguishing asci in every case. From this standpoint the ascus appears so far as yet known as a new structure, characteristic of the group of the Ascomycetes. By this I would by no means say that the process of cutting out the spores by free cell-formation as I have described it, may not have been developed from some other method of cell-division. Investigation of the method of spore-formation in such forms as *Exoascus*, *Dipodascus*, and *Eremascus* may perhaps throw further light on the matter. For the present the peculiar phenomena observed by Strasburger[1] in the swarm-spore formation in *Oedogonium* may be regarded as possibly analogous to and indicating a possible origin of the process in the ascus. Strasburger found that in a cell of *Oedogonium* which is about to form a swarm-spore, a hyaloplasmic-mouthpiece appears on the side of the cell-body, and that the nucleus, which up to this time may have been in any part of the cell, migrates to a position just beneath this mouthpiece. Rays now appear in the protoplasm at the base of the mouthpiece, and extend back into the protoplasm of the cell. The cilia also begin to bud out around the base of the mouthpiece. The cell-body now rounds up more and more to form the swarm-spore, which may be enclosed in a thin vesicle, when it escapes from the mother cell. Strasburger believes that this vesicle here, and in other cases of swarm-spore-formation, represents the cast off plasma-membrane of the mother cell. How the new plasma-membrane of the swarm-spore is formed is not certain. It is probable that the hyaloplasmic mouth-piece is a centre of formative activity at this stage. The migration of the nucleus to this region reminds one of what Haberlandt has found as to the position of the nucleus in cells whose walls are being thickened on one side. Haberlandt concludes that the nucleus is directly concerned in the forma-

[1] Zellbildung u. Zelltheilung, 3. Aufl.

tion of cellulose, and an analogous conclusion might be drawn here as to the relation of the nucleus to the formation of cilia. This view would be further strengthened by Strasburger's[1] observation on the swarm-spores of *Vaucheria*, where he found the cilia arranged in pairs over the whole surface of the spore, and a nucleus just beneath each pair.

Strasburger believes that in *Oedogonium* a centrosome lies at the base of the mouthpiece as the centre about which the rays above mentioned are oriented. The material for the cilia is undoubtedly formed in this region at the base of the mouthpiece, and if it is also the centre from which materials for a new plasma-membrane are diffused outward along the rays of the aster which is also formed at this place, we might consider the process as analogous to that in the ascus. Further details of the process in *Oedogonium* are necessary before the value of such a comparison can be tested.

The total dissimilarity of the process of cleavage in the sporangia described and the ascus as I have shown it in the above account, makes it necessary to look for the ancestors of the Ascomycetes elsewhere than in the lower Fungi. Thaxter's[2] studies of the Laboulbeniaceae have emphasized greatly the resemblance of that group to the Florideae and the hypothesis of the multiple origin of the Fungi from the Algae has gained correspondingly in strength. From this standpoint an attempt to homologize the swarm-spore-formation in such forms as *Oedogonium* with the free cell-formation in the ascus is not without value. Strasburger has pointed out a difference between ordinary cell-division and swarm-spore-formation in *Cladophora*, and it may be that this difference has been further developed in higher forms. The cleavage in the sporangia of the Phycomycetes described corresponds to the method of cell division in *Cladophora*, and it may be that the type of division shown in the swarm-spores has been developed into that shown in *Oedogonium* and the ascus. It would be especially interesting to know in detail

[1] Schwärmsporen, Gameten u. pflanzliche Spermatozoiden. Hist. Beitr., iii.

[2] Cont. to Mon. of Laboulbeniaceae, p. 251.

how the formation of spermatozoids in *Oedogonium* compares with that of the zoospores. The spermatozoids are known to have the structure of the zoopores, but more than one are produced in each cell. I have not been able to obtain sufficient material for the investigation of this point. Where we are to seek the origin of the ascus-type of spore-formation remains uncertain except perhaps for the facts in the swarm-spore-formation just noted. Brefeld has treated as practically one the question as to the presence of sexuality in the Ascomycetes and the relationship between the ascus and sporangium of the Phycomycetes. It is plain, however, that the sexual origin of the ascus-fruit and the relationship of ascus and sporangium are entirely distinct problems. The ascogonium may be the outgrowth of a fertilized egg and still the asci be homologous with the sporangia of *Sporodinia*, for example, where regularly the sexually produced zygospore germinates into a branching sporangiophore without the intervention of a mycelium. The analogy between such a germinating zygospore of *Sporodinia* and the egg of *Erysiphe* with its ascogonium and asci is quite striking. In *Sporodinia* however sporangia are produced later from vegetative mycelia without the preliminary formation of zygospores. There is thus no obligatory alternation of fruit-forms, an indefinite number of sporangial fruitings being followed by zygospore-formation. From this type of reproduction, which is common among the lower Algae and Fungi, we could pass to the condition of things we find in the Ascomycetes in two ways. First by the suppression of all production of sporangia which does not follow immediately on the germination of the fertilized egg so that the sexual and asexual fruiting bodies should gradually assume the relation of obligatory alternation; or it could be assumed that the fertilized egg gradually developes an intermediate stage of cell-division and growth, by which it produces a large number of asexual reproductive bodies before the mycelium which produces the non-sexual sporangia or conidia is developed. It is generally assumed that the alternation of generations of the Liverworts

and Mosses has arisen in this latter fashion. For the Ascomycetes it is impossible as yet to construct an ancestral line of development which shall settle this question. We can say however, as noted above, that the unlikeness in the method of spore-formation in the ascus and the sporangia which I have studied makes it impossible to assume any very direct relationship between the Phycomycetes and Ascomycetes.

To keep clear this distinction in the method of cell-division in the sporangium and ascus I would propose that the term *free cell-formation* be restricted to the method of cell-division in the ascus. The method of division by which the sporangiospores and conidia are formed may be described as cleavage by constriction. In *Synchitrium decipiens* and *Pilobolus*, where the division proceeds to the ultimate separation of the energides as protospores, the process can be called *progressive and complete cleavage.* In *Sporodinia* we have a restricted cleavage. In cutting off the gametes in *Sporodinia* and the conidia of the mildews we have division by simple constriction. The process is probably the same in the cell division of *Cladophora.* In cases such as the cutting off of the columella in *Sporodinia* and *Pilobolus* we have the process more or less modified by the appearance of internal cleavage by flattening and fusion of vacuoles.

Doubtless further types and modifications of the cleavage process will be found in investigating other, even nearly related Fungi, and it is hardly necessary to attempt further applications to the general theories of cell-division until a larger number of cases have been worked out for comparison.

EXPLANATION OF FIGURES IN PLATES XXIV—XXVI.

Illustrating Professor Harper's paper on Cell-Division in Sporangia and Asci.

All figures were drawn with the aid of the Abbe *camera lucida*, and with the Zeiss apochromatic objectives 8 mm., or 2 mm., Num. Ap. 1. 40.

PLATE XXIV.

Figs. 1–8. *Synchitrium decipiens.*

Fig. 1. Uninucleate cell almost full grown. × 250.

Fig. 2. Multinucleate stage showing progressive cleavage by furrows from the surface. Central mass of protoplasm not yet segmented. × 500.

Fig. 3. Portion of cell showing two nuclei and two cleavage-furrows. × 1500.

Fig. 4. Median section of cell showing cleavage farther advanced. × 250.

Fig. 5. Section from surface of a cell in early stage of cleavage. × 500.

Fig. 6. Cell after segmentation is completed, showing uninucleate protospores. × 250.

Fig. 7. Section of sporangium with spores after they have become polygonal by mutual pressure and contain several nuclei. × 500.

Fig. 8. Mature spores, multinucleated and with cell-walls. × 500.

Figs. 9–33. *Pilobolus crystallinus*, except figs. 28–32.

Fig. 9. Median longit. section of tip of sporangiophore bearing young sporangium. Figs. 9–12 show the nuclei merely as dots.

Fig. 10. Tip of slightly older sporangiophore with sporangium; section a little to one side of the middle.

Fig. 11. Median section of older sporangium, showing course of the protoplasmic flow from the mouth of the sporangiophore. *a*, section of protoplasmic ridge around the mouth of the sporangiophore.

Fig. 12. Median section of sporangium at stage when columella is forming, showing cleavage-furrows at base and flattened vacuoles above.

Fig. 13. Section of spore-plasma and sporangial wall just before cleavage begins. × 1250.

Fig. 14. Section of spore-plasma from base of sporangium, showing first appearance of superficial cleavage-furrows. *a*, collar; *b*, columella-cleft; *c*, section of protoplasmic lining of columella; *d*, angular vacuole in spore-plasma. × 750.

Fig. 15. Same as Fig. 14, but cleavage further advanced; *a*, sporangial wall. × 750.

PLATE XXV.

Fig. 16. Section through a portion of the upper part of a sporangium, showing irregular sausage-shaped bodies formed by cleavage of the spore-plasma; a little older than Fig. 15. × 750.

Fig. 17. Similar section to Fig. 16, but older, showing uninucleate protospores formed by cleavage. × 750.

Fig. 18. Protospores with dividing nuclei, young daughter nuclei connected by remains of spindle fibres. *a*, collar; *b*, protoplasm of columella. × 750.

Fig. 19. Four protospores, which have enlarged and become multinucleate. × 250.

Fig. 20. Embryonic cell, with nuclei arranging themselves in two groups with formation of hyaline equatorial zone. × 1250.

Fig. 21. Slightly older than the last. × 1250.

Fig. 22. Division of embryonic cell by constriction. × 1250.

Fig. 23. Mature binucleated sporangiospore with wall. × 1250.

Fig. 24. Outline drawing of a part of a median section through a sporangium with binucleated sporangiospores; *a*, sporangial wall; *b*, collar, showing its extension upward on the columella; *c*, intersporal homogeneous slime; *d*, columella-wall; , protoplasm of columella.

Fig. 25. Spore swollen and germinating in nutrient medium. × 1250, stained with ' carmalaun.'

Fig. 26. Outline of germinating spore at later stage with seven nuclei. × 1250.

Fig. 27. Spore with germ tube and numerous nuclei. × 1250.

Fig. 28. Drawn from living spore germinating in water. Figs. 28–32 drawn with apoch. obj. 2 mm. and oc. 8.

Fig. 29. Later stage of germination in water, which showed streaming protoplasm.

Fig. 30. Living spore germinating in nutrient medium.

Fig. 31–32. Later stages of germination.

Figs. 33–37. *Sporodinia grandis.*

Fig. 33. Median longit. section of young sporangium, showing the beginning of the differentiation of the spore-plasm. × 425.

Fig. 34. Median longit. section, showing the development of vacuoles which are to form the columella-cleft. × 425.

PLATE XXVI.

Fig. 35. Section showing dense spore-plasma and foamy protoplasm of columella and their separation by large and small vacuoles. × 1000.

Fig. 36. Section showing formation of columella-cleft and furrows passing upward to divide the spore-plasma. × 425.

Fig. 37. Median section of sporangium with ripe spores.

Figs. 38–48. *Lachnea scutellata.* Figs. drawn with obj. 2 mm. and oc. 8. Fig. 38 enlarged. Figs. 42 and 46 reduced.

Fig. 38. Ascus with nucleus dividing, daughter nuclei still connected by spindle fibres; *a*, nucleolus.

Fig. 39. Ascus with the two nuclei in equatorial plate stage.

Fig. 40. Later stage of division in binucleate ascus.

Fig. 41. Ascus with four nuclei in equatorial plate stage.

Fig. 42. Ascus in octinucleate stage. Five nuclei with asters shown in section.

Fig. 43. Nuclei with beaks, at whose apex is the aster.

Fig. 44. Formation of ascospores by free cell-formation.

Fig. 45. Later stage of same process.

Fig. 46. Ascus in section, showing five spores with their plasma-membranes just completed; interior aster rays still present.

Fig. 47. Ascus in section, with seven spores; beaks still present on part of the nuclei.

Fig. 48. Ascus with eight spores, still somewhat flattened upon each other.

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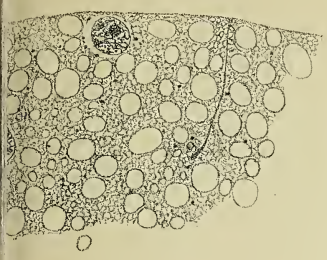

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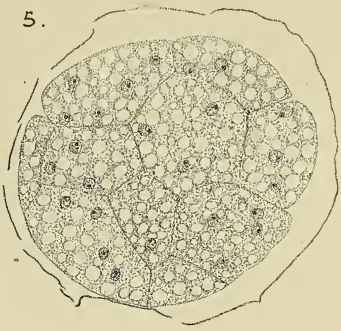

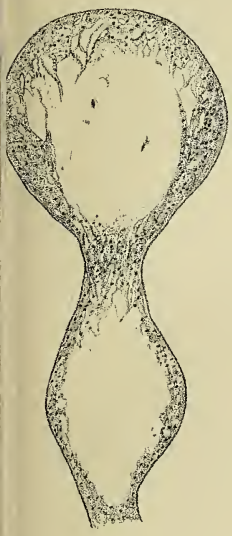

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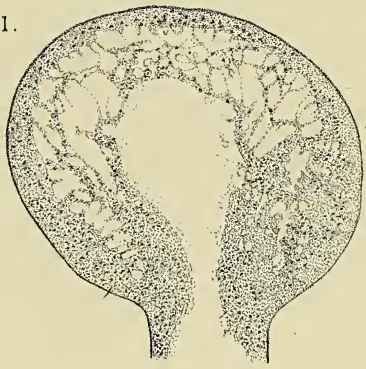

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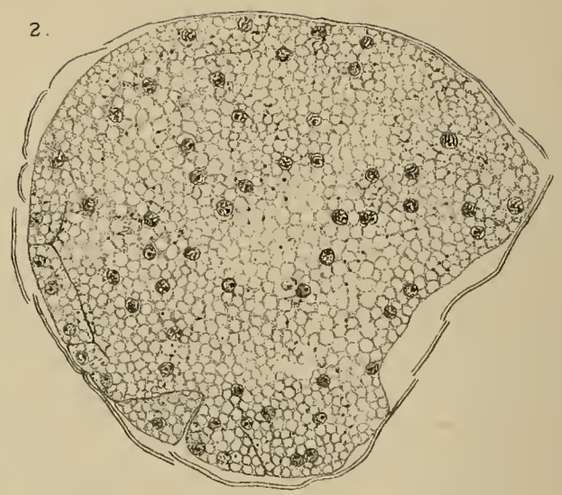

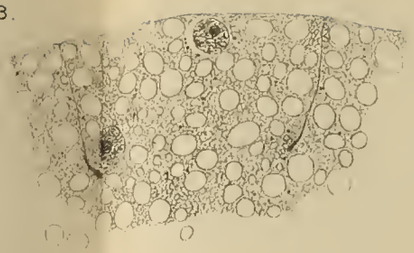

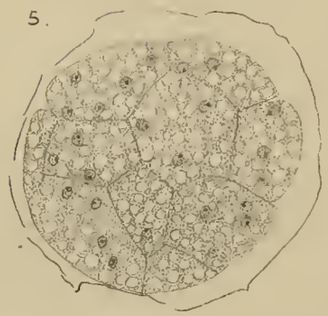

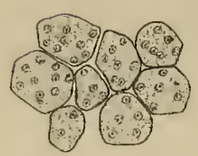

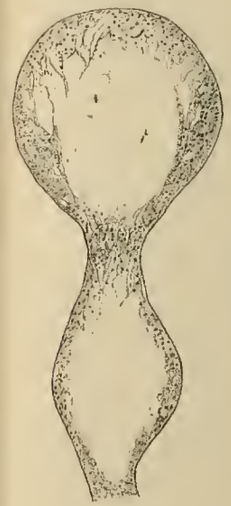

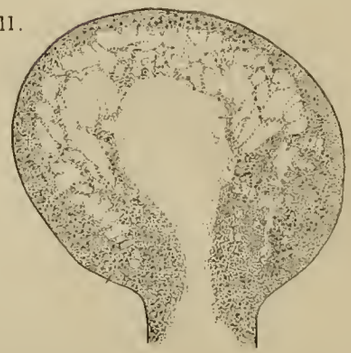

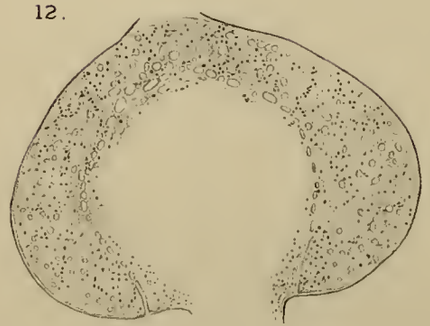

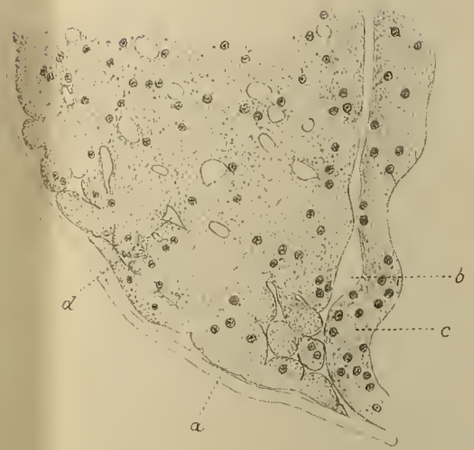

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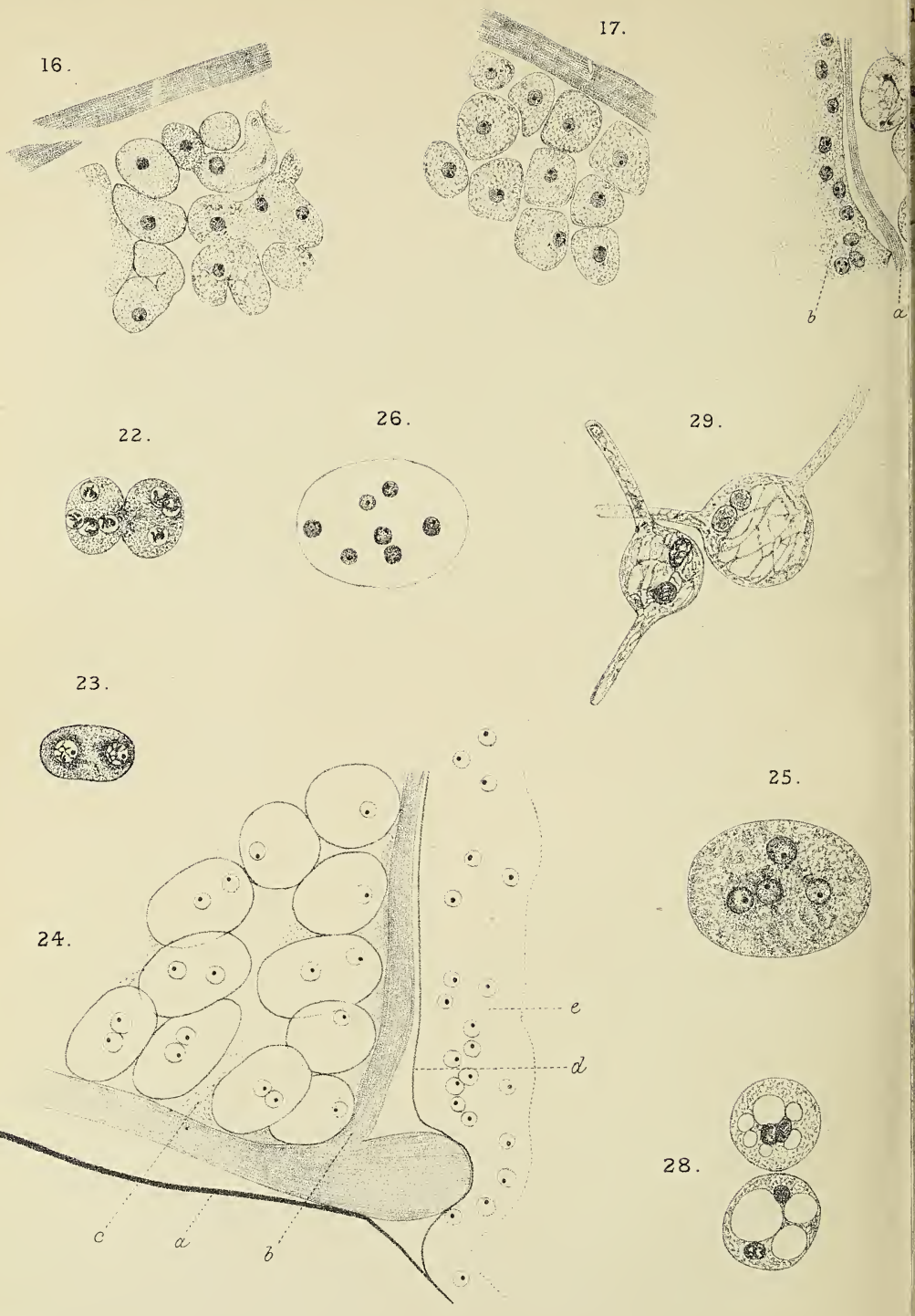

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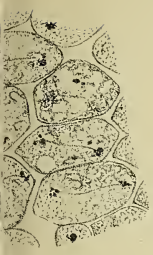

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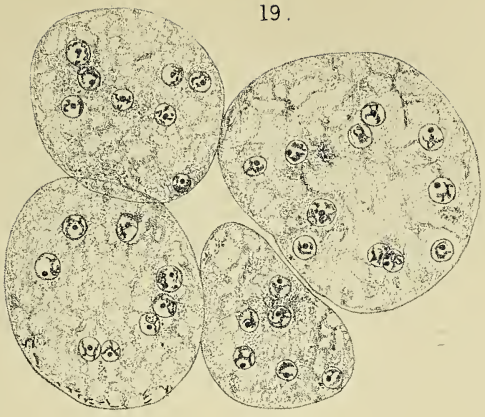

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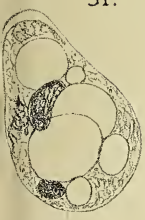

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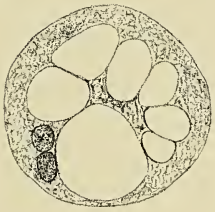

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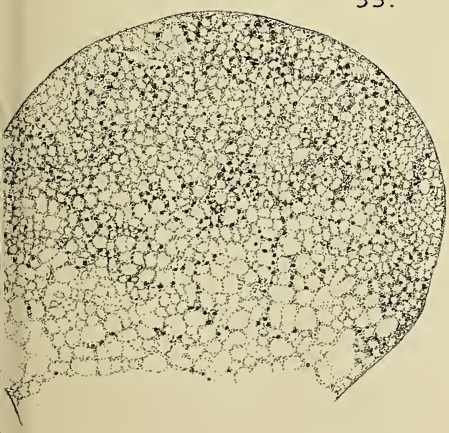

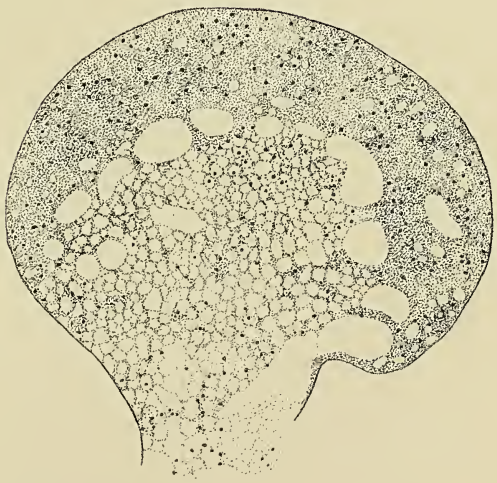

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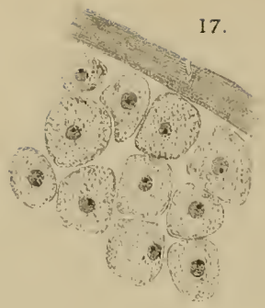

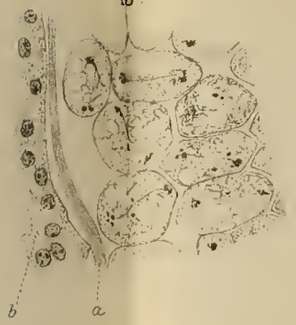

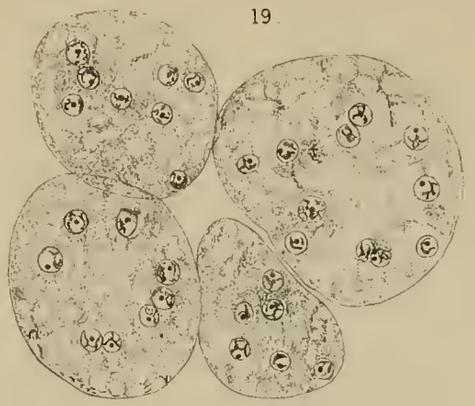

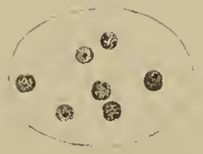

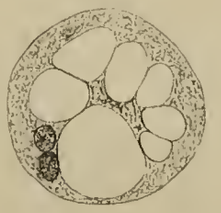

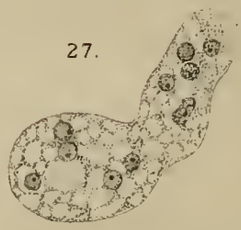

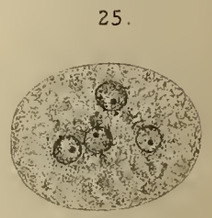

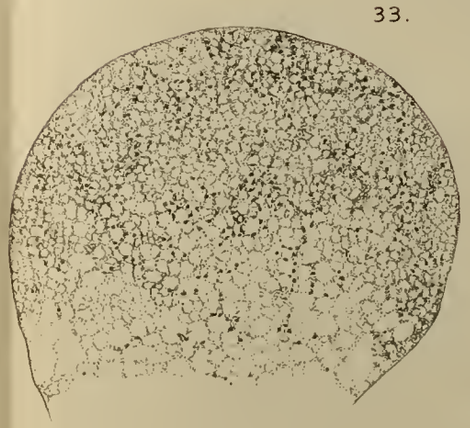

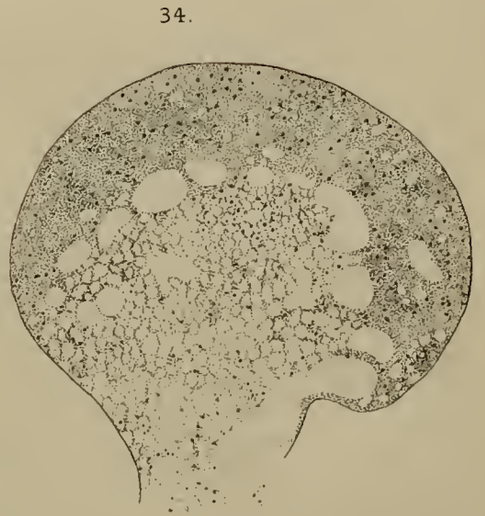

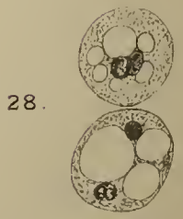

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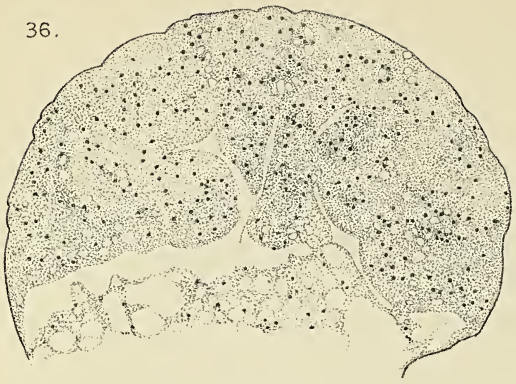

