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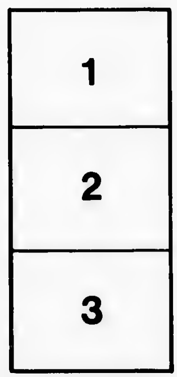
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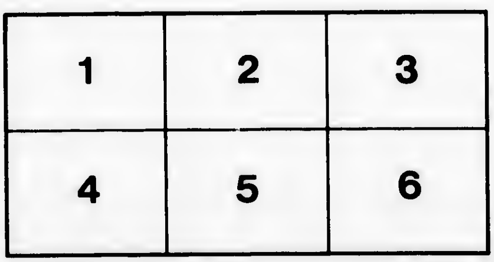
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ERRATA.

Instead of "latter" in 19th. line of page 6. read "former."
Instead of "*phlegmaria*" in 29th. line of page 6. read "*Phlegmaria.*"
Instead of "Rhabenhorst" in foot-note of page 12. read "Rabenhorst."
Instead of "*phlegmaria*" in 17th. line of page 14. read "*Phlegmaria.*"
Instead of "hypobasal" in 3rd. line of page 28. read "epibasal."
Figures 7, 8, 9, 10, 11, 12, 13 and 14 are all lithographed from photographs.

THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM.

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THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM.*

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I.

On account of their subterranean and inconspicuous prothallus and the slow germination of their spores, the literature on the subject of the sexual generation of the *Ophioglossaceæ* is somewhat scanty.

Hofmeister[1] was the first to give an account of the gametophyte in this group. His friend Irmisch sent him specimens of the very young sporophyte of *Botrychium Lunaria* in 1854. On visiting the spot where the young plants had been discovered, he found other examples, some of which were still attached to the maternal prothallus. The latter, he describes as being oval in shape and about a millimetre in length, of light brown colour externally, and yellowish white in section. The cells were filled with clumps of material not of a starchy nature. *Antheridia* were found mainly on the upper surface, the *archegonia* being situated below. Root-hairs were sparingly interspersed among the sexual organs. The antherozoids resembled those of the other *Filicineæ*, but were about one-half larger in size. The *archegonia* were sunk almost level with the surface of the gametophyte. One prothallus was found still attached to its spore, but attempts to germinate other spores, under observation, were unsuccessful. No young embryos were obtained, nor was it possible to study the development of the sexual organs. As a result of the inferior position of the *archegonia*, the young sporophyte appeared on the lower surface of the prothallus. The root grew out first, indeed two roots often made their appearance, before the first leaf became visible. The latter was bract-like and colourless. The two following leaves resembled it, but they had, either one or both of them, green tips. The fourth frond conformed to the usual type, and probably made its appearance in the next period of vegetation. From the situation of the embryo on the lower surface of the prothallus, the

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* Most of the material for this investigation was secured by means of a grant from the Elizabeth Thompson Scientific Fund.

1. Abhand, d k. Sächs. Gesellschaft d. Wissch. Bd. ii., pp. 657-662.

growing shoot was forced to make a half turn to assume its normal, negatively geotropic position.

In 1856, Mettenius[2] published an account of the sexual phase of *Ophioglossum pedunculosum*, which he found in considerable quantities, in the earth of the pots containing the adult spore-plants. Attempts to germinate the spores, under observation, failed also in this case. The youngest prothallia were tuber-like in shape, and one to three millimetres in thickness. Out of the tuber grew subsequently a conical process which elongated considerably (four to fifty millimetres), and sometimes branched. At the tip of the outgrowth, or of its ramifications, was found an apical cell, sometimes at least, of triangular pyramidal shape. The cylindrical portion of the prothallus grew upwards towards the surface of the soil, but, on reaching the light, became green and died away at the apex, or divided into two or three lobes which flattened out on the earth and developed no further. The tuber was composed of starch-laden parenchyma. In the process some textural differentiation was found, there being an axial, elongated, starch-free strand, surrounded by short starch-bearing cells. Both kinds of sexual organs were found in the same plant and not arranged in any definite order, but generally situated on the cylindrical process. The *antheridia* were large in size and their wall was generally two layers of cells in thickness. The antherozoids were large also, and composed of one and a-half to two spiral turns. The *antheridium* opened by a pore produced by the breaking away of two superimposed cells in its wall. The aperture was generally situated in that part of the wall nearest the apex of the prothallium. The spermatozoids swarmed out of the mother cells and about in the cavity of the *antheridium* before making their way out. The *archegonia* originated from two superficial cells, the upper of which gave rise by repeated divisions to a neck of three to five tiers of cells; the lower formed the axial row, which were not, however, made out individually by this writer. On account of the small number of embryos found, it was impossible to follow stage by stage, their development. Nothing was noted in regard to the formation of the first dividing walls. The youngest embryo was oval in shape and already segmented into a number of cells. The older ones were similar in configuration, but of larger size. The anterior end of the elliptical embryo grew through the tissues of the prothallium towards its apex, and bursting forth sooner or later, became the cotyledon, green in colour, and lanceolate in outline. The root developed more slowly and bored its way directly outwards. A rounded protuberance at the

2. Filices Horti Botanici Lipsiensis, pp. 119-120.

junction of the cotyledon and root, probably the foot, fastened the young sporophyte to the base of the *archegonium*. The apical bud appeared sometimes at the point of union of root and leaf, and sometimes further down on the root, thus simulating the adventitious buds arising from the roots of the adult plant.

The most recent contributions to our knowledge of this group is due to the discovery of the gametophyte of *Botrychium virginianum* by Professor Douglas Campbell[3] at Grosse Isle, Michigan, in 1893. The prothallia were unfortunately, like those of Hofmeister's *Botrychium Lunaria*, which they resembled in appearance, although larger in size, too old for the study of the development of the sexual organs and embryo. They are described as being flattened tubers with folded upper margins, covered with root-hairs and bearing the reproductive organs on the superior surface. Brown externally, white in section, the lower part of the gametophyte harboured an endophytic fungus. The *archegonia* had rather long and straight necks, while the *antheridia* were quite endogenous like those of *Equisetum* and *Marattia*. No young embryos were found, but only advanced young sporophytes, bearing already the first or a subsequent leaf.

Professor Campbell was the first to bring about the germination of the spores in this group. The process is exceedingly slow, requiring, even in the warm climate of California, for *Botrychium virginianum*, eighteen months or more, and for *Ophioglossum pendulum*, somewhat less than that time. The most advanced stages yet obtained by him, had only undergone two or three divisions. Chlorophyll was found in the young prothallium of *Botrychium virginianum*, and a suspicion of chlorophyll in that of *Ophioglossum pendulum*. This may have been due merely to the fact that germination took place in the light.

As there has been a tendency in recent years to associate the *Ophioglosseæ* with the isosporous *Lycopodineæ*, it is necessary to state briefly what is at present known concerning the gametophyte in the latter group. Fankhauser[4] discovered in 1872 the brown subterranean prothallus of *Lycopodium annotinum*. The examples found by him were lobed, tuber-like, and marked by numerous ridges and depressions. *Antheridia* and fully formed sporophytes were found on them, hence the prothallia must have been monœcious. In 1884, Bruchmann[5] found some much younger prothallia. These were of oval and flattened form,

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3. Trans. British Association, Oxford Meeting, 1894. Structure of Mosses and Ferns, 1895, pp. 224-228
4. Bot. Zeitung. 1873. No. 1.
5. Bot. Centralblatt. Bd. i., 1885, pp. 23-28.

the superior margin being raised so as to produce a depression in the centre. The *antheridia* occupied ridges in the bottom of this basin. No *archegonia* were present, nor did the plants show a definite apical meristem. The same observer remarked that the inferior cells of the prothallus were occupied by an apparently symbiotic fungus, the *mycelium* of which communicated with the outside by means of the root-hairs with which the plants was provided. He referred the symbiont to the genus *Pythium*. More recently Treub[6] has published a description of the prothallium of *Lycopodium cernuum*. Here the gametophyte, as in *Ophioglossum pedunculosum*, starts from a primary tubercule, and divides subsequently into green lobes. The sexual organs have no definite arrangement and are monœcious. The *archegonia* possess a single uninucleate canal-cell. The large *antheridia* have a single-layered outer wall and produce biciliate moss-like antherozoids. The embryo is peculiar in the possession of a rudimentary suspensor. The stem in the young sporophyte is at first represented by a parenchymatous mass which has been designated the primary tubercule. The first division in the embryo is transverse and gives rise to the epibasal and hypobasal cells. The latter originates first the cotyledon; the stem-apex apparently not developing till after several leaves have grown out. The first root also is derived from this segment, but only after a number of foliar organs have unfolded. The prothallus in this case was likewise occupied by a symbiotic fungus, which was considered by the author to be a species of *Pythium*.

Goebel[7] about the same time described the sexual phase of another species, *Lycopodium inundatum*. It closely resembled *Lycopodium cernuum* in structure, and also harboured a fungus resembling *Pythium*. Treub[8] has also published an account of another form, viz: *Lycopodium phlegmaria* which is slender, much branched, and entirely subterranean. It is especially interesting on account of the occurrence of a number of canal-cells in the *archegonium* and from the presence of paraphysis-like growths among the *antheridia*.

II.

In 1895, the writer came upon a large number of prothallia of *Botrychium virginianum* in a Sphagnum-swamp behind the village of Little Metis, in the Province of Quebec. The presence of these plants was revealed by the greenish-yellow cotyledons appearing above the surface

6. Etudes sur les Lycopodiacées. Annales du Jardin botanique de Buitenzorg. Tome iv., v., vii. viii., 1884-1890.

7. Bot. Zeitung. 1887. No. 11-12.

8. Op. Cit.

of a slight depression in the moss. On removing some of the overlying vegetation, numbers of the larger prothallia were easily obtained. It required, however, careful sorting of the peaty soil with the fingers to secure the younger and more interesting stages. Nearly a week was spent in working over about half the bed, the result being several hundred examples in all stages of development, of the gametophyte and attached sporophyte. Subsequently, in another season, a week was spent on the spot, and all the plants which careful sifting of the soil would yield, were removed. The second harvest amounted to over six hundred specimens, by far the larger number of which, however, were much too old for study. During the same summer, other and older plants were found in rich woods about two miles back of Metis. In the spring of 1896, additional discoveries were made in Foster's Flats, below the Whirpool, on the Niagara River, and on the east branch of the river Don, a few miles from Toronto. The last mentioned spot proved rich in interesting examples of older stages of the attached sporophyte. Most of these were removed last autumn (1897).

III.

One of the greatest difficulties in the way of the present research, was the proper preservation of the prothallia. They are singularly impermeable to fixing reagents on account of the thick external cuticle, and must be cut at intervals with a razor, to allow the preserving medium to penetrate. The presence of oil in large quantities in the tissues, also renders aqueous fluids useless, as they scarcely make their way in at all. A saturated solution of picric acid in thirty per cent. alcohol, gave fairly good results; but the best fixation was obtained by using a mixture of three parts of a saturated solution of corrosive sublimate in ninety per cent. alcohol, and one part of saturated solution of picric acid in the same menstruum, diluted with distilled water to reduce the alcohol to thirty per cent. strength. The same reasons which rendered the material hard to preserve, made it difficult to embed. Paraffine was mainly used, and the most satisfying results were obtained by infiltrating with benzole, in a vertical tubular dialyzer with a chamois leather diaphragm, revolved slowly by means of clockwork. It was found that the ordinary type of stationary dialyzer was quite unsuitable for these very delicate objects. When the prothallia in alcohol were placed in the top compartment, and the benzole below, the osmosis was exceedingly slow; and, if the position of the media was reversed, the weight of the benzole carried it through too rapidly, and injurious shrinkage was the result. The continued reversing of the relative positions of the two liquids by the clock movement, and the accompanying agitation, were found to overcome these inconveniences. Unfortunately, this device was hit upon only after numerous experiments, and when the investigation was almost completed. The transference from benzole to paraffine was effected in a stationary dialyzer, or by evaporating off the benzole in a water-bath, from a ten per cent. solution of paraffine in benzole. Celloidin embedding has also great advantages, but as the material has to be cut into slices not thicker than two millimetres at most, and as the prothallia were often nearly twenty millimeters in length, it was only employed for sections through certain regions of the gametophyte, and for the much less impenetrable young sporophyte. The stains chiefly used were either a combination of alum-cochineal and eosin, or aqueous saffranin, made by dropping a small amount of saturated alcoholic solution of equal parts of Grübler's alcohol and water soluble saffranins. This last method seems worthy of a wider application.

