THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM.

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Biological Series: edited by
Professor R. RAMSAY WRIGHT

NO. 1. THE GAMETOPHYTE OF BOTRYCHIUM VIRGINIANUM, BY E. C. JEFFREY

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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.

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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.

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

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I.

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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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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,

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 sub-form *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 *Entomopthoracæ*, 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 primitive 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. Farnkräutern. 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, e^1 and e^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 *Danea 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 *Lastræa 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 *pileorhiza*, 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 *pileorhiza* 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. Etudes 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

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

34. Studies in the morphology ot spore-producing members. Part 2. *Ophioglossaceæ*, p. 56, et seq.

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. × ⅔.

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

FIG. 12.—A prothallus bearing two further advanced sporophytes. × ¼.

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

FIG. 14.—An eight year sporophyte still attached to its prothallium. × ⅔.

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).

Fig. 1

Fig. 2

Fig. 3.

Fig. 4

Fig. 12

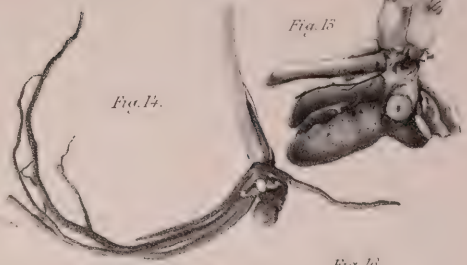
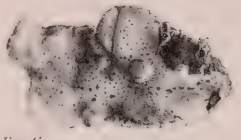


Fig. 14.

Fig. 13

Fig. 5



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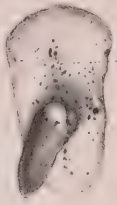

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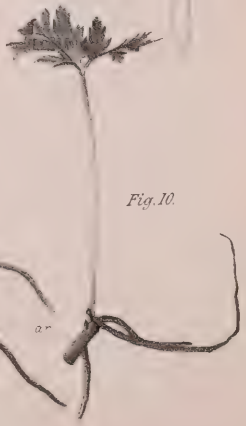

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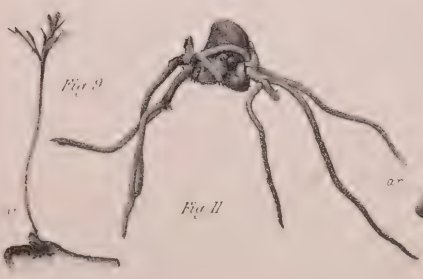
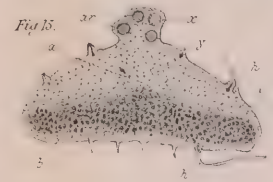


Fig. 9

Fig. 11



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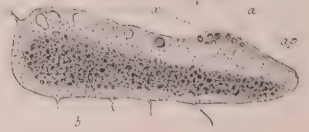

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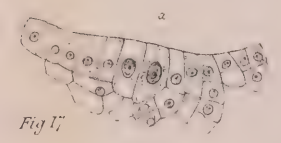
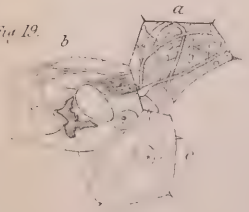


Fig. 17

Fig. 18.



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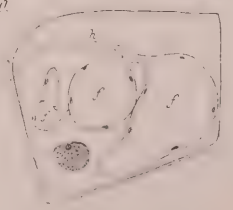

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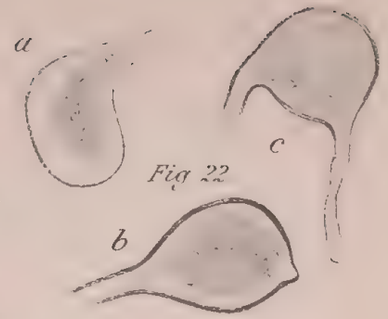