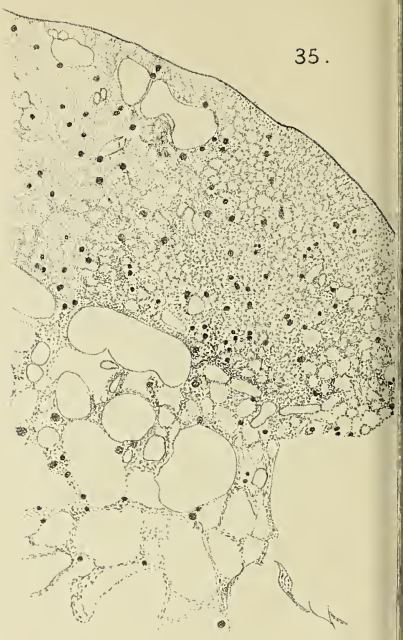

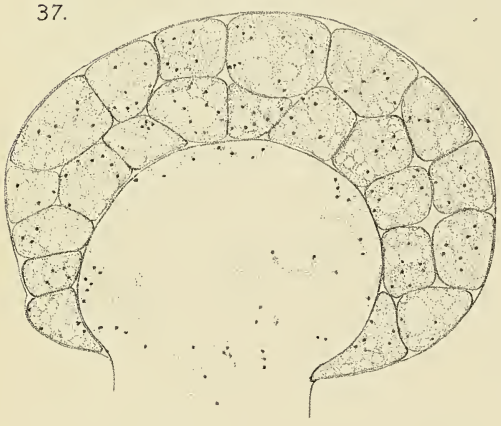

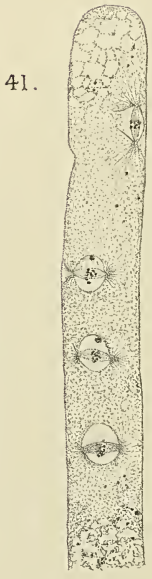

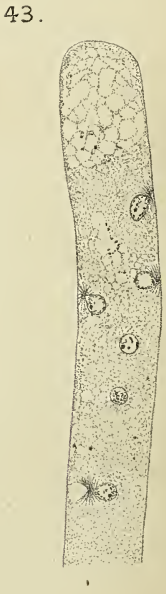

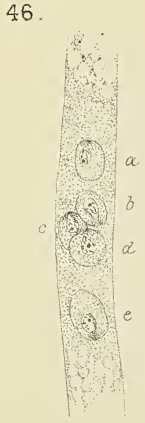

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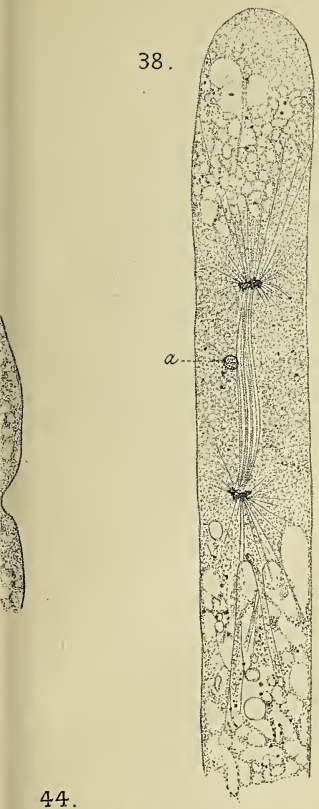

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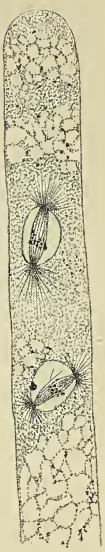

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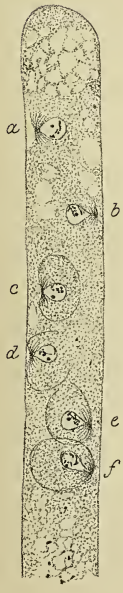

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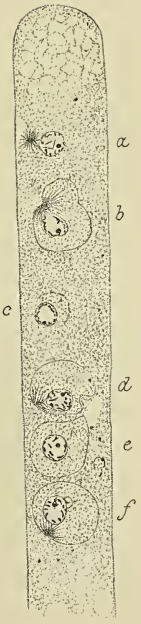

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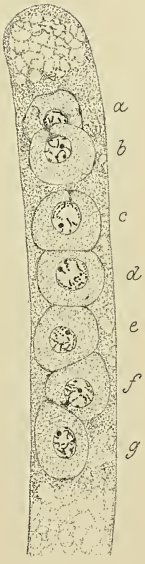

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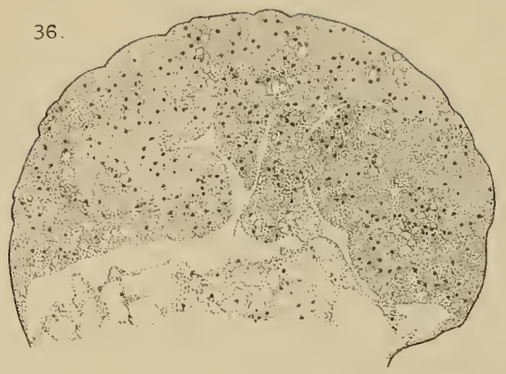

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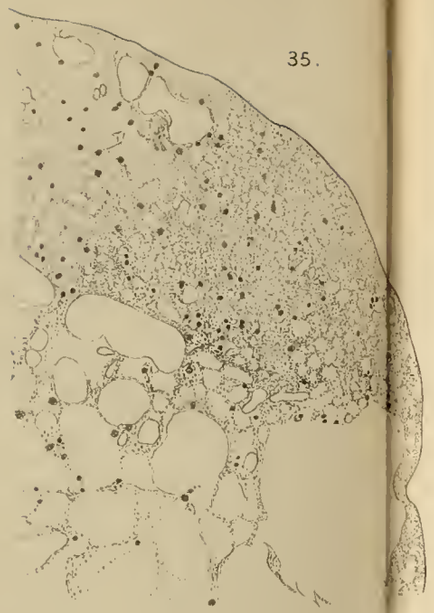

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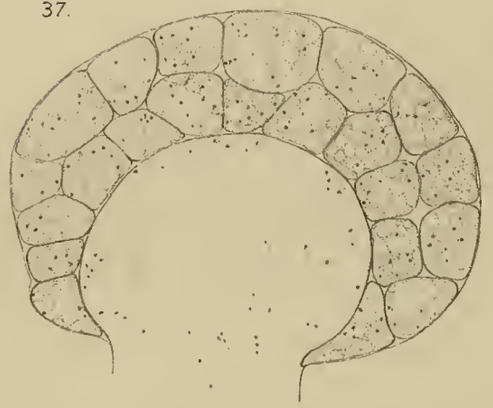

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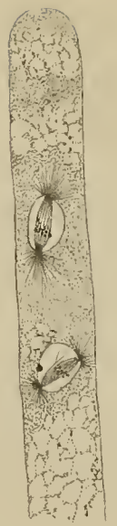

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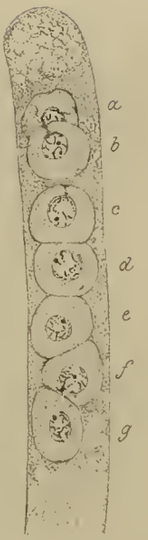

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HARPER. — ON CELL-DIVISION IN SPORANGIA AND ASCI.

Observations on the Vascular System of the Female 'Flowers' of Coniferae [1].

BY

W. C. WORSDELL, F.L.S.

With Plate XXVII.

THESE observations were made in order to determine whether a careful study of the course and structure of the vascular bundles would throw any light upon the vexed problem as to the nature of the female 'flower' in this order. While some interesting features have been brought out, but little new light, I have found, has been thrown on the question at issue as the result of these investigations. But this is only what might be anticipated, for the anatomy alone must necessarily be utterly inadequate to solve such a profound problem as the one before us [2].

Araucarieae.

Araucaria Cookii, R. Br.

A single bundle leaves the central cylinder of the axis of the cone, and runs obliquely upwards through a great many

[1] From the Jodrell Laboratory, Royal Gardens, Kew.

[2] A general discussion, from an historical point of view, of the question of floral morphology in Coniferae will be given in a forthcoming paper, together with full references to the literature.

[Annals of Botany, Vol. XIII. No. LII. December, 1899.]

internodes before entering the sporangium-bearing scale. About two-thirds of the way outward in the cortex this bundle divides into two ; a small bundle branches off and, turning on itself, passes slightly upwards (Fig. 1) so as to lie just above its parent-bundle, and with reversed orientation of its parts (Fig. 2). (This, therefore, is different from what occurs, according to Strasburger[1] in *A. brasiliana*, A. Rich., and *A. excelsa*, R. Br., where the bundle does not divide until it enters the scale, but agrees with his observations of what takes place in *A. Cunninghamii*, Sw.). During this transition the two bundles appear occasionally so fused with one another that the structure resembles that of a concentric bundle. The smaller bundle has about three layers of secondary xylem and is about three rows of tracheides in width : the large lower bundle has about four layers of secondary wood and is about four rows in width. There is naturally some variation in the point of branching, this taking place lower down in some cases than in others.

Immediately after this vertical branching, the lower (larger) bundle divides in the horizontal plane into three, of which the two lateral pass off somewhat to the right and left of the median one (Fig. 3). As soon as the bundles have entered the base of the scale, the upper one gives off a branch on either side, each of which *passes across* and takes up a position between the median and a lateral bundle of the lower group, so that this latter now consists of five bundles (Figs. 4 & 5). But, in some cases, only one such bundle may be given off. In other cases one of the lateral bundles of the lower group may divide, and, at the same time, a branch from the upper bundle may be given off and join the branch so formed. The upper bundle divides again into two which remain side by side (Fig. 5), and the outside bundle on each side of the lower group divides into two, of which the outermost revolves on its axis and comes to lie, with inverted orientation, on the same side as the upper bundle, so that there are now four bundles on this side and about five on the

[1] Strasburger : Die Angiospermen und die Gymnospermen ; 1879.

lower side [1]. In the above cases, where the two branches from the upper bundle are *intercalated between* the bundles of the lower group, the upper bundle subsequently gives off a branch, first on one side, then on the other, each of which fuses with the respective lateral bundles of the lower group. The lower bract-bundle, both before it cuts off the small upper inverted bundle, and at any time during its subsequent division, may exhibit centripetal xylem in small quantity; but this tissue is, perhaps, most frequently absent. Centripetal xylem occurs both in dorsal and ventral bundles in the stalk of the scale, as also in the region of insertion of the sporangium; the tracheides of this tissue are sometimes quite short, but often considerably elongated, and with abrupt horizontal or slightly oblique terminal walls; they have very dense, fine spiral thickenings just like those of the centrifugal xylem.

The sporangium has become imbedded in the tissues of what I regard as the seminiferous scale, and has thereby lost a portion of its individuality. This has probably taken place when the formerly orthotropous sporangium seated on the dorsal side of the scale became completely inverted, owing to intercalary growth arising below it; this change occurring in order to facilitate fertilization. When this happened, a portion of the tissues of the scale probably enwrapped it at the same time, enclosing everything except the extreme tip of the integument in the micropylar region. The tissues of the integument thus became completely fused with those of the seminiferous scale, so that no sharp line of demarcation any longer existed between them [2]. But the limit of the integument can easily be made out; the latter consists of two thick layers: an inner whitish, and an outer yellow tissue, formed of irregular, elongated, pointed cells, intermingled like the filaments of a fungus and containing elongated nuclei. This yellow layer is separated from the tissues of the

[1] A subsequent connexion may occur between one of the two median upper bundles and one of the intermediate bundles of the lower groups.

[2] According to my own view as to the nature of the seminiferous scale (see infra), this fusion is simply that between an inner and an outer integument.

seminiferous scale by a single layer of tangentially-elongated tannin-sacs. The tissue of the scale can be clearly seen extending as a *narrow* band round the *outer side* of the sporangium, and is perceived to be continuous with the inner portion of the scale (Fig. 6). The nucellus, throughout the greater part of its length, is quite separated from the integument and easily falls out; within it is the embryo-sac filled with clear and narrow-celled endosperm (Fig. 6). The nucellus is produced into a long black beak which protrudes beyond the integument and bends upwards externally to catch the pollen. It consists of contorted, elongated cells, like those of a stigma. Both bract and seminiferous scale are traversed throughout by great numbers of irregular stone-cells.

A double system of small bundles, belonging both to the bract and the seminiferous scale, occurs in the region of the insertion of the sporangium, running below and parallel to the long axis of the latter (Fig. 6). Both systems occur throughout the sporophyll up to a short distance beyond the insertion of the sporangium. At four points, viz., right and left of the sporangium both at its base and tip, some of the bundles of the seminiferous scale fuse together into a single large bundle, having two or three layers of xylem and very conspicuous phloem; at these points they may even assume a partially *concentric* structure, xylem and phloem occurring (as seen especially in one bundle) with inverted orientation, opposite the xylem of the large bundle. The reason for the increase in size of the bundles at these four points is not quite clear. These bundles lie slightly out of the row of small bundles of the seminiferous scale, for they are situated, two just above, and two just below, the insertion of the sporangium. Two or three of the bundles of the upper system, nearest the margin, perhaps die out. *Immediately opposite* the median portion of the sporangium there is a small blank space where no bundle occurs. The bundles of the seminiferous scale are, many of them, extremely small, some being quite rudimentary, with only an element or two of xylem. The bundles of the seminiferous scale appear to have a quite regular course from

its base until just below its free tip, without bending into the sporangium anywhere; indeed, I never saw any bundles within the sporangium nor showing any tendency to enter it. The fact that the nucellus forms a continuous tissue with the seminiferous scale may obviate the necessity for bundles being set apart to pass into the sporangium, for the tissues of the latter could be as well supplied by the bundles of the scale as are any of the ordinary tissues of the latter. The fact also that the function of supplying the sporangium is distributed throughout a number of small bundles instead of two or three, will account for the fact that these small bundles of the seminiferous scale are not specially differentiated from those of the bract; they have no unusual development of centrifugal xylem and phloem, and also frequently possess centripetal xylem. The large size of the bundles at the four points above-mentioned may be due to some mechanical function which they possess.

Thus, the course of the bundles in the seminiferous scale of this plant is somewhat different from that in *A. brasiliana*, A. Rich., as described by Van Tieghem [1] and Strasburger [2], and agrees more with what occurs, according to the latter author, in *A. excelsa*, R. Br. Van Tieghem is evidently in error in supposing that the sporangium has become reflexed on to the *ventral* side of the seminiferous scale, for the bundles of the latter, at least in *A. Cookii*, R. Br., run through the whole length of the organ *between the sporangium and the bract*, this fact proving beyond doubt that the sporangium is still situated on the *dorsal* side of the seminiferous scale, although slightly imbedded in the tissue of the latter. In this species there is also no sign, as above stated, of any bundles entering the sporangium, as described by Van Tieghem for *A. brasiliana*, A. Rich.

Above the insertion of the sporangium the two large bundles at either side of the latter again divide up into a number of smaller ones, which pass further across towards

[1] Ann. d. Sc. Nat., Bot., v^e^ sér., tome x, 1869.

[2] Angiospermen und Gymnospermen, p. 92, 1879.

the dorsal side of the seminiferous scale, and lie at equal distances apart. Somewhat higher up they die out, after gradually dwindling in size, and no bundles enter the short, narrow, free tip of the seminiferous scale.

In the lamina of the *bract* there are a number of small, parallel bundles, slightly curved into an arc. Each has two or three layers of centrifugal xylem, and a large and well-developed *centripetal xylem*, consisting, as seen in transverse section, of several large angular tracheides, of which the outermost are almost or quite as large as the whole of the centrifugal xylem. Many of those on the ventral side have numerous *very small* bordered pits on their transverse walls. Transfusion-tissue occurs at the sides of the bundle, but most of the tracheides are in the ventral position. In longitudinal section the centripetal xylem consists, on the inner side, of considerably elongated tracheides which are rather broad and have very fine reticulations, amongst which small pits with narrow borders and wide openings are scattered. Further out, the tracheides become shorter and wider and lose the reticulate thickenings, retaining only the small bordered pits. The tracheides resemble those in the leaf and seminiferous scale of *A. Bidwillii*, Hook.

Araucaria Bidwillii, Hook.

In this plant the bundles of the bract and seminiferous scale *arise quite independently from the central cylinder* of the cone-axis, and at a wide vertical distance apart. Each of these bundles is a compound structure, being formed respectively of two bundles branching off from two distinct strands of the cylinder. This is well seen in tangential section of the cone. The subsequent division of the two bundles in the outer cortex appears to be the same as obtains in *A. Cookii*, R. Br. Sometimes, however, one or the other bundle appears to arise from a single strand of the cylinder.

As in *A. Cookii*, R. Br., an oval ring of bundles enters the appendage from the axis. Of these, many of those belonging

to the seminiferous scale have an *inverted collateral* or a *concentric* structure, and this structure persists right through the organ from base to tip. Many of the bundles possess a concentric structure, in which the primary phloem occupies the centre, and xylem occurs in the form of two arcs on either side, or even extends right round the phloem (Fig. 7). Often, no xylem, but phloem only, occurs on the dorsal side of the scale: often, all the xylem of the bundle is on the dorsal inverted portion and there is none on the ventral side; the phloem is on this side also sometimes absent. The dorsal portion appears to be primary, as in some bundles very small tracheides were seen situated *between* two radial rows of phloem; some parts of the xylem are, doubtless, purely secondary, the primary portion having very likely become obsolete. Some of the *concentric dorsal structures* have the xylem in the centre entirely surrounded by phloem.

Throughout the whole length of the seminiferous scale, small bundles, exhibiting the above-named structure in varying forms, occur between the sporangium and the bract, as in *A. Cookii*, R. Br.

At each end of the vascular ellipse, in the lower part of the appendage, a large bundle occupies a lateral position, thus connecting the two systems of bundles, those of the bract and those of the seminiferous scale. In one part of their course some of the bundles of the bract assume a lateral position, one or two even becoming semi-concentric (in structure like those of the seminiferous scale), but this is exceptional. The bundles of the seminiferous scale pursue a continuous course from its base, past the insertion of the sporangium, up into the free laminar portion, with the exception of the four or five small bundles situated between the sporangium and the bract, which completely die out. All this is very similar to what obtains in *A. Cookii*, R. Br., although in the present species rather more easy of observation.

In the present case also no bundles were seen to turn off and enter the sporangium. But in radial section *two strands* of phloem-like tissue were seen penetrating the basal (chalazal)

part of the sporangium, but not extending as far as the nucellus; these strands were in both cases quite unaccompanied by tracheides, although a tracheide or two was observed on the edge of the tissue of the scale as if about to enter the sporangium.

In radial section it is distinctly seen how the integument has become fused with the tissues of the seminiferous scale; but on its inner side at the micropyle it is free for a very short distance. On its outer side the narrow strip of tissue pertaining to the scale appears to be continuous with the integument to the tip.

The tracheides of all the bundles have spiral thickenings, those of the secondary elements being very close and dense; the elements are extremely narrow and elongated.

Branched sclerides, as in *A. Cookii*, R. Br., are abundant everywhere, except in the tissue surrounding the sporangium, which is devoid both of these and of tannin-sacs, but the latter occur in the outer narrow portion of the scale.

In the *free* laminar portion of the seminiferous scale the bundles almost entirely lose their concentric structure, although some are curved towards their dorsal side. In some of these bundles a small quantity of centripetal xylem occurs, which here and there is fairly well developed (Fig. 8).

Holding the theory of the *axillary bud* as the explanation of the structure of the appendage of the cone in *Araucaria*, I believe, with Celakovsky, that the 'ligule' represents the seminiferous scale which is itself the vegetatively-developed outer integument of a sporangium situated *in the anterior position* on an axillary bud. This outer integument has become almost completely fused with the subtending bract in *Araucaria*, completely so in *Agathis*. The seminiferous scale has thus in this section almost entirely lost its individuality. Is there any evidence in the structure of the vascular bundle-system for the original separation of the ventral scale or ligule as a distinct organ? In *A. brasiliana*, A. Rich., *A. excelsa*, R. Br., *A. Cunninghami*, Sw., and *A. Cookii*, R. Br.,

there appears to be no sufficient evidence for such a former state of things, the bundle-system in these plants arising as a single unit from the central cylinder of the axis, and seemingly supplying a single appendicular organ. In *A. Bidwillii*, Hook., however, there is undoubted evidence forthcoming, for in this plant, as we have seen, there are two distinct sets of vascular strands arising at separated points in the central cylinder of the axis, one of which exhibits inverted orientation of its parts. This, assuredly, would not be likely to happen if the appendicular organ which they supply represented a single scale. Any anatomist would say that the uppermost of these two systems of bundles belonged to some organ axillary to the bract which the lower bundle-system supplied. This I hold to be the case, the axis of the axillary bud being suppressed, and the latter reduced to a single leaflet, representing the vegetative outer integument of the sporangium, the cylinder of bundles which originally supplied this now obsolete axis has also become reduced to an arc of bundles such as commonly constitutes the bundle-system of a leaf. And Van Tieghem is thus far right in the views he put forward. In *all* species of Araucaria, however, the two bundle-systems become, either in the cortex of the axis or subsequently in the stalk of the appendage, more or less intermixed, as we have seen. In the majority of species the two systems appear to exist as a unit at *their first origin from the central cylinder*. This state of things seems to indicate an adaptive modification of the structure of long standing.

As regards the structure of the ventral portion of the appendage, the occurrence of *concentric* bundles seems to show that *Araucaria* is an ancient type of plant, and approaches the Cycads in this respect, for in the sporophylls of these latter concentric bundles are very frequent. The structure of the bundles in the free ligular portion of the appendage clearly proves that this organ is of *foliar* nature, and refutes the opinion of Strasburger that it represents the terminal portion of the axillary *axial* organ.

ABIETINEAE.

Pinus sylvestris, L.

In the female cone of this plant, four bundles leave the central cylinder of the axis of the cone: a lower one with normal orientation, an upper one with inverted orientation (which arises by fusion of two bundles springing right and left from distinct strands of the cylinder), and two lateral ones orientated sideways (Fig. 9). These all pass through the cortex and enter the base of the appendage, *without undergoing further division or alteration in position.* The lower bundle enters the small bract which is adnate for a short distance to the much larger seminiferous scale. The three other bundles enter the latter, the two lateral moving up so as to form a single row with the upper bundle (Fig. 10); by subsequent division a considerable number of bundles is formed. The plant differs from *Araucaria* in the fact that four bundles, instead of two, leave the cylinder of the axis, the bundles which supply the seminiferous scale and bract respectively *thus arising independently from the cylinder of the axis*, and no connexions occurring between the two systems during their course through the cortex. But in *Araucaria Bidwillii*, Hook., as we have seen, the two systems also arise independently from the central cylinder of the axis, and they appear to preserve their independence during their subsequent course far more than is the case in *A. Cookii*, R. Br.; *A. Bidwillii*, Hook., however, would appear to be an exception to the general rule prevailing in that genus. The direct explanation of the structure in *Pinus sylvestris*, L., lies in the fact of the more complete separation of the bract and seminiferous scale than is the case in *Araucaria*, the almost complete fusion in the latter of the two foliar organs having occasioned a similar fusion between the two otherwise distinct vascular systems, and the latter fact not necessarily implying that the appendage in *Araucaria* is really a single organ.

A single bundle with normal orientation, leaves the cylinder

of the axis, in the basal part of the cone, to supply the *sterile* seminiferous scale. It is much larger than the lower bundle of the four which supplies the bract of the fertile scale. On entering, or immediately before entering, the common stalk of the two organs, this bundle divides into two, of which the upper bundle supplies the sterile seminiferous scale, and subsequently divides up into several bundles. It will be seen that here a complete fusion of the two bundle-systems prevails, offering a wide difference from what obtains in the fertile part of the cone. But this is probably due to the loss of individuality of the system belonging to the seminiferous scale arising from the complete loss of function of the latter; hence the more economical plan for the plant would be to unite, as far as possible, the two systems into one. Except at the base of the cone, the tracheides of the cylinder have dense, fine, spiral thickenings; this is an interesting fact, as this character is otherwise only met with, as a general one, in vegetative organs, in the *older* types, like the Taxineae.

In the lamina of the seminiferous scale of *P. excelsa*, Wall., many of the bundles exhibit a partially concentric structure. such as is frequently found in ovular *integuments* (Fig. 11).

Podocarpeae.

Microcachrys tetragona, Hook.

This plant, belonging to the Podocarpeae, possesses beautiful rosy-red sporangium-bearing appendages, which are extremely succulent in character and terete in shape. Each fructification resembles in appearance a small mulberry.

Celakovsky's view, which appears to me the most tenable of all, of the structure of the female 'flower' of the Podocarpeae is this: that the sporangium is the sole representative, as in all Conifers, of the sporophyll or carpel pertaining to a completely suppressed axillary bud; that this sporangium has become carried up, for a longer or shorter distance, on to the subtending bract; and that the outer integument is

present in a normal form and not, as in the Abietineae, vegetatively developed into the seminiferous scale. There is nothing in the following anatomical investigation to render this view anything but quite probably true.

The sporangium is seated near the tip of the appendage, and is inverted. The other integument, or aril, is represented by a small, scale-like outgrowth occurring on the upper, distal side only of the sporangium (Fig. 12).

The axis of the cone contains several small, widely separated bundles.

In the upper part of the appendage, at the level of the insertion of the sporangium, and where the red colour is most conspicuous, there are about two layers of cells, lying below the sclerotic hypoderm, which are palisade-like in form, containing a great number of oil-globules of very varying sizes, some of which are very amorphous in shape; these are coloured red by alkannin, and dissolved by potash. Many of these cells are yellow in colour from the tannin they contain, which substance appears to stain deeply with safranin. The epidermis possesses a fairly thick cuticle. In the rest of the organ the cells are uniform throughout in shape and contents. Immediately surrounding the bundle the cells are very much smaller.

As regards the vascular structure of the appendage, there are, in spite of the assertions of Eichler and others, two distinct systems of bundles, one pertaining to the sporangium, the other to the appendage or bract. But the former is extremely minute and rudimentary. At the junction of the bract-bundle with the cylinder of the axis the small bundle pertaining to the sporangium is only represented by a few scarcely visible, non-staining, narrow tracheides with loose spiral thickenings, following the course of the bract-bundle, and either separated by a short space from, or closely approximated to, its protoxylem. While the connexion between the bract-bundle and the cylinder is very often perfectly distinct, the same was never actually observed in the case of the small bundle of the sporangium. In one case the

latter bundle was perceived in the near neighbourhood of a bundle of the cylinder and evidently situated just at the side of the latter, as the two were observed in different foci. In a transverse section of the very base of the appendage, where it is quite free from the axis, the bract-bundle (which itself is exceedingly small compared with the entire size of the appendage) and the bundle of the sporangium are both distinguished, the latter with *inverted orientation* and closely approximated to the former. This sporangium-bundle consists of a small group of *well-staining* tracheides with, possibly, rudimentary protoxylem; its phloem is absent or extremely rudimentary (Fig. 13). The bract-bundle is normal in its parts. At a higher level, the smaller sporangium-bundle increases in size and possesses a small, rudimentary phloem-group. During their upward course the two bundles are here and there mutually connected by means of some primary centrifugal tracheides which pass round the protoxylem and unite between the two bundles.

The sporangium-bundle appears, in fact, to be undergoing gradual obliteration, as shown by its appearance at the base of the appendage and in the axis. Thus, although this bundle is continuous into the sporangium, the bract-bundle in all probability performs the greater part of the conduction: hence arises the need for union of the two at various points. At the level where the smaller bundle deserts the bract-bundle to pass into the sporangium, the phloem of the former consists of but three or four insignificant cells (Fig. 14).

In the upper part of the bract, in the region of the palisade-tissue, there is a large and very broad mucilage-sac extending along the dorsal side of the bundle, and of about twice the radial width of the latter (Fig. 12). On the ventral side, between the tips of the bract and the outer integument of the sporangium, occurs a curious hump-like projection, giving a peculiar shape to the bract as seen in radial section. The laminar portion of the bract, above the insertion of the sporangium, is extremely short and rudimentary.

The bract-bundle, as far as the insertion of the sporangium,

is of the normal endarch type, with (relatively to its size) well-developed xylem and phloem, and no transfusion-tissue. In the lamina it becomes excessively reduced and insignificant, acquiring there at the same time well-marked transfusion-tissue of (usually) shortly elongated tracheides, rather unequal in size. Throughout its course the transfusion-tissue is entirely lateral in position, like that in the leaf of *Podocarpus*; in transverse section the elements are small and compactly grouped.