IV.

The youngest prothallia obtained were already two millimetres in length by one and a-half in breadth. As may be seen from figure 1, they are of flattened oval shape, and covered with hairs. The growing point is at the narrow thin end, and the prothallium thickens and widens from thence backwards. *Antheridia* alone are found at this stage, and are entirely confined to the upper surface of the gametophyte. They form a cluster at the older end, but thin out into a narrow median row as they extend forward towards the growing point, figure 1, *ar.* In somewhat larger and older plants, the median row of *antheridia* is raised on the crest of a distinct ridge, and the *archegonia* begin to make their appearance upon its sides, figure 2. The antheridial ridge is a marked feature of most of the older prothallia, and must have the same significance in the process of fertilization as the inferior archegonial prominence possesses in the leptosporangiate *Filicineæ.* In more mature individuals the ridge is obliterated, especially in the posterior region of the prothallus, by the more rapid growth of the sides of the latter, which seems to be a provision for the nourishment of the fertilized *archegonia.* This phenomenon probably is the cause of the antheridial ridge not being noticed by Campbell [9]. Figure 3 shows a plant in which an embryo, *em.*, has already reached a considerable size. The antheridial prominence is still very marked; the root-hairs, however, have largely disappeared. In figure 5, we have a somewhat younger stage with the rhizoids still abundantly present, especially in the

9. Op. Cit.

younger anterior region of the prothallus. Figure 4 is of a lobed gametophyte; figure 6 shows a similar condition in which two embryos, *em.* 1, *em.* 2, are to be seen. The depression of the antheridial ridge in the posterior region by marginal growth is particularly well-marked. These lobed forms are quite abundant among the Metis specimens, but the Toronto plants did not manifest this peculiarity. I am inclined to believe that the conditions of life in the two cases may have been the cause of this difference. The Metis specimens were found in wet, peaty soil. The Toronto plants, on the contrary, grew in rich, yet rather dry, forest mould. Older lobed prothallia have almost invariably two sporophytes attached to them. In figure 7, is represented an example in which the first root of the young sporophyte has reached a considerable size. At this stage the axis of the young sporophyte, which, in earlier phases, is nearly always at right angles to that of the prothallus, becomes often more or less oblique, as in the example figured. This rotation of the axis is probably due to the continued growth of the prothallium after the formation of the embryo. Figure 8 shows a prothallium in which two roots of the attached sporophyte have grown to a considerable length, although the cotyledon is short and still unfolded. In figure 9, we have a small gametophyte with only one root, and yet having the cotyledon fully expanded. The first leaf may expand either after one, two, or three roots have been formed, according to the vigor of the plant, and may always be recognized by its seeming to grow out of the proximal end of the first and stoutest root. Figure 10, is of a strong plant with three precotyledonary roots. The lamina of the cotyledon is not bilaterally symmetrical, as in most of the *Filicineæ*, but of the palmate type represented by *Ophioglossum pedunculosum*. As may be seen from figures 9 and 10, the first leaf varies considerably in complexity in accordance with the greater or less robustness of the plant from which it originates. In the next drawing, figure 11, is represented a lobed prothallium, on which are two older sporeplants, deprived of the leaves of the year of their collection. Figure 12 shows a Toronto specimen, bearing two well-advanced sporophytes. Figure 13 is a representation of a bifurcated sporeplant, two examples of which have been found. Figure 14 is interesting, for it represents a sporophyte which has already developed the fertile ventral segment, and is yet still attached to the mother prothallium. The sporeplant in this case is eight years old, as indicated by the number of foliar lacunæ in the fibro-vascular cylinder. There seems to be little danger of error in drawing this inference, for a considerable acquaintance with the young sporophyte enables me to state positively, that never more than one leaf is developed at a time, and in all probability, only one in a year. Attached sporophytes, five or six years old, are sufficiently common, as has been already stated in the preliminary notice.[10]

The prothallia described in the foregoing account were from two to twenty millimetres in length, and from one and a-half to fifteen millimetres in breadth. The gametophyte of *B. virginianum* is thus considerably larger than any geophilous prothallus which has yet been described. Attempts have been made to germinate the spores of this species, but although these are still undecayed, no signs of growth have yet made their appearance after eighteen months. Professor Douglas Campbell got them to sprout in less time than this, but doubtless the warmer climate of California had some influence in hastening the process. He found a few large chloroplasts in the young plants; but it seems probable that the presence of chlorophyll here is accidental, and depends on the spores being sown contrary to the natural conditions, in the light. An analogous phenomenon occurs when potato tubers are grown under conditions of illumination. Most of the prothallia collected by the writer were found ten centimetres or more below the surface of the soil. Mature sporophytes have been dug up, with the foot-tubercle still intact, and buried often thirty centimetres in the ground. These facts make it very difficult to imagine that the tubercular, deeply subterranean, gametophyte of *B. virginianum* can have been preceded by a green aerial phase as are the quite superficial, colorless, gametophytic buds of *Vittaria*, *Trichomanes* and *Hymenophyllum* described by Goebel, or the larger tuberlike, resting phase of the liverwort *Geothallus* recently studied by Campbell. It is perhaps worth while to suggest that the slow germination of the spores in the case of Pteridophyta, with subterranean prothallia is an adaptation to enable the former to reach a favorable depth in the substratum, before beginning their growth.

V.

A cross-section of the prothallus, such as is represented in figure 15, reveals a number of important features. The antheridial ridge, *x.*, is seen above, containing several *antheridia*. On its sloping sides are the *archegonia*, *y.* Multicellular hairs are often found attached to the ridge, to its flanks and to the base of the prothallium. The position of several of these is indicated in the figure at *h.* The internal cells, *a.*, of the upper part of the plant appear light in color, and contain protoplasm and small quantities of starch. The lower cells, *b.*, both in fresh and stained sections, are dark-colored, and in their natural condition, filled

10. Can. Inst. Proceed. Vol. i. Pt. 1, p. 10. Annals of Botany. Vol. xi., p. 485.

with a heavy oil which is not readily soluble in alcohol. They are likewise occupied by a filamentous fungus which is presently to be described. Figure 16 illustrates a median long-section of the prothallus. At *x.* is seen the antheridial ridge cut lengthwise, and showing the *antheridia* in various stages of development. The younger ones are found nearer the anterior, sloping, apical region, *a.p.* The distribution of the fungiferous tissue is represented in this figure. It is to be noted that it extends forward gradually, as the prothallus increases in length, by the activity of the apical meristem. The fungus never occupies all the cells on the lower side of the prothallus, but leaves free always a few of the lower tiers. Above, as has been already stated, there is a considerable mass of cellular tissue underneath the reproductive organs, quite free from infection and containing a small amount of starch. The symbiont is always present, as it has never been missed in the four or five hundred plants which have been minutely studied. It is not possible to state whether it is indifferent or beneficial to its host; it certainly does not seem to be injurious. The infected cells do not apparently suffer, and perhaps the presence of oil in them, may be interpreted as an indication of improved nutrition. Only experimental cultures can settle this important question.

The growing region of the prothallus is always on the upper side, figure 16, *a.p.* It is marked by the presence of a superficial layer of high columnar cells like those found at the base of the apical incision of the leptosporangiate gametophyte. These are represented in figure 17. One of the columnar cells, *a.*, is in all probability, the initial cell. It is very difficult to secure exactly horizontal sections of the apical region except in very young plants, of which my supply was somewhat limited. These were all used up for longitudinal and transverse series, and I am accordingly unable to describe the horizontal configuration of the initial cell.

The root-hairs are from one to four millimetres in length and are often multicellular, especially when they arise from the crest or flanks of the prothallium. Those which originate from the base are unicellular and longer than the others. These rhizoids are generally about twenty micra in width and are more or less completely cutinized. It is chiefly through them that the symbiotic fungus makes its way into the prothallium. The passage of the fungal *hyphæ* through the cutinized wall of the root-hair, is marked by the formation of thick sheaths which surround the *hyphæ* for ten or more micra of their course. These sheaths are apparently only formed where the fungus has to penetrate an already cutinized wall, and one does not find the phenomenon repeated as the *hyphæ* pass successively through the walls of the internal cells of the host-plant. Figure 21 represents a broken root-hair, the basal wall of which has become cutinized and consequently forms a sheath where the *hypha* is passing through. The penetration of the next cell-wall inwards is unaccompanied by this phenomenon. In figure 18 can be seen part of a root-hair, *c.*, on the lateral walls of which are two sheaths, and the hair in this case being intact, sheaths are not formed in the uncutinized basal wall. In the same figure sheaths can be seen at *b* and *d*, where the fungus has passed in through ordinary superficial cells of the prothallus. This is apparently of rare occurrence.

After penetrating about two or three layers of cells, *y*, the symbiotic filaments, which are from two to four micra in diameter, begin to grow luxuriantly, and fill the succeeding strata of cells, *x*, with a much-coiled *mycelium*. If this be examined with a good apochromatic objective, it is possible to discover that it is by no means always filamentous, but that in many cases, the *hyphæ* expand into large thin-walled vesicles, which are often so abundant that they fill the cells with a botryose mass resembling a *Completoria*, figure 19, *b* and *c*. In other cells the filaments prevail, *ibid. a.* It is not difficult to satisfy oneself that the *hyphæ* and vesicles belong to one and the same *mycelium*, figure 19, *b*. Frequently some of the vesicular structures become ruptured and shrivel up, *ibid.* Figure 20 shows a freshly infected cell of the prothallus, highly magnified, in which the vesicular structures have just begun to form. Often the advance of the symbiont through the prothallus is marked by the penetration of filaments or by a mixed growth of *hyphæ* and vesicles into new cells. Another kind of organ is also found in the *mycelium*, viz., *conidia*. These are thick-walled and from fifteen to twenty micra in diameter. They are generally formed at the end, but sometimes, though rarely, in the course, of a *hypha*, and are filled with a dense, coarsely granular protoplasm. The contents of the *conidium* are not separated from the filament by a septum and thus resemble the *conidia* of the subform *Aphragmium*[11] of the genus *Pythium*. The *conidium* germinates *in situ*, forming a tube which often makes its way into the adjoining cells of the host-plant. I have never been able to detect the formation of zoöspores from these *conidia*, and indeed it is difficult to imagine how they could serve as a means of distribution for so completely endoparasitic a fungus. The stages of formation and germination of the *conidia* are shown in figure 22, *a*, *b* and *c*.

It will be seen from the above account that the symbiont of *Botrychium virginianum* presents several rather remarkable characteristics. In its mode of penetration it resembles *Completoria complens*, as described by

11. Rhabenhorst, Krypt, Flora. Fischer, Phycomyceten, p. 397.

Leitgeb[12] in the prothallia of *Pteris cretica*, *Aspidium falcatum*, and other ferns; the formation of the dark brown sheath from the cell-wall of the host-plant being very characteristic. Atkinson[13] has described a similar phenomenon for a *Completoria* found in the same species of prothallia in America. In the filamentous portion of the undivided *mycelium* as well as in the formation of its *conidia* it markedly resembles a *Pythium*. In the botryose vesicular masses completely filling the cells of the host, it again strikingly simulates *Completoria*. It may perhaps fairly be considered as a form uniting the genera *Pythium* and *Completoria*. If, on further investigation, the above view proves to be correct, it may possibly be necessary to remove *Completoria* from the vicinity of the *Entomopthoraceæ*, where it has been placed on account of its ejaculatory *conidia* by Nowakowski and Thaxter, and toreplace it with the *Peronosporaceæ* where Leitgeb, as a result of his careful investigation, considered it to belong.

The endophyte of the prothallium of *Botrychium virginianum*, unlike that of *Lycopodium cernuum*, described by Treub,[14] and that of *L. annotinum*, described by Bruchmann,[15] is always intracellular and never becomes intercellular, in the deeper layers of the host-plant. Treub's description is somewhat brief, but from the fuller account of Bruchmann, the structure of the *mycelium* in the symbiont of *Lycopodium* seems to be quite different from that of the form found in *Botrychium virginianum*.

Only further study of the fungus can settle whether it is a distinct species of *Completoria* or *Pythium*, or, on the other hand, an intercalary species. Before leaving this subject, there is one more interesting fact to record. In older prothallia bearing well-advanced sporophytes, the symbiont is shrunken and dead. Whether this state of affairs is rightly comparable to the similar phenomena observed by Frank in the *mycorhizæ* and *mycodomatia* of various Phanerogamia, at the time of flowering or seeding, and is to be considered as a digestion of the symbiont by its host, must for the present be left in suspense. The prothallia often continue to live long after the death of the endophyte. Nothing of the nature of an *oögonium* has yet been observed in any stage of development of the fungus.