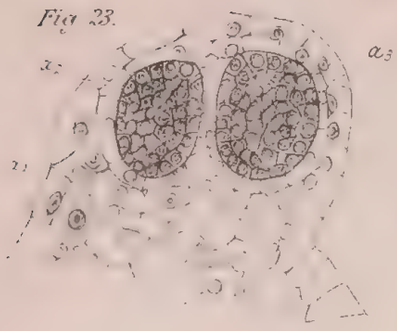

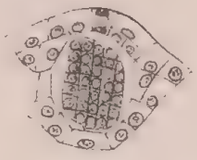

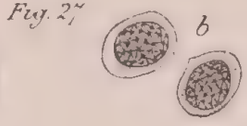

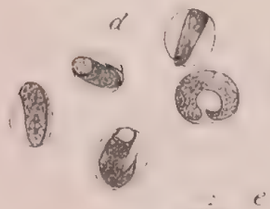

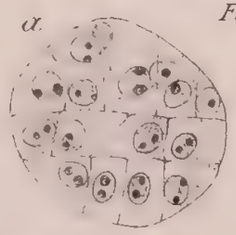

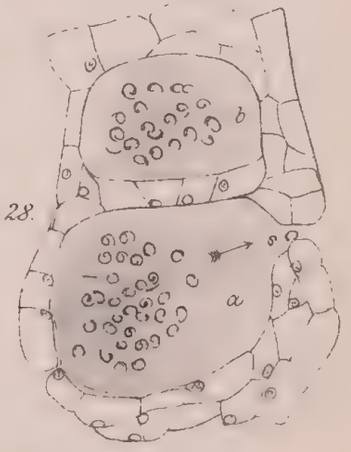

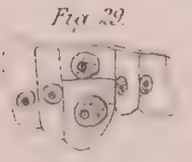

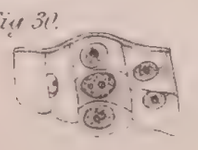

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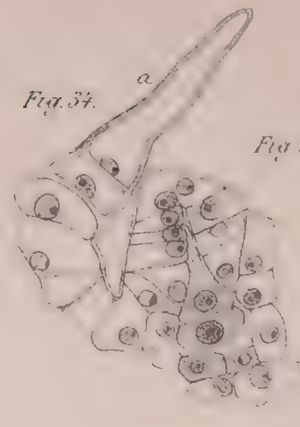

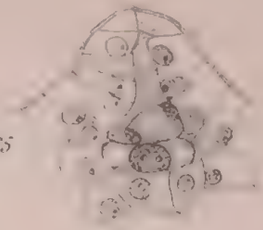

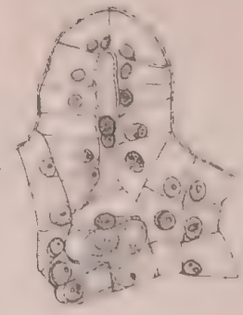

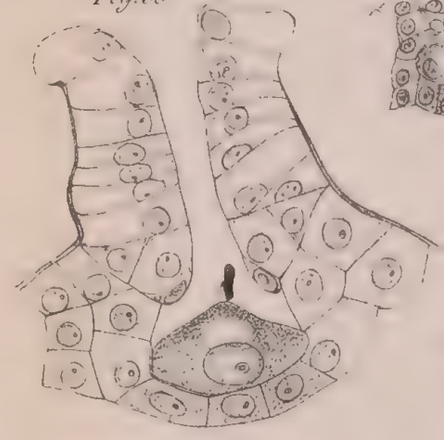

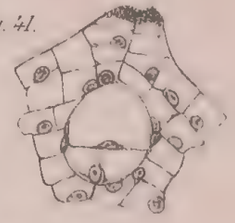

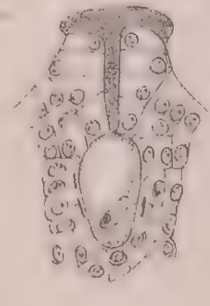

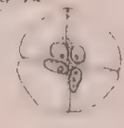

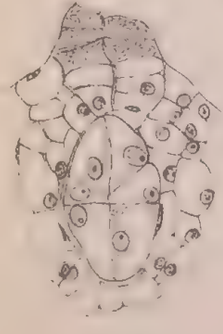

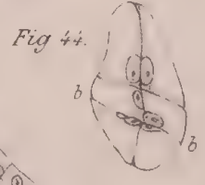

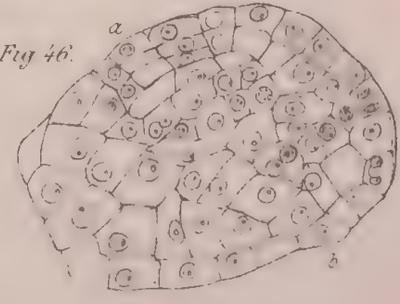

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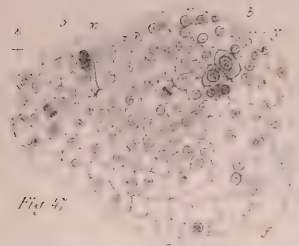

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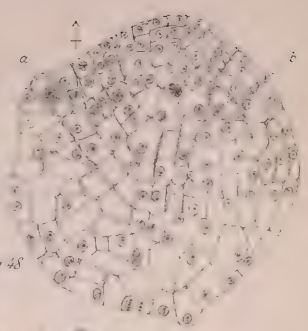

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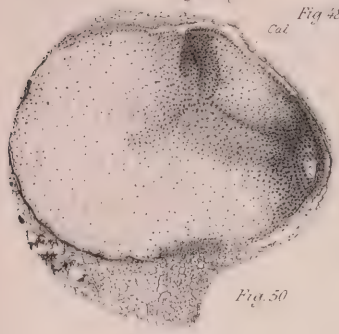

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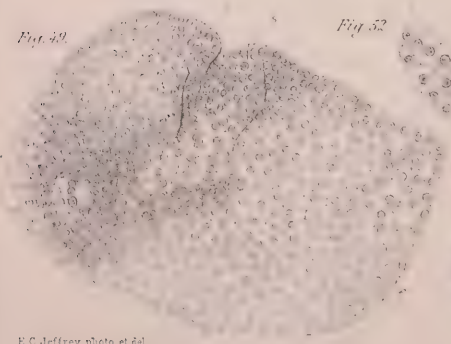

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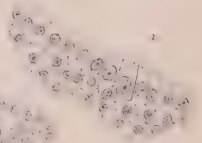

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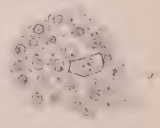

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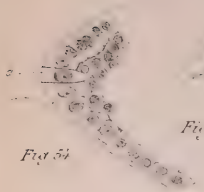

Fig. 54.