Taxeae.

Taxus baccata, L.

In this plant I was unable to perceive the slightest anatomical evidence for Van Tieghem's view, that the sporangium represents the first leaf of a tertiary axis. On the contrary, Celakovsky's idea that the sporangium is *terminal* to the secondary axis bearing the bract, appears by far the most probable theory, and well supported by every character of the organs, as well as by the course of the bundles. The anatomy of the bundles of the *sterile bracts* below the sporangium is very interesting on account of the presence of well-developed *centripetal xylem*, a tissue which is (quite naturally) scarce in the bundles of the foliage-leaves (Fig. 15).

Taxodineae.

Sciadopitys verticillata, Sieb. & Zucc.

The sporangiferous scale of this plant consists, as in Cupressineae, of the bract and seminiferous scale very intimately united, but towards the apex the two parts are quite distinct, the latter organ extending some distance beyond the former. It is thick and fleshy and bears a great number of sporangia overlapping one another at its base, and on the upper (dorsal) surface (Fig. 16). About three bundles leave the central cylinder of the axis, two of which belong to

the seminiferous scale, and one to the bract; the former have inverted orientation (Fig. 17).

On entering the seminiferous scale the two bundles branch up into a number, and spread right across the whole width of the scale (Figs. 20, 21). Branches from these bundles bend back, about two-thirds of the way up the scale, towards the place of insertion of the sporangia, but none were observed to enter these latter (Fig. 16). These bundles which supply the sporangia have a peculiar structure. They are all more or less *concentric*, most of them being incompletely so (Fig. 20); often several small ones are grouped together in an irregular fashion. The bundles which do not supply the sporangia are normally collateral, but some are of horse-shoe form. All possess a fair amount of *centripetal xylem*, consisting of similar tracheides to those of the abundant transfusion-tissue, viz. of angular contour and large size.

The bundle which enters the bract also branches. The larger of these bundles have *centripetal xylem*; many rudimentary strands, consisting of xylem only, are irregularly scattered about, many of them contiguous to the mucilage-canals.

Cryptomeria japonica, D. Don.

The female cones of this plant are rounded in shape and present appendages which are clearly divided into dorsal and ventral portions, the latter, as in *Sciadopitys*, being somewhat longer than the former, and representing the 'seminiferous scale'; it bears two elongated sporangia at its base.

Two or three bundles are given off from the central cylinder of the axis. Simultaneously one of the bundles gives off a branch, which, passing to the upper side, becomes, with its inverted orientation, the bundle of the seminiferous scale. The other bundle (when two only arise from the cylinder) branches almost at the same time, so that, eventually, four bundles enter the appendage grouped together as a small cylinder.

After entering the appendage, the two lateral bundles

divide each into two, and then into three, one or two passing towards the ventral side of the appendage so as to lie in the same plane with the bundle of the seminiferous scale, which has by this time divided into two. A very interesting fact to notice is that in the upper part of the seminiferous scale the bundles assume more or less of a *concentric* structure, and in the lower part of the appendage a concentric bundle appeared to arise quite independently of the other bundles, and ended blindly without fusing with those adjoining it; this is of great interest, as similar concentric bundles, of independent origin and course, are found in the sporophylls of the Cycads. The bract or dorsal portion of the scale eventually possesses a system of three bundles, of which two exhibit transfusion-tissue and a little centripetal xylem, while the other is completely *concentric* in structure. The only difference between this plant and *Sciadopitys*, as regards the character of the bundle-system in the scale, lies in the fact that in the former the bundles of the appendage are chiefly formed by branching of the laterally-placed bundles, in the latter of the ventrally-placed ones.

Cupressineae.

Cupressus Lawsoniana, Andr. Murr.

The bract and seminiferous scale are in this group so completely fused together that it is not easy to imagine each scale as in reality consisting of two, of which the tips only are free, that of the bract being reflexed (Fig. 21).

Three bundles leave the central cylinder of the axis; a median anterior one, and two from the lateral region of the cylinder and at a rather higher level than the first; the median bundle supplies the bract (Fig. 22).

Immediately on entering the double scale, one of the two lateral bundles (the orientation of which is at right angles to that of the bract-bundle) gives off a small branch which passes to the ventral side, assuming at the same time an

orientation the reverse of that of the bract-bundle (Figs. 23 and 24). This bundle then branches further (Fig. 25).

The two lateral bundles give off a number of branches which eventually spread round the whole contour of the double scale, the original median bundle of the three which left the cylinder of the axis remaining right in the centre of the scale (Figs. 25 and 26). Strasburger[1] suggests from this that the tissues of the seminiferous scale completely enwrap those of the bract.

There are thus a number of bundles both in the bract and the seminiferous scale. The structure of all appears to be endarch. No bundles were observed to enter the sporangia. A bundle or two also passes down into the lower reflexed lobe of the bract.

Conclusions.

Although, as was a foregone conclusion, the study of the anatomical characters of the female organs of Coniferae has failed to solve the problem of the real nature and morphology of these organs, it has, nevertheless, not been without its value, both as a means of here and there opening the way for plausible suggestions for the solution of the problem, and also as throwing some light upon the phylogenetic relationships of the order as a whole.

In *Araucaria Cookii*, R. Br., and *A. Bidwillii*, Hook., the very distinct presence in the sporangiferous appendage of the cone, of a double system of bundles, each system being independent of the other almost (or quite) from the central cylinder of the axis outward, the upper system, moreover, having its bundles orientated the reverse way to those of the lower, suggest strongly, that the appendage consists, not of one, but of two foliar organs, corresponding to the bract and the seminiferous scale in the Abietineae, which are only adnate at the very base, and whose bundles originate as

[1] Angiospermen und Gymnospermen, p. 83.

distinct systems from the central cylinder of the axis. If the ligule-like outgrowth of *Araucaria* were merely a ligule or an emergence, we should expect to find the bundles which supply it, branching off from those of the underlying organ somewhere near the insertion of the ligule, which, as we have seen, is very far from being the case.

In the Podocarpeae, again, it is supposed by many that we have to do with a single axial appendage bearing a sporangium on its upper surface. But, as I have clearly shown in *Microcachrys*, there are, as in *Araucaria*, two quite distinct systems of bundles in the appendage, with mutually inverted orientation of their parts, and which arise independently from the cylinder of the axis. Here then it appears also highly probable that we have to deal with two foliar organs, and not merely with a single one.

It is the same in this respect with *Sciadopitys*, *Cryptomeria*, and *Cupressus*. The axial appendage bearing the sporangia most clearly contains two perfectly distinct systems of bundles which arise as such from the cylinder of the axis, and have mutually inverse orientation of their parts. The two organs in the Cupressineae are so intimately fused together, being separable only at their tips, and even then in an obscure sort of way, that there is as much temptation as in the case of the Araucarieae to regard them as constituting but a single organ.

In the Taxodineae, to which we may regard *Sciadopitys* as also belonging, the compound nature of the appendage is more obvious than in the last-mentioned order, there being usually a distinct difference in size between the two organs composing it.

The Abietineae exhibit by far the best illustration of the compound nature of the axial appendage of the female cone, for in this group the two foliar organs composing it are distinct and separable except at the very base, where they are adnate to one another. The bundle-systems which supply each arise, as in the preceding case, independently from the cylinder of the axis. The whole course and

structure of the bundles of the appendages is similar to that of the bundles in the other groups.

The Taxeae differ from the other groups in the fact that the sporangia occur in a position *terminal* instead of lateral to the axis on which they are borne. The anatomy points clearly to the fact that no axial foliar appendage of any kind exists upon which the sporangia are inserted, the cylinder of the axis being directly continuous into the base of the sporangium. This latter difference, however, amounts to very little if we regard, with Celakovsky, the seminiferous scale of the other groups, as being the morphological equivalent of the outer integument of the Taxeae, which has become, with the exception of the Podocarpeae, vegetatively developed. In the Podocarpeae the relationship is precisely the same as in the Taxeae, with the exception of the axillary, instead of the terminal position of the sporangium. In this order the bundle-system belonging to the sporangium (which is, *in all the groups*, the sole representative of the sporophyll, according to the view I here adopt) becomes obvious owing to the fact that the latter gets carried, by the basal intercalary growth, on to the upper part of the bract. In the four other groups the bundle-system pertaining to the sporangia becomes very apparent owing to the vegetative development of their outer integuments which, in the form of the widely-expanded seminiferous scale, possesses a pronounced vascular tissue.

Hence, a study of the course of the vascular bundles in the female 'flowers' of this order tends towards a unification of the apparently, and from a superficial view, diverse structures which we find them exhibiting, and, although taken by itself, it will not explain their morphological significance, it will, nevertheless, greatly aid us towards some comprehension, if only a partial one, of the composition of these curious structures.

An examination into the structure of the individual bundles traversing the appendages of the cone or 'flower' affords us, as it seems to me, some clue as to the wider relationships of the plants concerned.

The bundles of the 'bract' or lowermost of the two

appendages in all the groups are in structure, as a rule, similar to those of the foliage-leaves. Sometimes they exhibit characters rather more ancient than obtain in the latter. A notable instance of this occurs in the bundles of the *sterile bracts* or *sporophylls* of the rudimentary cone of *Taxus*, where, contrary to what we know to be the case in the foliage-leaves, a large amount of *centripetal xylem* is found.

That ancestral characters should turn up in preponderating measure in those organs which are intimately associated with the most primitive parts of a plant, viz., the reproductive organs, is what one would naturally expect. Hence the mesarch structure of the bundles of the sterile bracts or sporophylls of *Taxus*. But the most ancient characters would surely be expected to be found in the actual reproductive organs themselves. In my recent paper on the sporophylls of Cycads [1] I made a special point of remarking and figuring the *concentric* structure (which I regard as *ancestral*) of many of the bundles which directly supply the megasporangia in most of the genera. While in the other parts of the sporophyll the bundles were nearly always of the collateral type of structure, *in every case* some of those which were sectioned immediately below the insertion of the sporangium [2], and some of those (and this is the most important point) in the *integument* of the sporangium, exhibited in unequivocal form a more or less complete *concentric* type of structure. Now, as has been described in the foregoing pages, bundles exhibiting a *concentric* type of structure occur in the seminiferous scale (the vegetatively-developed *outer integument*, according to Celakovsky's view) of *Araucaria*, *Pinus*, and *Sciadopitys*. No doubt, if further investigations had been made, they would have been found in the other genera also. The bundles exhibiting this structure are usually those in closest connexion with the sporangium, the others exhibit the ordinary collateral structure.

[1] Worsdell: 'The Vascular Structure of the Sporophylls of the Cycadaceae;' Ann. Bot. Vol. XII, 1898.

[2] loc. cit., Figs. 4 and 20.

The importance of this concentric structure of the bundles in the outer integument (seminiferous scale) of the megasporangium of Coniferae, as also of the mesarch structure of the bundles in their cotyledons and foliage-leaves (which I regard as a half-way stage from concentric towards endarch structure), lies in the fact of its pointing to the primitive stock of Fern-like plants, organisms to which this concentric or stelic structure of the vascular tissues is peculiar, as the source and origin of the great order of Coniferae, just as it is now all but universally admitted to be, of the allied order, Cycadaceae.

EXPLANATION OF FIGURES IN PLATE XXVII.

Illustrating Mr. Worsdell's paper on the 'flowers' of Coniferae.

The following are the abbreviations used in the lettering of the figures:—*int.*, integument; *nc.*, nucellus; *b. b.*, bundles of bract; *b. s. s.*, bundles of seminiferous scale; *s. s.*, seminiferous scale; *x.*, xylem; *ph.*, phloem; *px.*, protoxylem; x^1., centripetal xylem; x^2., centrifugal xylem; *s.*, sclereide; *b. o.*, bundle of the ovule; *b.*, bract; *o. int.*, outer integument; *ov.*, ovule; *ax.*, axis.

Figs. 1–5. *Araucaria Cookii.* Successive stages in the orientation and branching of the bundle-system from the central cylinder of the axis outward to the appendage. Diagrammatic.

Fig. 6. *Araucaria Cookii.* Diagrammatic transverse section of the appendage at the level of the sporangium, showing the two distinct bundle-systems of the bract and the seminiferous scale.

Fig. 7. *Araucaria Bidwillii.* Transverse section of a concentric bundle of the seminiferous scale. × 220.

Fig. 8. *Araucaria Bidwillii.* Transverse section of a bundle in the free ligule-like portion of the seminiferous scale. × 285.

Fig. 9. *Pinus sylvestris.* The four bundles leaving the central cylinder of the axis. Diagrammatic.

Fig. 10. *Pinus sylvestris.* The four bundles entering the appendage. Diagrammatic.

Fig. 11. *Pinus excelsa.* Transverse section of a bundle in the expanded laminar portion of the seminiferous scale. × 220.

Fig. 12. *Microcachrys tetragona.* Radial section of the appendage of the cone, showing the insertion of the sporangium and the bundle-system. × 35.

Fig. 13. *Microcachrys tetragona.* Transverse section of the bundle-system at the base of the appendage. × 430.

Fig. 14. *Microcachrys tetragona.* Transverse section of the bundle-system of the appendage at the level of insertion of the sporangium. × 430.

Fig. 15. *Taxus baccata.* Transverse section of a bundle in a sterile sporophyll of the rudimentary cone. × 220.

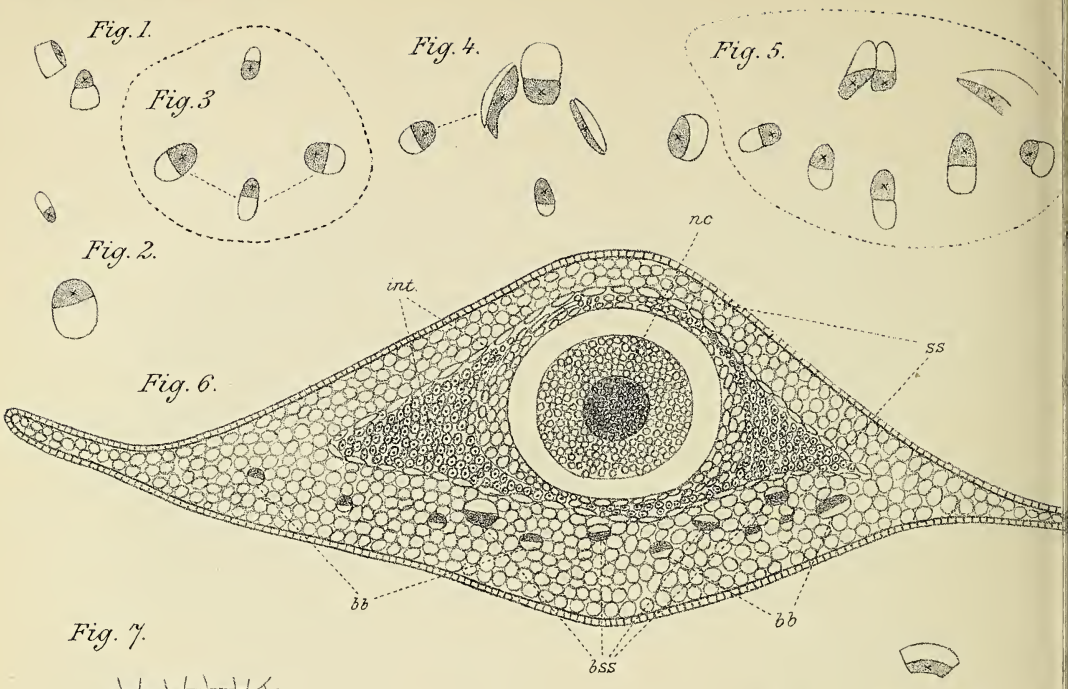
Fig. 16. *Sciadopitys verticillata.* Radial section of the appendage showing insertion of the sporangia and the bundle system. Diagrammatic.

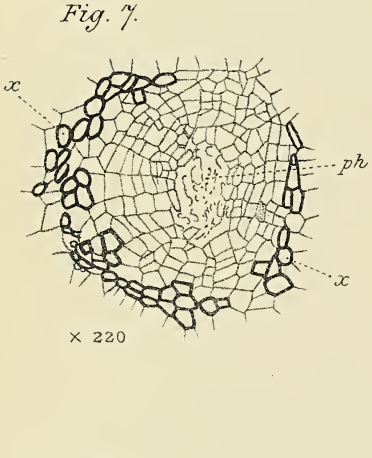
Figs. 17, 18 and 19. *Sciadopitys verticillata.* Successive stages in the orientation and branching of the bundle-system from the central cylinder of the axis outward into the appendage. Diagrammatic.

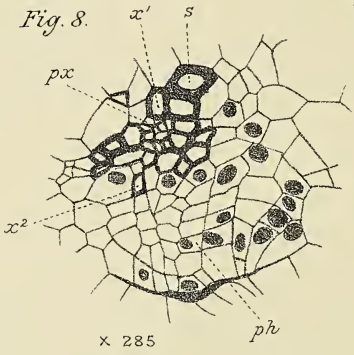
Fig. 20. *Sciadopitys verticillata.* Transverse section of a partially concentric bundle in the seminiferous scale. × 430.

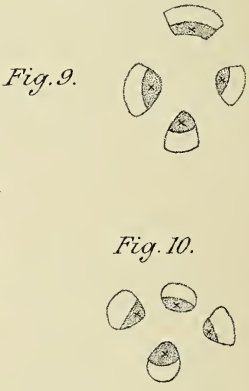
Fig 21. *Cupressus Lawsoniana.* Radial section of the appendage showing the insertion of the sporangia and the bundle-system. Diagrammatic. × 15.

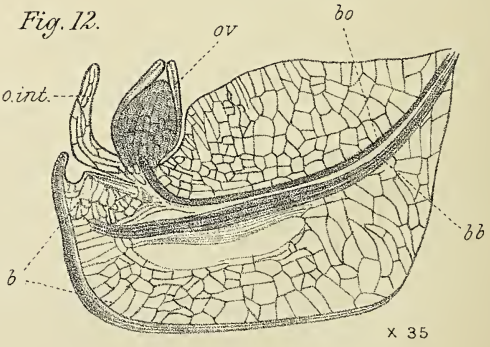
Figs. 22–26. *Cupressus Lawsoniana.* Successive stages in the orientation and branching of the bundle-system from the central cylinder of the axis outward into the expanded peltate portion of the appendage. Diagrammatic.











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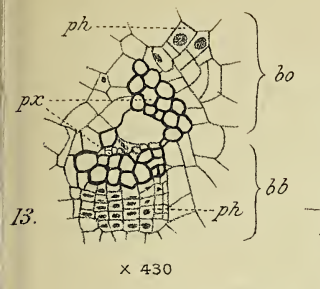
WORSDELL.— VASCULAR SYSTEM OF FEMALE FLOWERS OF CONIFERÆ.



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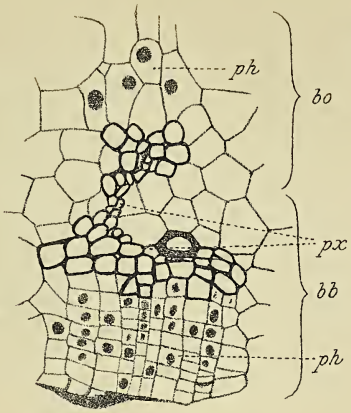

Fig. 14.

Fig. 15.

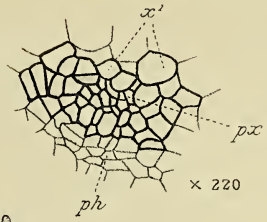

Fig. 17.

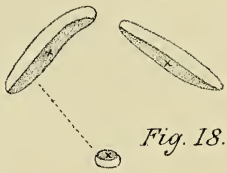

Fig. 18.

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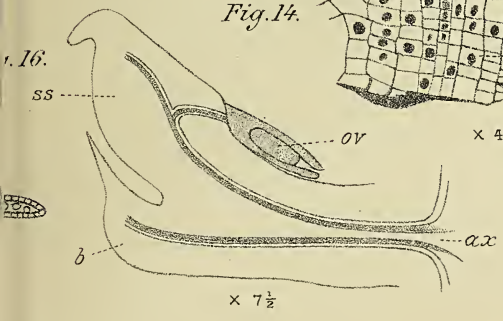

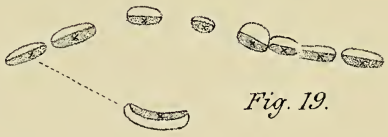

Fig. 19.

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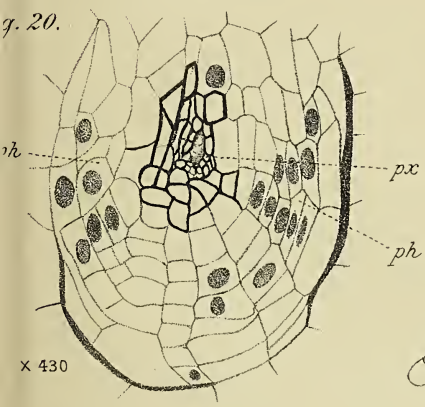

Fig. 21.

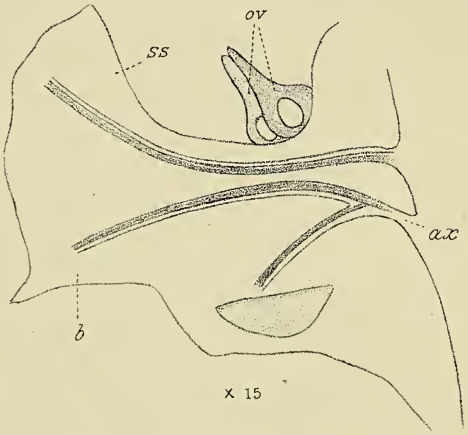

Fig. 23.

Fig. 24.

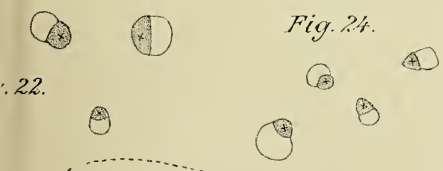

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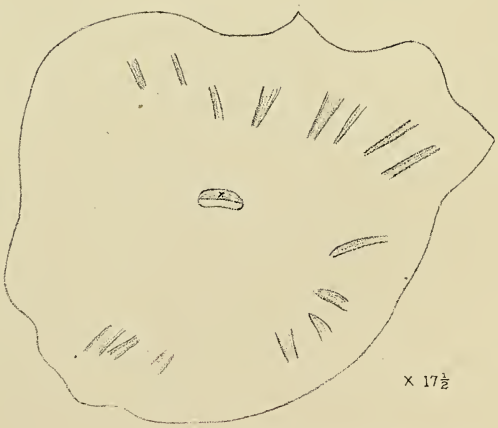
Fig. 26.



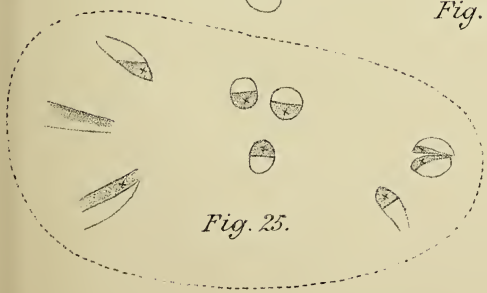


Fig. 25.

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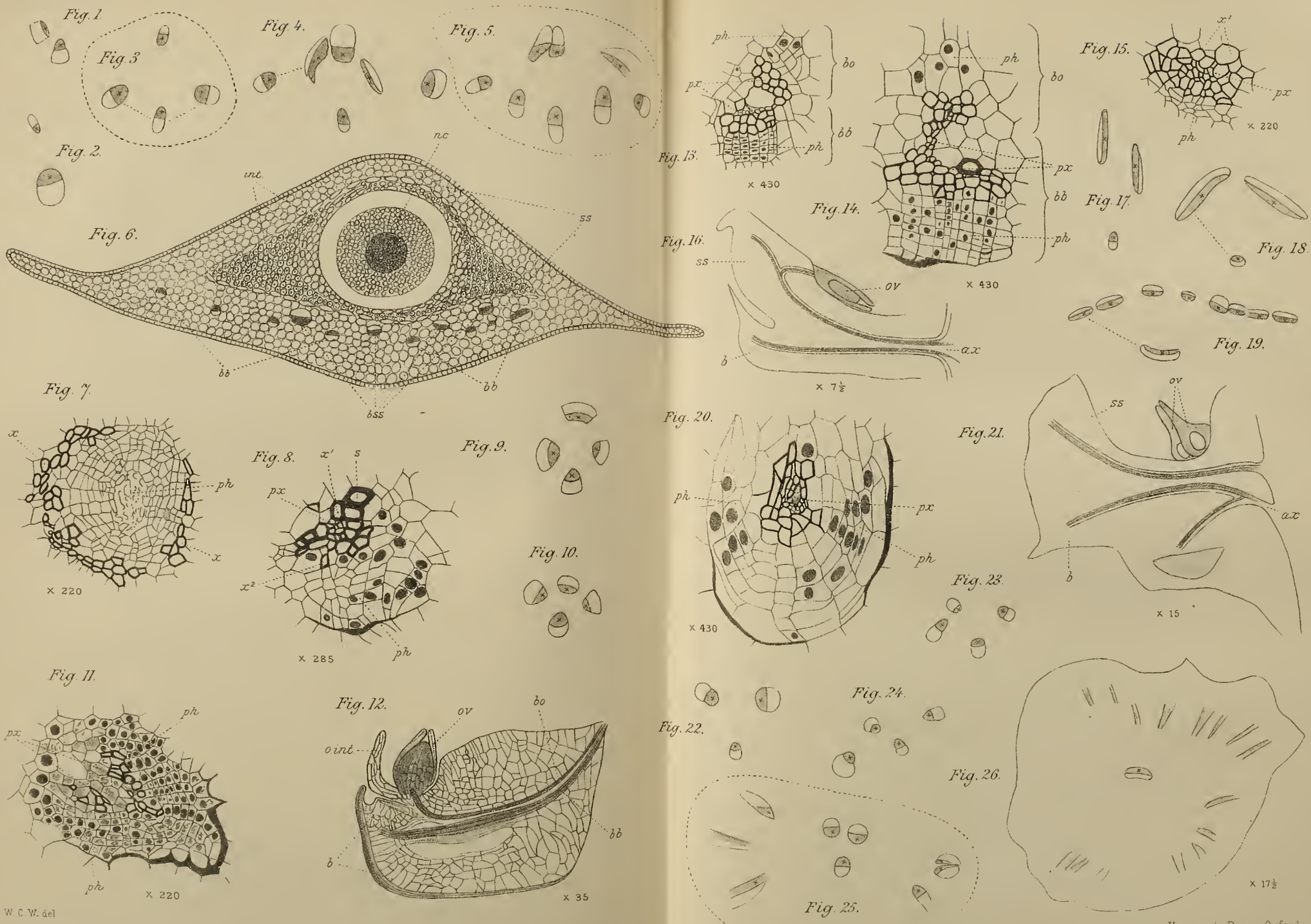

WORSDELL.— VASCULAR SYSTEM OF FEMALE FLOWERS OF CONIFERÆ.

University Press, Oxford

Symbiosis [1].

BY

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Synopsis [2].

ORIGIN of the idea and of the term. Differences between parasitisim and symbiosis (1).

Lichens, previously regarded as autonomous plants, are shown to be dual organisms, a symbiosis of Alga and Fungus (2). Controversy regarding the Lichen theory, and establishment of the latter by means of synthetic cultures (3).

Other cases of symbiosis known previous to 1880. Algae in the stems of *Gunnera*, and the roots of *Cycas*, in the thallus or fronds of *Anthoceros* and *Blasia*, *Azolla*, *Lemna*, &c. (4).

Extension of the idea of symbiosis: insect-fertilization, epiphytes, &c. (5).

Galls not necessarily due to insects, but may be due to the irritating action of Fungi or Bacteria. Phytocecidia of the Aleppo pine, &c. (6).

Symbiosis in animals. Green Infusoria, *Hydra*, sponges, &c. (7).

Mycorhiza, the roots of many humus plants curiously

[1] Paper read before a joint meeting of the Chemical and Botanical Sections of the British Association at Dover, 1899.