VI.

The *antheridia* arise, after the first basal cluster has been formed, figure

12. Sitzungsberichte d. Akad. d. Wissch. Wien. Math.—Natwissch. Classe. Bd. 84. Abth. i., 1881, p. 291 and p. 307.

13. Bull 94. Cornell Experimental Station, p. 52, 53.

14. Op. Cit. i., p. 124.

15. Op. Cit. pp. 310-313.

1, always on the crest of the antheridial ridge, figure 23. The older *antheridia* are found generally higher on the ridge than the younger ones, figure 23, a^1, a^2, a^3. The first indication of the male organ is a richly protoplasmic superficial cell, which divides transversely, giving rise to a shallow outer cell and a deep inner one, figure 23 a^1. The former becomes transformed into the outer wall of the *antheridium*, and the latter originates by repeated divisions, the mother-cells of the antherozoids. In figure 24 is represented a young stage in which both the inner and outer cells have already undergone several divisions. When the *antheridium* attains about a third of its ultimate size, its outer wall is doubled by periclinal divisions. In figure 25 these are represented as just beginning. Subsequently, the mass of spermatocytes is shut off internally from the prothallium cells by further periclinal divisions, figure 23, a^2, a^3. Often the *antheridia* are accompanied by short multicellular hairs, resembling those found on the rest of the surface of the prothallus and comparable to the paraphyses described by Treub in *Lycopodium phlegmaria*, figure 26, *par.* The more pri itive mother-cells of the antherozoids possess large nuclei with numerous nucleoli, figure 27, *a.* After a number of simultaneous divisions of the spermatogenic tissue, the definite spermatocytes are formed. In these the reserve chromatin in the form of nucleoli has disappeared. The filar chromatin is arranged in what appears to be a true *reticulum.* When the formation of the antherozoids begins, the nucleus contracts somewhat and the bars of the chromatic *reticulum* become thickened, figure 27 *b.* The nucleus then assumes a lateral position, and begins to flatten out, figure 27 *c.* This process is continued, and by the lengthening out of the nucleus, the condensation of its chromatin, and the curvature produced by its position in the cell, the antherozoid is formed, figure 27 *d.* The interesting structure to which Webber[16] in his recent studies on the antherozoids of the *Cycadeæ*, has applied the name *blepharoplast*, and which he compares with the cilia-forming body lately discovered by Belajeff[17] in the *Filicineæ* and *Equisetineæ* has been looked for in the developing antherozoids of *Botrychium virginianum*, but has not been made out. This is probably due to the fact that osmic acid fluids could not be used as fixing reagents on account of the oil in the tissues, and because the stains employed were not those used by Belajeff, but either a combination of alum-cochineal and eosin, or aqueous saffranin alone. The material illustrative of spermatogenesis was somewhat limited in amount, and it was not thought advisable to risk the series by removing their covers

16. Bot. Gazette. Vol. xxiv., p. 233.

17. Ueber Nebenkern in Spermatog. Zellen u. d. Spermatogenese d. Farnkraütern. Berichte d. deutsch. Bot. Gesell. Bd. xv , pp. 337-339. Idem—Die Spermatogenese d. Schachtelhalm. Ibid. Bd. xv. pp. 339-342.

and re-staining with the reagents employed by Belajeff. The writer hopes to secure more young prothallia in the coming summer, in which event it will be possible to come to a decision on this important point.

The fully developed antherozoid forms a spiral of one and a-half turns and has the structure usual in the *Filicineæ*. The cilia come off from the attenuated, anterior end of the spiral. I could not decide, from the preserved examples which were the only ones I had the opportunity of examining under high magnification, the exact length of the ciliary region. The antherozoids, like those of *Ophioglossum pedunculosum* described by Mettenius[18], escape from the mother-cells while still within the *antheridium*. They swim about freely in its cavity, figure 28, *a* and *b*: sometimes still retaining their protoplasmic vesicles and in other instances being already freed from them, figure 27, c^1 and c^2. The spermatozoids make their way out by means of an aperture formed by the disappearance of two superimposed cells of the outer wall of the *antheridium*. They do not escape all at once, as is quite generally the case, but seem to be voided in several swarms, at intervals, under undiscovered conditions. The cavity of the *antheridium* is filled with a thin gelatinous matrix, resulting, probably, from the disintegration of the spermatocytic walls, figure 28, *a* and *b*.

VII.

As has already been stated, the *archegonia* originate on the flanks of the median ridge of the prothallia, figure 15, *y*. The youngest stage of the *archegonium* is a single, richly protoplasmic, superficial cell, which, as in the *antheridium*, divides subsequently into an outer shallow cell and an inner deeper one, figure 29. The former gives rise to the neck of the *archegonium*, and the latter to its axial row of cells. The next stage is the horizontal division of the inner rudiment which separates from it the large basal cell, figure 30. The superficial rudiment subsequently begins to divide, first, by anticlinal walls, figure 31; and then by periclinal ones, figure 32; thus forming the neck. The richly protoplasmic basal cell divides, figure 32; and then the upper axial cell undergoes a division, which results in the formation of the cervical canal-cell and the ventral cell; figure 33 and figure 34. In the latter figure is seen a paraphysis, *a*, which is in reality, only one of the multicellular hairs common over the whole surface of the younger parts of the prothallium. In figure 35, the nucleus of the cervical canal-cell has divided, and as may be seen in the next figure 36, the nuclear division

18. Op. Cit.

is not followed by the formation of a cell-wall, such as has been described by Farmer and Campbell in *Angiopteris*, *Marattia*, and *Osmunda*. From the study of many hundred *archegonia* in this stage of development, the statement is made with some confidence that such a wall is never present in *Botrychium virginianum*. In figure 37, is represented an *archegonium* in which the ventral canal-cell has made its appearance. One very rarely finds this canal-cell intact, as it quickly disintegrates and in preserved material, at any rate, is represented by an indistinct mass thrust against the wide base of the cervical canal-cell. In figure 38, is seen a ripe *archegonium* which has ejected its canal-cells. The apical cells of the neck are, as is usual in the Pteridophyta, thrust outwards. At the same time one frequently notices chromatolysis in the nuclei of the upper cells of the archegonial neck, figure 37, although this phenomenon is by no means invariably present.

The mature egg is large and possesses a very dense protoplasm, which however, generally encloses a hydroplastid. The free surface of the oösphere rises into a median elevation, the receptive prominence. Figure 38, was drawn from a preparation in which a single spermatozoid had entered the canal of the *archegonium*. It has not been possible to follow the stages of union of the sexual nuclei. After fertilization, the canal is generally occluded by the closing together of the neck cells, figure 39, although this is by no means invariably the case, figure 40. The oöspore grows to many times its original size before the first division takes place. Figures 39 and 40, represent two stages of the yet undivided oöspore. In figure 41, the first segmentation has occurred, and the basal wall is horizontal, as in the other eusporangiate Pteridophyta. In figure 42, the embryo has become divided into quadrants by the median wall, which is the next to appear, and which, in the majority of cases at least, is parallel to the long axis of the prothallium. The transverse wall next makes its appearance at right angles to the other two. In figure 43, is represented an embryo which has already undergone further divisions. The upper octants have been sub-divided before any similar activity has appeared in the lower segments. There is no indication of a suspensor, and as the lower part of the embryo is not loaded with food materials, it seems probable that the earlier divisions in the upper octants, are for the purpose of thrusting the young sporophyte deep into the prothallium, that it may be more easily nourished and attain its characteristically large size without exposure to injury. The divisions are not always so regular, as in the case of the embryo represented in figure 43. In some instances, the basal wall is rather oblique, and corresponding differences exist in the orientation of the ensuing divisions, figure 44. Quite often, too, no regular course of segmentation can be made out at all, as in figure 45. When the embryo is only a little larger than those figured in 43, 44 and 45, the basal, median, and transverse walls are quite obscured by subsequent divisions. It is not possible to detect any indication of apical initials such as commonly occur in the early phases of the leptosporangiate sporophyte, and such as have also been described in some, at least, of the eusporangiate Pteridophyta. The next phase which is chosen for representation, is that in figure 46. Although no apical cells could be made out in this preparation and others of the same age, there is in the example figured, a very considerable formation of periclinal walls in the upper internal region of the embryo. The whole lower portion of the young sporophyte forms the foot, figure 46 *f.* In figure 47, is shown an embryo in which the root and shoot have already become differentiated. The periclinal activity already referred to, has led to the formation of a large amount of tissue in the upper portion of the embryo, and this is supported on the broad basis furnished by the foot. A high merismatic epidermis has already become differentiated at *x*, the cells of which are very rich in protoplasm and have the elongated columnar configuration of the shoot meristemeta of most of the Pteridophyta. Among these, the one marked *a* seems to be the initial cell. At *y*, is a protuberance which is the outward indication of the first root. Within this, at *b*, is the apical cell of the root, distinguished by its darkly-stained protoplasm, and by the fact that it has just undergone its first periclinal division. The condition of the embryo of *Botrychium virginianum* at this stage, is remarkable in that the stem-apex appears before the first leaf. The cotyledon is consequently derived from the shoot meristem, just as the later leaves are, but as in the case of the latter, it is not possible to follow the changes in the meristem leading to the formation of the foliar rudiment. The difficulty is greater in the case of the cotyledon, on account of the comparative paucity of younger embryos which have been cut exactly axially. For this investigation nearly three hundred series o prothalli, from two to twenty millimetres in length, have been sectioned. In spite of this not inconsiderable labor, less than twenty per cent. proved to be of value, either because no embryos were present, which is very commonly the case; or being present, they were not cut in a truly median plane. The surface of the gametophyte presents such irregularities that the proper orientation of the younger phases of the embryo is entirely a matter of chance. So far as I am aware the embryo of the *Equisetaceæ* presents the only other case yet described, in which the primitive foliar organ is secondarily derived from the shoot-apex. Sadebeck[19] makes the following statement concerning the equisetaceous embryo:—"Nach meinen Untersuchungen bin ich vielmehr zu dem Resultat gekommen, dass die obere Hälfte des noch zweizelligen Embryo ganz unmittelbar die primäre Axe darstellt, aus welcher sich in gleicher Weise, wie später bei der erwachsenen Stammknospe die Blätter erzeugen."

The embryo of *Isoëtes echinospora*, as described by Campbell,[20] also resembles in a measure that of *B. virginianum.* It has a large foot originating from *both* the hypobasal quadrants, which by its position and size, at least, somewhat strikingly resembles that of *Botrychium.* In the case of the latter, it is quite impossible to state from which of the primitive divisions of the fertilized egg, the foot takes its origin. A resemblance also exists in the formation of the root and shoot from the upper part of the embryo. In *I. echinospora*, however, the cotyledon is the first shoot-organ to appear, and the stem-meristem does not definitely develop until later, although there is an indication of its existence from the first.

It is not to be supposed, however, that these resemblances are in any way to be considered as indicative of relationship, for the development of the embryo may vary greatly in the same natural group. In the *Marattiaceæ*, for example, both *Angiopteris* and *Marattia*, as described by Farmer[21] and Campbell,[22] are distinguished by the precocious development of the cotyledon. In *Danæa*, [23] on the other hand, it is the root which first shows considerable development. A somewhat similar state of affairs has been observed by the writer in the *Equisetaceæ. Equisetum arvense* and *E. hiemale* have a precocious root, whilst *E. limosum* and *E. palustre* develop first the shoot-organs. Among the *Ophioglossaceæ* themselves, in *Ophioglossum pedunculosum*, the cotyledon is the first organ to rupture the *calyptra.* In *Botrychium virginianum* and *B. Lunaria*, the root is prior in appearance.

In figure 48, is represented an embryo, which, although larger, is yet younger than that in figure 47. At *a* and *b* are probably the root and shoot initials. Figure 49 is an older stage than figure 47. The root, *r*, is already well advanced and its apical region is fully developed. Behind

19. Die Entwick. d. Keimes d. Schachtelhalme. Pringsheim. Jahrbucher f. Wiss. Botanik. Bd. xi., p. 582.

20. Annals of Botany, vol. v., p. 244.

21. Annals of Botany, vol. vi., p. 265.

22. Annals of Botany, vol. viii.

23. Brebner, G. On the Prothallus and Embryo of *Danæa simplicifolia.* Annals of Botany, vol. x., p. 109.

its terminal meristem are elongated cells which, later, give rise to fibro-vascular tissues. The cotyledon, *c*, is also for the first time visible, and beside it is the stem-meristem, *s*. Below is the very massive foot, *f*. Figure 50, lithographed from a photomicrograph, represents a still later stage of development. Here the root is almost ready to burst the *calyptra, cal.* The cotyledon is distinctly seen, and at this stage, for the first time, covers over the stem-apex, which now lies on the side of a transverse fissure. No vascular tissue appears till the root has grown to a length varying from five to twenty millimetres, and has burst the *calyptra*. The first tracheides arise in the proximal region of the root after it has emerged from the prothallium. Subsequently they make their appearance in the cotyledon and the stem-axis.