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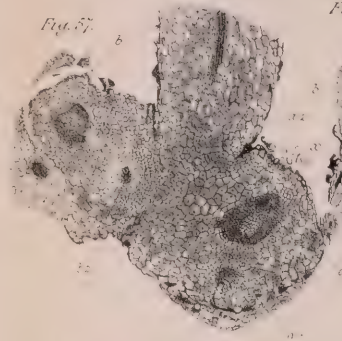

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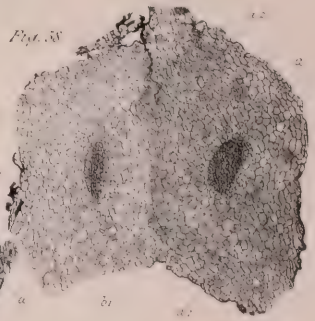

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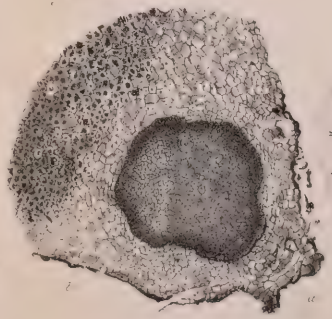

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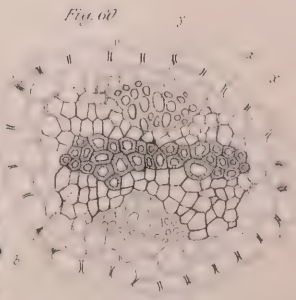

Fig. 60.

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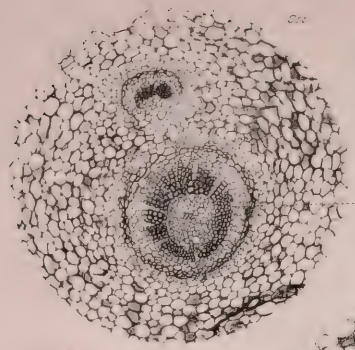

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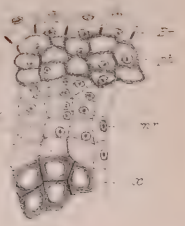

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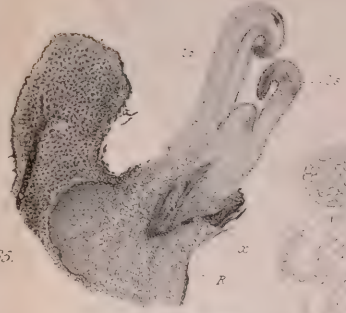

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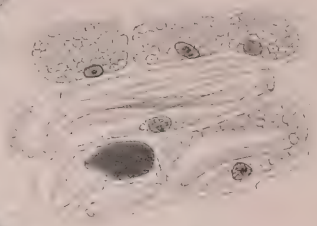

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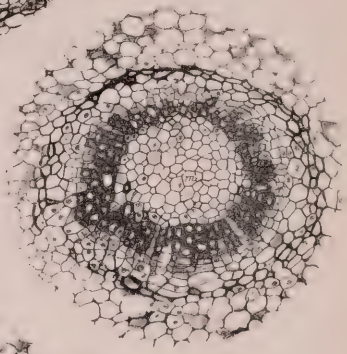

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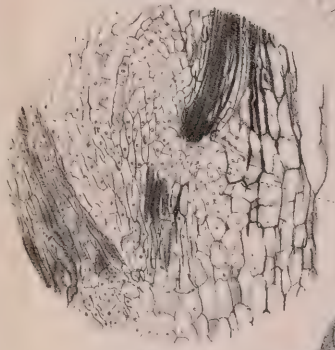

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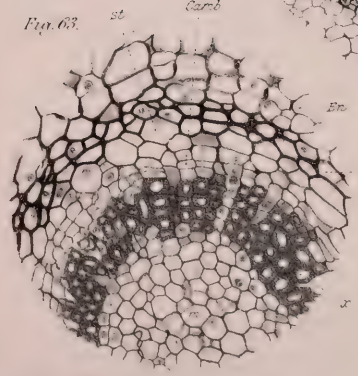

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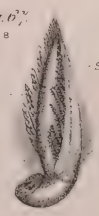

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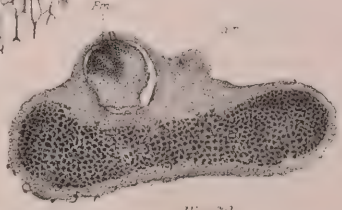

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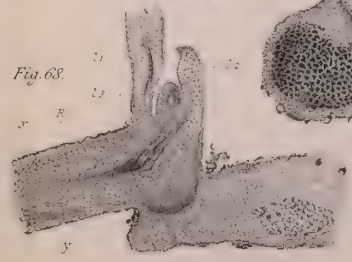

Fig. 68.

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BIOLOGICAL SERIES

NO. 2. THE ANATOMY OF THE OSMUNDACEÆ

BY J. H. FAULL

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UNIVERSITY OF TORONTO STUDIES.

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NO. 2. THE ANATOMY OF THE OSMUNDACEÆ

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THE UNIVERSITY LIBRARY, 1902: PUBLISHED BY THE LIBRARIAN

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PREFATORY NOTE

The work embodied in this article was done by Mr. J. H. Faull, as a graduate student in Botany, in the Biological Department of the University of Toronto during the winter of 1900-01.