[2] The figures in parentheses refer to the bibliography collected at the end.

[Annals of Botany, Vol. XIII. No. LII. December, 1899.]

swollen and modified owing to the presence of Fungi, which do not injure the plant, but link its roots to the decomposing leaves around. Explanation as an instance of symbiosis. Evidence partly anatomical and partly experimental (8).

'Budding' and 'grafting' are processes involving the establishment of a symbiosis.

The nodules on the roots of leguminous plants (9). Discovery and controversy as to their nature. They contain living bacteroids, which penetrate the root hairs and flourish in the living cells. Universality of these nodules on healthy roots. Hellriegel and Willfarth's cultures, and evidence as to the fixation of nitrogen (10). Laurent and Schloesing's proof that nitrogen is fixed from the air (11).

The leguminous nodules a case of symbiosis, comparable to galls.

Other instances not yet explained. Nodules on the roots of *Juncus*, *Myrica*, and other plants (12).

Symbiotic fermentations (13). All natural fermentations mixed. Pure cultures and the importance of synthetic cultures.

Kephir (14), the ginger-beer plant, and other instances of symbiotic ferments (15). Decomposition of cellulose (16). Nitrifying and de-nitrifying organisms (17). The direct alcoholic fermentation of starch by the simultaneous action of two Fungi (18).

Return to the idea of symbiosis. Necessity of limiting the term. Antibiosis (19) (antagonism). Metabiosis (20). Difficulty of distinguishing in given cases. Hypothetical considerations, and importance of further investigations.

Particular Cases.

The above may be accepted as affording general headings under which the subject of symbiosis might be treated.

For the purposes of this discussion, I proceed to consider some special cases, and limit myself—as requested to do—to certain aspects of symbiotic fermentations.

Several cases of symbiosis among Bacteria are now known. Apart from numerous instances of temporary association between pathogenic micro-organisms and animals such as earth-worms, rats, flies, ticks, and mosquitoes, which disseminate their germs and infect cattle, sheep, horses, and men (21) (reminding us of the transference of the spores of *Botrytis* by bees, which carry this parasite with the pollen and infect the stigmas of bilberries with the parasite (22)) or which act the part of intermediate hosts to the disease germs, much as certain pond snails do to the liver-fluke of sheep (23), we now know several cases of symbiosis between two species of Bacteria or of Fungi, or between a Bacterium and a Fungus, where each symbiont is incapable of carrying on alone the work which the symbiotic association is able to perform—a point which is essential to the definition of symbiosis in the narrower sense, i. e. the co-operation of two associated organisms to their mutual advantage.

A striking example is afforded by certain Bacteria concerned in the destruction of cellulose in ponds, bogs, rivers, &c. (24). Van Senus found that a certain anaërobic Bacterium, resembling, if not identical with, Van Tieghem's *B. Amylobacter* (25), though incapable of dissolving cellulose by itself, can do so if associated with another Bacterium, also incapable of itself attacking cellulose. *B. Amylobacter* can ferment pectose compounds, and is thus capable of isolating cells one from another, but cellulose is not attacked by it.

Van Senus believed that the one Bacillus destroys certain products of fermentation excreted by *B. Amylobacter*, which inhibit its cellulose-fermenting powers.

I may remark here, that if a sound potato, rhizome, or other underground organ is placed in water and the air exhausted as completely as possible, I almost invariably find its cellulose walls destroyed in a few days by a mixture of Bacteria, and with the symptoms found in many kinds of 'wet rot.' There is no reason to believe that these organs would rot if merely wet and not deprived of air, since they lie in ordinary soil—even moist soil—for weeks or months, with plenty of water in

their tissues, and respire oxygen, as is well known. The presumption is that the anaërobic conditions set up in the experiment described favour certain forms of soil Bacteria, such as Van Senus worked with, and enable them to co-operate in the destruction of the cell walls.

An even more remarkable example is given by Winogradsky, who found that the anaërobic Bacterium known as *Clostridium Pasteurianum* is able, if supplied with abundance of dextrose and protected from the access of oxygen, to fix atmospheric nitrogen (26). In the cultures, and presumably in the soil, the *Clostridium* was found to work when protected by a mantle of aërobic bacteria. In fact, the nitrogen-fixing *Clostridium* was working in the meshes of the oxygen-consuming species, and forming gelatinous flocks like the well-known grains of kephir, or of ginger-beer plant.

Yet another striking instance of symbiotic association has recently been given by Omeliansky (27). In experiments on nitrification at Bonn (28), the assertion had been made that the nitrifying organisms, i. e. the Bacteria known to oxidize ammonia to nitrous acid, and nitrous acid to nitric acid, could be grown and made to do their specific work in media containing proteids or other organic nitrogenous bodies. Now this was directly contradictory of the experience of Warington (29), Winogradsky (30), and other workers, who had found that one great peculiarity of these nitrifying organisms is that they refuse to grow on such media: they are incapable of using organic nitrogen. Several workers (31) then showed that the Bonn observers had inadvertently employed mixtures of two or more species, and Omeliansky undertook a critical re-investigation of the whole subject, and has put forward the following explanation of the tangle.

If *Nitrosomonas*—the Bacterium which oxidizes ammonia to nitrous acid—and *Nitrobacter*—the Bacterium which further oxidizes nitrous to nitric acid—are sown together or separately on a medium containing organic nitrogen, no growth or change occurs.

But if a Bacterium capable of decomposing the organic

nitrogenous medium, e. g. *Bacillus ramosus*, is added to the above-mentioned *Nitrosomonas* and *Nitrobacter*, the associated three organisms are able to carry out all the processes and complete the cycle of nitrification. That is to say, *B. ramosus* breaks down the gelatine and ammonia is formed, this is then oxidized to nitrous acid by *Nitrosomonas*, and the nitrous acid is further oxidized to nitric acid by the *Nitrobacter*.

If *B. ramosus* and *Nitrosomonas* only are sown together, then only nitrous acid is formed, because the latter organism is only capable of carrying the oxidation the one stage.

If *B. ramosus* and *Nitrobacter* only are used, then only ammonia is formed, because the latter organism cannot oxidize ammonia.

If we try to imagine the working of this association of organisms in the soil, and bear in mind the frequent co-existence and action of the de-nitrifying Bacteria which Gayon and Dupetit (32), Giltay and Aberson (33), Warington (34) and others have familiarized us with, a glimpse is obtained of the very complex symbioses which may be concerned in the circulation of nitrogen in Nature. Moreover, some of these de-nitrifying Bacilli appear to be anaërobic, and can only work in the surface soil if protected from the access of oxygen ; say, by an associated aërobic Bacterium.

Another interesting case is the following. Perdix a few years ago isolated from water an anaërobic Bacterium which converts starches into sugars, which with the aid of a yeast can be fermented, the whole process going on in association (34 *a*).

Other cases of symbiotic associations of Bacteria exist among the forms concerned in the reductions of sulphates (35) and the oxidation of sulphuretted hydrogen (36), the iron bacteria (37), &c. ; but I propose to mention only one or two further examples, taken from the true Fungi.

Symbiotic associations of Fungi are probably common, but only a few cases are as yet established, and these principally among the ferment-Fungi (38).

Van Laer has called attention to the symbiotic co-existence

of two yeasts in many beers, explaining certain peculiar after-fermentations as due to the results of one yeast acting on the medium improved for it by the other (38 *a*).

The Japanese have long been in the habit of brewing a peculiar fermented liquor known as rice-wine, or Saké (39). Rice grains are steamed, and when cool are infected with a mould fungus now known as *Aspergillus Oryzae.* When the rice is quite mouldy, at which time it emits a peculiar odour like that of pine-apples, the starch is found to be rapidly turning to sugar, under the action of a diastatic enzyme secreted by the Fungus.

This decomposing rice is then placed in water and exposed to the action of a yeast, which rapidly ferments the sugar, and the alcoholic Saké results.

So closely is the yeast associated with the *Aspergillus*, that, in practice, the alcoholic fermentation commences soon after the enzyme of the *Aspergillus* begins to hydrolyze the starch of the rice, and for some time a controversy existed as to whether the yeast was not really part of the life-history of the *Aspergillus*. Several observers have now shown, however, that we have here a striking case of symbiosis (40).

On reviewing these examples, we shall find that very different degrees of association of the organisms are to be met with.

At the one end of the series we find two organisms merely associated for a short time, e. g. Bacilli and worms, bees and *Botrytis*-spores, and, so far as we may speak of symbiosis at all in these cases, it is merely temporary or disjunctive.

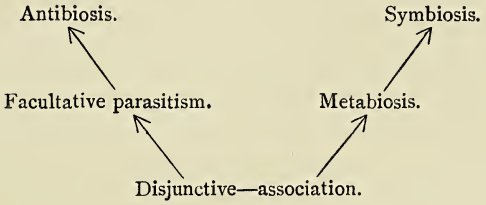
At the other end of the series we have a close permanent combination of the two organisms working in unison, e. g. the Lichens, and Winogradsky's *Clostridium* with its protective mantle of aërobic Bacteria ; also the ginger-beer plant and kephir.

But between these extremes it is possible to find all stages, the half-way house being met with in cases such as the Saké ferment, where the *Aspergillus* evidently prepares the way for the *yeast.*

It has been proposed to apply the term *Metabiosis* to such cases.

It must not be forgotten that there are extremes in another direction, where one of the two associated organisms is injuring the other, as exemplified by many parasites, but these cases I leave out of account here. This state of affairs has been termed *Antibiosis.*

It seems not impossible that the biological relationships of these cases one to another could be shown thus :—



The Physiology of Symbiosis.

It will be an interesting exercise to see if we can get any further glimpses into the physiology of the phenomenon of Symbiosis.

When we come to inquire as to the processes which lead to enhancement of the functional activity of one organism by another living symbiotically with it, the matter presents many difficulties; for it is at the outset quite obvious that many things are possible, and it soon becomes evident that a tangle of complexities lies before us, as always in the inter-relations between associated biological units. We need go no further than the examination of the possibilities in the inter-relations between a weed and a cultivated plant, or between two trees in a forest, for illustrations of this truth.

Confining attention for the moment to closely associated symbionts, such as those composing a Lichen, the ginger-beer plant, or a clump of symbiotic Bacteria or Fungi, researches have made it practically certain that the provision of definite

food-materials by the one symbiont for the other may be an important factor; e.g. an Alga supplies a Fungus with carbohydrates, or a Fungus converts starch into the fermentable sugars which the associated yeast needs. In other cases the advantage derived is one of protection from some injurious agent—e.g. the aërobic Bacterium prevents the access of oxygen to the anaërobic one. But there is evidence which suggests that mere nutrition or protection is not the only or even the principal factor involved. It is well known that the products of fermentative actions are frequently poisons, and we all know of cases where such poisonous excreta of living cells act as stimuli to other living cells, if supplied to them in minimal doses and very gradually: I need only instance the effects of tobacco or alcohol on man, in illustration of this.

Several observers have shown that in presence of a particular food-substance the living cell is stimulated to produce and excrete a particular enzyme, while the substitution of another food stimulates the organism to excrete a totally different enzyme.

Now let us see if there is any evidence to support the hypothesis that some such stimulative action is exerted by one symbiont on another. To a certain extent we find such evidence in the remarkable vigour and large size of the algal cells in a Lichen as compared with the same cells living an independent life, and in the persistent zone of brilliant green and often hypertrophied cells of leaves in which certain Fungi are living, the gigantic cells of the nodules on leguminous roots in which the bacteroids are living, and many other cases; but since it is impossible to say how far these are cases of merely enhanced nutrition, we will pass them by and seek for other instances.

One of the earliest I can find is Hugo Schulz's demonstration in 1888 (41) that minute quantities of poisons such as corrosive sublimate, iodine, iodide of potassium, bromine, arsenious acid, chromic acid, sodium salicylate, or formic acid, when added to yeast in 10 per cent. grape-sugar solution, immediately raise the fermentative activity of the organism—

as measured by the amount of carbon-dioxide evolved. Effront, in 1894 (42), showed that hydrofluoric acid acts similarly on yeasts, butyric ferments, and *Mycoderma*, and, later, that the same is true of formaldehyde, salicylic acid, picric acid, &c.

The results of Johannsen's experiments with seeds, buds, &c., treated with ether or chloroform, look like another case in point: respiration is increased, and the whole course of metabolism so altered that in some cases buds of flowers can be stimulated to open long before their normal period (43).

The results obtained by Farmer and Waller with carbon-dioxide, which was found to induce an initial acceleration of the movement of the protoplasm in *Elodea*, may be a further instance (44).

Pfeffer has recently called attention to a still more remarkable instance—that it is possible by etherizing the living cells of *Spirogyra* to alter the type of nuclear division from *mitotic* (indirect) to *a-mitotic* (direct). Massart had shown that callus, the hypertrophied tissue developed under stimulation by mites, fungi, exposure to air, &c., is formed of cells which divide with *a-mitotic* nuclear division; and other cases occur. But it is even more to the point for my purpose that Gerassimoff, in Pfeffer's laboratory, found *Spirogyra* driven to *a-mitotic* division by associated Bacteria and other organisms, which he regards as a case of symbiosis (44 *a*).

Now it may be regarded as certain that if a cell can be thus stimulated to alter the details of so fundamental and complex a morphological process as its cell-division, by the action of associated organisms, the metabolic activities of its protoplasm are being driven into very different channels from the normal, and many physiological processes must be affected.

Of course I am here raising questions which concern the border-line between health and disease, and much investigation is still required as to the meaning of these matters; but I ought to add that according to Pfeffer the etherized cells can be again restored to their normal state if the traces of the anaesthetic are washed out, and those familiar with Klebs' experiments on

other algae will appreciate the significance of this one with *Spirogyra*.

However feeble the evidence may be, we can at least say, then, that there is some evidence in support of the hypothesis that one symbiont may stimulate another by excreting some body which acts as an exciting drug to the latter—just as truly as certain drugs act as stimulants to some cell or organ of a higher animal, and no doubt in a fundamentally similar manner (44 *b*). It will be noted that such drugs are frequently excreta from vegetable cells.

But there is another, perhaps more indirect way in which one symbiont may enhance the activity of another. It has long been known that the accumulation of the products of metabolism of a cell tend to inhibit the activity of that cell, and that if by any means we can destroy or remove the metabolite as it is formed, the cell concerned can go on working. Similarly with ferments, and even with enzymes, the accumulation of the products gradually inhibits the action, as Tammann (45) showed in the case of Amygdalin and emulsin, and Brown and Morris (46) and Lea (47) in the case of starch and diastase, to mention two illustrations only.

Now suppose we have two organisms *A* and *B* living in symbiosis, and suppose that *A* is capable of hydrolysing starch by the excretion of diastase, while *B* removes the product of hydrolysis, by fermenting the sugar as fast as it is formed; in this case there is every reason to expect that *A* will push its hydrolysing action to the utmost, not only because it is of advantage to *A* to be relieved of the inhibiting sugar, but because the diminution of the sugar re-acts as a stimulus to the secretion of more enzyme.

There is yet another point to be considered. Katz, in 1898 (48), published some results confirming in many points the discoveries of Wortmann (49), Brown and Morris (50), and others, that Fungi, Bacteria, embryos, and other enzyme-secreting organisms not only vary the extent and kind of enzyme secreted, but can be stimulated to vary the enzyme according to the quantity or quality of food materials at hand.

I think this line of inquiry may lead to results in the present connexion, as it is obvious that the products of fermentation of an organism *A* must be favourable, or without effect, or deleterious to the action of another, *B*, in its immediate neighbourhood. Moreover, it is shown that a product which is, *per se*, devoid of either favourable or deleterious action, may acquire the power of exerting one or the other if the concentration increases.

Katz regards the action of sugars as not a purely chemical one, but as a physiological stimulus; and without pretending to understand the distinction in detail, we may admit the importance of the experimental facts, and not only seek for, but also hope for, more light.

Here, then, is a brief sketch of some of the salient features of symbiosis, and of some of the physiological factors concerned in the processes; and though it is far from exhaustive, it may serve our purpose to-day of starting a discussion, and of showing some lines along which further investigation is desirable.

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48. KATZ: Die regulatorische Bildung von Diastase durch Pilze. Jahrb. f. wiss. Bot., xxxi, p. 599 (1898).
49. WORTMANN: Ueber den Nachweis, das Vorkommen und die Bedeutung des diastatischen Enzyms in den Pflanzen. Bot. Zeit., 1890, p. 581.
50. BROWN and MORRIS: Researches on the germination of some of the Gramineae. Journ. Chem. Soc., vol. lvii, 1890. Trans. p. 458.

Some Methods for Use in the Culture of Algae[1].

BY

H. MARSHALL WARD, D.Sc., F.R.S.,

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With Plate XXVIII.

THE following notes are somewhat of the nature of suggestions, since much has still to be done, no doubt, before the efficacy of the treatment and the faults and difficulties of the methods in detail are fully demonstrated; but since the author has found they can be used with some measure of success, the various workers interested in the culture of Algae may care to take the methods up and try to improve them.

1. If agar is swollen in dilute acetic acid, and then washed very thoroughly so that every trace of soluble salt is removed, it can be used, mixed with the necessary culture fluids, as a convenient medium for the growth of some Algae, as Beyjerinck had already observed. But, so far as the author knows, the use of such a medium for separating Algae in plate culture and for observing their growth in hanging drops has not been attempted. It can be done, however, though the high melting-point of the agar and the sliminess of some Algae occasionally cause difficulties.

The author has also succeeded in separating Algae by the following methods:—

2. Shake them up in sterilized nutritive mineral solution, mix rapidly with silica jelly, also sterilized, and pour into

[1] Paper read before the Botanical Section of the British Association, at Dover, Sept. 1899.

[Annals of Botany, Vol. XIII. No. LII. December, 1899.]

glass dishes. With species of *Oscillaria* and of *Palmella*, certain Protococcoideae, &c., the author has observed growth in hanging drops of this silica-jelly medium under high powers, and has seen sufficient to make it hopeful that even Diatoms may be cultivated this way; and it is not impossible that some modification of the process could be utilized for the culture of marine Algae.

Another device is as follows:—

3. Shake the Algae up in the nutritive solution and rapidly mix with sterilized plaster of Paris, and pour into dishes. The fixed Algae grow *in situ* in some cases, but others appear to be too sensitive for such treatment.

Experiments have also been made as follows, with some success:—

4. The Algae are shaken up in the culture medium, and a large quantity of lime-water quickly added. Then carbon dioxide gas is passed rapidly through, and the Algae are thrown down with the precipitate of calcium carbonate; this is poured into dishes as if it were plaster of Paris. Perhaps this method could be utilized in the study of calcareous Algae, but with some forms it appears too drastic. Baryta seems to act as a poison, and cannot be substituted for lime.

It is clear that if once we obtain a pure colony on a glass dish, a trace fished out with a needle may be used to start other cultures. Season, temperature, intensity of light and other factors, are of importance in these matters.

As an illustration of what may be done with the hanging drop culture I take the following example:—

On August 21 a quantity of *Oscillaria—O. tenerrima* (Ktz.) apparently—was shaken up in Knop's mineral solution, added to silica jelly and poured with a drop of sodium chloride into a plate, where it soon set. On the 22nd I made a hanging drop of a small portion of this, and fixed a small piece of the Alga under a 4 mm. dry apochromatic lens with No. 2 eye-piece with micrometer. The piece measured 13·5 μ long $\times$ 2·25 μ broad, and next day showed no perceptible change. Nor could I detect any growth on the 24th, or

following days; but by the 25th it was evident that growth was taking place in other pieces scattered over the field, and on the 26th I fixed another segment, measuring 45 μ long × 2·3 μ broad, and obtained the following measurements. See Plate XXVIII.

Date.	Hour.	Temp.	Length.	Elongn.
Aug. 26.	11.30 a.m.	22° C.	45 μ	
,, 27.	3.10 p.m.	25	58·5 μ	13·5 μ
,, 28.	10.20 a.m.	21·5	68·4 μ	9·9 μ
,, ,,	12.50 p.m.	21·5	72·9 μ	4·5 μ
,, ,,	3.30 ,,	22	76·5 μ	3·6 μ
,, ,,	6.15 ,,	21·5	78·75 μ	2·25 μ
,, 29.	10.0 a.m.	18·5	85·5 μ	6·75 μ
,, ,,	12 noon	21	90·45 μ	4·95 μ
,, ,,	3.35 p.m.	21	99·0 μ	8·55 μ
,, ,,	8.0 ,,	20·5	105·75 μ	6·75 μ
,, 30.	9.0 a.m.	19·5	109·0 μ	3·25 μ
,, ,,	1.15 p.m.	23	113·5 μ	4·5 μ
,, ,,	3.30 ,,	23	123·5 μ	10·0 μ
,, ,,	6.45 ,,	22	132·75 μ	9·25 μ
,, 31.	9.0 a.m.	18·5	137·25 μ	4·5 μ
,, ,,	12 noon	20	148·5 μ	11·25 μ
,, ,,	3.30 p.m.	21	162·0 μ	13·9 μ
,, ,,	6.50 ,,	20	171·0 μ	9·0 μ
Sept. 1.	9.15 a.m.	17	175·5 μ	4·5 μ
,, ,,	12.30 p.m.	19·5	189 μ	13·5 μ
,, ,,	3.5 ,,	20	198 μ	9 μ
,, ,,	6.40 ,,	21	207 μ	9 μ
,, ,,	9.45 ,,	20	211·5 μ	4·5 μ
,, 2.	5.25 a.m.	17·5	211·5 μ	0
,, ,,	10.15 ,,	18	213 75 μ	2·25 μ
,, ,,	1.10 p.m.	20	216·0 μ	2·25 μ
,, 3.	10 0 a.m.	16·5	235·0 μ	19 μ

From the fact that this filament was held by the tail, as it were, I could not determine whether one only or both ends were growing.

It took from 11.30 a.m. on Aug. 26 to the same hour on Aug. 29 (i. e. 72 hours) to double its length—from 45 to 90 μ—and this appears to be very slow growth, though I am not aware that measurements exist to help us in deciding *how* slow.

The next doubling of the length—90–180 μ—was completed about 10 a.m. on Sept. 1—i.e. in about 70½ hours.

In a diagram made at the time (Plate XXVIII), the curve of growth shows very clearly that no elongation took place during the hours of darkness, but that growth and assimilation are coincident, a point which appears worthy of note in view of what we know as to the effect of light on growing organs.

It may also be added that I have been able to observe the division into zoospores of certain Protococcoideae in hanging drops of agar, and that this appears to occur only during the night.

In connexion with the action of light on green Algae, experiments in which ordinary (reflected) light was allowed to act on agar plates of Protococcoideae, covered with a stencil letter, showed its effect in a few days; the Algae in the area exposed to light developed normally, but those in the non-illuminated parts remained undeveloped. Consequently a faint green letter appeared in a colourless ground.

When the light was more intense, however, the exposed Algae were killed, whereas those in the covered parts of the agar developed fairly well, and thus the surprising phenomenon of a colourless letter in a green matrix appeared. The development in the shaded parts was probably due to the reflection of some of the intense light at the back of the plate, and this diffused over the non-exposed area was strong enough to enable the Algae to grow, but not sufficiently intense to kill them.

Many questions involving the use of quartz and coloured screens, and of different sources and intensities of light arise out of these experiments, and are held over for further investigation.

EXPLANATION OF PLATE XXVIII.

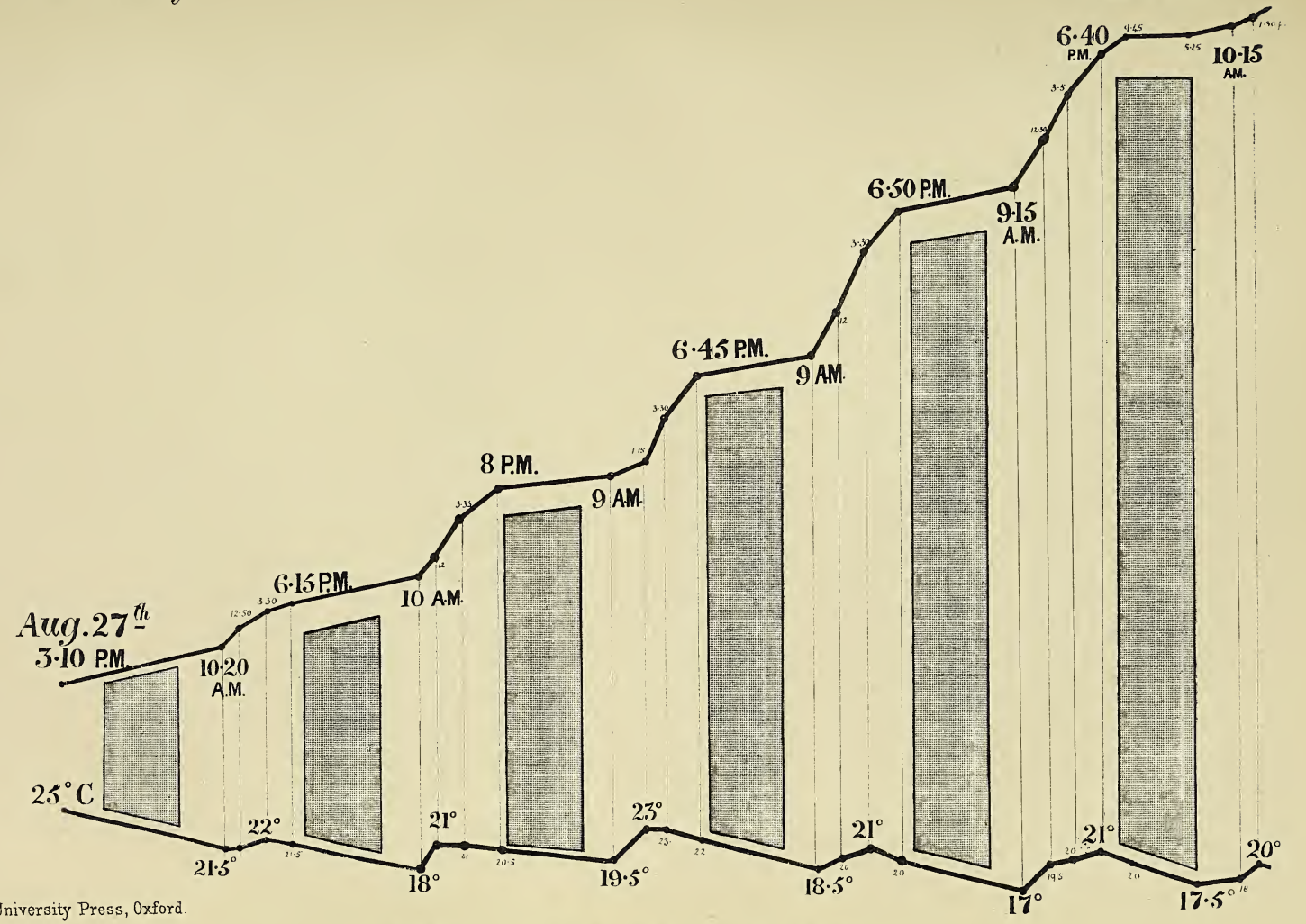
Illustrating Prof. Ward's paper on the Culture of Algae.

The upper curve represents the growth of the filament referred to on p. 565, and the lower curve the changes of temperature.

The curves start at 3.10 p.m. on Aug. 27, when, as seen from the table (p. 565), the filament measured 58·5 μ in length. The shaded areas represent the hours of darkness of each successive night.

It will be seen that the period of maximum growth coincides with that during which assimilation is active—i.e. from 9-10 a.m. to 6-7 p.m.—and that little or no growth occurs at night.

On comparing the curve of temperature we see that it does not explain the ups and downs of the upper curve; maximum growth occurs with falling temperature.



University Press, Oxford.

On Geotropism and the Localization of the Sensitive Region[1].

BY

FRANCIS DARWIN, M.B., F.R.S.

With Plate XXIX.