Before referring to the further developmental changes in the nascent sporophyte, it will be well to consider an interesting abnormality. In figure 51 is represented part of a prothallus in which tracheides are present, near a region of superficial decay. The decayed spot probably marks the position of an embryo which has been injured and in consequence has rotted away, So far as I have been able to learn, by reference to the literature on the subject, such prothallial tracheides are the invariable accompaniment of apogamy. Their presence was first described in connection with this phenomenon by Farlow[24] in the apogamus prothallia of *Pteris cretica*. They have since been seen by many observers under similar conditions. Lang[25] has recently found them in the interesting reduced, apogamous, sporangiferous sporophytes of *Lastrea dilatata*, Presl, var. *Cristata gracilis*, Roberts and *Scolopendrium vulgare*, L., var. *ramulosissimum*, Woll. According to Bower, tracheides also occur in the prothallia [endosperm] of certain Cycads. In view of the recent discoveries of antherozoids in the pollen-tubes of this group, it would be interesting to know if the Cycads also manifest the phenomenon of apogamy.

The example figured is the only occurrence of prothallial tracheides which has come under my notice in examining a large number of gametophytes. In this case both *antheridia* and *archegonia* were present. Recently an example of apogamy in *Pteris aquilina* has come under my observation in which an apogamous and a normal embryo were produced side by side on the same archegonial pad. The former was accompanied by a single prothallial tracheid. The apparent rarity of the phenomenon in *Botrychium virginianum* may be due to the conditions under which the Metis specimens, which I have almost exclusively

24. Quarterly Journal of Microscopical Science, vol. xiv., N.S., p. 266.

25. Annals of Botany, vol. xi., pp. 157-168; also, Proc. of Royal Society of London.

investigated, grew. They were found as has been already stated, virtually submerged in a peat-bog, and as a consequence, absence of proper water supply which has been noticed as a predisposing cause of apogamy, would not make itself felt. Possibly prothallia from the rich, rather dry soil of the Don valley might yield a greater number of examples. If we may infer apogamy from the presence of prothallial tracheides, the gametophyte of *Botrychium virginianum* is unique among the eusporangiate vascular Zoidogama, in this respect; unless the phenomenon is shown to be present in the tracheid-bearing Cycad endosperms described by Bower, and apogamy can no longer be considered as peculiar to the leptosporangiate *Filicineæ*.

Returning to the young sporophyte, the shoot-organs and the root possess fairly well marked apical cells, as is shown by Campbell[26] to be true also of the mature spore-plant. Figure 52 represents the terminal meristem of the young stem in vertical section. At *a* is probably the apical cell. In figure 53 the same region is shown in horizontal section. In figure 54 is the apex of the cotyledon in longitudinal section. Figure 55 represents a long section of the apex of the first root in an embryo which has not yet broken through the *calyptra*. A large primary segment is found on the side of the *pilcorhiza*, a state of affairs rarely seen in later stages of the root, as subsequently the small cells of the inner part of the root cap abut immediately on the apical cell. This is possibly to be explained by the comparatively slight development of the *pilcorhiza* which consequently requires only very occasional contributions from the apical initial. The root of *Botrychium virginianum* is an endotrophic *mycorhiza* and, as has been shown by Frank, there is a tendency to degeneracy in the root-cap of roots of this type. The apical cell is much more active on its flanks although even here it divides slowly, compared with the apical initial of the leptosporangiate *Filicineæ*. In figure 56 the root-apex is seen in transverse section, and unlike that of the stem, its initial cell is triangular in this plane.

Figure 57 shows an interesting case of polyembryony corresponding to that described by Treub[27] in *Lycopodium cernuum*. It was first noticed after a series had been made of what appeared externally to be a bifurcated embryo. The central cylinders of two plants, *a* and *b*, are shown; *a* is larger and much more abundantly supplied with reserve food-materials, which cause it to stain more intensely; *b* is smaller, less developed, and in a condition of malnutrition as is indicated by a corresponding paleness of hue; a^2 is the second root of embryo *a*, and is

26. Campbell. Mosses and Ferns; pp. 232, 235.

27. Études sur les Lycopodacées; Extrait vi., p. 11.

quite fully matured; a^3 is the foliar trace of the cotyledon, which is just being separated by a layer of decidual periderm; x is the central cylinder of a, with the trace of the second leaf just making its appearance; b^2 is the still embryonic second root of the smaller embryo b; b^3 is the young cotyledon and y is the central cylinder. Figure 58 represents a lower section in the same series with the same lettering as before; a^1 is the primary root of the better developed embryo, and b^1 is that of the smaller embryo. At a^2 is a prominence indicating the point of origin of the second root of the larger embryo. Figure 59 is of a section still lower down and passes through the common foot of the geminal sporophytes. The staining alone indicates the boundary between the two plants. Their central cylinders are separate throughout, but the fundamental tissues appear to be in textural continuity. A quite sharp demarcation, however, is produced by the different condition of nutrition of their cells; those on the side of a being loaded with starch; those of b, on the other hand, containing only a very small amount. Unwillingness to sacrifice the series prevented the use of the ordinary methods of demonstrating protoplasmic continuity for the purpose of discovering whether the protoplasm of the two was in reality continuous. The phenomena of nutrition would seem to negative such a supposition. Figures 57, 58 and 59 have been lithographed from photomicrographs.

The first root of the young sporophyte is sometimes diarchous, but just as often triarchous. There seems to be no relation between the vigor of the root and the number of protoxlyem-strands; as depauperate plants sometimes have three strands, and, on the other hand, robust individuals often have only two. I have not found a single example of a monarchous root in the large number of specimens which I have examined. Figure 60 is a drawing of a section of a diarchous primary root in aqueous analinsulphate. The endodermis a is quite distinct, and shows plainly the characteristic radial lignified zones. Between it and the vascular tissue are one or more layers of pericycle cells. The protoxylem tracheides, x, are reticulate in their sculpture and not ringed or spiral as is generally the case. The metaxylem elements almost always meet in the centre. The bast, y, is made up of thick-walled elements, some of which are sieve-tubes and the rest elongated parenchyma cells. Between the bast and the vessels, is a considerable amount of wood parenchyma. Often two or three diarchous roots are formed, but sooner or later triarchous, and finally tetrarchous ones are produced.

The central cylinder of the stem becomes fully differentiated below the point of origin of the cotyledon. From the very first it has a well-marked pith, figure 61 *m*. The pith communicates with the external fundamental tissue through a gap caused by the exit of the cotyledonary trace, as has been described by Van Tieghem[28]. The internal endodermis discovered in the younger portion of the stem of *Botrychium Lunaria* and others of the *Ophioglossaceæ* by Van Tieghem[29] and Poirault[30], is not present in this species, although the external endodermis is well-marked, only disappearing opposite the foliar gaps. The bast-tissue originates first in the young central cylinder and seems never to have any secondary additions from the activity of the *cambium*. Graf zu Solms[31] has thrown doubt on the existence of secondary wood in the *Ophioglossaceæ*, but in this species there can be no uncertainty as to its presence; in fact, the wood is practically all secondary, as may be learned from the radial arrangement of its matured elements and by following the course of its development, figure 63 *x*, and figure 64 *x*. The first-formed wood-elements are reticulately sculptured and are never of the ringed or spiral type. In this respect they resemble those of the stem of the *Marattiaceæ*, and, in fact, also those of the *Osmundaceæ*; for the groups of typical protoxylem elements found in the upper region of the bundles of the latter, really belong to the leaf-traces. It is more than probable that the absence of typical primitive tracheary tissue in all these cases, is due to the very slow growth of the stem, a phenomenon which renders their presence unnecessary. The writer has noticed the absence of these elements in the slowly growing stems of species of so-called polystelic *Primulæ*, viz:—*P. Auricula* and *P. farinosa*.

During this investigation, the rather interesting observation has been made, that the periderm-tissue first described in the *Ophioglossaceæ* by Russow[32] and Holle[33], is formed in *Botrychium virginianum* at the bases of defunct leaves, and thus is merely an abciss-layer. Figure 65, from a photomicrograph, shows a young sporophyte still attached to its prothallium; *r* is the first root and *x* the base of the cotyledon; l^2 and l^3 are developing leaves. As may be seen from the figure, the course of the cotyledonary bundle *x*, has been interrupted by the interca lation of a layer of periderm. Figure 66 shows the tissues in question under a sufficiently high magnification to make clear the details of periderm formation. By the continued growth of the latter the distal part of the

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28. Remarques sur la structure de la tige des Ophioglossées. Journal de Botanique, iv., Année; p. 407
29. Op. Cit.
30. Recherches sur les Cryptogames vasculaires. Annales de Sci. Nat. Bot. Tome xviii.; p. 170.
31. Fossil Botany, p. 223.
32. Mém de l'Acad. Imp. des Sciences de St. Petersbourg. vii. Serie. Tome xix., No. 1, p 117.
33. Bot. Zeit. 1875. Ueber Bau u. Entwicklung der Ophioglosseen, p. 12.

leafstalk is forced continually outwards and eventually decays, leaving no trace of its existence. This is the reason that, in transverse sections of older stems, the foliar bundles of fallen leaves apparently disappear before reaching the external cortex. The periderm formation of *B. virginianum* is thus connected with the occlusion of the leafstalks, and is probably to be explained as an adaptation for protecting the subterranean stem from infection by the fungi of the soil.

In a transverse section through the older region of the stem, the periderm is never found to form a continuous investiture as in the higher plants, but is strictly localized in areas representing the points of origin of former leaves. The writer has not yet had an opportunity of investigating whether the mode of cork formation obtaining in *B. virginianum* is common to the whole group, but it seems probable that this may prove to be the case. Periderm is also often formed both in the sporophyte and in the gametophyte where surface injuries have occurred: a striking case of correspondence between the two generations.

The cotyledonary trace originates from the central cylinder as a single strand, figure 61, *cot.*; but separates shortly after reaching the petiole into two approximately collateral bundles. These pass upwards through the long leafstalk into the lateral lobes of the lamina, one of them giving off a bundle for the median lobe, exactly as in the postcotyledonary leaves of many *Filicineæ*. The endodermis is never quite continuous on the inner side of the cotyledonary trace, and in subsequent leaves becomes less and less marked, till at the stage in which there are four petiolar bundles, it is entirely absent. Figure 67 represents the laminar portion of the ninth leaf of a sporophyte which was still attached to its prothallium. The fertile segment, *f. s.*, of the lamina is already present. This plant was at the same time the oldest sporophyte still in connection with the gametophyte, and the youngest already producing spores, which has come under my notice during the present investigation.

In figure 68 is a still attached young sporophyte. Its prothallium is infected with the already defunct symbiont, *a*. The spore-plant still bears its cotyledon l;[1] and two younger leaves, l^2 and l^3 are in the process of formation. In the primitive root, *r*, can be seen at *x* and *y*, certain dark spots which are cells occupied by the sporophytic endophyte. There is no resemblance between the latter and that of the gametophyte as its mycelial filaments are much larger, being generally about eight micra in diameter. There are no vesicles nor *conidia* present, and in fact the sterile *mycelium* is uniformly filamentous in character. These features are reproduced in figure 69. The occurrence of a symbiont in the roots of the *Ophioglossaceæ* has long been known, and is mentioned by Russow and Holle in the works already cited. The latter refers to its presence or absence, the varying number of protoxylem groups in the larger and smaller roots of *Botrychium matricariæfolium*. In *B. virginianum* this explanation cannot be accepted, as, although the first formed roots vary greatly in the number of archixyles, it is only in rare cases like that figured in 68 that the fungus is present.

VIII.

The results of this investigation may be summarized as follows :—

(1). The gametophyte of *B. virginianum* is entirely subterranean, without chlorophyll and probably symbiotic. It is from two to twenty millimetres in length by one and a-half to fifteen millimetres in breadth, and oval in outline, whether viewed from above or from the side.