E. C. Jeffrey,
Lecturer on Biology.

THE ANATOMY OF THE OSMUNDACEAE

By J. H. Faull, B.A.

THE ANATOMY OF THE OSMUNDACEAE.

(*Reprinted from the Botanical Gazette, Vol. XXXII**)

(WITH PLATES XIV–XVII)

INTRODUCTORY.

THE cauline vascular system of the Osmundaceae has attracted considerable attention on the part of morphologists, since it is exceptional among the leptosporangiate ferns in exhibiting a type of structure presented by the phanerogams. Thus DeBary, the exponent of the "bundle system," states that "collateral bundles" are with rare exceptions characteristic of the stems and leaves of the phanerogams, but are likewise found in the Osmundaceae,[1] and that in their arrangement in the stems of the Osmundaceae they follow the "dicotyledon type."[2] Later we find Van Tieghem, the first enunciator of the "stelar theory" expressing himself as follows:[3]

> La tige des *Osmundes* et des *Todées* diffère de celle des autres Fougères. La stèle axile et sans moelle du jeune âge, au lieu de se diviser en restant grêle, demeure simple en s'élargissant progressivement à mesure que la tige grossit; elle prend une moelle de plus en plus large, à la périphérie de laquelle sont rangé en cercle un certain nombre de faisceaux libéroligneux à bois séparés, mais à libers confluents, entourés d'un péricycle commun et d'un endoderme général. En un mot la tige de ces plantes demeure monostélique à tout âge, comme celle de la plupart des Phanérogames.

Plainly enough, therefore, these eminent botanists, starting from very different conceptions, have arrived at the same conclusion, namely, that the central cylinder of the Osmundaceae resembles that of the phanerogams.

It is important to note, however, that heretofore all anatomical researches in this family have been confined to the tropical genus Todea and the cosmopolitan *Osmunda regalis;* and that hence the conclusion just stated has been based on the phenomena

*The original pagination is as follows: page 3 of this separate corresponds to page 381 of the *Botanical Gazette*, Vol. 32, December, 1901.

[1] DEBARY: Vergleichende Anatomie der Vegetationsorgane der Phanerogamen und Farne 331.

[2] DEBARY: *op. cit.* 246.

[3] VAN TIEGHEM: Traité de Botanique 1373.

presented by these alone. When Van Tiegham proposed his "stelar hypothesis" several cryptogams besides the Osmundaceae were cited as exceptionally possessing medullated monostelic central cylinders. Since then more extended researches have been made which have yielded important results. Thus it has been shown that the central cylinder of Ophioglossum and of Botrychium instead of being medullated monostelic is in reality "gamodesmic;"[4] that the central cylinder in the entire family Equisetaceae, some of whose species were included in the exceptions, is of the same kind;[5] and that the central cylinder of the genus Helminthostachys is also of the "gamodesmic" type.[6] It is true that Strasburger holds[7] that the internal endodermis and endodermal sheaths about individual bundles are of intrastelar origin, and not of cortical as is the external endodermis, and that therefore these exceptions still stand; but this objection may be advantageously left for subsequent consideration. Of the apparent exceptions, the family Osmundaceae has remained untouched, and I have undertaken the present research on this anomalous case, with the primary object of furnishing data that will help determine the proper morphological interpretation of its vascular system.

The family Osmundaceae is a very limited one in point of numbers, consisting of but two genera, Osmunda with eight species, and Todea with six, and therefore constitutes a very small part of the fern flora of the earth. But this does not seem to have always been the case,[8] for the Marattiaceae, although overwhelmingly predominant in the Coal period, constituted but 4 per cent. of the total filicineous flora in the Lower Jurassic, the remainder being composed of Osmundaceae and Cyatheaceae, with the related families Matonineae and Protopolypodiaceae. As to distribution, the first genus is confined to the northern hemisphere, and the Todeas are with one exception found only in Australasia. Five Osmundas belong exclusively to restricted areas in east Asia and the adjoining islands; *O.*

[4] POIRAULT: Ann. Sci. Nat. Bot. VII. 18:113. 1893.

[5] JEFFREY: Mem. Boston Soc. Nat. Hist. 5:155. 1899.

[6] FARMER: Ann. Bot. 13:421. 1899.

[7] STRASBURGER: Histologische Beiträge. 3:—. 1891.

[8] SCOTT: Studies in fossil botany 304. 1900.

Claytoniana occurs in the Himalayas and North America; *O. cinnamomea* in eastern Asia, North and South America; and *O. regalis* in every continent except Australasia. Of the Todeas, *T. barbara* is a native of Australia, New Zealand, and South Africa; and the remaining species, the so-called "filmy" Todeas (Leptopteris of some authors), belong to oceanic islands in the eastern south-tropical region.[9]

Of these species I have had the opportunity of studying five, namely, *O. regalis*, *O. cinnamomea*, *O. Claytoniana*, *T. barbara*, and *T. superba*. Nevertheless, in the following pages most attention will be devoted to *O. cinnamomea*, not so much because its anatomy has not previously been described, as because the writer, for reasons which will become apparent, believes it retains a more primitive type of skeletal axis than any of the family so far investigated. The material of the species of Osmunda studied was collected from several different localities, and in large quantities. Of *O. cinnamomea* specimens from fully a hundred and fifty plants were preserved and examined, and of each of the others perhaps one-third of that number. The more important points were verified from specimens taken from three different localities.