TWO well-known cases of localization of heliotropic and geotropic sensitiveness are described in the Power of Movement in Plants. In the case of heliotropism the method is simple and the conclusion clear. The seedlings of *Phalaris* are extremely sensitive to light, but when little caps of an opaque material are placed on the tips of the plants they no longer bend towards the light, or only do so in a very slight degree, as Rothert[2] has shown. Now since, in this experiment, the part which is capable of curvature is fully exposed to light, it follows that the power of perceiving light resides chiefly in the tip, which thus plays the part of a nerve-centre specialized so as to receive a stimulus and to transmit it to the motor apparatus.

In the case of the gravitation stimulus the case is not so simple: we cannot shelter part of a plant from gravitation as we can from light, and the only plan which at first suggested itself was the removal of the sensitive part by

[1] Paper read before the Botanical Section of the British Association, Dover, Sept. 1899.

[2] Cohn's Beiträge, Bd. vii, 1894.

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amputation. The result was that the roots so treated lost nearly or completely their power of curving geotropically.

The conclusion drawn from this experiment, namely, that the root-tip is a sense-organ for gravitation, was much criticized, sometimes unjustly, but in one respect with justice. It was said that amputation might act as a shock and thus prevent curvature. It was this—the difficulty of distinguishing between the removal of the sense-organ, and the disturbing effect of the *operation* of removal—that made Rothert [1] (in his admirable summary of the question) despair of any solution. However, in August, 1894, Pfeffer [2] read a paper at the Oxford meeting of the British Association, in which he explained his well-known method, now generally associated with the name of his brilliant pupil Czapek [3]. The root is forced to grow into a little glass stocking, that is to say, a tube closed at one end and bent at right angles, so that while that part of the root which is capable of curvature is horizontal the tip is vertical. Under these circumstances no fresh curvature takes place; the sense-organ at the tip of the root is not stimulated, since it points vertically downwards: it is, as it were, satisfied with its position, and consequently transmits no influence to the bending region. This beautiful method has not been found applicable to other organs, and thus it happens that apogeotropic sensitiveness [4] has not been localized as far as I know. Rothert [5] and Czapek [6] have indeed shown some reason to believe that in the case of *Avena* and *Phalaris* the tip is the percipient part; but neither method is, in my opinion, absolutely convincing.

[1] Flora, 1894, vol. lxxix.

[2] Annals of Botany, Sept. 1894, p. 317.

[3] Czapek, Prings. Jahrb., 1895, p. 243.

[4] In the case of diageotropic flowers, where the part which is horizontal takes no part in the curvature, there must be transmission of stimulus, as Vöchting (Bewegungen d. Blüthen und Früchte) and Schwendener and Krabbe (Abhand. d. k. Preuss. Akad., Berlin, 1892) have shown. Czapek (Pringsheim's Jahrb., xxxii, 1898, p. 274) shows that diageotropic sensibility is to a great extent localized in the lamina of a *Tropaeolum* leaf.

[5] Cohn's Beiträge, Bd. vii, Heft 1, 1894, p. 187 of reprint.

[6] Pringsheim's Jahrb., 1898, p. 254.

Rothert's argument is as follows. When a heliotropically curving *Phalaris* is carefully watched, a certain relation between the form of the curvature and the distribution of growth-rate is found to exist. And since the same relation is found in a geotropically curving *Phalaris*, Rothert argues that this relation, being correlated with a certain localization of heliotropic irritability in one case, is probably correlated with similar localization of geotropic irritability in the other case. Whether or no the arguments are sound, the fact is as Rothert and Czapek suppose.

Let us first take the case of an apogeotropic organ in which the power of perceiving the gravitation-stimulus is not confined to one part of the organ, but distributed through all the growing parts, such an organ, in fact, as Sachs describes as typical. Imagine two such organs placed horizontally, one end being supported and the other projecting freely into the air. Let us further imagine that one specimen (*normal*) is fixed by its basal end, while the other (*inverted*) is fixed by its apical end. What will happen? The behaviour of both specimens will be the same, i. e. the free ends of both will bend up and will ultimately point vertically upwards.

An experiment of this sort was made with young dandelion stalks by Frank[1] in 1868, from a different point of view, namely, to show that the geotropic curvatures are not affected by the morphological position of the organ, that is, by whether the free end is morphologically the base or the apex. Noll[2] has made similar experiments with the flower stalks of *Aconitum* in his researches on the relation between epinasty and geotropism.

All this seems to have little bearing on the question before us, but its connexion is real enough. Imagine that precisely a similar experiment is made with organs in which only the apex is sensitive to gravity, while the basal part is not directly sensitive, but merely bends upwards when it receives a trans-

[1] Beiträge zur Pflanzenphysiologie: I. Ueber die durch die Schwerkraft verursachten Bewegungen von Pflanzentheilen, p. 80, 1868.

[2] Flora, 1893, p. 360.

mitted stimulus—in fact, imagine that our apogeotropic organ has the qualities which actually exist in roots. What will happen? The normal plant in which the apex is free will behave like the above described specimens—it will bend upwards, and when the sensitive apex is vertical it will cease to be stimulated, and will therefore cease to transmit a stimulus to the bending region.

Now take the other case in which the tip is fixed and the base free: the tip being horizontal is stimulated, and an influence is transmitted to the motor region, so that the basal end begins to rise. But now observe the difference between the two: the curvature of the normal specimen brings the tip nearer and nearer to, and finally into the vertical position, the position of equilibrium. But no amount of movement in the inverted specimen has any effect on the tip, which remains irritated because it remains horizontal. It is clear, therefore, that the motor part of the inverted specimen ought to continue curving *ad infinitum*.

I had concluded that this ought to take place with roots, and made many fruitless efforts to demonstrate the fact, but was foiled by the difficulty of fixing the root by its slippery tip and also by the difficulties caused by the weight of the cotyledons. It was only when I turned to grass seedlings that I succeeded. Rothert[1] has shown that in the seeds of the grasses *Setaria*, *Panicum*, and *Sorghum* the localization of the heliotropic sensitiveness is more definite than in *Avena* or *Phalaris*. The structure of these seedlings is remarkable from the presence of what may be called a hypocotyl—a relatively long stalk between the sheath-like cotyledon[2] and the grain. This hypocotyl is the part which bends, but according to Rothert it is not sensitive to light, while the cotyledon is sensitive to light but does not bend. These

[1] Cohn's Beiträge, loc. cit.

[2] I use the word cotyledon (in the sense in which it was employed in the Power of Movement) to mean the hollow sheath-like leaf-structure which precedes the true leaves. Rothert has adopted the term cotyledon in this sense, leaving on one side all questions as to the morphology of the part in question.

species having well-marked localization of light-sensitiveness seemed well suited for my purpose. My procedure is as follows: seedlings of *Setaria*, *Sorghum*, &c., are carefully removed from the sawdust in which they have germinated and placed immediately in water; the best are then selected, the roots are cut off, and each seedling is fixed by pushing its cotyledon into a capillary glass tube, which is supported horizontally in damp air; the seedlings are further supplied with water by means of a fine spray occasionally applied. They grow fairly well, but no doubt owing to the unnatural circumstances, they have not the full vigour and regular behaviour of properly rooted seedlings. A large number of experiments were made in this way, and though many cases occurred in which the seedlings either refused to grow or refused to geotrope, yet on the whole the result was abundantly convincing.

The result was precisely what was expected on the hypothesis that the cotyledon is the sense-organ for gravitation. The free end continued to curve in one direction for days, producing a series of coils like a tendril, or even tying itself in knots. Three or four complete circles is the most I have seen; but if the conditions were more favourable, I see no reason why more turns should not be made. The figures here given (Plate XXIX) are reproduced from accurate drawings kindly made for me by Miss Pertz while the experiments were in progress. The specimens were usually drawn when it was clear that growth, and therefore curvature, had ceased: they are not therefore any guide to the minimum time required for the production of spirals. In the figures the grains are shaded for the sake of distinctness; the limit of the cotyledon is marked by a transverse line in Figs. 1, 3, 4, 5 (*Sorghum* and *Setaria*); the cotyledon in Fig. 2 (*Phalaris*) extends to the grain. The roots were cut off to make the free end of the seedling lighter; adventitious roots, however, often appear as shown on the hypocotyls in Figs. 3 and 4. Fig. 2 shows the simplest case of curvature, Fig. 3 the most complex. Fig. 1 is interesting as showing that, when the cotyledon is only

some 10°–20° from the vertical, the curvature is in the right direction. Figs. 3 and 4 show that the cotyledon has some power of geotropic movement, there is not therefore an absolute distinction between the motor and sensitive regions. It should be especially noted that the occurrence of prolonged curvature in the hypocotyl does not disprove the existence of some independent geotropic sensitiveness in that part, it merely proves that sensitiveness is overwhelmingly present in the cotyledon.

To those who wish to repeat the experiments, I recommend *Sorghum* seedlings, as they are larger and more vigorous than *Setaria*, and give a higher percentage of corkscrew-like specimens. *Phalaris* also gives the result with considerable certainty. Most of the experiments were made in the dark, but some heliotropic experiments were made. It being known that the heliotropic sensitiveness is, in the species used (*Setaria*, *Sorghum*, *Phalaris*), either quite or nearly confined to the cotyledon, it is clear that the same sort of continued curvature ought to be obtainable by one-sided illumination. For this purpose seedlings were fixed on a klinostat in such a way that they were heliotropically stimulated, though removed from the action of gravity.

Under these conditions they bent over to the light and continued curving just as in the case of the geotropically affected plants. But it is not easy to cultivate the seedlings on the klinostat, and probably on this account the heliotropic experiments did not give such good spirals as those obtained geotropically. Fair results were also obtained without a klinostat.

As a control, seedlings were also grown on a klinostat in a dark room; under these circumstances the seedlings should remain straight. In the first trials this was not the case, and this may possibly have been due to my having examined them too often with a light.

When the jar in which the seedlings were contained was wrapped in black cloth and the klinostat kept in the dark room, the curvatures were almost absent. But this question

needs further experimentation. Another point is worth mention, though here again more work is wanted. If a seedling is fixed by the tip in a vertical glass tube, the apex of the cotyledon being upwards, there seems no reason why curvature should occur, since the sensitive part, the cotyledon, is in the position of non-stimulation. But curvature does occur in this situation, and this may be due to two causes: (1) the tube can only be approximately vertical, and it is possible that the slight stimulus (which analogy suggests must accompany slight obliquity) *being continuous* may be enough to make the hypocotyl bend; (2) it is possible that a certain amount of geotropic sensibility may exist in the hypocotyl; if this were so, it would behave like an ordinary apogeotropic organ, and these are well known to curve up when fixed with all available accuracy, with the free end down [1]. But this would only account for a movement through 180°, which would bring the free end vertically upwards. In many of my preliminary experiments the tubes were certainly not strictly vertical; and when especial care was directed to this point, the effect was small, as the following observations show. Seven *Sorghum* seedlings were fixed by their upwardly directed cotyledons, and after two days only gave an average deviation of about 30° from the vertical. The tubes holding the cotyledons were then fixed horizontally, and by the next day six out of the seven had curved through 180° more or less.

Theoretically the method here described is an ideal one for discovering in what position a geotropic organ is most strongly stimulated by gravitation. Sachs [2], as is well known, supposed that the horizontal is the position of maximum stimulation, a conclusion which has been controverted by Elfving [3] and Czapek [4]. In 1888 Miss Bateson and I [5] pointed out that as

[1] Frank, loc. cit., p. 80. Czapek, however, shows that if a geotropic organ is placed in gypsum, so that it cannot circumnutate, it is not geotropically stimulated when the apex is vertical.

[2] Arbeiten, i, p. 454; ii, p. 240.

[3] Acta Soc. Sc. Fenn., 1880.

[4] Czapek, Prings. Jahrb. xxvii, 1895; xxxii, 1898.

[5] Annals of Botany, 1888, p. 65.

soon as a stem or root begins to curve the line of gravitation strikes it at a new angle, and therefore the stimulus is not constant. But with *Setaria* or *Sorghum* the sensitive part remains in one position, so that the stimulus is constant. I have made a number of experiments with grass seedlings at various angles, but I have not been able to come to a definite conclusion, although the result certainly favours the belief that a seedling fixed with the cotyledon pointing obliquely downward is more strongly stimulated than when it is in the opposite position [1].

The method which I have described I hope to apply to a number of other plants, especially those in which sensitiveness to light is known to be only partially localized at the apex.

I have already tried it with leaves and with diageotropic flowers, but the results are not yet complete enough for publication.

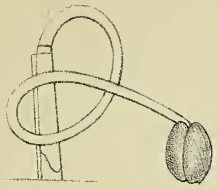
[1] Miss Pertz has tested this point by means of the intermittent klinostat without being aware that Czapek had used this method. With grass-haulms she obtained results similar to Czapek, and there can be no doubt that his result is correct. See Miss Pertz's communication in the present number of the Annals of Botany, p. 620.

DESCRIPTION OF FIGURES IN PLATE XXIX.

Illustrating Mr. Darwin's paper on Geotropism.

Figs. 1 and 5. *Setaria*: seven days' exposure.
Figs. 3 and 4. *Sorghum*: six days' exposure.
Fig. 2. *Phalaris*: ten days' exposure.

For further details see p. 571.



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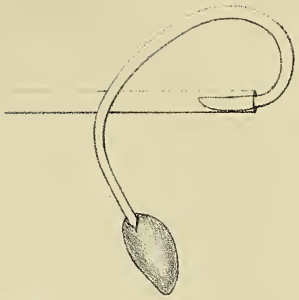

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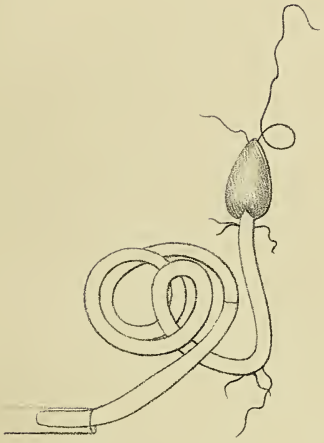

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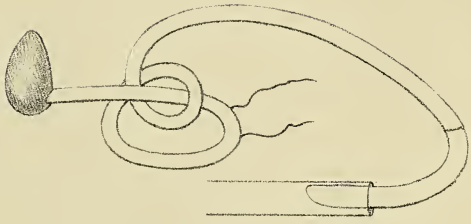

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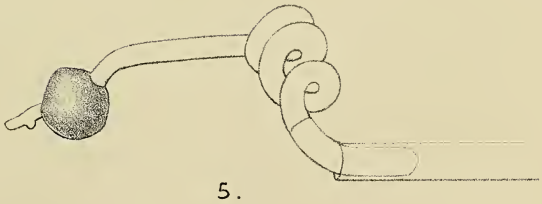

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D. Pertz del.

University Press, Oxford.

DARWIN. — ON GEOTROPISM.

The Sexuality of the Fungi[1].

BY

HAROLD WAGER.

AMONG the numerous remarkable and interesting observations which have, in recent years, been made upon the cytology of the lower plants, those which throw light upon the sexual processes in the Fungi take a prominent place. Fifteen years ago[2] we knew that the Phycomycetes as a group were sexual, that the Ascomycetes, according to one school, exhibited phenomena which could be regarded as sexual, but that the other groups of Fungi exhibited no sexual features at all, unless certain cell-unions occurring in some forms could be regarded as such. We were practically ignorant of their cytology, and even the presence of nuclei was regarded by many observers as doubtful.

Now we are not only acquainted with the minute details of nuclear and cell division in a number of forms belonging to the different groups, but the cytological features of fertilization in the Phycomycetes have been investigated and the phenomena brought into line with those occurring in the higher plants and animals, so that we may say of this group, as of the higher plants and animals, that 'the act of fertilization

[1] Afternoon lecture delivered before the Botanical Section at the Dover meeting of the British Association, 1899.

[2] See Marshall Ward, on the Sexuality of the Fungi, Q. J. M. S., vol. xxiv, 1884, for an excellent account of what was known at that time.

[Annals of Botany, Vol. XIII. No. LII. December, 1899].

consists in the definite fusion of a male nucleus with a female nucleus to form the primary nucleus of the embryo or new generation.'

In the higher groups (the Ustilagineae, Uredineae, Ascomycetes, and Basidiomycetes) the question of their sexuality has recently been brought prominently forward by the discovery that in certain of their cells a fusion of nuclei takes place at a period just antecedent to, or during, the formation of spores, which is regarded by some observers as a true sexual process, and which in some respects certainly does appear to take the place of, and produces the same results as, the ordinary phenomena of sexuality.

In the following pages I propose to deal first of all with the phenomena of fertilization occurring in the lower Fungi, secondly with the nuclear fusions in the higher Fungi, and thirdly with some theoretical considerations concerning the phenomena of sexuality and nuclear fusion in the whole group.

Phycomycetes.

The Peronosporeae[1] exhibit a distinct sexual differentiation in the formation of male and female elements in distinct cells, oogonia (ooangia) and antheridia. The oogonium is produced as a terminal or intercalary swelling on a hyphal filament inside the host-plant. The antheridium is a tubular outgrowth which may be formed on the same filament as the oogonium or on another one in its immediate neighbourhood. Both contain protoplasm and numerous nuclei. The antheridium comes into contact with the oogonium, and curves closely around it. The nuclei of the oogonium, and probably of the antheridium, then divide by a process of karyokinesis each into two, so that there are now twice as many. The contents of the oogonium separate into two distinct parts—a peripheral portion (the periplasm), which contains all the nuclei, and a central spherical portion (the gonoplasm), which

[1] Wager, Ann. Bot., iv, 1889, and x, 1896; Berlese, Jahrb. f. wiss. Bot., Bd. xxxi.

contains no nuclei. In the central part of the gonoplasm a dense, deeply stainable substance then appears, sometimes spherical, sometimes irregular in outline, which is regarded by Swingle[1] as a definite organ or organoid of the cell, but which appears to be nothing more than a condensation of granular protoplasm[2]. As soon as it has been produced, one of the nuclei from the periplasm passes into the gonoplasm, and comes into contact with the central body. In *Peronospora parasitica*[3] this nucleus becomes slightly elongated in the direction of the central mass before it reaches it, and sometimes several of the nuclei in the periplasm exhibit a slight elongation in the same direction, so that it is possible that the central mass exerts some attractive power on the periplasmic nuclei, resulting in the passage of one of them bodily through the gonoplasm to come in contact with it.

The antheridium puts out a fertilizing tube, into which one, or perhaps two nuclei pass; this tube then penetrates the periplasm and passes through the gonoplasm until it comes nearly into contact with the female nucleus; and then through an opening at the apex of the fertilizing tube, the male nucleus makes its way into the gonoplasm, and comes into close contact with the female nucleus. The two nuclei may at once fuse to form the zygote-nucleus (*Cystopus candidus*, *C. Portulacae*, *Peronospora Ficariae*, &c.), or the fusion may be delayed for some time, until the thick membrane surrounding the zygote has been partly formed (*Peronospora parasitica*).

The zygote-nucleus, of those species in which the fusion of the sexual nuclei takes place at an early stage, begins at once to divide, and this division is repeated until as many as thirty-two nuclei may be formed; and in this condition it enters on its period of rest. The ripe zygote is therefore multinucleate[4]. In *Peronospora parasitica*, on the other hand,

[1] W. T. Swingle, Two New Organs of the Plant Cell. See Bot. Gazette, Feb. 1898, p. 110.

[2] Wager, loc. cit., 1896.

[3] The following observations on *P. parasitica* are from an unpublished paper by the writer.

[4] Wager, loc. cit., 1896; Berlese, loc. cit.

in which the fusion of sexual nuclei does not occur until a much later stage, subsequent division of the zygote-nucleus does not take place, so that the ripe zygote contains only one nucleus.

This difference in the behaviour of the nucleus during the maturation of the oospore is probably connected with the mode of germination. De Bary[1] has already pointed out that in *Cystopus* and some other species the oospore on germination produces at once a mass of zoospores. In *Peronospora Valerianellae* and others the oospore at once develops a germ-tube. It may be therefore that the uninucleate condition of the zygote indicates germination by a germ-tube, the multinucleate condition germination by the formation of zoospores.

In the Saprolegnieae it has not been found possible to obtain perfectly satisfactory information as to the nature of the sexual process. According to the views of De Bary[2], Marshall Ward[3], Hartog[4] and others, the antheridial tube never opens into the oosphere, although it becomes firmly attached to it. Trow[5], on the other hand, maintains that in some species at any rate a true fertilization occurs by the passage of a nucleus into the oosphere. The facts which he brings forward to support his view are: (1) The young oosphere at first contains only one nucleus. At a later stage two are to be found. (2) These two nuclei fuse together into one, the embryo-nucleus. (3) In one case the apex of an antheridial tube containing a nucleus was observed penetrating the oosphere. (4) The second nucleus of the oosphere appears at an early stage just beneath the wall of the oosphere, and in close proximity to the antheridial tube outside.

From my own observations I am able to confirm (1), (2),

[1] Fungi, Eng. edit., p. 135.
[2] De Bary, Fungi, Eng. edit.
[3] Marshall Ward, loc. cit., p. 272, also Q. J. M. S., 1883.
[4] Hartog, Cytology of the Veg. and Rep. Organs of the Saprolegnieae. Trans. R. Irish Acad., vol. xxx, 1895.
[5] Trow, Ann. of Botany, ix, 1895, and xiii, 1899.

and (4), but I have never seen the penetration of the oosphere by an antheridial tube, although I have observed hundreds of sections of oospheres in all stages of development. At the same time it seems difficult to escape coming to the conclusion that fertilization does take place, and Hartog himself says:—'In a few cases, however, I have seen two nuclei of uneven size in the young oosphere; and from this I might have inferred sexual fertilization, had not the ooangium is these very cases happened to be free from all signs of antheridial branches, let alone "fertilizing tubes[1]." ' Hartog contends that the nuclei of the oogonium fuse together in groups to form the nuclei of the oospheres, and that this is in fact a fusion of potential gametes or sexual cells, which not only takes the place of the morphologically sexual fusion, but is equivalent to it in the sense that it rejuvenates the cell. The presence of two nuclei in the oospheres represents to him the last stage in this fusion of nuclei, and is not a fertilization process as Trow describes. The whole matter evidently requires further investigation before we can come to any definite conclusions as to the exact nature of the phenomena of fertilization in this group.

Among the Chytridineae the only form in which the cytological features of fertilization have been observed is *Polyphagus Euglenae*. Individual plants of this species are unicellular and uninucleate. The formation of the zygote takes place by the fusion of the protoplasm and nuclei from two slightly unequal cells, which are brought into communication with one another by means of a pseudopodium-like process, put out from the smaller (male) cell, which comes into contact with the larger (female) cell. At the point of contact the apex of the pseudopodium swells up to form the zygote cell. The protoplasm and nucleus from the male cell then pass into it, and subsequently the protoplasm and nucleus from the female cell. The two nuclei are at first unequal in size, the male nucleus being the smaller; and it

[1] Hartog, The Alleged Fertilization in the Saprolegnieae. Annals of Botany, vol. xiii, 1899, p. 450.

also contains a smaller quantity of stainable substance. The male nucleus increases in size until it attains the same size and staining properties as the female nucleus, and in this condition the zygote enters on a resting stage. Fusion does not definitely take place until germination begins, and in all cases where it has been seen, it occurs not in the zygote itself, but in the young sporangium which is at once formed when the zygote begins to germinate [1].

In the Mucorineae the zygotes are formed by the fusion of multinucleate cells (apocytia), which are, so far as can be seen, identical with one another. The investigations of Dangeard and Maurice Léger [2] on *Sporodinia grandis* show that the sexual cells are distinctly isogamous and contain several hundreds of minute nuclei. On fusion taking place the protoplasm and nuclei of the two cells become mixed together. Changes then take place leading to the disappearance of all the nuclei. At the same time two groups of deeply stained bodies appear, one at each end of the zygote. According to Léger [3] each group is composed of from fifteen to thirty small spheres (nucleoli?), 'sphères embryogènes.' They arrange themselves into a spherical layer surrounding a globule of oil and then fuse together, producing a hollow sphere, 'sphère embryonnaire,' full of oil. In the process of germination these two 'sphères embryonnaires' increase in size and fuse together. The fused mass becomes clear, and numerous nuclei appear in it, which pass into the sporangiferous mycelium and begin to divide. The same phenomenon is observed in the azygospores, except that only one sphere is produced.

The union of the 'sphères embryogènes' is the sexual process. The azygospores are therefore truly sexual, and

[1] These observations are taken from an unpublished paper by the writer. See also Brit. Ass. Reports, 1898, p. 1064.

[2] Dangeard and Maurice Léger, (1) Recherches sur la Structure des Mucorinées; (2) La Reproduction sexuelle des Mucorinées. Le Botaniste, vol. iv, 1894–5, pp. 4 and 7.

[3] Léger, Structure et développement de la zygospore du *Sporodinia grandis*. Revue générale de Botanique, t. vii, 1895, p. 481.

the process of conjugation is of secondary importance, and not sexually significant in the group [1].

Dangeard [2], however, does not agree with Léger's interpretation of the 'sphères embryonnaires.' For him the ultimate fate of the nuclei has not been determined.

In the Entomophthoreae we also have fusion of similar cells formed on different filaments, but the only genus in which the cytology of the process of conjugation has been investigated is *Basidiobolus* [3], which presents a feature of peculiar interest, in that its zygotes are normally formed by the fusion of adjacent cells (as sometimes occurs exceptionally in the Alga *Spirogyra*), which are uninucleate. Unlike *Spirogyra*, however, the nuclei before fusion undergo mitotic division into two. One daughter nucleus in each cell becomes cut off and degenerates, the other two nuclei with their protoplasm fuse together to form the zygote. The fusion of nuclei takes place at a late stage, and Raciborski has shown that it may even be delayed until germination has taken place; in such cases the germ-tube then contains two nuclei.

Hartog [4] regards the adjacent cells as progametes, 'each of which by unequal divisions forms two gametes, the apical one arrested, the other functional.'

Higher Fungi.

In the Ascomycetes the single nucleus of the young ascus is produced by the fusion of two or more cells derived from the mycelium [5]. Dangeard, who has investigated a large

[1] Léger, loc. cit.

[2] Dangeard, Considérations sur les phénomènes de reproduction chez les Phycomycètes. Le Botaniste, vol. iv, 1894–5, p. 249.

[3] Eidam, Cohn's Beiträge zur Biol. d. Pflanzen, Bd. iv, p. 181. Fairchild, Ueber Kerntheilung und Befruchtung bei *Basidiobolus ranarum* Eidam, Jahrb. f. wiss. Bot., xxx, 1897, p. 285. Raciborski, Ueber den Einfluss äusserer Bedingungen auf die Wachsthumsweise der *Basidiobolus ranarum*. Flora, 1896, p. 107. Chmielewskij, see Bot. Centralbl., vols. xxxviii and l.

[4] Hartog, loc. cit., Q. J. M. S., p. 27.

[5] Dangeard, La Reproduction sexuelle des Ascomycètes. Le Botaniste, sér. iv, 1894–5. Harper, Beiträge zur Kenntniss der Kerntheilung und Sporenbildung im Ascus, Ber. d. Deut. Bot. Ges., 1895.

variety of forms, states that in the Exoasci the mycelium becomes divided into cells, each of which possesses two nuclei and has the value of an oogonium. The two nuclei fuse together, and the cell becomes an ascus, the single nucleus of which divides to form the nuclei of the spores. In *Peziza*, *Helvella*, *Morchella*, and *Acetabula* the oogonium appears to be formed by apical fusion of two filaments, but this is not the correct interpretation, for Dangeard finds that it is an appearance brought about by the curving of a single filament. But he admits that it may be due to a terminal fusion of two filaments in the case of *Eremascus*[1] and *Dipodascus*[2]. The oogonia always contain two nuclei; two gametes which fuse together to form the nucleus of the oospore.

In *Endocarpon miniatum* the papilla which forms the ascus contains two nuclei, and in *Aspergillus glaucus* the young asci contain two nuclei which apparently fuse together.

Harper[3] shows that in *Peziza Stevensoniana* the ascus-nucleus is formed by a fusion of four nuclei.

Dangeard also states that in the Truffle[4] the cells which form the asci contain two nuclei. These cells appear frequently to arise from the fusion of two different threads, but Dangeard thinks that, as in other cases, it may be simply due to the curving of a single filament. The two nuclei fuse together and at once begin to divide to form the spore nuclei.