(2). The whole surface of the plant is beset with rhizoids, which are generally multicellular. The upper part of the gametophyte is occupied in most prothallia, which have not yet produced embryos, by a median ridge. The reproductive organs are found exclusively on the superior surface, the *antheridia* being situated on the crest of the ridge, and the *archegonia* on its flanks.

(3). The gametophyte grows by a well-marked apical meristem which is situated on the upper side, anteriorly, and apparently originates from a single initial cell.

(4). There is present in the lower part of the prothallus, an endophytic fungus, possessing characteristics which will perhaps, on further study, justify its recognition as a form intermediate between the genera *Pythium* and *Completoria*. The symbiont is accompanied by a large amount of oil, and probably advantageously affects the nutrition of the prothallus. The fungus dies after one or more embryos have reached a considerable size.

(5). The *antheridium* originates from a single superficial cell and is characterized by possessing a double outer wall. The antherozoids are of the ordinary filicineous type and are rather large in size.

(6). The *archegonium* likewise takes its origin from a single superficial cell. The neck consists of seven or eight tiers of cells. The cervical canal-cell is binucleate, but is never represented by two cells. A stratum of basal cells is present.

(7). The first division of the fertilized egg is transverse, as in the other eusporangiate Pteridophyta. The identity of the octant walls which are formed in the usual way, is early lost, and the embryo grows to a relatively large size before the organs make their appearance. The root and shoot originate from the upper part of the embryo; and it may perhaps be inferred that, like those of *Isoetes echinospora*, they are derived from the upper octants. The foot is formed from the whole of the lower region of the embryo. The cotyledon is apparently derived secondarily from the shoot meristem.

(8). The root, the stem, and the cotyledon grow by the segmentation of a single apical cell, as in the adult plant. The root develops more rapidly than the other organs; and the second or third root may make its appearance before the cotyledon unfolds. The latter is green and capable of assimilation, as in *Ophioglossum pedunculosum*.

(9). The root-system of the young sporophyte is soon occupied by a symbiotic fungus, which differs in the size of its filaments and in several other respects, from that found in the gametophyte.

(10). Evidence of apogamy has been found in the form of prothallial tracheides.

(11). One example of polyembryony was observed.

(12). The sporophyte remains for a long time attached to the gametophyte. It is an open question whether this is a primitive characteristic, or merely an adaptation. The fact that the young sporophyte of the much less robust *B. Lunaria*, according to Hofmeister's account remains for a very short period attached to its gametophyte, would seem to justify the latter assumption.

IX.

In coming to any conclusions as to the bearing of this research on the phylogenetic position of the *Ophioglossaceæ*, due weight should be given to the fact that the present species is the only one which has been somewhat fully investigated; and the results of recent observations on the *Marattiaceæ*, *Lycopodiaceæ*, and *Equisetaceæ* show that a very considerable variety of development may exist even within the same natural group. Moreover the saprophytic habit of the gametophyte of *B. virginianum* has in all probability more or less profoundly modified its structure.

It will be convenient to consider first the position of *B. virginianum* in regard to the other representatives of the *Ophioglossaceæ* which have been studied. Its prothallus resembles very closely that of *B. Lunaria*, and shows indications of being only a more specialized type. That this is the case is rendered probable by the strict localization of the *antheridia* on the antheridial ridge, and by the occurrence of the reproductive organs on the upper surface of the gametophyte. It is interesting in this connection to note the scattered disposition of the *antheridia* in the very young prothallus; for this is probably to be regarded as a primitive feature. An embryological comparison between the two forms is not possible, as the embryology of *B. Lunaria* is at present unknown. The young sporophyte of *B. virginianum*, in that it is attached to the upper surface of the prothallus, and has a completely developed and assimilatory cotyledon, differs from the sporophyte of *B. Lunaria.* The young spore-plant also remains much longer attached to the gametophyte than is the case in the latter species. *B. virginianum* seems, of all the representatives of the genus in Canada at least, to be the most completely adapted to modern conditions; for it is everywhere abundant in rich woods, and always outnumbers the other species.

The prothallus of *Ophioglossum pedunculosum* does not very closely resemble that of *B. virginianum.* The presence of a primary tubercle and the formation of green prothallial lobes are its characteristic features. It should be remembered, however, that within the single genus *Lycopodium*, *L. annotinum* resembles in its prothallus *B. virginianum* and *B. Lunaria*, whilst *L. cernuum* and *L. inundatum* have a gametophyte like that of *Ophioglossum pedunculosum.* It is possible that a species of *Botrychium* may yet be found in which the prothallus is like that of *Ophioglossum pedunculosum.* The *antheridia* and antherozoids of the present species quite exactly resemble Mettenius' description of those of *Ophioglossum pedunculosum.* The *archegonia* correspond, too, in so far as the earlier description offers points of comparison. In the development of the embryo, the account of Mettenius is rather too meagre to allow of any exact inferences in regard to points of likeness in the successive phases of segmentation. The young sporophyte of *Ophioglossum pedunculosum* develops its cotyledon early, and the primary root is slow in pushing its way out, which exactly reverses the course of events in *B. virginianum* and probably also in *B. Lunaria.*

Bower[34] has recently fully discussed the relationships of the *Ophioglossaceæ* to the other groups of the Pteridophyta. He comes to the conclusion that the ventral fertile leaf-segment of the *Ophioglossaceæ* is the morphological equivalent of the single ventral sporangium of the homosporous *Lycopodineæ*, and derives it from the former by a process of septation and branching. He also compares the two groups in

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regard to the structure of the vegetative organs of the mature sporophyte, and finds that in this respect they also show a marked resemblance to one another. Lastly, the organization of the gametophyte and the development of the sporophyte, are discussed in the same connection with a like conclusion.

It is only necessary in considering the results of the present investigation, to examine the latter features. In regard to the structure of the prothalli, the two groups certainly do present marked likenesses; *e.g.*, the gametophyte of *Ophioglossum pedunculosum* to those of *Lycopodium cernuum* and *L. inundatum*, and the gametophytes of *B. Lunaria* and *B. virginianum* to that of *L. annotinum.* It is quite possible, however, that the resemblance in these cases is due to a similarity in environment.

The male organs of the two groups are in some important features quite different. The *antheridium* has a double outer wall in the *Ophioglossaceæ* and the antherzoids are spiral and multiciliate. In the homosporous *Lycopodineæ*, the *antheridium* has a simple outer wall, and the antherozoids have the general configuration and the two cilia of the antherozoids of the Bryophyta.

The *archegonia* of *B. virginianum* at least, resemble those of the *Filicineæ*, (excluding *Isoetes*, which probably does not belong here), in having a basal cell and a single binucleate canal-cell, or at most two neck canal-cells. On the other hand the *Lycopodineæ* and *Equisetaceæ* are without the basal cell and have a decided tendency to increase the number of cervical canal-cells. Too much importance should not, however, be attached to these structural features of the *archegonia.*

The embryo of *B. virginianum* and apparently that also of *O. pedunculosum*, lacks the suspensor and primary sporophytic tubercle which are so characteristic of most of the isosporous *Lycopodineæ*, and in these defects resembles the *Filicineæ.* So far as the facts in the case of *B. virginianum* go, it seems probable that the *Ophioglossaceæ* are much more closely allied to the eusporangiate *Filicineæ* than to the isosporous *Lycopodineæ*, although they may be possibly the nearest of the megaphyllous Pteridophyta to that group. In all probability, the *Ophioglossaceæ* are more primitive than the *Marattiaceæ* which they in some respects resemble.

As a result of the fuller knowledge in recent years of the segmentation of the embryo of the Pteridophyta, it is scarcely possible to retain any longer the conception of octants propounded by Leitgeb and others when the leptosporangiate *Filicineæ* were practically the only ferns in which anything of the embryology was known. In the homosporous *Lycopodineæ* the apex of the stem, the cotyledon, and the root, are all according to Treub's description, derived from the hypobasal half of the embryo. In *Isoetes, echinospora*, the same three organs, according to Campbell's account, originate from the epibasal octants, the foot being formed from *all* the hypobasal octants. No recent complete investigation of the embryology of the *Selaginelleæ* is available, but the phases of development described by Pfeffer can only be harmonized with the octant theory by something like a *tour de force*. In the *Equisetaceæ*, according to Sadebeck, the shoot originates from the upper octants, and the root and foot from the lower octants, the primitive leaves being derived secondarily from the shoot meristem. The *Ophioglossaceæ*, as represented by *B. virginianum* resemble embryologically *Isoetes echinospora*. The segmentation of the *Marattiaceæ* alone, agrees fairly well with the stages of development found in the leptosporangiate *Filicineæ*, and it is not very difficult in this group to refer the organs to definite pairs of octants. But of all the eusporangiate forms, the *Marattiaceæ* come closest to the leptosporangiates, and this probably is the explanation of their embryological agreement.

If we are to accept the hypothesis that the eusporangiate Pteridophyta are primitive, and if we follow Bower in deriving their sporophytic phase from the progressive sterilization of the potential sporogenous tissue of intercalary sporogonium-like forms, the axis is certainly to be regarded as primitive, and the leaves and roots must be considered as secondary outgrowths from the axis; either by eruption as Bower surmises, or by some other undiscovered process. According to this conception, foot and shoot are the primitive organs, and leaf and root are subsequently derived from the latter. This view of the matter harmonizes with what is known of the embryology of the lower eusporangiates. In the highly specialized leptosporangiates on the other hand, a process of acceleration and rearrangement has been carried out and the organs appear precociously, in definite relation, to the earlier segmentations of the embryo.

In conclusion, the writer wishes to express his special obligations to Professor G. L. Goodale of Harvard University for very kindly putting at his disposal the books of the Gray Herbarium.

EXPLANATION OF PLATES.

PLATE I.

FIG. 1.—Youngest prothallium found, *ar.* antheridial ridge. × 8.

FIG. 2.—An older stage in which the antheridial ridge has become more marked. × 16.

FIG. 3.—A considerably older gametophyte on which is a developing embryo, *em.* The antheridial ridge, *ar*, is particularly prominent. This prothallium is lithographed from a photomicrograph. × 7.

FIG. 4.—A lobed prothallus from a photomicrograph. × 4.

FIG. 5.—From a photomicrograph; represents a younger phase in which the root-hairs are abundant. × 8.

FIG. 6.—A lobed prothallus lithographed from a photomicrograph, and bearing two embryos, em^1 and em^2. × 4.

FIG. 7.—A young sporophyte showing the first root. × 8.

FIG. 8.—A young sporophyte showing two roots; the cotyledon is still unexpanded. × 4.

FIG. 9.—A young sporophyte with the primary root and the cotyledon. × 1.

FIG. 10.—A stouter sporophyte with three roots and the cotyledon. × $\frac{2}{3}$.

FIG. 11.—A lobed prothallus bearing two advanced sporophytes. × 1.

FIG. 12.—A prothallus bearing two further advanced sporophytes. × $\frac{1}{4}$.

FIG. 13.—A bifurcated sporophyte still attached to its prothallium. × 4.

FIG. 14.—An eight year sporophyte still attached to its prothallium. × $\frac{2}{3}$.

FIG. 15.—A cross-section of a prothallus showing the antheridial ridge, *x*; the fungiferous cells, *b*: and the uninfected cells, *a*. At *y* are the archegonia, and *h*, root-hairs. × 16.

FIG. 16.—A long-section of the prothallus; lettering the same as in the preceding figure. *ap*, apical region. × 16.

FIG. 17.—Apical meristem. *a*, apical cell of prothallus. × 250.

FIG. 18.—Showing the penetration of the fungus into the gametophyte. *c*, root-hair; *b* and *d*, superficial cells, in which the cutinized sheaths have been produced; *x*, fungiferous cells; *y*, uninfected cells; *a*, conidia. × 250.

FIG. 19.—Fungiferous cells; *a*, with purely filamentous mycelium; *b* and *c*, mixture of filamentous and vesicular mycelium. × 600.

FIG. 20.—Cell showing the formation of vesicles, *f*, as outgrowths from a hypha, *h*. × 1,000.

PLATE II.

FIG. 21.—Base of a broken root-hair; *s*, cutinized sheath; *b*, hypha of penetrating fungus. × 1,000.

FIG. 22.—*a*, formation of conidium; *b*, ripe conidium; *c*, germinating conidium. × 1,000.

FIG. 23.—Antheridial ridge showing three antheridia in different phases of development, a^1, a^2, and a^3. × 250.

FIG. 24.—An older antheridum. × 250.