Observations have been mainly restricted to the mature root, stem, and leaf trace. Some young plants of Osmunda were studied, and the growing points of the older stems have been sectioned. But the mature stem, especially the region at which it branches, has proved to be of chief interest from the standpoint of questions of comparative anatomy.

THE STEM.

GENERAL ANATOMY.—The mature stems are very stout rhizomes, exceptionally so in *T. barbara*, which grow in a direction somewhat oblique to the horizontal. The leaves are in a closely set tuft at the anterior end, for they are annual and the internodes are very short. The broadly winged, overlapping bases with their sclerenchymatous sheaths resist decay long after the remaining portion of the leaf has perished, and these, together with the roots, which are very numerous, greatly add to the bulk of the stem. The stem usually bifurcates once into two

[9] DIELS: Engler and Prantl's Natürlichen Pflanzenfamilien I[4]: 377. 1900.

branches of equal size, which lie in a horizontal plane. A few specimens of *O. regalis* were found, however, in which one of the forks was much larger than the other, but the larger almost immediately divided again, so that there were three branches of about the same size lying in the same plane. The forking bears no relation to the number of leaves produced, counting from the cotyledons, nor to the age of the plant. Occasionally there is no branching at all, though maturity has long since been attained, while in rare cases it has taken place comparatively early in the life of the fern.

The rhizome exhibits a very characteristic appearance in cross-section (*fig. 1*). The outer portion, the thick external cortex (*ex. c.*), consists of very resistant, dark-brown sclerenchyma, in *O. cinnamomea* of a rich red-tinted brown, in *O. regalis* and the Todeas of a black, and in *O. Claytoniana* of a dull brown hue. The cortex is marked by leaf-traces (*lt*), which form a close spiral, and at the nodes by the escaping roots (*r*). In *O. cinnamomea* sclerification of the cortical tissue is later in taking place than in the other species. The internal cortex (*i. c.*) is parenchymatous, comparatively narrow, roughly pentagonal, and its cells are heavily loaded with starch grains. Passing the pericycle and the bast region, which form a complete sheath, the wood (*x*) of the stele is seen to be broken up into bundles of various shapes arranged in a circle, and separated from one another by the so-called medullary rays. These medullary rays extend out from a large pith. The pith or medulla in *O. Claytoniana* and *T. superba* is apparently homogeneous. In *O. regalis* it is often discolored and may contain one or more strands of brown sclerenchyma; in *O. cinnamomea* it is very frequently characterized by some brown sclerenchymatous tissue, and in *T. barbara* there is a large axial strand of this supporting tissue.

Histology.— But we turn now to acquire a more intimate acquaintance with the stem as revealed by a study of its histological features. For this purpose several sets of transverse and longitudinal series were prepared, and a great many microtome sections examined. The material cut included stems of various ages. As development proceeds rather slowly, all the tissues are mature only at a considerable distance from the apex of the plant.

The cortical part of the stem has little of interest for us other than in the respects already mentioned. The sclerenchyma consists of elongated, thick walled cells, with a small lumen containing starch grains. The walls are brownish, and marked by simple pits, which are round or slit-like. According to Strasburger,[10] the endodermis is not the innermost cortical layer, but I am unable to verify this. He has made the statement that the innermost cortical layer at a certain stage divides by tangential walls to form several layers of cells; of these, the outermost becomes differentiated as the endodermis, and the remaining layers lie between this and the phloem, filling the place of a pericycle. The somewhat elongated cells of the endodermis are marked in every case by the characteristic cuticularization of the radial walls, which in transverse section shows as the "radial dot" (*fig. 6, e, e*). The "radial dot" is distinctively brought out by treatment with phloroglucin and hydrochloric acid, and also with dilute sulfuric acid. In *O. Claytoniana* the radial markings are generally not as distinct as in the rest of the species studied, and the cells are reduced in size in comparison with those of the layers in contact (*fig. 8, e*). The contents in this species, too, are meager, consisting of granular protoplasm, a nucleus which as a rule stains a deeper red with saffranin than those of surrounding cells, and a few starch granules as shown by treatment with iodin solutions. Sometimes the endodermal cells of *O. cinnamomea* are likewise apparent by the lack of contents, in contrast to the heavily-laden cells, both ectad and centrad. Generally in this species, as in the remaining ones, *T. superba* excepted, the cells are filled with tannin, so that the endodermis stands out very distinctly.

The pericycle is entirely parenchymatous and consists of several layers—in *O. Claytoniana* and Todea of two or three, in *O. cinnamomea* of three or four, and in *O. regalis* of one to three. The cells are elongated, cylindrical, provided with large nuclei, and filled with finely granular contents, part of which is starch. Haematoxylin imparts to this tissue a light blue color. Tangential sections show that the orientation of the cells is very irregular (*figs. 3* and *9, p*). Immediately opposite the point of origin of a leaf trace, and for a short distance below, the long axes of

[10] STRASBURGER, *op. cit.* 449.

the cells run parallel with the long axis of the stem, but for the most part in the remaining regions of the stem there is considerable disturbance, though only in tangential planes. This disturbance is commonly so marked that the long axis of the cell is at right angles to the stem axis, and between this and the parallel position there is every gradation. Therefore in transverse section these cells are either round or more or less tangentially lengthened (*fig. 8, p*). This variation in orientation is of interest, as it is connected with a similar phenomenon in layers lying nearer the cauline axis, namely in the phloem region.