This fusion of nuclei in the ascus, or in the cell from which the ascus arises, is therefore apparently general throughout the group, although it is desirable that this should be confirmed, and is regarded by Dangeard as a true sexual process, a fusion of two nuclei of different origin into a single sexual nucleus. He is thus opposed to De Bary's views as to the nature of the sexual process in this group.

[1] Eidam, Zur Kenntniss der Entw. bei den Ascomyceten. Beitr. z. Biol. d. Pflanzen, Cohn, iii, 1883.

[2] Lagerheim, *Dipodascus albidus*, eine neue geschlechtliche Hemiascee, Jahrb. f. wiss. Bot., xxiv, 1892.

[3] Harper, loc. cit.

[4] Dangeard, La Eruffe: Rech. sur son développement, sa structure, sa reproduction sexuelle. Le Botaniste, iv, 1894–5, p. 63.

De Bary's views, however, have been confirmed by the researches carried on by Harper[1] on some of the simpler forms of the Ascomycetes. In *Sphaerotheca*, for example, Harper finds that the organs regarded by De Bary as sexual, oogonium and antheridium, each contain one nucleus, and that the antheridial nucleus passes into the oogonium, the two nuclei then fusing together to form the sexual nucleus of the oospore. The antheridial nucleus is at first slightly smaller than the nucleus of the oogonium, but before fusion takes place it attains practically the same size.

The fertilized oogonium (oospore) becomes surrounded by filaments arising from the basal cells. Then the nucleus divides, and a row of cells forming the ascogonial filament is formed; one of these, the subapical cell, contains two nuclei, and from this cell the single ascus arises. This cell increases in size, and the two nuclei which it contains fuse together into one, and this subsequently divides to form the nuclei around which the ascospores are formed.

Dangeard[2], however, does not accept Harper's results as to the primary sexual fusion of nuclei, although he agrees in general with him as to the subsequent phenomena. In an exhaustive paper he attempts to prove that only one fusion of nuclei takes place, viz. in the ascus. He affirms that the antheridial cell undergoes degeneration at an early stage, which extends both to protoplasm and nucleus; that the ascogonium often contains one nucleus only, even when the covering branches begin to surround it; that ascogonia with two nuclei sometimes show a nucleus still in the antheridial cell, and that an appearance of fusion which he once observed was due not to actual fusion, but to one of the covering hyphae lying alongside the antheridial cell.

From a careful examination of the figures given both by Dangeard and Harper, it appears to me that not only is the

[1] Harper, Die Entwickelung des Peritheciums bei *Sphaerotheca Castagnei* Lev. Ber. d. Deutsch. Bot. Ges., xiii, 1895, p. 475. Ueber das Verhalten der Kerne bei der Fruchtentwickelung einiger Ascomyceten. Jahrb. f. wiss. Bot., xxix, 1896, p. 655.

[2] Dangeard, La Reproduction sexuelle dans le *Sphaerotheca Castagnei*. Second Mémoire sur la Reproduction sexuelle des Ascomycètes. Le Botaniste, v, 1895–6.

evidence strongly in favour of regarding these organs as sexual, but that it supports Harper's conclusions as to fertilization. Dangeard [1], in fact, himself, in speaking of his own view of the sexuality of *Sphaerotheca*, says :—' Cette fécondation (nuclear fusion in the ascus) laisse même toute liberté d'interprétation au sujet des archicarpes et des branches anthéridiennes ; elle exige simplement que ces organes soient devenus inutiles ; s'ils remplissent encore leur fonction, s'il y a fertilisation, l'asque doit en provenir directement ; et c'est bien le cas, ainsi qu'en témoignent les *Eremascus* Eidam et les *Dipodascus*.'

It is of course possible that fertilization may not always take place in individuals of this species, which would account to some extent for the discrepancy between the observations of these two authors ; and in this connexion the observations made by Mary M. Nichols [2] on certain pyrenomycetous Fungi, which distinctly support the views maintained by Harper, are of considerable interest. The author states that in *Ceratostoma brevirostre* and *Hypocopra* the antheridia fuse with the archicarps in some cases, whilst in others the archicarps appear to develop without fertilization. In *Teichosporella* there remains only a possible rudiment of an antheridium, while in *Teichospora* this has often entirely disappeared.

In the Ustilagineae Dangeard [3] states that a fusion of two nuclei takes place in the young resting spores (brand spores) of *Doassansia Alismatis* and *Entyloma Glaucii*, Dang. *Doassansia Alismatis* forms brown pustules on the leaves of *Alisma Plantago*, and its slender mycelium is found in abundance in the intercellular spaces of the mesophyll. Each cell contains several nuclei. In *Entyloma Glaucii* [4] also the mycelium is found abundantly in the intercellular spaces of the host-plant, and the cells are multinucleate. In both species the

[1] Dangeard, loc. cit., p. 252.

[2] Mary M. Nichols, The Morphology and Development of certain pyrenomycetous Fungi. Bot. Gazette, vol. xxii, 1896, p. 301.

[3] Dangeard, Recherches sur la Reproduction sexuelle des Champignons. Le Botaniste, iii, 1893–4, p. 221.

[4] Dangeard, La Reproduction sexuelle de *l'Entyloma Glaucii* (Dang.). Le Botaniste, iv, 1894–5, p. 12.

resting spores are formed as intercalary swellings on the hyphae, or as terminal swellings on short lateral branches. In the young condition each resting spore contains two nuclei, which fuse together into one. The spores then become surrounded by thick cell-walls. Dangeard has also observed two nuclei in the young spores of *Ustilago*, and he thinks there are two in *Urocystis*. In *Tilletia* he could not obtain stages young enough for the examination of the young spores.

In the Uredineae the researches of Sappin-Trouffy [1] and Poirault and Raciborski [2] give a very full account of the cytology of numerous species of this group in all stages of development. In the aecidia the sporiferous filament contains two nuclei, which both divide at the same time, and by a successive series of parallel divisions a row of spores and intercalary cells are produced, each of which contains two nuclei. Each aecidiospore thus contains two nuclei of different origin. The uredospores and teleutospores also contain two nuclei of different origin. These are regarded by Sappin-Trouffy as the last stages of a series of parallel divisions which started during the formation of the aecidiospores, and which now fuse together in the cell of the teleutospore into a single nucleus. This final fusion is regarded by Sappin-Trouffy as an act of fertilization, and the teleutospore is therefore equivalent to a zygote.

Massee [3] described in 1888 the presence of sexual organs, antheridium and oogonium, in the *Aecidium* found on *Ranunculus Ficaria*, but this has not been confirmed [4].

In the Basidiomycetes a fusion of nuclei has been observed to take place in the basidia of a large number of species

[1] Sappin-Trouffy, La pseudo-fécondation chez les Urédinées et les phénomènes qui s'y rattachent. Le Botaniste, iii, 1893–4. Sur la signification de la Fécondation chez les Urédinées. Le Botaniste, v, 1896–7, p. 32. Recherches Histologiques sur la Famille des Urédinées. Le Botaniste, v, 1896–7, p. 59.

[2] Poirault et Raciborski, Sur les noyaux des Urédinées. Compt. Rend. d. l'Acad. d. Sci., cxxi, p. 308, and Jour. Bot., ix, 1895.

[3] On the Presence of Sexual Organs in *Aecidium*. Ann. Bot., vol. ii, 1888, p. 47.

[4] See Nypels, Bull. d. l. Soc. Belge Microsc., 1885, p. 70.

belonging to the orders Hymenomycetes, Tremellineae, and Auricularieae.

The number of nuclei which fuse together appears to vary in the different species. In *Stropharia stercoraria*[1] two or more ; in *Amanita muscaria*[2] two or three ; in *Mycena galericulata*[3] four, and in *Lepiota mucida*[4] six to eight. Dangeard[5], however, who has extended his researches to species of *Dacryomyces*, *Calocera*, *Craterellus*, *Bovista*, *Nyctalis*, *Hydnum*, and *Polyporus*, states that the young basidium only contains two nuclei. In all these cases the nuclei appear to pass into the basidium from the hymenium, but Rosen comes to the conclusion, from an examination of *Psalliota campestris*, that they may be produced in the basidium.

The details of the nuclear fusion have been observed in *Stropharia stercoraria*[6]. The two nuclei come into contact with one another, and a flattening of each takes place where they touch. A dumb-bell-shaped structure is thus produced. The membrane separating them then disappears, and the nuclear network of the one becomes intermingled with that of the other. The fused nucleus is at first oval in shape, and the two nucleoli for a short time remain separate, but they ultimately fuse together into a slightly elongated mass, which soon becomes spherical, and the nucleus then exhibits no trace of its composite structure. The single basidium-nucleus then divides by mitosis, and the daughter nuclei formed pass through the sterigmata into the spores[7]. The fusion of two nuclei has been observed in *Tremella mesenterica* by Dangeard[8], and in *Auricularia* by Sappin-Trouffy[9]. In both cases the

[1] Wager, Annals of Botany, 1892, p. 146, and 1893, p. 489.

[2] Wager, ibid., 1894, p. 321.

[3] Rosen, Beiträge zur Kenntniss der Pflanzenzellen, ii. Cohn's Beitr. zur Biol. d. Pflanzen, vi, 1892–3, p. 237.

[4] Wager, loc. cit., 1893, p. 321.

[5] Dangeard, Sur la Reproduction sexuelle des Basidiomycètes. Le Botaniste, iv, 1894–5, p. 119.

[6] Wager, loc. cit., p. 496 (1893).

[7] Rosenvinge, Sur les noyaux des Hymènomycètes, Ann. d. Sci. Nat., Bot., sér. vii, tome 3, 1886.

[8] Dangeard, loc. cit., p. 125.

[9] Sappin-Trouffy, Recherches mycologiques. Le Botaniste, v, 1895–6, p. 44.

basidium divides up after the nuclear fusion into a number of cells, each of which contains one nucleus. In *Tremella* the cell divisions are longitudinal; in *Auricularia* they are transverse. Each cell produces a sterigma, at the apex of which a spore is formed, and the nucleus then passes into it.

This phenomenon of nuclear fusion in the Basidiomycetes is regarded by Dangeard as sexual and the basidium as an oospore [1].

Theoretical Considerations.

We have now to consider the general bearing of these facts upon the question of sexuality.

Sexual reproduction may be regarded as a process by which (1) the energy of division is restored, and (2) two independent lines of descent blended into one.

According to many observers the essential end of sexuality is rejuvenescence. The cell in some way becomes enfeebled, loses 'the capacity of carrying on the vital processes by itself[2],' and requires some stimulus to reinvigorate it to further growth. Hence the need for fertilization. But whether this is a primary attribute of living matter or has been secondarily acquired in order to ensure a mixture of germ-plasms derived from different sources has not been determined[3].

A study of the process of fertilization in the Phycomycetes lends support to the view that the fusion of the two cells and nuclei is primarily for purposes of reinvigoration simply. In all the higher forms of life the only mode of reproduction is sexual. In the Fungi and lower forms of life generally we have in addition asexual reproductive organs, which, as Strasburger says[4], 'are especially concerned with the rapid multiplication of the individuals under favourable external conditions; whilst sexual reproduction is of importance in

[1] Dangeard, loc. cit.

[2] Hertwig, The Cell, Eng. edit., p. 291.

[3] See Wilson, The Cell in Inheritance and Development, pp. 129, 130.

[4] Strasburger, The Periodic Reduction of the number of the Chromosomes in the Life-History of Living Organisms. Ann. Bot., viii, 1894, p. 282.

maintaining the existence of the species under circumstances which are unfavourable to the vegetative existence of the individual.'

The number of cases in which there is a blending of two distinct lines of descent into one is rare, and sometimes the conjugating cells are closely related to one another. In *Basidiobolus* the fusion always takes place between adjacent cells. In the Peronosporeae the same filament often gives rise both to the male and female organs, and in some cases this appears to be the rule. In Mucorineae the conjugating cells are borne on separate filaments, but these may be, and often are, branches of the same plant. In *Polyphagus Euglenae* two distinct individuals fuse together, but in *Zygochytrium* the conjugating cells are produced on different filaments proceeding from the same individual. Thus so far as sexual differentiation is concerned, all we can say is that although the conjugating nuclei are in most cases removed at some distance from one another in their development, yet the required stimulus may be obtained when they are as closely related as in *Basidiobolus*.

It has been shown that the need for fertilization in the simpler forms of Fungi and Algae depends to a certain extent upon external conditions, which affect transpiration, atmospheric pressure, and food supply. The connexion between food supply and sexuality in the Fungi was suggested by Marshall Ward [1] in 1884; and Eidam [2] showed that if the conidia of *Basidiobolus* are sown in a nutrient fluid, a firm mycelium is produced, which forms simultaneously both asexual and sexual cells. In an exhausted nutrient medium, on the contrary, the conidia produce only a loose mycelium, which immediately and exclusively gives rise to sexual cells, which unite together to form zygotes.

Again, Raciborski [3], in 1896, working with the same Fungus,

[1] H. Marshall Ward, On the Sexuality of the Fungi. Q. J. M. S., vol. xxiv, 1884. [2] Loc. cit.

[3] Raciborski, Ueber den Einfluss äusserer Bedingungen auf die Wachsthumsweise des *Basidiobolus ranarum*. Flora, 1896, p. 107.

showed that at a low temperature, 6–7° C., only sterile mycelium is formed; that the formation of conidia demands free access of air; and that the formation of zygotes is connected with bad conditions of vegetation, such as the transport of a prosperous culture into an unfavourable medium, dilution of the nutrient medium, or too strong a solution of it, or an elevated temperature.

Klebs[1] showed in his studies on *Sporodinia grandis* that carbohydrates are needed to form zygotes, and that sporangia may be formed luxuriantly in nitrogenous media. Increased transpiration tends to the formation of sporangia. When transpiration is checked within certain limits, zygotes are formed in addition, and when still further checked, zygotes only are formed. When the air-pressure is reduced parthenogenesis results, and if still further reduced no sexual organs are formed at all, and ultimately the production of sporangia also is stopped.

Some observations of my own are also interesting as bearing upon this point. In the case of *Polyphagus*, when there is an abundant food supply in the form of fresh *Euglena* cells, sporangia only are produced. In a very short time, however, as this special food supply becomes exhausted, sexual organs are also formed, and in the later stages of a culture, when the food supply is much reduced, sexual organs only are formed.

In *Peronospora parasitica* and *Cystopus candidus* I find that oospores are mostly developed in those parts from which the food supply has not been absorbed by sporangia, and which are still succulent and full of sap. The portion of the stem which contains them is large and succulent, with few asexual organs present, or they are contained in the young succulent tissues at the apex. Those parts of the plant which are covered with asexual spores and which appear white—those parts where the Fungus is visible to the observer on a cursory examination—very rarely contain sexual organs in abundance.

[1] Klebs, Zur Physiologie der Fortpflanzung einiger Pilze. I. *Sporodinia grandis*. Jahrb. f. wiss. Bot., xxxii, 1898. See also II. *Saprolegnia mixta*. Jahrb. f. wiss. Bot., xxxiii, 1899.

Again, in *Peronospora effusa* the sexual organs are found in abundance in the leaves of the host-plant, but always in greater abundance in the young leaves near the apex. Also in *Peronospora Arenariae*, which is found upon many of the Caryophyllaceae, I soon found that it was useless to look for sexual organs in those parts of the plant which are covered with a luxuriant growth of asexual organs, but that they were only to be found in certain parts of the stem which were slightly differently coloured from the rest, and on which asexual organs were not found in any quantity.

Hartog strongly supports the rejuvenescence theory[1]. He brings forward a considerable body of evidence to show that replacement theories of fertilization are inadmissible, since all fail to account for one or more of the many phenomena involved in the various types of sexual fusion of nuclei. He points out that rejuvenescence is brought about by (1) change of the mode of life, (2) plasmodium-formation, (3) isogamy, involving the fusion of two or more gametes and their nuclei, and (4) oogamy. He further points out that many cases of parthenogenesis involve the fusion of sister nuclei, and that this 'replaces the advent of a male nucleus.' Among the Fungi he instances *Saprolegnia* as a case in which this occurs, but this requires confirmation, as does also the case of *Sporodinia* described by Léger[2], in which he describes the same phenomenon as occurring in the formation of the azygospores.

In animals the polar bodies formed during the maturation of the egg are now generally regarded as reduced ova, or, as Hartog says, they represent true gametes arrested in their development. In certain cases the second polar body remains in the egg, and Boveri discovered 'that in *Ascaris* the second polar body might in exceptional cases remain in the egg, and there give rise to a resting nucleus indistinguishable from the egg-nucleus or sperm-nucleus.' He was thus led to the interesting suggestion that parthenogenesis might be due

[1] Hartog, Some Problems of Reproduction, Q. J. M. S., 1891.
[2] Léger, loc. cit.

to the retention of the second polar body in the egg and its union with the egg-nucleus. The second polar body would thus, in a certain sense, assume the rôle of the spermatozoon, and it might not without reason be said: *Parthenogenesis is the result of fertilization by the second polar body*[1].

This conclusion was confirmed by the observations of Brauer on the parthenogenetic eggs of *Artemia*, in which the second polar body is actually formed, but remains in the egg, and 'here plays the part of a sperm-nucleus precisely as maintained by Boveri[2].'

Here, then, we have a clear case of rejuvenescence taking place by the fusion of two sister nuclei replacing definitely the sexual fusion and producing an egg capable of germination.

Such cases as this are not only very instructive as throwing a light upon the phenomena of sexuality in the lower Fungi, but are very significant when we come to consider the fusion of nuclei which takes place in the reproductive organs of the higher Fungi. That this fusion is not merely a vegetative one, and therefore of little significance, is proved by the fact that not only does it occur generally in all the groups of the higher Fungi, but it takes place at a definite stage in the life-history of the individual, and at a period which immediately precedes the formation of spores. To this there is apparently no exception, and it is therefore evident that we have here a phenomenon of considerable importance in the life-history of the higher Fungi.

Dangeard's view that it is a definite 'sexuality which differs in nothing in its essential characters from that of other plants and animals[3]' is not, I think, justified by the facts, even if we compare it to the sexual act in the higher plants when reduced to its lowest terms, viz. *the fusion of two nuclei* simply. For we find that in some cases a multiple fusion takes place—three, four, and even eight nuclei fusing together to produce the single nucleus of the basidium or ascus. In this respect, therefore, it does not resemble the act of fertilization as we

[1] See Wilson, loc. cit., p. 202. [2] See Wilson, loc. cit., p. 205.
[3] Loc. cit., 1894–5, p. 167.

know it in those cases which have been fully investigated. For there are, I think, no cases in which multiple fusion of nuclei is found as a definite sexual act or accompanying a sexual act, if we except some few cases of polyspermy, in higher plants and animals. And in the lower plants the cases in which it is said to occur are doubtful, if we except those cases of multiple union of motile gametes. In *Sphaeroplea*, for example, it is said that the oosphere-nucleus is formed by the fusion of several nuclei[1]; but Klebahn[2] in a recent paper states that even when the oosphere contains more than one nucleus, they do not fuse together to form one, but that fertilization takes place by the fusion of the spermatozoid-nucleus with one only of the oosphere-nuclei. The presence of more than one nucleus in the oosphere of this plant is remarkable, but, as Klebahn says, it does not alter our views as to the essential phenomena of fertilization, for even here it consists in a fusion of the male nucleus with one nucleus of the oosphere, the other nuclei remaining unchanged[3].

In *Dasycladus* it is said that the nucleus of each gamete is formed by a fusion of several vegetative nuclei[4].

In the Mucorineae, according to Dangeard and Léger[5], a fusion of nuclei apparently takes place in the zygote, but this is not certain, and Istvanffi[6] states that the zygote is 'thatsächlich mehrkernig.' The other cases of multiple nuclear fusion which have been described in the Fungi do not accompany a definite sexual act, although it is suspected to occur in some forms, the cytology of which has not been investigated.

The objection to Dangeard's view which is often made is that the nuclei which fuse are too nearly related to one

[1] See Hartog, Some Problems of Reproduction, p. 19.

[2] Klebahn, Die Befruchtung von *Sphaeroplea annulina* Ag. Sonderabdruck aus der Festschrift für Schwendener.

[3] Klebahn, loc. cit., p. 101.

[4] See Hartog, loc. cit., p. 18.

[5] Loc. cit., Le Botaniste, 1894, p. 10.

[6] Istvanffi, Ueber die Rolle der Zellkerne bei der Entwickelung der Pilze. Ber. d. Deutsch. Bot. Ges., 1895, Bd. xiii, p. 454.

another, coming as they do from one cell, to allow its being regarded as sexual. But, as has already been pointed out, this objection does not hold if we regard the fusion of adjacent cells in *Spirogyra* and *Basidobolus* as sexual. For in these cases the two nuclei may be as closely related as are those of the basidium or the ascus. And the objection would certainly not hold in the case of the Uredineae if Sappin-Trouffy's observations are correct, for the nuclei are far enough removed in relationship, although they are found in the same cell. If with Sachs we could regard each nucleus with its protoplasm as representing a separate unity (Energid), it seems to me that, in the Uredineae at any rate, and possibly in the Ustilagineae and Basidiomycetes, we might consider these nuclear fusions as indicating a sexuality nearly equivalent to that in *Basidiobolus*.

Or we might regard these nuclei with Hartog[1] as the centres of potential gametes, in which case the nuclear fusions would be of the nature of those parthenogenetic fusions which take place in the eggs of *Artemia*, in *Saprolegnia* (if Hartog's observations are correct), in the azygospores of *Sporodinia* (according to Léger[2]), and in *Derbesia*, where the zoospores have nuclei constituted by the fusion of several vegetative nuclei, a process which, according to Hartog[3], replaces the formation and union of gametes. But it seems to me that the evidence before us is not sufficient to enable us to come to any definite conclusion one way or the other, and a most serious objection to Dangeard's view that it is a true sexual process is found in Harper's observations on *Sphaerotheca* and other simple forms of the Ascomycetes. These observations show that we have in these cases two distinct nuclear fusions. The first appears to be morphologically a true sexual fusion of two nuclei from different cells, resulting in the production of an ascogonium; the second, a fusion of two or more nuclei in the ascus which occurs at a definite stage in the life-history, and results in the formation of ascospores. What is the meaning of this second nuclear fusion?

[1] Hartog, loc. cit. [2] Léger, loc. cit. [3] Hartog, loc. cit.

One explanation which seems to me possible is that it is brought about by the subsequent development of the oospore into an ascogonial filament of cells instead of being simply transformed into an ascus, as in *Eremascus*, and that the energy imported by the sexual fusion having become used up in the production of the ascogonial cells, the necessary energy to produce another reproductive cell, the ascus, can only be obtained by a further nuclear fusion.

In the higher Ascomycetes this second nuclear fusion has probably replaced altogether the morphologically sexual fusion of the simpler forms, and cannot therefore be regarded as a true sexual phenomenon, though perhaps, as Groom [1] points out, presenting some analogy to the sexual process, but not homologous with it.

The only phenomenon which bears directly upon this double fusion of nuclei in the Ascomycetes is that described by Chmielewskij in *Spirogyra crassa* [2]. In this Alga the two sexual nuclei fuse together in the zygote; the resulting nucleus then divides, by karyokinetic division, into four, of which two break up into fragments and disappear, while the other two, the secondary nuclei, again unite into the definite nucleus of the zygote, which remains till germination.

If the phenomenon, as described by Chmielewskij, really takes place, it resembles in a striking manner what occurs in *Sphaerotheca*. For in both cases we have first a fusion of sexual nuclei, then division into four or more, and subsequent fusion of two of this again into a single nucleus. Looked upon in this light, the second nuclear fusion would then be a sexual phenomenon in *Sphaerotheca*, equivalent to the second fusion in the zygote of *Spirogyra*. And it is conceivable, I think, that an equivalent of this second fusion may have replaced altogether the primary fusion, so that in the higher Ascomycetes the second is the only one which

[1] Groom, On the Fusion of Nuclei among Plants: A Hypothesis. Trans. and Proceedings of the Botanical Society of Edinburgh, 1898, p. 132.

[2] Chmielewskij, Materialien zur Morphologie und Physiologie des Sexualprocesses bei den niederen Pflanzen. Charkow, 1890 (Russisch), Referat in Bot. Centralbl., 1, 1892, p. 264.

occurs; that is to say, the ascogonial nucleus, although unfertilized, may still possess sufficient energy to produce the ascogonial filament, but the necessary stimulus for the production of ascospores must be brought about by the fusion of two nuclei in those ascogonial cells from which asci arise.

We might also regard the fusion in the Basidiomycetes as having arisen in the same way, although in this group we have no indication of the primary sexual process.

Groom [1] concludes that the nuclear fusions in question are asexual in character, and superposed upon and subsequent to the sexual act. They are concerned with the alternation of generations, and take place in a small fructificative degenerate generation.

In the Uredineae and Ustilagineae we have no indication whatever of a primary sexual process; but from the fact that these groups have become so completely parasitic—a parasitism of a peculiarly high order, as Marshall Ward [2] puts it, by which the organism has adapted its life to the habits of its host—we should expect also some modification in the sexual organs, so that here, as in the Basidiomycetes and Ascomycetes, we are probably dealing with a degenerative nuclear fusion, not homologous with the sexuality of the Phycomycetes.

It is not possible to come to any final conclusion as to the exact significance of these nuclear fusions in the higher Fungi. That they take the place of a sexual act, and lead to the reinvigoration of the cell, cannot, I think, be doubted.

As Strasburger says: 'If the nuclei which fuse proceed from parts of the plant far removed from one another, one could see in this fusion a re-establishment of equilibrium necessary for the preservation of the species, which would be comparable in its physiological effects to fertilization [3].'

But even if the nuclei are found not to 'proceed from parts

[1] Groom, loc. cit. [2] Loc. cit., p. 296.

[3] Strasburger, Ueber per. Red. der Chromosomenzahl im Entwicklungsgang der Organismen. Biol. Centralbl., xiv, 1894, p. 864. See also Hartog, loc. cit., Q. J. M. S., p. 69.

of the plant far removed from one another,' it seems to me that we could still regard it as comparable physiologically to fertilization, just as in *Artemia* the fusion of the second polar body with the egg-nucleus may be regarded as physiologically a process of fertilization. In the present state of our knowledge, however, we cannot, I think, regard it with Dangeard as a morphologically sexual phenomenon, especially in the light of Harper's researches.

But it explains, I think, in a satisfactory manner, what has always been somewhat of a mystery—how it is that the asexual reproductive cells of the Fungi become stimulated to further growth and development; and Strasburger's statement that 'these arrangements for asexual reproduction were so efficient in the Fungi that the result was the disappearance of the sexual organs and of sexual reproduction [1],' receives a further interpretation.

Conclusions.

The following summary of facts and conclusions drawn therefrom indicates the scope of this paper.

1. In the Phycomycetes we have a true sexuality, consisting in the fusion of two nuclei derived from separate more or less completely differentiated cells. In its essential characters it does not appear to differ from that of higher plants and animals.

2. Before fusion takes place there may be a preliminary division of the sexual nuclei (Peronosporeae, *Basidiobolus*), or it may be absent (*Polyphagus*), but in this case the nuclei before fusion lose a considerable amount of stainable substance, which appears to pass into the surrounding cytoplasm.

3. This preliminary division may be connected with chromosome reduction, but the evidence before us is not sufficient to enable us to come to any definite conclusions as to its theoretical significance. Moreover, the reduction in the number

[1] Strasburger, The Periodic Reduction of the number of the Chromosomes, &c., Ann. Bot, vol. viii, 1894, p. 283.

of the chromosomes is stated to take place on the germination of the zygote in the Peronosporeae (Berlese).

4. Before fusion the two nuclei, which at first may differ very much in size (*Polyphagus*), attain the same size and staining properties.

5. Fusion may take place at once, or may be delayed until after germination has taken place (*Basidiobolus*, *Polyphagus*), so that apparently the formation of the oospore membranes and the early stage of germination are independent of nuclear fusion.

6. The fusion of nuclei takes place in the resting condition (*Cystopus candidus*, *P. parasitica*, *Basidiobolus*), or possibly, in some cases, in the chromosome stage (species of *Cystopus* and *Peronospora* (Berlese), *Polyphagus* (Wager)).