FIG. 25.—A still older phase in which the outer wall is undergoing division. × 250.

FIG. 26.—Antheridial ridge showing the formation of paraphyses, *par*. × 90.

FIG. 27.—Development of antherozoids; *a*, young sperm-cells. × 500. *b*, definite spermatic mother-cells; *c*, a later phase of the same, the nucleus is beginning to become crescentic; *d*, young antherozoids within the mother-cells; *e*, ripe antherozoid. In e^1, the protoplasmic vesicle is still retained; in e^2, it has disappeared. × 1,000.

FIG. 28.—Matured antheridia showing the doubled outer wall; within, the antherozoids are swimming in a gelatinous matrix. In *a*, they are escaping. × 250.

FIG. 29.—First stage in formation of the archegonium. × 250.

FIG. 30.—A later phase showing formation of the basal cell. × 250.

FIG. 31.—Anticlinal division of the cervical rudiment. × 250.

FIG. 32.—Periclinal divisions of the cervical portion of the archegonium. × 250.

FIG. 33.—Nuclear division of the axial cell. × 250.

FIG. 34.—The same completed. A paraphysis at *a*. × 250.

FIG. 35.—Nuclear division of the cervical canal-cel × 250.

Fig. 36.—The same completed. × 250.

FIG. 37.—Ripe archegonium, showing the ventral canal-cell. × 250.

FIG. 38.—Opened archegonium with penetrating antherozoid. × 500.

FIG. 39.—Fertilized egg. × 250.

FIG. 40.—The same older and larger. × 250.

FIG. 41.—First division of the embryo. × 250.

FIG. 42.—Formation of the median wall of the embryo. × 250.

FIG. 43.—An older embryo in which anticlinal divisions are present in the upper octants. × 250.

FIG. 44.—Another embryo of the same age, with oblique walls. × 250.

FIG. 45.—The same age as the foregoing, showing irregular segmentation. × 250.

FIG. 46.—A more advanced phase showing periclinal activity in the upper cells of the young embryo at *a*; *b* is the foot region. × 250.

PLATE III.

FIG. 47.—An older embryo; *y*, the root; *x*, the shoot; *f*, foot; *a*, initial cell of shoot; *b*, initial cell of root. × 250.

FIG. 48.—A younger, but larger embryo than the foregoing, with the same lettering. × 250.

FIG. 49.—An advanced embryo; *r*, root; *c*, cotyledon; *s*, shoot; *f*, foot. × 160.

FIG. 50.—From a photomicrograph. Lettering as before; *cal*, calyptra. This embryo is considerably older than the foregoing. × 50.

FIG. 51.—Part of a prothallium containing tracheides; *a*, decayed spot where an embryo has probably disappeared; *t*, tracheides. × 250.

FIG. 52.—Apical region of the shoot in vertical section; *a*, the initial cell. × 250.

FIG. 53.—The same, in horizontal section; *a*, the apical cell. × 250.

FIG. 54.—Longitudinal section of the apex of the cotyledon; *a*, apical cell. × 250.

FIG. 55.—Apical region of the primary root; *a*, apical cell. × 250.

FIG. 56.—Tranverse section of the same; *a*, apical cell. × 250.

FIG. 57 Transverse section of two united embryos, *a* and *b*. a^2 is second root of *a*; a^3, cotyledon of *a*; *x*, central cylinder of *a*; b^2, second root of *b*; b^3, cotyledon of *b*; *y*, central cylinder of *b*. × 50. (From a photomicrograph).

FIG. 58.--The same, a section through a lower region. Lettering as in the previous figure. a^1, first root of *a*; b^1, first root of *b*. × 50. (From a photomicrograph.)

FIG. 59.—Section through the foot-region of the same embryos. Lettering as before. × 50. (From a photomicrograph.)

FIG. 60.—Transverse section of a diarchous primary root: *a*, endodermis; *x* xylem; *y*, phloëm; *b*, parenchyma. × 250.

PLATE IV.

FIG. 61.—Transverse section of the young stem, above the exit of the cotyledonary trace: *cot*. cotyledonary trace; *c.c.*, central cylinder; *m*, medulla. × 50. (From a photomicrograph).

FIG. 62.—The same, more highly magnified. × 160. (From a photomicrograph).

FIG. 63.—Part of the central cylinder of the foregoing, more highly magnified; *en*, endodermis; *y*, phloëm; *x*, xylem; *camb*, cambium; *s. t.*, sieve-tube; *m*, medulla. × 220. (From a photomicograph).

FIG. 64.—Part of central cylinder of quite a young plant; *en*, endodermis; *ph*, phloëm; *camb*, cambium; *x*, xylem; *m.r.* medullary ray.

FIG. 65.—Longitudinal section of an attached sporophyte; *r*, primary root; *x*, remains of cotyledon; l^2 and l^3, developing leaves. × 20. (From a photomicrograph).

FIG. 66.—The base of the cotyledon from the preceding, more highly magnified, showing the formation of abciss-periderm at *j*. × 160. (From a photomicrograph).

FIG. 67.—Lamina of an attached sporophyte, eight years old, showing the fertile segment, *f. s.*, and sterile segment, *s. s.* × 8.

FIG. 68.—Longitudinal section of an attached young sporophyte; l^1, cotyledon; l^2 and l^3, developing leaves; *r*, primary root; *x* and *y*, endophytic fungus of the sporophyte. × 20. (From a photomicrograph).

FIG. 69.—Cells of the primary root, containing the fungus of the sporophyte. × 420.

FIG. 70.—Transverse section of a prothallus: *ar*, antheridial ridge; *em*, an embryo. × 20. (From a photomicrograph).

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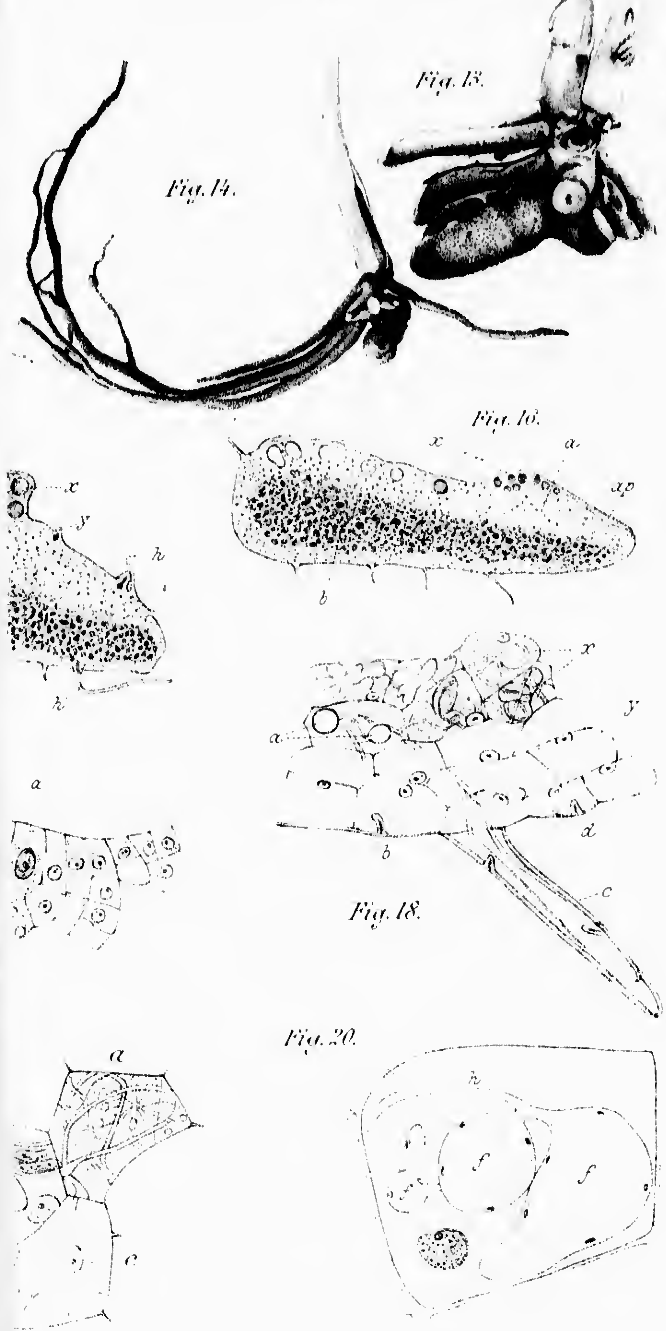

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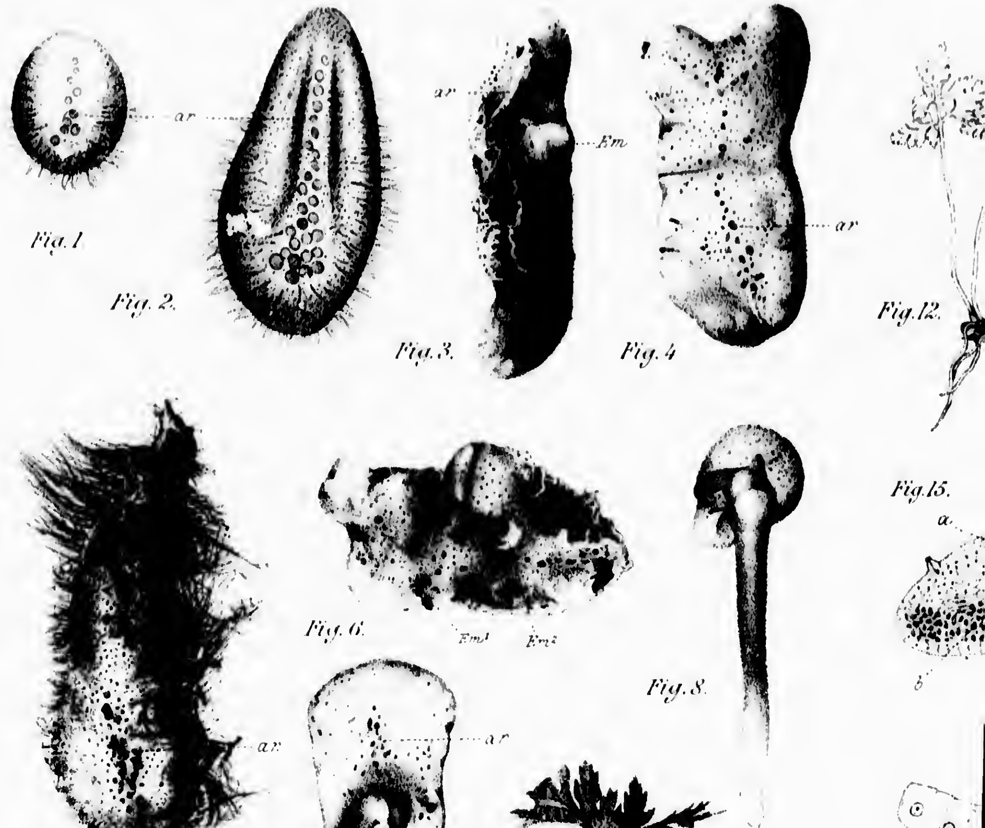

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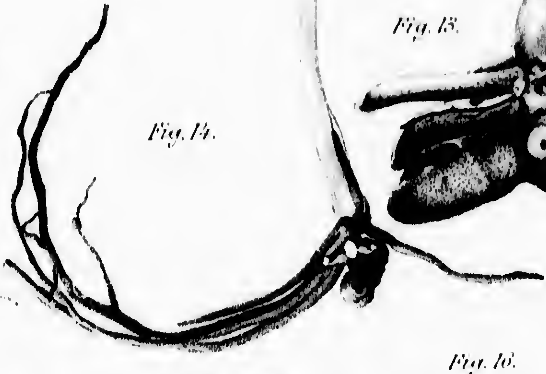

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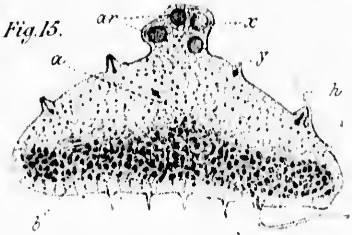

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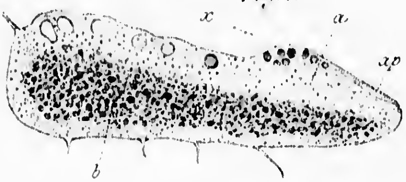

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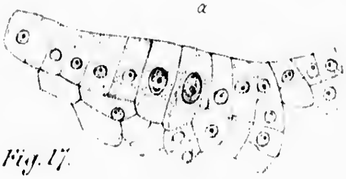

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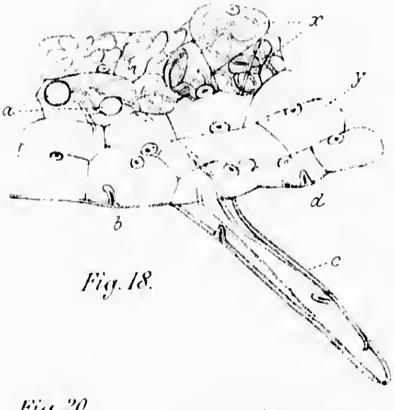

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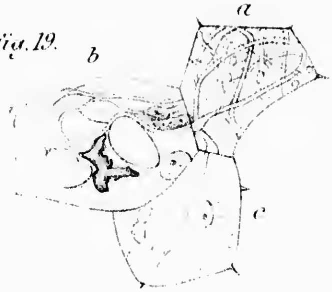

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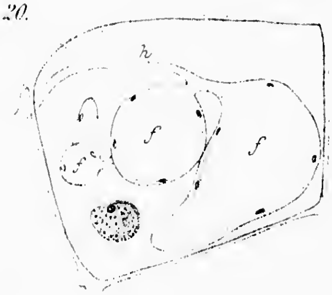

Fig. 20.