XYLEM.—Before dealing with the phloem, however, it will be convenient to describe the xylem. The wood elements are of two kinds, namely, small ringed and spiral elements constituting the protoxylem, and scalariform tracheids which are of later development constituting the metaxylem. Occasionally a parenchymatous cell is found among the tracheids. A transverse section shows, as mentioned before, a ring of variously shaped bundles; and by tracing these up and down, or by boiling a piece of stem in potash and then removing the softer tissues, there is shown to be a network forming the wall of a hollow cylinder, the strands being the "bundles" of DeBary, and the meshes the spaces occupied by the "medullary rays." Though there is a great deal of regularity in the apparent construction of this network, as proved by DeBary and Zenetti in *O. regalis*, yet a study of development shows that the "bundle theory" is inadequate for giving the right conception of the vascular system. In the young stem of the Osmundaceae the wood forms a completely closed cylinder, and Van Tieghem, basing his conclusions on Todea and *O. regalis*, has stated this to be the case for the whole family. I am able to state that the phenomena in the young stem of *O. cinnamomea* and *O. Claytoniana* are in accordance with his general conclusions in this respect.

Now directly above the point at which a leaf trace leaves the stele the wood is not developed for some distance. This gap is filled by parenchyma chiefly, except at the outer part, which is occupied by sieve tubes. There are exceptions in *O. cinnamomea* to be described later. Thus a transverse section of the stele, just above a node, shows a ring of wood broken at one place, the break being occupied by the tissues just referred to; in other

words, the stele here has one medullary ray. *Fig. 23* shows a transverse section of the stem of *O. Claytoniana* through this region. Still further up the internode the ring is complete again. There is the same sort of gap above the second node. However, as the nodes become more frequent, that is, as the internodes become shorter, a leaf gap extends through more than one internode, and in a transverse section there is more than one medullary ray, until in the full grown stem, where a leaf gap extends through several internodes, a transverse section shows several gaps cut across, or in other words shows several medullary rays. It is therefore evident that the number of medullary rays seen in any transverse section depends on the frequency of the nodes and the length of the gaps. In well nourished stems the number is greatest in *O. Claytoniana* (*fig. 17*), there usually being about twenty, and in *T. barbara* (*fig. 24*) the fewest. In this species the gaps are quite short, so that while the wall may be thin in many places at any given level, there are not more than two to six medullary rays seen in the cross section (*fig. 24*).

The persistent portions of the cylinder of wood, the "bundles," present various contours in cross section, the shape of any particular portion lying between two adjacent gaps, that is, of any strand, varying with the level at which it is cut. Just below where the leaf trace is given off, the wall is hollowed out on the side towards the pith, so that the transverse section of the strand presents a horseshoe shape (*fig. 17*). The middle of the inner surface of the strand at this level is occupied by protoxylem, which consists of about a half dozen small ringed and spiral vessels. Following the strand down, it is seen that the arms of the horseshoe thicken on the sides facing one another, especially towards the ends of the arms (*fig. 15, s*). Finally, the opening between the ends is fully closed and a small group of parenchymatous cells lying exactly centrad of the protoxylem is thereby enclosed (*fig. 17, s*). The parenchyma is more and more encroached upon by the xylem, until lower down it is seen no more. Not far below where the parenchyma vanishes, the protoxylem in that strand likewise disappears. Somewhat above the level at which the parenchyma is enclosed the strand begins to thin out on the outer side, a sharp trough-like indentation appearing, but not in the same radius as that in

which the protoxylem lies. This trough continues to deepen until a few nodes down the strand is cut through, the point at which the break occurs being, indeed, the apex of a leaf gap. Thus neither the outer nor the inner surface of the cylinder of xylem is smooth ; the lower part of a leaf gap can be traced as a hollow on the inner surface just below where the leaf is given off, ending as a blind tube amongst the tracheids, while the upper end of the gap may be traced as a furrow on the outer surface of the cylinder, gradually becoming more and more shallow.

The protoxylem occurs in small groups of six to eight cells each, and a transverse section of the central cylinder shows from five to seven of these groups. Each group of protoxylem elements passes out in its entirety into a leaf trace, and on following back from the leaf trace each vanishes as already described. The protoxylem is therefore not continuous throughout the stem, but is in small, discontinuous strands. This fact has been recorded for *O. regalis* by Zenetti.[11]

Lying externally to the wood are from four to six layers of elongated parenchymatous cells, rich in protoplasmic contents and in small starch grains. They are continuous with the parenchymatous cells of the medullary rays and do not materially differ from them. Those occupying the middle of the medullary rays have more meager contents, and towards the stem axis they become larger. That there is a "xylem sheath" characterized by cells of greater size and richer contents such as Zenetti describes for *O. regalis*, I cannot affirm, and certainly there is not such a sheath in *O. Claytoniana*.