7. The formation of sexual organs depends to some extent upon the conditions under which the Fungus is grown.

8. Centrosomes have not been observed taking part in the process of fertilization. Definite centrosomes have not been observed in the Phycomycetes.

9. The sexual elements which fuse together in any given case are generally derived from one and the same individual, often from the same filament; and in *Basidiobolus* the two nuclei which fuse are derived from adjacent uninucleate cells.

10. In the higher Fungi nuclear fusions occur at a definite stage in the life cycle, resulting in the production of spores either directly (Ustilagineae and Uredineae) or indirectly (Ascomycetes and Basidiomycetes).

11. These nuclear fusions are probably not morphologically sexual, but they replace the sexual act, and are physiologically equivalent to it, in that the cell is thereby reinvigorated to further development, and this accounts for the continued asexual reproduction of these forms.

12. Among the higher Fungi the simpler Ascomycetes only, such as *Sphaerotheca*, exhibit a true sexual fusion, accompanied, however, by a subsequent fusion of nuclei in the ascus.

NOTES.

ON THE INFLUENCE OF THE TEMPERATURE OF LIQUID HYDROGEN ON THE GERMINATIVE POWER OF SEEDS[1]. By Sir William T. Thiselton-Dyer, K.C.M.G., C.I.E., F.R.S.—The Comptes Rendus for August 28 (p. 434) contains a communication from Professor Dewar to M. Henri Moissan, 'relative à la solidification de l'hydrogène.' It concludes with the following sentence, which may be easily overlooked:—'Des graines refroidies dans de l'hydrogène liquide conservent toute la propriété de germer.'

This is the first announcement of an interesting experiment in which Professor Dewar did me the honour to ask me to assist him. He has further suggested to me to put on record the facts, as far as they came under my observation, and any physiological conclusions to which they seem to point.

With this suggestion I have no alternative but to comply. Botanists will naturally expect some more detailed account than is contained in the brief announcement which I have quoted. But as my share in the research has been of the smallest, I should have much preferred that Professor Dewar should have given the result of the whole investigation himself.

When Professor Dewar first suggested the experiment to me, he pointed out that it would be a costly one, that it would only be possible to operate on very small quantities of seeds, and that the number of kinds must also be few.

[1] From the Proceedings of the Royal Society, vol. lxv, 1899.

The dozen seeds experimented upon by Messrs. Brown and Escombe, which were submitted to the temperature of liquid air, were apparently selected as belonging to different natural families, and also in some degree as to their composition [1]. My choice was much more restricted. I took two out of their list for the sake of comparison: barley and vegetable marrow. I added wheat, which had more than once been made the subject of experiment. This gave me two farinaceous seeds and one oily one. I then took shape and bulk into account. Wheat and barley are roughly ellipsoidal and medium in size. The vegetable marrow is relatively large but flattened. I therefore added another oily seed, mustard, which is small and spherical. I followed Messrs. Brown and Escombe in taking a pea, which is also spherical in shape but nitrogenous in composition. Finally, I sought a very minute seed, and pitched upon musk.

The list then ultimately stood:—

Brassica alba.
Pisum sativum.
Cucurbita Pepo.
Mimulus moschatus.
Triticum sativum.
Hordeum vulgare.

The next point seemed to be to eliminate the source of error which might arise from defective germinative power. I therefore communicated the list to Messrs. Sutton & Sons, of Reading, and asked their assistance. With their invariable kindness in any scientific inquiry, they willingly complied, and sent the samples required, with the following report:—

'We now have pleasure in sending a packet of each of the seeds you name. They are all of last year's growth, and of good germination.

'For your information we append the germinations arrived at by our tests made in March last of the various parcels from which these samples are taken.

'We have no doubt that each grain of wheat is a germinating seed, as specially fine seeds have been picked out.

'In the case of musk a good growth was obtained, but the germination was not counted.

[1] Roy. Soc. Proc., vol. lxii, p. 161.

'Germinations:—

Mustard	100 per cent.
"Bountiful" Peas . . .	100 „
Vegetable marrow . . .	96 „
Musk	Good.
Wheat	96 „
Barley	100 „ .'

I forwarded the samples (which were small) to Professor Dewar, and suggested that they should be each divided into two portions, one for a control experiment under ordinary conditions, the other to be returned to me after being subjected to cooling. Owing to some misunderstanding, this was not done; but, as will be seen in the result, the omission proved immaterial. The seeds, it should be stated, were simply air-dried: they were ordinary commercial samples, and no attempt was made to further desiccate them.

I pointed out to Professor Dewar the advisability of exposing the seeds to extreme changes of temperature as gradually as possible, a precaution which Messrs. Brown and Escombe carefully observed[1]. He promised 'to consider what can be done to avoid any disaster from this cause.'

On July 21 he wrote to me:—'In spite of the weather I have carried out my promise, and cooled some seeds in liquid hydrogen for half an hour. I had to seal them up in a glass tube, cool first in liquid air, and then transfer to the hydrogen. They have, therefore, been cooled to $-250°$ C., or $-252°$ C., while being in a vacuum (seeing the air left had no appreciable tension). The seeds, in other words, have been transferred to a condition resembling that of moving through space. Another set of the seeds have been cooled only in liquid air for comparison.'

On July 22 he added, on returning the seeds:—'There can be no doubt about the seeds being cooled, as they were in the hydrogen for more than an hour. In fact I used nearly 600 c.c. of liquid hydrogen.'

The seeds came to me in the small packets of tinfoil in which they had been placed in the tube. On opening these it was observed that the seeds were as fresh and bright as before being subjected to the treatment. There was not the slightest discoloration observable

[1] Loc. cit., p. 161.

in the green tint of the peas. This practically disposed of the only anxiety which Professor Dewar felt as to the success of the experiment, and expressed to me on July 25 :—

'My own impression is that unless the sudden vacuum caused by the liquid hydrogen cooling has produced physical rupture of the seeds, they will germinate as usual. If they survive this awful strain, then I believe no increase of the time of cooling could produce any effect other than results from one hour's exposure to such severe cold.'

The seeds were sown in a cool greenhouse, without heat, on July 27. On August 1 they had all germinated. In the case of the mustard, 136 young plants were produced from 155 seeds; the remainder had, however, germinated, but the seedlings had damped off. One of the packets of wheat, for some reason, germinated slightly more slowly than the rest.

On August 5, I received a further packet of the seeds (the musk excepted) indiscriminately mixed. Professor Dewar wrote the same date:—'I have sent you seeds to-day which, if the treatment with cold can kill, ought to be dead. They have been immersed in liquid hydrogen for upwards of six hours, and no attempt was made to graduate the cooling. They were placed in the vacuum vessel into which the liquid hydrogen could drop from the apparatus, and had to take their chance. The seeds have been soaked in liquid hydrogen, and in this respect differ from the last that were cooled in a vacuum from being sealed in a glass tube.'

In this instance again the seeds did not show the smallest visible trace of the ordeal to which they had been subjected. They were sorted out and immediately sown, under the same conditions as before. By August 9 the seeds had all germinated without exception. I communicated the result to Professor Dewar, and he informed me, August 15 :—'The temperature Fahrenheit to which the seeds were cooled was -453° F. below melting ice.'

These are the details of the experiment. As it is not likely to be often repeated, I have thought it worth while to place them on record as precisely as possible.

The first question that suggests itself, is what evidence we have for believing that the seeds have actually been brought to the almost inconceivable temperature with which they were surrounded. That they were so brought, Professor Dewar himself has not a shadow of

doubt. That substances at widely extreme temperatures can remain in juxtaposition at least for some time, and still maintain them, is illustrated by a striking experiment shown by Professor Dewar at the Royal Institution on April 1, 1898. Liquid air poured into a large silver basin heated to redness, remained apparently as quiescent at this high temperature as in cooler vessels, and maintained a spheroidal condition. This is well understood. But the fact remains that liquid air with a temperature of about $-190°$ C. was contained in a vessel which had a temperature of 800° C., the difference in temperature between the two being 1000° C.

If we turn for a moment to the effect of heat on living structure, we know that a temperature of 75° C. is fatal to all protoplasm, because at that temperature its proteids are coagulated. Yet there is good evidence for the fact that seeds have been exposed for prolonged periods to a temperature above 100° C., and yet have subsequently germinated. It may be taken as absolutely certain that in this case that temperature never reached the embryo, but must have been intercepted by the imperfect conducting power of the seed-coats. Cohn again has found that the spores of *Bacillus subtilis* survive prolonged boiling [1], and a similar observation applies.

It is probable that plant structures are deficient in thermal transparency, and they are notoriously indifferent conductors. Nevertheless, it is difficult to believe that in the case of such small bodies as seeds, their being brought to the temperature with which they are surrounded can be more than a question of time.

That the thermal opacity of at least the seed-coats may be really considerable is not, however, impossible, even at low temperatures. The following remarks by Professor Dewar have an obvious bearing on this point:—

'Pictet, after an elaborate investigation, concluded that below a certain temperature all substances had practically the same thermal transparency, and that a non-conducting body became ineffective at low temperatures in shielding a vessel from the influx of heat. Experiments, however, prove that such is not the case, the transference of heat observed by Pictet appearing to be due not so much to the materials themselves as to the air contained in their interstices. Good exhaustion in the ordinary vacuum vessels used in low temperature work reduces the influx of heat to one-fifth of what

[1] See Vines, Physiology of Plants, p. 283.

is conveyed when the annular space of such double-walled vessels is filled with air[1].'

It is to be noticed that in Professor Dewar's first experiment the seeds were practically in a vacuum. It is obvious, from what has been quoted above, that this would help them to retain their heat. Any hesitation in accepting the results of the experiment on this ground is, however, swept away by the second experiment, in which the seeds, with absolutely no protection at all, were actually soaked in liquid hydrogen for six hours. The extremity of caution can hardly resist the conclusion that they must have been brought to the same temperature.

Professor Dewar finds 'that silica, charcoal, lampblack, and oxide of bismuth all increase the insulation to four, five, and six times that of the empty vacuum space.' It might possibly be worth while to try how far a packing of small air-dry seeds would compare, say, with charcoal. And this would in some degree be a measure of the thermal transparency of seed structures.

Professor Dewar suggested to me that I should supplement this statement by some remarks on the physiological bearing of the experiment. This has already been discussed by Messrs. Brown and Escombe, and there is perhaps little of moment to add to their conclusions.

The real interest of the whole investigation obviously lies in the question how far it modifies our conceptions of the nature and properties of living matter. Protoplasm, whatever its source, has physical properties and an ultimate chemical composition which are practically uniform. This uniformity, however, overlies a potential diversity which is not to be measured. Such diversity cannot be accounted for by any purely physical conceptions, as physical conceptions are understood.

We not merely know the ultimate constitution of protoplasm, but we also know a good deal about its proximate constitution. Yet the properties of living protoplasm are very far removed from the mere sum of those of its constituents, and no light can be derived with respect to them in this direction. And what we know about the constituent bodies themselves is at present not a little obscure. They belong, as it were, almost to the fringe of possible chemistry, and almost elude the methods of chemical research. But they, complex as

[1] On Liquid Air as an Analytic Agent, Roy. Inst., Apr. 1, 1898, pp. 7 and 8.

they are, are not themselves protoplasm. Their cumbrous molecules are built up and broken down by ordinary chemical processes. They are not in themselves, in any intelligible sense, living, though essential to the exhibition of vital phenomena.

There our analysis of living matter by physical methods for the present stops. But we are justified in pushing, at any rate, semi-physical conceptions as far as we dare. We conceive, therefore, the physical constituent molecules of protoplasm as aggregated into larger molecules which, as they are unlike anything we know as purely physical, we call physiological [1]. Of the properties of such molecules we have some faint conceptions. The first is their instability. They are *kinetic*; 'living substance is continually breaking down into simpler bodies, with a setting free of energy; on the other hand living substance is continually building itself up, embodying energy into itself, and so replenishing its store of energy [2].' This kinetic condition is essentially life; when it ceases, we have hitherto believed that the constituents of protoplasm come under the sway of purely inorganic conditions.

If we pause for a moment to attempt a quasi-mechanical explanation of the more developed phenomena of living organisms, such for example as are included under heredity, we are led to suppose that the physiological molecules may themselves be grouped into larger aggregates. And each stage of aggregation introduces us into a new order of phenomena. All that we can say is, that beyond the first stage the properties which are characteristic of higher molecular aggregrates are ultra-physical, taking physical in its ordinary signification. That does not imply, however, that physical conditions are ever in abeyance. Each stage of aggregation is conditioned by every one that precedes it. In this sense life rests *au fond* on a physical basis.

A continuous kinetic condition appears to be one distinctive property of physiological molecules. This not merely manifests itself in continuous chemical activity, but under appropriate conditions in actual visible motion. And it is to be remarked of the former, that though chemical in kind, it is undoubtedly ultra-chemical, as chemistry is understood in the laboratory. A further characteristic of the

[1] Identical with Foster's 'somacula,' Textbook of Physiology, Part I, fifth ed., p. 6.

[2] Foster, loc. cit., p. 41.

physiological molecule is that it possesses the power of breaking up chemical combinations and reuniting their constituents in a way which absolutely eludes the methods available to the chemist, and entirely outstrides the pace at which he can preceed. There is the same kind of difference between the two methods as there is between arithmetic and the calculus in the solution of a mathematical problem.

The question then is, how far the effects of Professor Dewar's experiments, and of those who have preceded him in the same field, require us to modify our conception of the physiological molecule. Are we obliged to admit with Professor C. de Candolle and Messrs. Brown and Escombe that it may descend to a purely *static* condition?

This is really bound up with another question. The kinetic condition depends on the constant liberation of energy by chemical change. Of this the most important is that due to oxidation. But we now know that this is not the only source of energy in living matter, or in all cases the indispensable one. The late Dr. Romanes showed that neither a high vacuum nor subsequent exposure for twelve months to absolutely indifferent gases, such as hydrogen or nitrogen, or even poisonous ones, such as hydrogen sulphide, had any effect on the germinative power of seeds. Professor Pfeffer has, however, informed me in conversation that an injurious effect is ultimately produced.

Vital processes have their optimum point as regards temperature. Their superior limit, for the reason already pointed out, is tolerably sharp; but the inferior is by no means equally so. According to Boussingault the decomposition of carbon dioxide by green plants may take place nearly at o°C.[1] Below the optimum there is then some evidence of a 'slowing down.' While some processes reach their limit, can we assume that all do?

The question would be peremptorily answered for us by those who assert that all chemical action is in abeyance at such temperatures as I am discussing. Photographic action still takes place at the temperature of liquid air, though this may be due to phosphorescence. But a jet of hydrogen will burn in it.

Messrs. Brown and Escombe sum up the two methods of explaining what has been called 'dormant vitality' with sufficient accuracy in their paper. According to the one view, metabolic and its resultant kinetic activity is 'slowed down' indefinitely. In such a case as now

[1] Sachs, Textbook, second ed., p. 729.

described, it might be said that this takes place along an asymptotic curve, continually approaching but never becoming equal to zero.

According to the other, protoplasm passes absolutely from the kinetic to the static condition. Its locked-up energy becomes purely potential, and Professor C. de Candolle has not hesitated under these circumstances to compare it to an explosive.

It has been pointed out that such a conclusion is absolutely in conflict with Mr. Herbert Spencer's well-known definition of life. But it appears to me that that definition was only intended to apply to higher stages of the aggregation of living matter than that of the physiological molecule on which I have endeavoured to fix the discussion. The question seems to me to be simply whether it is admissible to regard *that* as capable of being brought to an absolutely static condition.

Conceive two such molecules, one known to be living, but static, and the other dead, and both to be maintained in a condition in which they are not immediately susceptible to chemical change. What is the criterion of life? There is none. It seems to me then that the question I have propounded does not admit of any positive answer in the present state of our knowledge.

A problem, perhaps somewhat scholastic, which once vexed the souls of biologists was—whether life was the cause of organization or organization of life. What is to be our answer if our starting-point is no more than a possible 'explosive'?

ON THE STRUCTURE OF THE STEM OF A RIBBED SIGILLARIA[1]. By Professor C. E. Bertrand.—The structure of certain species of *Lepidodendron*, e.g. *L. selaginoides* and *L. Harcourtii*, is well known, and we are in possession of some facts as to the anatomy of *Sigillaria spinulosa* and *S. Menardi*, species which belong to the section of the genus characterized by a smooth bark (*Leiodermaria*). On the other hand, we are still in want of data with regard to the structure of the *Rhytidolepis* section of *Sigillaria*—the species with a ribbed bark. It has been suggested that some of the stems described under the name *Diploxylon* may very probably be partially decorticated Sigillarias.

In March of this year (1899) I received from the colliery of

[1] Abstract (translated) of a paper read before the Botanical Section of the British Association, Dover, Sept. 1899.

Glaneuse, in the Haidinghen district, Pas de Calais, a piece of a ribbed Sigillarian stem, which presented a recognizable external surface, while, at the same time, the wood of the central cylinder was clearly preserved. The specimen may probably be referred to *Sigillaria elongata*; the possible error does not exceed the difference which separates *S. elongata* from *S. scutellata*. It was found by M. Ludovic Breton and his son, M. Eug. Breton, in a seam of coal known as the 'Veine perdue.' The fragment measures 100 mm. in diameter and 60 mm. in height, and the surface is traversed by seventy-two ribs, of which forty-eight are visible and twenty-four are hidden by a fold of the surface. The structure of the wood, which is in places perfectly preserved, agrees on the whole with that of a *Diploxylon*, and the primary xylem constitutes a continuous, centripetally developed corona. Externally, this is enclosed by a continuous zone of centrifugal secondary wood, but the cambial and phloem regions, and also the central tissues, have completely disappeared. The carbonized peripheral region of the stem consists of sclerous tissue, of which the elements have thin brown walls and contain an amorphous yellow substance.

The continuous corona is made up of ten to thirteen rows of large scalariform tracheides, without any free lignified elements internal to the primary xylem, which consists solely of tracheides without any interposed primitive fibres ('fibres primitives'); in the latter respect the French specimen differs from one of the *Diploxylon* stems from the coal-measures of Oldham. The external face of the corona is characterized by very prominent teeth corresponding to the furrows of the external surface. The prominent teeth alternate regularly with the sinuses. The smallest xylem-elements are situated in the projecting teeth. In a region of the corona, at some distance from the point of exit of a leaf-trace, the spiral tracheae are arranged in two superficial groups laterally placed in relation to the projecting teeth; but in the immediate neighbourhood of the origin of a leaf-trace, the spiral elements form a median band in the middle of a sinus. The large tracheides of the primary wood represent its cauline portion ('partie réparatrice'). The leaf-traces arise from the external face of the corona, and each is detached from the middle of a sinus. The leaf-traces of every alternate sinus are cut almost at the same level, an arrangement which points to an almost regularly verticillate disposition of the leaves.

Each leaf-trace springs from the small external elements of the xylem, and consists of six tracheae in the form of a tangentially extended group, and of six to eight radially disposed centripetal bands of scalariform tracheides, each band including four elements. The foliar bundles consist of primary xylem only, and follow a radial course through the medullary rays of the secondary wood.

The secondary wood is twenty-three elements in thickness; the arc opposite each sinus of the corona is composed of ten to twelve radial bands of tracheides, while the arc opposite a projecting tooth of the course includes four to six bands. There are thus thirty-two radial series of tracheides between two outgoing leaf-traces; the elements opposite the teeth are somewhat smaller in diameter than in the xylem opposite the sinuses, but the difference is very slight.

In *Sigillaria spinulosa* from Autun the leaf-traces arise from the same point in the corona as in the ribbed stem from Haidinghen; their arrangement and the structure of the wood is also the same; but in the former species the primary wood occurs in the form of distinct groups or islands occupying positions corresponding to the sinuses in the corona of the ribbed *Sigillaria*. In *S. spinulosa* there is no primary xylem in the positions corresponding to the teeth on the surface of the corona of the Haidinghen stem. The polar regions ('régions polaires'), which tend to be differentiated in the projecting teeth of the corona of the ribbed *Sigillaria*, are not represented in the corona of the Leiodermarian type of stem. Moreover, as Renault has stated, the leaf-traces of the latter type of *Sigillaria* consist in part of secondary xylem. The arrangement of the spiral elements is also different in the two species. In *S. spinulosa* there are ten to fourteen radial bands of secondary tracheides opposite each group of primary xylem, and four to six bands of smaller elements opposite each interval separating the detached groups of which the corona is composed. In the number (thirty-two to thirty-four) of radial series between two outgoing traces, the two species agree. We see, therefore, that there is a close agreement in the structure of the *Leiodermaria* and *Rhytidolepis* types as regards the manner of exit of the leaf-traces; but the disposition of the small protoxylem-elements affords a striking distinction. In the Autun *Sigillaria* there is also a tendency to differentiate the secondary wood opposite the sinuses from that opposite the teeth.

A comparison of the ribbed *Sigillaria* of Haidinghen, with the

Diploxylon stems from Halifax and Oldham, and the Lepidodendroid stem from Burntisland, shows that the primary xylem becomes broader in this series of forms. The polar groups of small elements, which still form strongly prominent teeth on the surface of the corona in the stems from Halifax and Oldham, are considerably reduced in the Burntisland plant, just as in *Lepidodendron selaginoides*. The leaf-trace, which is still given off from the middle of a sinus in the Halifax and Oldham stems, arises laterally, in relation to the teeth of the corona, in the Burntisland stem, approaching *Lepidodendron selaginoides* in this point also. The secondary wood of the Burntisland form shows no indication of any differentiation of the secondary wood into segments or arcs corresponding to the sinuses and teeth of the primary wood. In fact, certain English types of *Diploxylon* only accentuate the differences which separate the Haidinghen *Sigillaria* from *S. spinulosa*.

The central axis of the ribbed *Sigillaria* differs from that of the Phanerogamic type in the manner of origin of the leaf-traces and in the structure and centripetal development of the primary xylem; these structural features characterize the radial form of vascular axis, and are in short those of a well-defined Cryptogamic type.

THE JURASSIC FLORA OF BRITAIN[1].—The Jurassic plant-bearing strata exposed in the cliff sections of the Yorkshire coast, between Whitby and a few miles south of Scarborough, have afforded unusually rich data towards a restoration of the characteristics and composition of a certain facies of Mesozoic vegetation. Since the publication of A Geological Survey of the Yorkshire Coast, by Young and Bird, in 1822, the numerous species of Inferior Oolite plants from Gristhorpe Bay, Scarborough, Cloughton Wyke, Haiburn Wyke, Whitby and other localities have been described by Phillips, Brongniart, Lindley and Hutton, Morris, Göppert, Leckenby, Saporta, Zigno, Nathorst, Carruthers and other writers, but no detailed account of the flora has been published. The names of Bean, John Williamson, his son William Crawford Williamson, Phillips, Murray, Leckenby and others will always be closely associated with the earlier investigations of the fossil flora of east Yorkshire. The British Museum unfortunately possesses but few of the type-specimens of these Jurassic plants;

[1] Read before the Botanical Section of the British Association, Dover, Sept. 1899.

some appear to have been lost, but several have been recognized in the Museums of Cambridge, Scarborough, Whitby, York, Newcastle, Paris and elsewhere.

The Author has been engaged during the last few years in examining the Yorkshire Inferior Oolite Species, and an account of the flora—to be published as a British Museum Catalogue—is now passing through the press. The determination of the species is in nearly all cases based on the external characters, as the conditions of preservation were unfavourable for the petrifaction of the internal tissues. Cycadean plants are especially numerous, being represented by some species which must be classed with the Bennettiteae and by others of which the exact position cannot be definitely determined. The Ginkgoaceae include species of the genera *Ginkgo* and *Baiera*, while *Brachyphyllum mamillare* Brongn., and *Pagiophyllum Williamsoni* (Brongn.) may be mentioned as two of the more abundant Conifers.

Among the ferns there are examples of the Matonineae, Schizaeaceae, Osmundaceae, Cyatheaceae, and other families, and *Equisetites columnaris* Brongn. and *Lycopodites falcatus* L. and H. represent the Equisetaceae and Lycopodiaceae respectively. A few more or less unsatisfactory fossils have been referred to the Bryophyta and Thallophyta. The absence of any Monocotyledons and Dicotyledons is a striking feature, while the flora as a whole presents a marked agreement with floras of Rhaetic and Lower Jurassic age described from various European and extra-European localities. The following list may serve as a guide to the nature of the vegetation which existed in the North-West of Europe during the latter part of the Jurassic Epoch:—

Bryophyta: *Marchantites erectus* (Leck.).

Equisetaceae: *Equisetites columnaris* Brongn., *E. Beani* (Bunb.).

Lycopodiaceae: *Lycopodites falcatus* L. and H.

Filicineae: *Matonidium Goepperti* (Ett.), *Laccopteris polypodioides* (Brongn.), *L. Woodwardi* (Leck.), *Todites Williamsoni* (Brongn.), *Klukia exilis* (Phill.), *Ruffordia Goepperti* (Dunk.), *Coniopteris Hymenophylloides* (Brongn.), *C. quinqueloba* (Phill.), *C. arguta* (L. and H.), *Dictyophyllum rugosum* L. and H., *Cladophlebis lobifolia* (Phill.), *C. denticulata* (Brongn.), *C. haiburnensis* (L. and H.), *Taeniopteris vittata* (Brongn.), *T. major* L. and H., *Sphenopteris princeps* Presl., *S. Murrayana* (Brongn.), *S. Williamsoni* Brongn., *Sagenopteris Phillipsi* (Brongn.).

CONIFERAE: *Cryptomerites divaricatus* Bunb., *Cheirolepis setosus* (Phill.), *Araucarites Phillipsi* Carr., *Taxites zamioides* (Leck.), *Brachyphyllum mamillare* Brongn., *Pagiophyllum Williamsoni* (Brongn.), *Czekanowskia Murrayana* (L. and H.), *Nageiopsis anglica*, sp. nov.

GINKGOACEAE: *Ginkgo digitata* (Brongn.), *G. Whitbiensis* Nath., *Baiera Lindleyana* (Schimp.), *B. gracilis* Bunb., *B. Phillipsi* Nath., *Beania gracilis* Carr.

CYCADALES: *Williamsonia gigas* (L. and H.), *W. Pecten* (Phill.), *Otozamites Beani* (L. and H.), *O. acuminatus* (L. and H.), *O. graphicus* (Leck.), *O. Bunburyanus* Zign., *O. obtusus* (L. and H.), var. *oolitica*, *O. Feistmantelli* Zign., *O. parallelus* Phill., *Dioonites*, sp., *Nilssonia compta* (Phill.), *N. mediana* (Leck.), *N. tenuinervis* Nath., *Anomozamites Nilssoni* (Phill.), *Ptilozamites Leckenbyi* (Leck.), *Ctenis falcata* L. and H., *Podozamites lanceolatus* (L. and H.), *Pachypteris lanceolata* Brongn.

A. C. SEWARD, Cambridge.

A NEW GENUS OF PALAEOZOIC PLANTS[1].– The following description is based on the examination of ten sections prepared from a fragment of stem in the Binney Collection (Woodwardian Museum, Cambridge). The type-specimen occurs in a calcareous matrix associated with the shells of Goniatites, and was originally obtained from the Lower Coal-Measures of Lancashire; it consists of a cylinder of secondary xylem, 2 cm. in breadth, enclosing a central region, 1·9 cm. in diameter, occupied by primary xylem. The wide primary stele is made up chiefly of groups of unusually large tracheids with their walls covered with bordered pits, associated with thin-walled parenchyma; the tracheids are characterized by their isodiametric or horizontally elongated form, while a few are distinguished by their greater length. In the peripheral region of the primary stele the tissue assumes various forms; the large short tracheids and parenchyma extend in places close up to the inner edge of the secondary wood, but more or less compact groups of narrower and longer tracheids occur here and there in the peripheral zone and constitute leaf-traces.

[1] Read before the Botanical Section of the British Association, Dover, Sept. 1899. For a more complete account of the genus *vide* Proceedings of the Cambridge Philosophical Society, Vol. x, Part III, p. 158, 1899.