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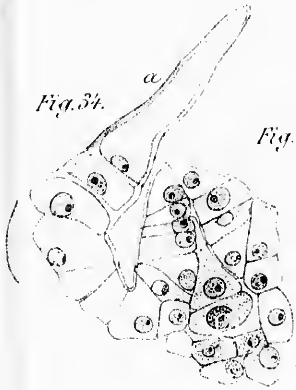

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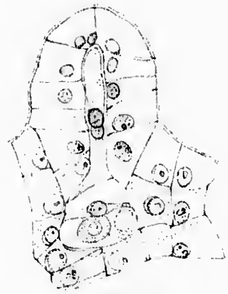

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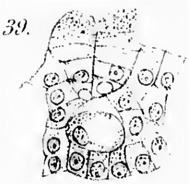

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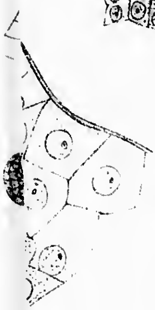

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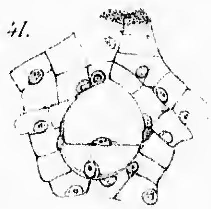

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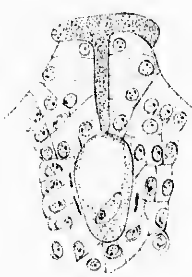

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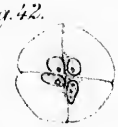

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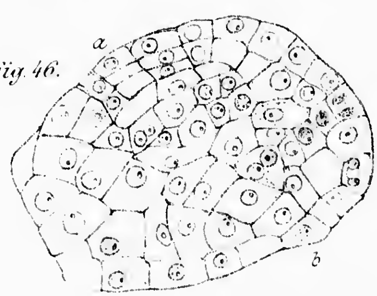

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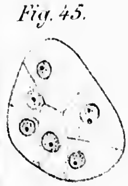

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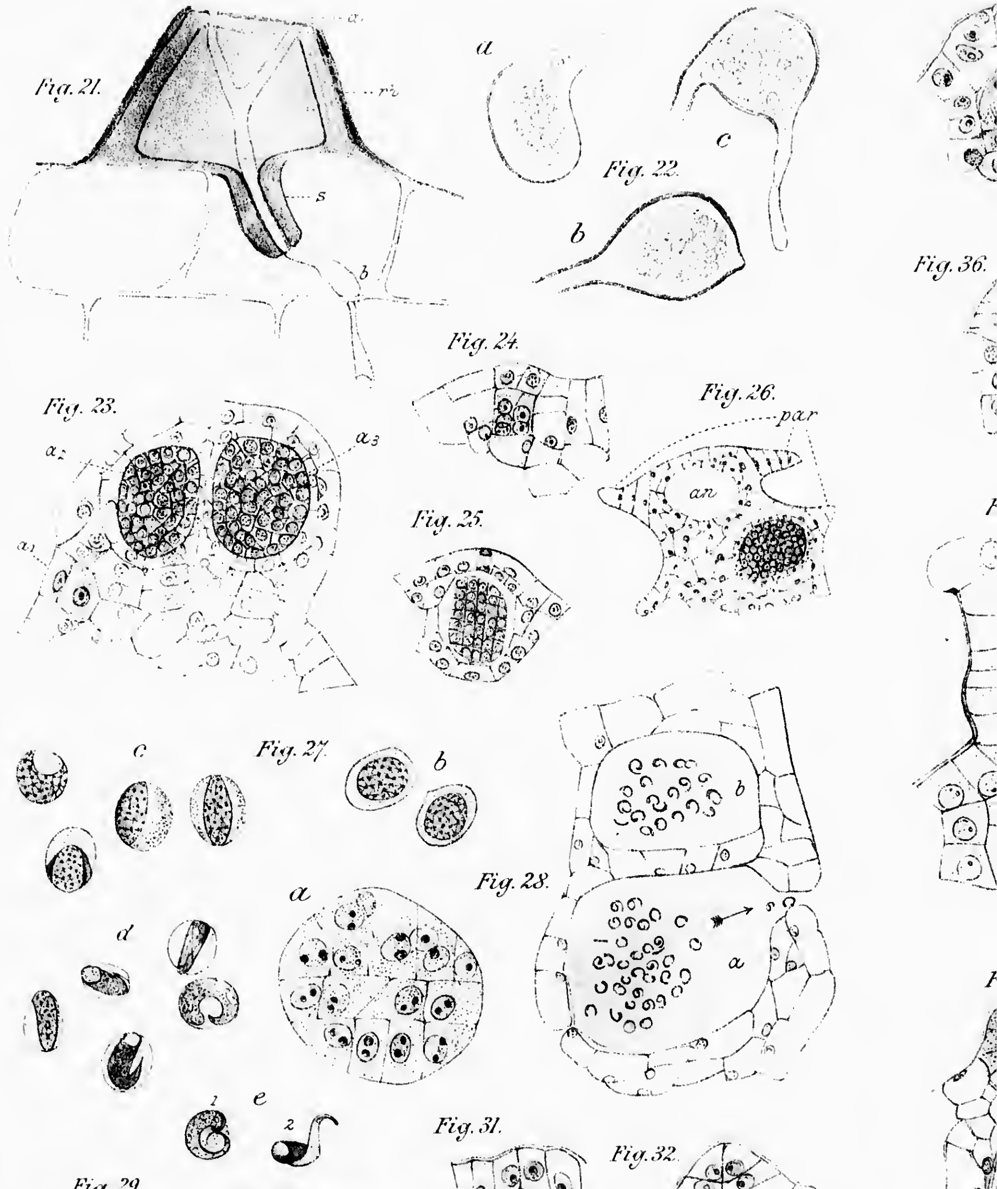

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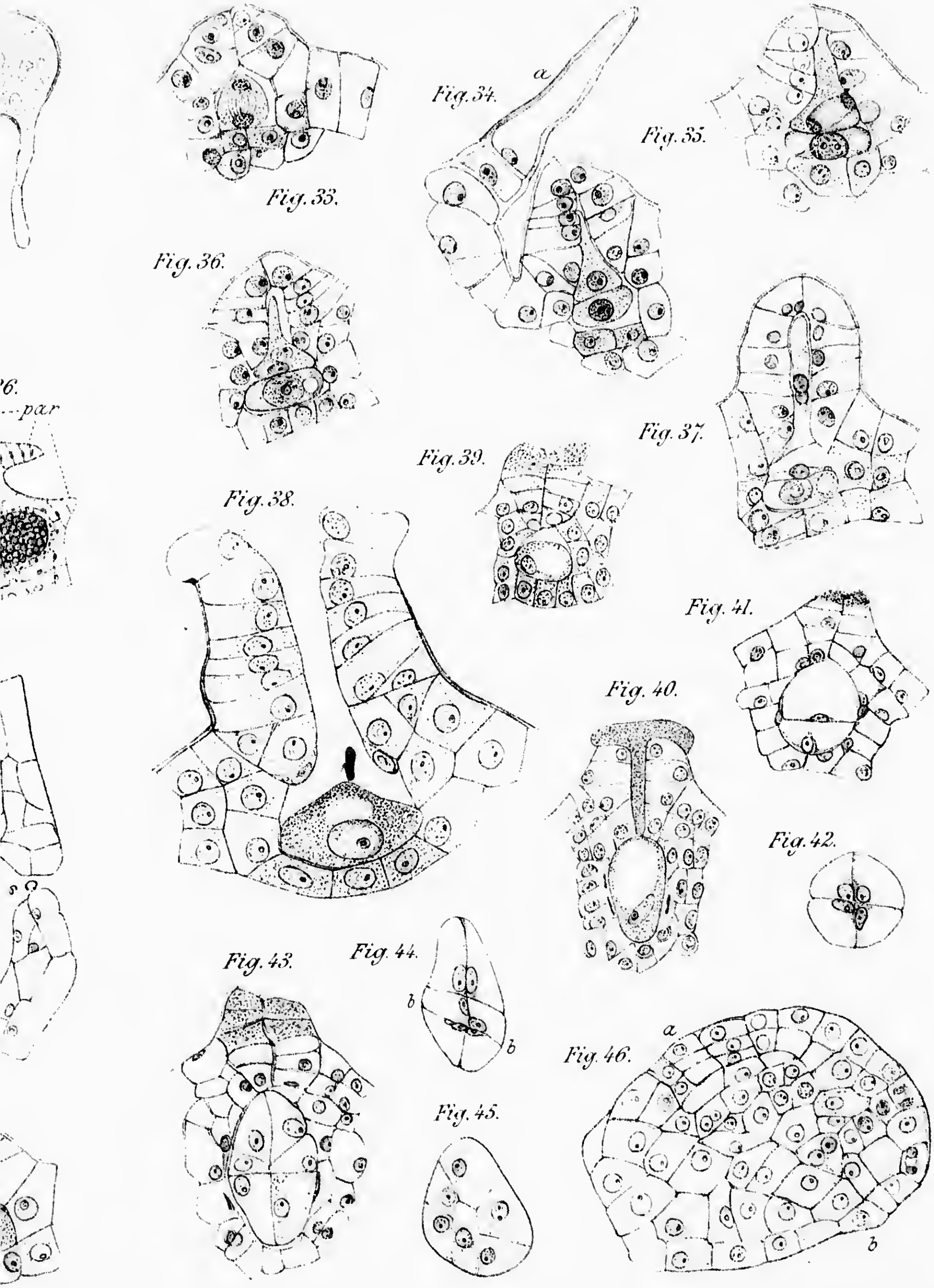

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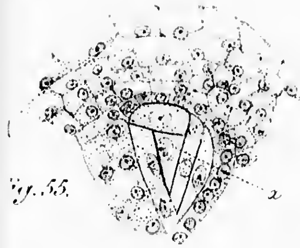

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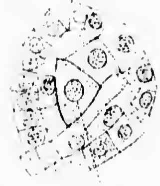

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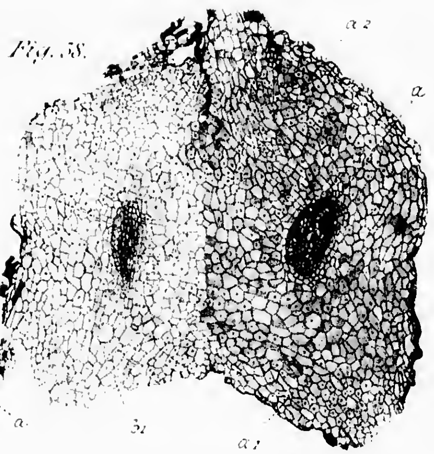

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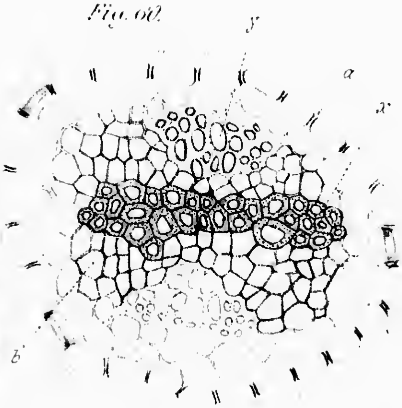