PHLOEM.—The tissues that have just been described are bordered by the phloem, which consists chiefly of sieve tubes Parenchymatous cells are sometimes met with in isolated positions in the metaphloem, and between the metaphloem and the protophloem they constitute a more or less broken layer, most pronounced in *O. Claytoniana*, and least constant in *O. regalis* and *T. barbara*. The sieve tubes are strongly developed and are of the "type vigne" of Lecomte. They are large, have thin walls of unmodified cellulose lined with a delicate layer of protoplasm, and are devoid of nuclei. They are provided with oblique

[11] ZENETTI: Das Leitungssystem im Stamm von *O. regalis*. Bot. Zeit. 53:63. 1895.

terminal walls and are furnished with sieve plates both simple and compound. The sieve plates are covered with "globules brillants," and by treatment with proper reagents callus plugs may be demonstrated in them. The sieve tubes of the protophloem are smaller than those of the metaphloem and their terminal walls are not as oblique.

As there has been considerable difference of opinion regarding the disposition of the phloem in *O. regalis*, it will be well to define a sieve tube. Both Russow and Janczewski have studied sieve tubes very carefully, and Poirault has more recently reinvestigated the subject in the vascular cryptograms. The investigator last named summed up his observations on sieve tubes in the roots of vascular cryptogams in the following terms :[12]

> Les tubes criblés peuvent se rapporter à deux types : le premier caractérisé par des cloisons transverses perpendiculaires aux faces principales et ne portant qu'un seul crible (*type Courge*, Lecomte) ; le second reconnaissable à ses cloisons transverses très obliques portant d'autant plus de cribles que leur obliquité est plus grande (*type Vigne*, Lecomte). On trouve, en outre, sur les faces longitudinales des ponctuations isolées ou réunies en très petits groupes, constituant rarement des cribles aussi développés que ceux des faces transverses. Le contenu de ces tubes est un liquide hyalin tenant en suspension de nombreuses sphérules réfringentes, rassemblés surtout au niveau des cribles et des ponctuations isolées. Il n'y a pas de noyau. La membrane est cellulosique.

He further adds that two substances occur as a rule — (1) the "globules brillants" already mentioned, and (2) "les *bouchons calleux* qui font corps avec la membrane et peut-être la traversent entièrement."

In dealing with the sieve tubes of the stem and petiole he does not point out any other peculiarities, but deals at length with the callus plugs, and the perforation of the sieve plates.[13] The observations of Russow, Janczewski, and Poirault agree for the most part, except in reference to the callus plugs.

The following criteria would seem to be distinctive in determining the presence of sieve tubes in the Osmundaceae; the existence of sieve plates, the absence of nuclei, and the presence of "globules brillants." Less distinctive and rather as a confirmatory test I have sought for callus. Russow made this test

[12] POIRAULT: Récherches sur les cryptogames vasculaires. Ann. Sci. Nat. Bot. VII. 18: 138. 1893.

[13] POIRAULT: *op. cit.* 191.

one of paramount importance, but it seems best in dealing with the vascular cryptogams to give it a second place, and for the following reasons: (1) the callus, so-called, in the seive tubes of the vascular cryptogams may not be identical with that found in phanerogams; (2) it occurs in minute quantities only, and in some plants (*e. g.*, the Ophioglossaceae) probably does not occur at all; (3) its presence is determined by delicate microchemical means, and then only by limited color reactions.

Janczewski[14] claimed to have found callus in *Pteris aquilina* alone of all the vascular cryptogams he examined, and states that it does not occur in *O. regalis*. The reagents he used were Schulze's solution (or chlor-zinc-iodin) and rosolic acid. On the other hand, Russow found callus in all of the sieve tubes he examined. The reagent he used was a mixture in variable proportions of chlor-zinc-iodin and potassium iodid-iodin. It should be stated that in the vascular cryptogams callus occurs in the wall of the sieve plate, appearing as if it were a part of the wall. After staining with a suitable iodin solution, the callus shows in face view as one or more round brown spots, and in section as rods or granules occupying the entire thickness of the lamella. Poirault has largely corroborated Russow's observations. He disagreed with Russow's generalization that it is a constant feature of sieve tubes, for he states that he has been unable to find any trace of callus in Angiopteris and Ophioglossum.[15] In view of these investigations, therefore, it becomes a matter of interest to know if the sieve tubes of Osmundaceae show the phenomena of callus as described by Janczewski for Pteris, and by Russow and Poirault for many others of the vascular cryptogams.

Accordingly tangential and transverse sections about five microns thick, of the three Osmundas studied and of *T. barbara* were cut from mature pieces of stem embedded in celloidin. In the present research the writer has tried several stains, such as ruthenium oxid, Hofmann's blue, rosolic acid, and Russow's mixture. These have been applied to sieve tubes of plants from widely separated groups, such as Vitis (summer and winter sieve tubes), Tilia, Pinus, Pteris, and the mixture of chlor-zinc-iodin

[14] JANCZEWSKI: Tubes cribreux. Ann. Sci. Nat. Bot. VI. 14: 50. 1882.

[15] POIRAULT: *op. cit.* 192.

and potassium iodid-iodin proved to be by far the most satisfactory reagent for the demonstration of callus. The two constituents of this reagent were prepared fresh, and then mixed in different proportions until one giving the best results was obtained. The proportions vary with the different kinds of plants tested. In using this stain, though the presence of the celloidin is not a serious objection, it is preferable to dissolve out the celloidin, wash in alcohol, then in distilled water, and examine in stain on the slide.