A leaf-trace, as seen near its entrance into the secondary wood, presents the appearance of an oval group of comparatively narrow elongated tracheal elements and vertical rows of parenchyma, with about six external protoxylem groups. Each leaf-trace on passing vertically downwards through the primary tissues of the stem becomes less compact and spreads laterally in a fan-shaped manner; the elongated tracheids become shorter and more irregular in shape, and finally merge into the short and large tracheids of the more central region or metaxylem. Between the metaxylem and the xylem of the leaf-traces there is no sharp line of division, as each foliar strand in its downward course gradually loses its individuality and becomes indistinguishable from the metaxylem. An examination of the transverse sections leads to the conclusion that the stem had a phyllotaxis of two-fifths. The secondary wood agrees in structure with that of recent Cycads and with *Lyginodendron*, *Medullosa* and other Palaeozoic genera. The characteristic features of the plant, which it is proposed to name *Megaloxylon Scotti*[1], may be summarized as follows:—

Megaloxylon Scotti, gen. et sp. nov. The primary single stele consists of a peripheral leaf-trace region and a central metaxylem region; the metaxylem consists of tracheids varying in shape from isodiametric and somewhat flattened to more or less elongated elements with numerous bordered pits on their walls. With the large isodiametric or even horizontally elongated tracheids occur some smaller short tracheids and occasionally irregularly-shaped longer tracheal elements. The metaxylem tracheids occur in groups of varying size and form scattered through a parenchymatous ground-mass, which includes small secretory cells.

At the periphery of the primary stele numerous strands of spirally pitted protoxylem tracheids occur in an exarch position; these strands of protoxylem occupy different positions in regard to one another in different parts of the stem, according to the position in its vertical course at which a leaf-trace is seen. A leaf-trace has the sectional form of an elliptical mass of long tracheids—with bordered pits on their walls and of somewhat larger diameter than the tracheids

[1] I have associated this new species with the name of my friend, Dr. D. H. Scott, whose researches have so materially extended our knowledge of the Cycadofilices and demonstrated the importance of this extinct group from a phylogenetic standpoint.

of the secondary wood, but much narrower than the large metaxylem tracheids—associated with short parenchymatous cells; several groups (at least six) of protoxylem elements occur on the external edge of the trace. As a leaf-trace passes deeper into the stem the tracheids become less compactly arranged, and the whole leaf-trace becomes wider and less well defined; its long and narrow tracheids are gradually replaced by shorter elements of more irregular and variable form, and these are eventually linked on to the short and large tracheids of the metaxylem region; the peripheral leaf-trace region and the axial metaxylem regions of the stele are in close organic connexion.

The secondary wood of the stem is made up of regular rows of tracheids, with multiseriate bordered pits on their radial walls, and broad and deep medullary rays composed of short parenchymatous cells.

As a leaf-trace passes through the secondary xylem of the stem its primary tissues become enclosed by a zone of secondary tracheids and medullary rays.

The structure of the primary wood recalls that of *Heterangium* and *Medullosa anglica* Scott, but there are certain important peculiarities in the present species which constitute well-marked differences and render advisable the institution of a new generic name. The primary peri-medullary strands in the stele of *Heterangium*, as also in *Medullosa anglica*, are distinctly mesarch in structure, whereas in *Megaloxylon* the protoxylem groups occupy an exarch position. Another distinctive feature of the new type is the unusually large size and the peculiar short form of the metaxylem tracheids—elements which probably served for water-storage rather than for transport.

In *Megaloxylon* we have a type of stem in which the primary xylem is distinctly of the fern type; the protoxylem is external, and not internal as in *Heterangium*; but in recent ferns the xylem may be endarch, mesarch or exarch, and no great importance from the point of view of affinity to the ferns as a group should be attached to this point. On the other hand the mesarch structure of the xylem of *Heterangium*, *Lyginodendron* and other *Cycadofilices* affords an important Cycadean character, which is not met with in *Megaloxylon*.

Megaloxylon adds another connecting link between the Palaeozoic Cycadofilices and recent ferns; in anatomical characters the two

genera *Lyginodendron* and *Heterangium* approach most nearly to the Osmundaceae and Gleicheniaceae respectively; in *Megaloxylon*, on the other hand, the structure of the primary xylem affords evidence that the *Lygodium* type of stem was also represented in the Cycad-fern alliance, which played so prominent a part in Palaeozoic vegetation.

A. C. SEWARD, Cambridge.

ON THE PRIMARY WOOD OF CERTAIN ARAUCARIOXYLONS.—The genus *Araucarioxylon* of Kraus (*Araucarites*, Goepp., *Dadoxylon*, Endl.) is used to include those fossil Gymnospermous woods which have approximately the structure of the recent *Araucaria* or *Dammara*. The characters of the genus as given by Kraus are as follows: 'Lignum stratis concentricis distinctis vel obsoletis; cellulis prosenchymatosis porosis; poris magnis rotundis, rarius uniserialibus contiguis, creberrime pluriserialibus spiraliter dispositis compressione mutua hexagonis; cellulis ductibusque resiniferis nullis; radiis medullaribus uni- rarius pluriseriatis[1].'

The genus is admittedly an artificial and provisional one. From the investigations of Grand'Eury and Renault we know that many, though not necessarily all of the Palaeozoic Araucarioxylons were identical with the wood of the Cordaiteae, that remarkable extinct Order of Gymnosperms which those observers have revealed to us. Other specimens, and especially those of Mesozoic age, no doubt belonged to true Coniferae; in fact the secondary wood, by itself, is of little value as a guide to affinities. Where other tissues, such as the pith and primary xylem, are also preserved, the case is a good deal more favourable, for we then have the anatomical ground-plan of the organ before us. The study of the primary tissues will no doubt lead in the future, as it has done in the past, to the gradual breaking up of these artificial genera into more natural groups.

In the Cordaiteae and in the more typical Araucarioxylons generally, the primary wood of the stem, where it has been investigated, has proved to be purely *centrifugal* in development, the first-formed spiral tracheides lying at the inner edge of the wood, adjacent to the pith[2].

[1] In Schimper, Paléontologie Végétale, vol. ii, p. 380, 1870.

[2] I leave out of account, for the moment, such stems as those of *Protopitys* or *Lyginodendron*, which were at one time included under *Araucarioxylon*, but have long since been separated.

In fact the primary structure is *endarch*, just as in the stems of recent Coniferae and Cycadaceae [1].

In March of this year my friend Mr. R. Kidston, F.G.S., called my attention to certain sections of *Araucarioxylon* in his possession, which showed distinct strands of primary wood in the pith of the stem. Mr. Kidston, with his accustomed generosity, lent me his sections and specimens for further investigation. Only the chief results can be given here; a full illustrated description will, I hope, appear later on.

The specimens in question are of two very distinct types. The one, which we will first consider, will be named provisionally *Araucarioxylon fasciculare*. Mr. Kidston's specimen came from the Loch Humphrey Burn in the Kilpatrick Hills, Dumbartonshire, where it was found by Mr. John Renwick in 1898. Its horizon is given as that of the Calciferous Sandstone series, and it is thus of about the same antiquity as the well-known Lower Carboniferous fossils of Burntisland and of Arran. I find in the Williamson collection, sections (C. N. 1378–80; 1391–93) of a stem showing a perfectly similar structure; this specimen was derived from the Carboniferous Limestone, near Haltwhistle in Northumberland.

The pith of *A. fasciculare* is small, having a maximum diameter of about 2 millim. in the Kidston specimen, and about 3 millim. in that from the Williamson collection. The pith itself consists of short-celled parenchyma, and presents nothing remarkable, but around its periphery is a ring of eight or nine distinct strands of primary wood. These strands show a gradation in size; the smaller are imbedded in the outer layers of the pith; the larger are beginning to enter the surrounding zone of secondary wood, through which they can be traced for some distance in the different sections. These strands, which are thus on the point of exit, are most conspicuous objects in the transverse sections, attaining a diameter of from ·8 millim. to 1 millim., with an approximately circular contour. The smallest elements lie almost in the middle of the strand, or slightly nearer its outer surface; in one case their spiral markings were clear. The structure of these primary strands is thus *mesarch*, as in those of *Lyginodendron Oldhamium*. The large primary tracheides surrounding the protoxylem are spiral, reticulate or pitted.

[1] *Exceptis exceptandis*; cf. Scott, On Peduncle of Cycadaceae, Annals of Botany, vol. xi, 1897.

The smaller circum-medullary strands, which clearly represent leaf-traces at a greater distance below their point of exit, also show mesarch structure, but in the smallest of them the protoxylem-group approaches the inner edge of the strand. It is evident that the outgoing leaf-trace became much enlarged on approaching its point of exit. The same thing is seen in *Poroxylon*, and in *Lyginodendron Oldhamium*, though less conspicuously. The arrangement of the larger outgoing traces agrees with a 2/5 phyllotaxis. The internodes were presumably short, for in the Williamson specimen three bundles are seen passing out in one transverse section. The bundles soon assumed a nearly horizontal course, for they are sometimes cut almost transversely in tangential sections of the wood.

The structure of the secondary wood is in all respects that of a typical *Araucarioxylon*; the rays are narrow, usually uniseriate, occasionally two cells thick in the middle. In height they vary as a rule from one to about twelve cells, but a few are higher. The pits are limited to the radial walls of the tracheides; they are ranged in three or four alternating rows, and have an hexagonal outline, with the slit-like pore often beautifully preserved. The medullary rays are of typically muriform structure.

This stem thus combines the primary xylem-structure of the Lyginodendreae with the secondary wood of a typical *Araucarioxylon*. The name *fasciculare* is proposed for this form of stem on account of the extreme prominence of the primary bundles.

The second species is from the same horizon as the first. Mr. Kidston's sections, on which the following account is entirely based, bear the inscription: '*Araucarioxylon*, Lennel Braes, Berwickshire. Calciferous Sandstone Series, B. N. Peach, 1883.'

This stem differs strikingly from the foregoing in the size of its pith, which is nearly an inch (22 millim.) in diameter. The pith itself is remarkable; it consists of large, very short cells, with abundant 'secretory sacs' among them. There are horizontal lenticular gaps in the tissue, suggesting an approach to the discoid '*Sternbergia*' structure so characteristic of the pith of *Cordaites*.

The primary xylem-bundles in this case are small, ranging from ·15 to ·3 millim. in diameter, but are very numerous. Forty-six were counted in a transverse section, but the number no doubt varies. They are ranged in an irregular ring round the periphery of the pith, in which most of them are imbedded, very few being in actual contact

with the secondary wood. At two or more points in the transverse section a pair of these small bundles was observed just entering the zone of secondary wood. At each of these points there was another bundle a little way to the interior, apparently a 'faisceau réparateur' about to replace the outgoing strands. At another place a strand was observed passing out through a large ray of the secondary wood.

The primary xylem-strands show in most cases a very distinct mesarch structure. The smallest elements are near the middle of the strand, and are shown by the longitudinal sections to be spirally thickened, while the surrounding tracheides are reticulate. Anastomosis appears to occur frequently among the primary xylem-strands.

The secondary wood, which is exquisitely preserved, though only present in small quantity, is of the type characterizing the subgenus *Pissadendron*[1] (*Pitus* of Witham, *Palaeoxylon* of Brongniart). The numerous medullary rays attain a great height, and are commonly four cells in thickness, though small uniseriate rays also occur. Towards the pith the rays are much dilated, and the woody wedges correspondingly restricted.

The pitting of the secondary tracheides is preserved with astonishing perfection, and is of the *Araucarian* type; there are usually from three to five rows of the hexagonal pits, each with a narrow, horizontal, or inclined pore, on the radial wall of each tracheide. Tangential pits also occur in places. The innermost secondary tracheides show a spiral thickening, which, however, as in the tracheides of the Yew, appears to co-exist with a pitted structure.

The organization of this stem is, so far as I am aware, quite unique. The numerous small circum-medullary xylem-strands, for the most part independent of the zone of centrifugal wood, appear to be without any near parallel among recent or fossil Gymnosperms as at present investigated.

Yet I believe that the fossil in question is one that has long been known. It will be remembered that Lennel Braes, on the Tweed, from which Mr. Kidston's specimen comes, was one of Witham's localities, whence he obtained specimens of his *Pitus antiqua*[2]. Witham's plant agrees so well with Mr. Kidston's specimen, as shown by a comparison

[1] Kraus, in Schimper, loc. cit., p. 384.

[2] Witham of Lartington, Internal Structure of Fossil Vegetables, Edinburgh, 1833, pp. 23, 37, 71.

of the sections, that, considering the identical locality, I have no doubt they are one and the same thing, and therefore refer the specimen here described to *Araucarioxylon antiquum* (Witham sp.). The Craigleith Tree (*Araucarioxylon Withami*, Lindl. and Hutt. sp.) is doubtfully distinct[1]. No observations appear to have been made hitherto on the primary structure of these fossils.

If we now compare the two species described, it is noticeable that *Araucarioxylon fasciculare* has a distinctly Coniferous or Cordaitean rather than Cycadean type of secondary wood. The rays are narrow, and the elements of moderate size. It is significant that in this stem (as also, in some degree, in *Protopitys Buchiana*) this type of secondary wood co-exists with a Filicinean or Cycadofilicinean primary structure.

In *A. antiquum* the larger elements and broader rays give the wood a more Cycadean character, but the general anatomical habit suggests a Cordaitean stem rather than anything else. The primary bundles, which afford the connecting link with more primitive forms, are here a much less conspicuous feature than in the former species. In fact the primary structure of *A antiquum* is much less like Cycadofilices than that of *A. fasciculare*, while as regards the secondary wood the reverse is the case. Personally, I put all the weight on the primary structure, and suspect that *A. fasciculare* may still have belonged to the more primitive group, while *A. antiquum* may have been already far on the road towards Cordaiteae.

The facts described in the present note establish a further link between Cordaiteae and Cycadofilices, and so far tend to support the hypothesis of the Filicinean origin of the Gymnosperms generally.

The further discussion of the question of the affinities of these fossils must be reserved for the fuller communication which is to follow. In the mean time I will only add that both *Araucarioxylon fasciculare* and *A. antiquum* will certainly require generic separation, on the basis of their primary characters.

D. H. SCOTT, Kew.

[1] See Goeppert, Revision meiner Arbeiten über die Stämme der fossilen Coniferen (under *Pitys*). Bot. Centralblatt, vols. v and vi, 1881. In one of Witham's original sections of the Craigleith fossil, kindly lent by Prof. I. Bayley Balfour, I find distinct remains of the primary xylem-strands around the pith, agreeing with those of Mr. Kidston's specimen of *Araucarioxylon antiquum*.

ON THE GRAVITATION STIMULUS IN RELATION TO POSITION.—When an apogeotropic organ is placed on the intermittent klinostat [1] it is subjected to alternate stimuli tending to make it curve in opposite directions. If the organ is fixed say at an angle of 45° to the horizontal axis of rotation, the organ will, during half the time, point obliquely upwards, and during the other half it will point obliquely downwards. Are the gravitation stimuli equal in these two positions? If so, no curvature will occur, but if Czapek [2] is right in believing that 45° below the horizon gives a stronger stimulus than 45° above, it is clear that the organ must gradually curve towards the horizontal.

Thirty-four experiments were made with grass-haulms (principally those of *Lolium perenne*) fixed at angles varying between 35° and 55° to the horizontal axis of the intermittent klinostat. In four cases no bending occurred, in twenty-seven cases the haulms bent from 2° to 19° towards the horizontal, while in three instances they bent in the opposite direction or laterally. There can therefore be no doubt that grass-haulms obey Czapek's Law in being more strongly stimulated at angles of about 45° below the horizontal than at corresponding angles when the free end points obliquely upwards [3].

The above observations were made some time ago in ignorance of the fact that Czapek [4] has used the same method in a cognate experiment.

D. F. M. PERTZ.

Physiological Laboratory, Cambridge,
October, 1899.

SOME OBSERVATIONS BEARING ON THE FUNCTION OF LATEX [5].—The author has lately returned from a year's sojourn in Ceylon, where he has been acting as scientific assistant to Mr. Willis, the Director of the Royal Botanic Gardens. During his

[1] For a description of the instrument see F. Darwin and D. F. M. Pertz in Annals of Botany, 1892, p. 245.

[2] Pringsheim's Jahrbücher, XXVII.

[3] The facts also agree, broadly speaking, with Elfving's results, Acta Soc. Sci. Fennica, 1880.

[4] Sitzb. K. Akad. Wien, Bd. civ, 1895.

[5] Abstract of paper read before the Botanical Section of the British Association, Dover, Sept. 1899.

time there he has been principally engaged in investigations on caoutchouc-yielding trees, chiefly *Hevea brasiliensis* (Para Rubber), and *Castilloa elastica var.* (a Central American Rubber-tree). The results of this research are contained in a recently-published circular of the Royal Botanic Gardens, Ceylon, entitled 'Caoutchouc or Indiarubber,' intended primarily for those interested in rubber cultivation.

The purpose of this paper is to draw attention to some of the observations and experiments recorded in the Circular, which, besides their practical value, have a general botanical interest, and also to make public other observations which may throw light on the functions of laticiferous tissue. It is arranged in six sections. The main features of these are here briefly given.

Section I is occupied chiefly with the coagulation of the latex of *Hevea*. Coagulation is now known to be brought about by the proteid contained in the latex passing from a soluble to an insoluble state. The latex of *Hevea* is not coagulable by heat or slight additions of alkalies, but is coagulable in the cold, by small quantities of acids. The approximate weight of acid required to clot completely 100 c.c. of latex has been worked out for sulphuric, hydrochloric, nitric, acetic, oxalic, tartaric, and citric acids. Experimental evidence points to the proteid in question being alkali-albumen rather than ordinary albumen. It has previously been called albumen.

The behaviour of the latex towards certain saline solutions has also been investigated. Mercuric chloride is shown to be a powerful coagulator.

Section II contains observations and remarks relating to the carbohydrates of latex.

Sugar in variable proportions is of frequent occurrence in latex. The little contained in the trunk-latex of *Hevea* seems invariably to be cane-sugar.

It is suggested that the sugar may arise, in part at least, from the surrounding injured tissues, and may not be always originally present in the latex.

The starch-rods so characteristic of the laticiferous tubes of *Euphorbia* and allied genera have been found still present in the turned and fallen leaves of the following species examined: *Euphorbia pulcherrima*, *E. Bojeri*, *E. rothiana*, *Pedilanthus tithymaloides*, *Hura crepitans*, *Excaecaria bicolor*, and *Sapium biglandulosum*. This fact

is somewhat opposed to the view of these tubes functioning as conductors of starch from the leaf.

In Section III reasons are given for thinking that in some caoutchouc trees the latex of the young stems and leaves differs in the composition of its globules in suspension from that of the trunk and main branches. While the latter yield rubber free of stickiness, the former give a somewhat viscous substance with feeble elasticity. Such is the case with *Hevea*, *Castilloa*, *Landolphia Kirkii*, *Ficus elastica*, and *Urceola esculenta*.

Section IV treats of an important fact connected with the tapping of *Hevea* trees, viz., that wounding the bark causes a greater flow of latex from subsequent injuries, a point first indicated in the experiments of Mr. Willis, who found that the weight of rubber obtained from the second tapping was about double that from the first. The author has followed this up with some instructive results.

In Section V a peculiarity in the exudation of latex from the severed base of the petiole of *Hevea brasiliensis* and *Plumiera acutifolia* is described and discussed.

In Section VI a special laticiferous system developed in the immature seed of *Hevea brasiliensis* is brought to notice.

The paper concludes with general remarks and suggestions on the origin and functions of laticiferous tissue.

J. PARKIN, Cambridge.

INTUMESCENCES OF HIBISCUS VITIFOLIUS (L.)[1].

I. *Anatomical Part.*

The plants on which the following observations were made, were grown, directly or indirectly, from seed from Somaliland. The intumescences, which vary in size and shape, occur on the leaves, stems, green parts of the flower, and on the young fruit. Some are entirely colourless; others are green at the base. Those on (1) the leaf differ from those on (2) the stem.

1. *On the leaf* the intumescences are of two types.

(α) Purely epidermal.

(β) Partly sub-epidermal.

[1] Abstract of paper read before the Botanical Section of the British Association, Dover, Sept. 1899.

a. The purely epidermal and smaller type consists of one or two tiers of much elongated, thin-walled cells, usually twisted spirally round one another. At the apex is a stoma, which may or may not lead into an intercellular space.

β. The larger outgrowths contain basal prolongations of parenchyma.

2. *On the stem* the outgrowths are more complex, and usually larger. The basal part consists of elongated sub-epidermal cells divided by periclinal walls. The upper part is made up of much enlarged, thin-walled epidermal cells, similarly divided. The outgrowths later become cut off by cork, which arises in the lowest row of daughter-cells derived from the original epidermis, i. e. in the lowest colourless cells; after suberization of these cells the outgrowth shrivels.

II. *Experimental Part.*

Seedlings were raised in the Tropical Pit, and eight of them were planted, each in a separate pot, and allowed to grow on under identical conditions. They all developed intumescences, and were all very much alike. When each had about nine or ten leaves, and was beginning to flower, the plants were placed under different conditions, and examined at the end of six weeks:—

The plant grown in the open was entirely free from intumescences; it was particularly vigorous, and had strong lateral branches.

The plant in the temperate house had outgrowths only on the *under* sides of the leaves, and on the flowers and fruits.

The plant in the filmy fern-house was very unhealthy, but had no outgrowths.

All the other plants had outgrowths on one or both sides of most of the leaves, on the stems, the green parts of the flowers, and on the young fruits.

Conclusions.

As far as the evidence goes at present, it seems to point to the conclusion that the intumescences are pathological, and are due neither to insects nor to fungi, but to the direct effects of environment. The formation of outgrowths appears to be caused by excessive moisture combined with a high temperature. If the temperature is low the plants do not appear to have strength to form them. The

production of outgrowths seems to be a response on the part of the plant to insufficient transpiration.

Note.—Similar, but less well-marked, outgrowths were observed on the leaves of plants of *Ceratotheca triloba*. As in the case of *Hibiscus vitifolius*, they were not formed in a plant placed in the open ground.

Outgrowths which may prove to be of the nature of those in *Hibiscus vitifolius* have been described by Sorauer in *Dracaena* (*angustifolia*, &c.), *Cassia tomentosa*, *Acacia* (*semperflorens*, &c.).

E. DALE, Cambridge.

STEM-STRUCTURE IN SCHIZAEACEAE, GLEICHENIACEAE, AND HYMENOPHYLLACEAE[1].—There is a wide difference between the types of stem-structure shown by the different members of the *Schizaeaceae*[2]. Thus *Lygodium* has a stele in which the xylem forms a solid mass in the centre of the stem, and is surrounded by a continuous ring of phloem, pericycle, and endodermis.

Aneimia Phyllitidis, on the other hand, has a ring of separate bundles (or steles), which may be compared with those of *Aspidium* or other *Polypodiaceae*; each of them consisting of a band of xylem surrounded by a phloem, pericycle, and endodermis of its own.

Mohria resembles *Aneimia Phyllitidis* in type. Certain species of *Aneimia*, e. g. *A. mexicana*, have in the internodes a complete ring of xylem bounded on the inner and outer side by a ring of phloem, pericycle, and endodermis, with a central pith, and thus resemble *Marsilia*. *Schizaea* has a ring of xylem surrounding a central pith, but no internal phloem or endodermis.

The above four genera, which make up the *Schizaeaceae*, agree in having a stem-protoxylem, which is not well-marked, as it consists of elements which are not annular or spiral, and are usually not specially small. *Lygodium*, *Aneimia*, and *Mohria* are exarch; in *Schizaea*, however, the relative position of the protoxylem has not been made out with certainty.

In their main points the types of stem-structure found in the *Schizaeaceae* agree with the structures shown at successive levels in the stem of a 'seedling' plant of *Polypodium*, i. e. at successive stages

[1] Abstract of paper read before the Botanical Section of the British Association, Dover, Sept. 1899.

The main structural points in this Order and the *Hymenophyllaceae* are described by Prantl, Morphol. d. Gefässkrypt., 1, 1875, and 2, 1881, Leipzig.

in the ontogeny of such a fern[1]. Hence the *Aneimia* type (which corresponds with that of a mature *Polypodium*) may be regarded as the more specialized type among the *Schizaeaceae*, and *Lygodium* (which corresponds in structure with the base of the stem of *Polypodium*) as the more primitive type.

The *Gleicheniaceae* and *Hymenophyllaceae* also include forms with a solid central mass of xylem, but differing in some details from *Lygodium*. The protoxylem is well-marked and composed of annular and spiral elements in both orders. *Gleichenia* is mesarch and closely resembles the fossil genus *Heterangium*.

In the *Gleicheniaceae* the only advance on the *Lygodium* type is found in *Platyzoma* (a sub-genus of *Gleichenia*), in which there is a ring of xylem surrounding a central pith[2], as in *Schizaea*, but differing from the latter plant in having an inner endodermis.

In the larger species of *Trichomanes* there is a solid xylem-mass, but with a group of parenchyma in connexion with the one or two protoxylems, which are more or less centrally placed. In *Hymenophyllum* the corresponding parenchymatous mass is large in proportion to the amount of xylem. In the smallest species of *Trichomanes* the stele of the rhizome takes the form of a collateral bundle. The protoxylem of *Trichomanes spicatum*, unlike the other species examined, resembles that of the *Schizaeaceae*.

The solid stele may be regarded as primitive, the *Aneimia* type being derived from it by the following steps :—

1. Solid central xylem-mass surrounded by phloem, &c.
2. Ring of xylem surrounding a central pith.
3. Ring of xylem with internal phloem, endodermis, and pith.
4. Ring of separate bundles formed by the breaking up of the above vascular ring, owing to large leaf-gaps.

The *Aneimia* type thus explained would not be polystelic, in the morphological sense of the word, but the separate bundles would represent peripheral parts of an originally solid stele, in which the central part has been replaced by parenchyma, additional pieces of phloem and endodermis having been differentiated to complete the concentric bundles.

The full results of this investigation will be published subsequently.

L. A. BOODLE, Kew.

[1] Leclerc du Sablon, Annales des Sci. Nat., Bot., sér. vii, t. xi, 1890.

[2] Poirault, Ann. des Sci. Nat., Bot., sér. vii, t. xviii, 1893.

A PECULIAR EMBRYO-SAC IN PEPEROMIA PELLUCIDA.—During a preliminary study of the development of the ovule and embryo-sac of *Peperomia pellucida*, I was struck by certain remarkable peculiarities, which seemed to be perfectly constant, and while all the details have not yet been worked out, it is evident that in this plant we have a form which differs remarkably from the usual Angiospermous type.

The origin of the embryo-sac shows nothing unusual, but after the first division of the primary nucleus a difference is manifested. The young embryo-sac is small, and broadly oval in outline, with a relatively very large nucleus. After the first division of the latter, the two daughter-nuclei remain close together, and after the next division, the four resulting nuclei are arranged at equal distances from each other, very much as in the ordinary tetrad-division of spore-mother-cells. This stage is followed by one with eight nuclei, arranged equally about the periphery of the sac, which is completely filled with granular cytoplasm without the usual central vacuole. Absolutely no trace of the polarity, so characteristic of the usual embryo-sac, can be detected, nor is there any sign of a definite egg-apparatus, of antipodals, or of polar nuclei. About this time the central vacuole is formed, and soon afterwards another nuclear division takes place, resulting in sixteen free nuclei, distributed equally about the periphery of the sac, in the rather thick cytoplasmic layer.

Whether in any case more than sixteen nuclei are present before fertilization remains to be seen—nor is it yet quite clear just what is the origin of the ovum.

That the structures here described are formed *before fertilization* is not to be doubted. The closely set flowers make it very easy to follow the successive stages in longitudinal sections of the flower-spike, and unbroken series of stages have been repeatedly followed.

The further development also presents some apparent anomalies, but these have not yet been studied with sufficient care to warrant any positive statements.

The material used was collected at the Royal Gardens, Kew, and my sincere thanks are due to the Director for this, as well as much other material.

D. H. CAMPBELL.

DRESDEN, *November*, 1899.

Vol. XIII. No. XLIX. MARCH, 1899. Price 14s.

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Oxford

CLARENDON PRESS DEPOSITORY, 116 HIGH STREET

1899

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