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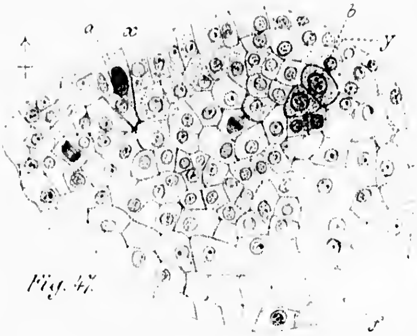

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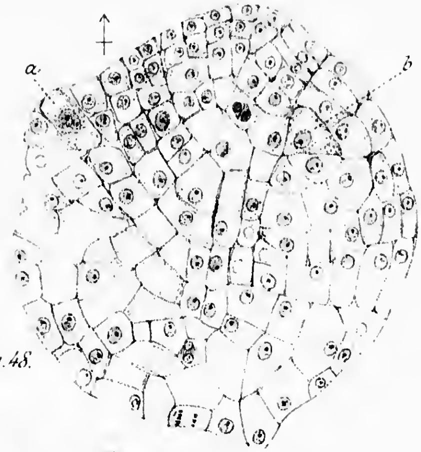

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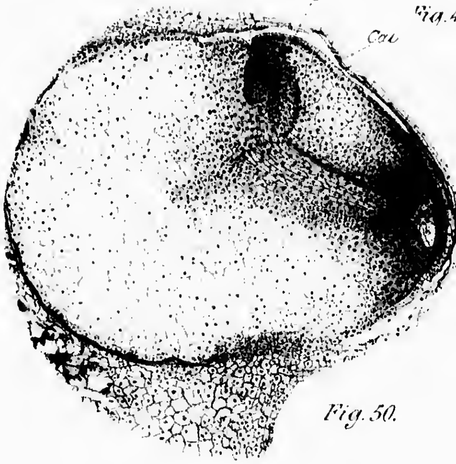
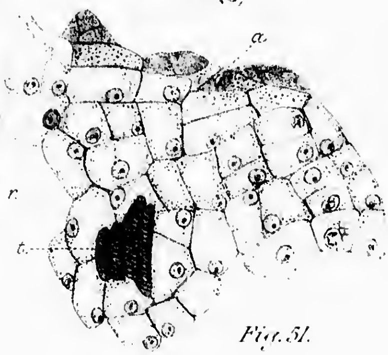


Fig. 50.



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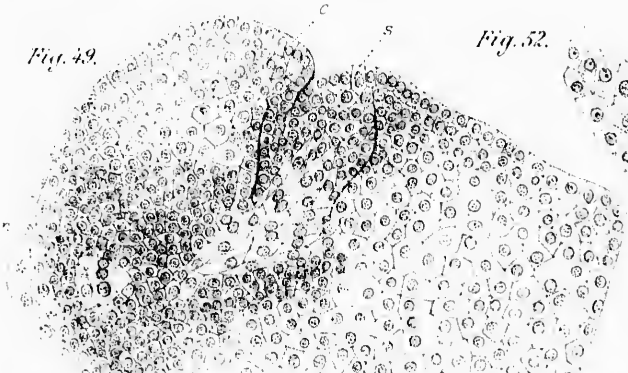

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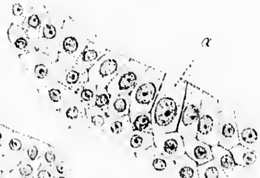
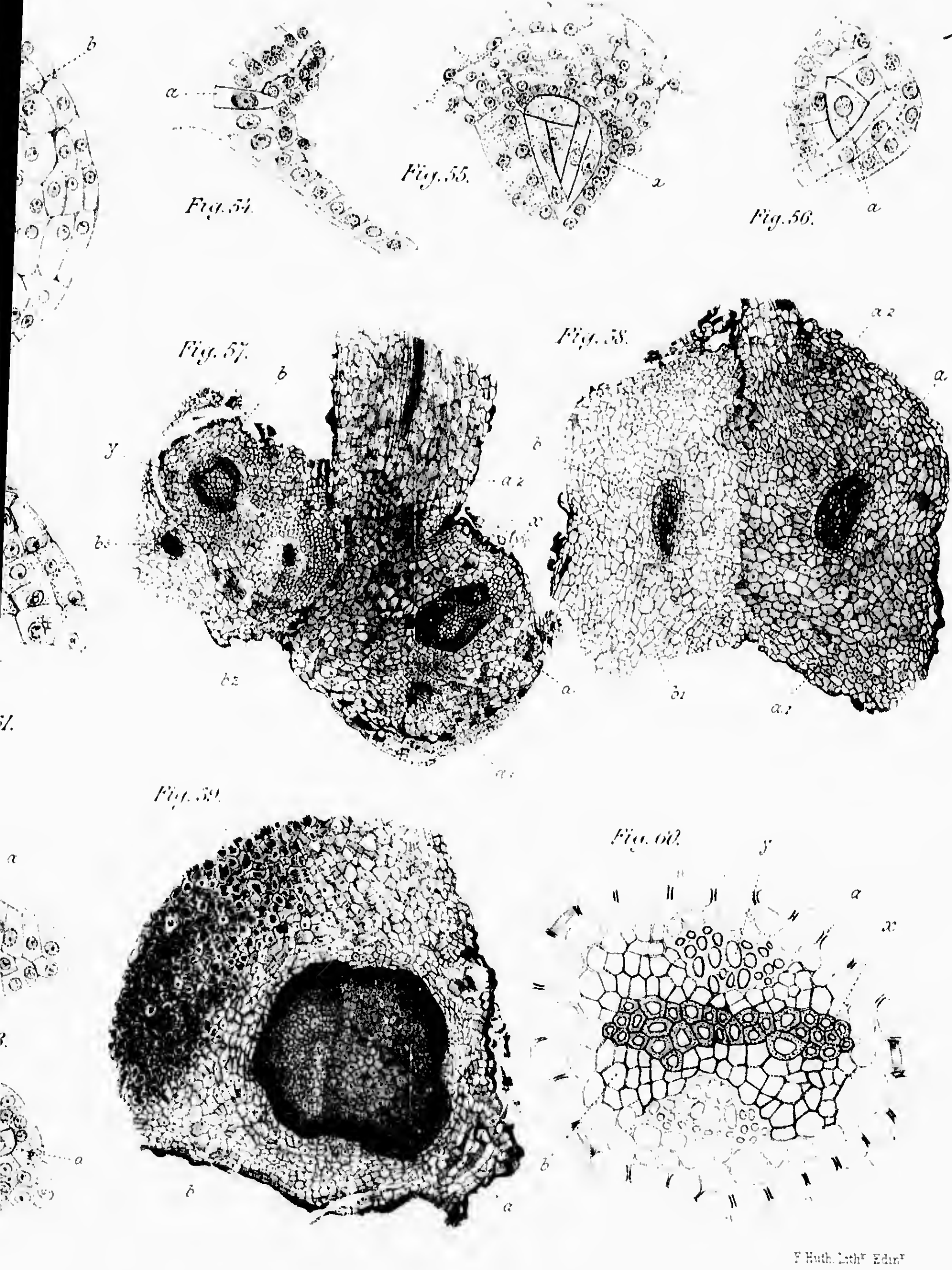


Fig. 52.

Fig. 53.

37



F. Huth, Lith^r Edin^r

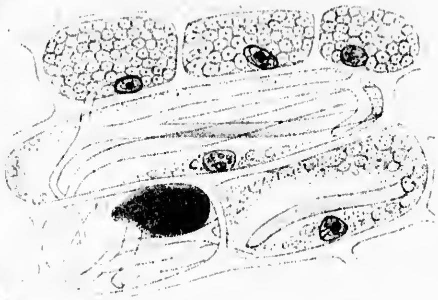


Fig. 69.

Fig. 66.

Fig. 70.

F Huth, Lith^r Edin^r

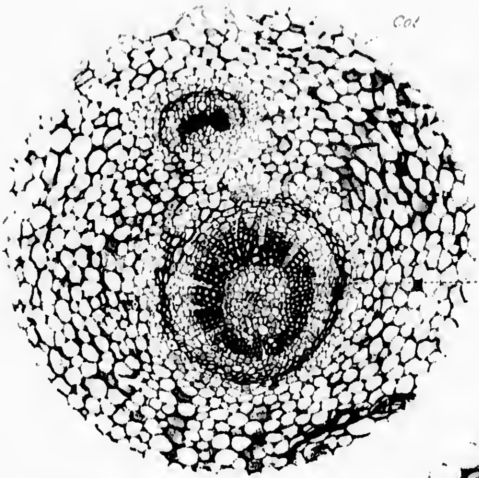


Fig. 61.

Fig. 64.

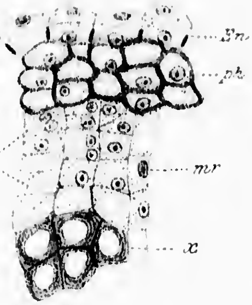
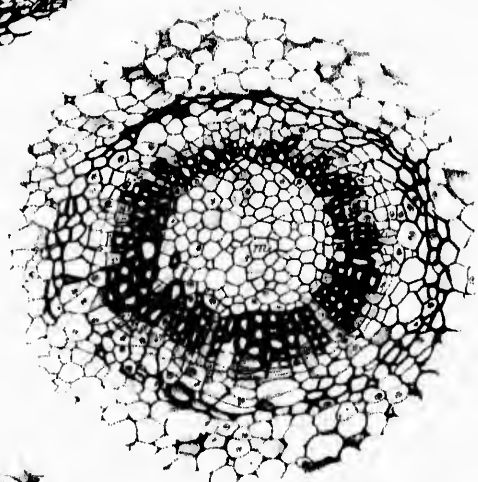


Fig. 65.

Fig. 62.



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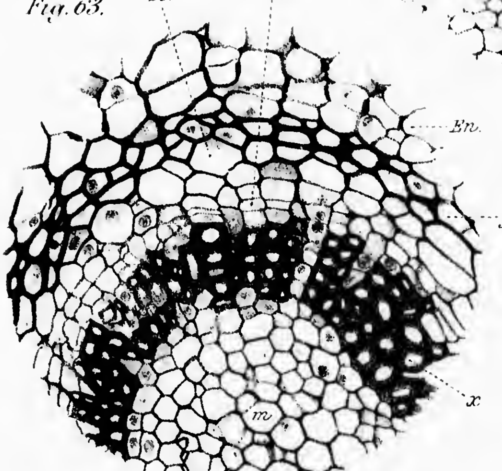

Fig. 67.
× 8

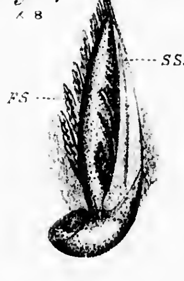

Fig. 68.

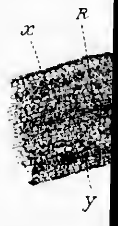

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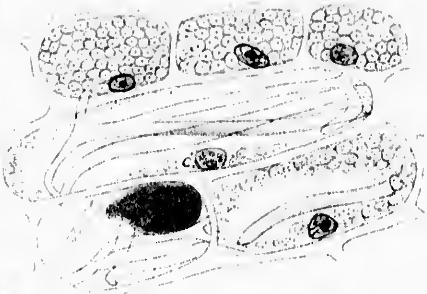
Fig. 65.



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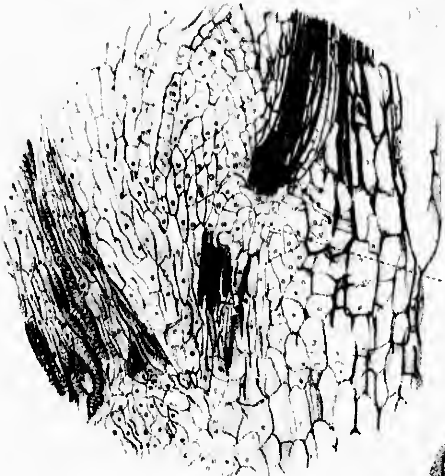

Fig. 66.

Fig. 70.

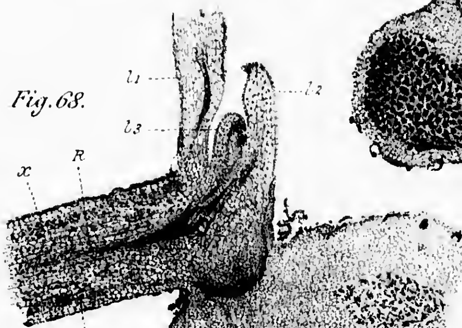

Fig. 68.

F Huth, Lith^r Edin^r