In face view it is difficult to make observations on account of the "globules brillants," hence the most reliable observations can be made on sectioned plates. Almost at once after applying the stain, the callus plugs become evident, staining a dark red-brown (*fig.* 7). They appear as more or less fine rods, completely traversing the sieve plate, and their number in a sieve plate depends on its size. The cellulose is slower in staining; at first it is light blue or a violet, and later a deep blue. Hence the callus plugs are to be seen most clearly in the early stages of the staining process. The stain unfortunately is not permanent. Callus was clearly demonstrated in the species under investigation, but on account of the size of the cells and of the sieve plates, *T. barbara* proved the best subject for the purpose. As one of the characters of the sieve tubes of the Osmundaceae, we record, therefore, the regular occurrence of callus plugs in the sieve plates.

The "globules brillants" are exceedingly abundant in the sieve tubes, and especially in the older ones (*fig.* 7). While they adhere to the protoplasm of the cell and may be found in any part of the cell they are by far most abundant about the sieve plates, dotting their surface, filling the pits, and surrounding the entrance to the pits. They are evidently not homogeneous, but appear to consist of two substances, one of which is more refractive than the other, for by slight focusing up and down they change from a dark looking, opaque granule to a light semi-translucent spherule. Iodin solutions stain them brown, not appreciably different from the callus plugs. Occasionally irregular fragments of matter are found in the cell, which also stain a similar brown. The relation between these fragments, the "globules brillants," the callus, and the disappearance of the nuclei calls for further investigation.

The sieve plates are very numerous, and vary in size and form. The walls of the pits are abrupt, and the number of pits varies with the size of the plate. The larger plates are irregularly oblong and the smaller ones are round. In Todea they are largest and most numerous. Having described the sieve tubes at some length, we shall now examine their distribution.

The phloem forms a continuous sheath, the outer portion of which is the protophloem. To this peripheral part of the phloem I find that DeBary and Zenetti alone make specific reference. The former states[16] that this region of the central cylinder is characterized by "quergestreckte Zellen," but he offers no opinion as to the nature of the tissue in question. Zenetti divides the zone into strands of typical protophloem and connecting portions of "quergestreckte Zellen." The typical protophloem is in short strands one cell thick, lying ectad of strands of xylem which are about to give off the leaf traces. The "quergestreckte Zellen" he cannot recognize as sieve tissue, because of the "quergestreckt" form and the position of the cells. This tissue in *Osmunda regalis*, consequently, forms a cylinder interrupted by the strands of protophloem only, but Zenetti found it to be generally two cells in thickness and opposite the medullary rays often several cells deep.

I have ascertained that the "quergestreckte Zellen" are devoid of nuclei; their walls are of cellulose, staining violet or blue with iodin solutions. They are rounded, elongated elements, with more or less oblique terminal walls; and are characterized by the possession of sieve plates which show the callus reaction when treated with Russow's reagent; the cells contain an abundance of "globules brillants" which are aggregated especially about the plates; the protoplasm is reduced to a thin parietal layer. The characters of the typical protophloem cells are the same as those of the "quergestreckte Zellen" except in regard to orientation (*fig. 6*).

Transverse sections show that the long axes of the so-called "quergestreckte Zellen" are tangentially placed, and never radially to any degree. To determine the slant of the long axes, therefore, with reference to the axis of the stem, tangential sections must be made. If such be examined it is seen that some are

[16] DEBARY: *op. cit.* 360.

exactly at right angles to the axis, others are almost or entirely parallel, and between these extremes there is every gradation. This at once explains the difference in "width" of the "quergestreckte Zellen" in transverse section. It is further to be noted from the tangential sections that the ends of typical protophloem cells never abut against the long sides of the "quergestreckte Zellen," but there is a gradual change in the direction of the latter so that their ends communicate with the protophloem and it is quite impossible to say where the typical protophloem ends and the "quergestreckte Zellen" begin.

The root and leaf have been examined for "quergestreckte Zellen," for if these elements constitute a characteristic textural feature of the Osmundaceae they would naturally occur elsewhere than in the stem. They are not present at all in the appendicular organs. Further, in the young sporophyte, where the leaf gaps are far apart, they are absent from considerable portions of the stem. The real nature of the "quergestreckte Zellen" will be discussed after observations on their development and their relation to the leaf traces have been described.

The "quergestreckte Zellen" and the typical protophloem cells form a continuous sheath in all the species studied. In *O. Claytoniana* the elements of this sheath are very much smaller, and so it is easier to distinguish them from the metaphloem. In *T. barbara* their histological characters are best studied because of their relatively large size. Frequently in *O. cinnamomea* and *O. regalis* it is difficult to decide in the mature stem whether or not certain cells belong to this sheath or to the metaphloem. But evidently in all of the species the sheath is rarely more than two cells in thickness, and often, especially in *O. Claytoniana*, there is but a single layer. Opposite outgoing leaf traces the sheath is reduced to a single stratum.

The *metaphloem* forms a hollow cylinder consisting of large sieve tubes such as have already been described. They are thin-walled, and especially in *O. regalis* in the older parts of the stem have often collapsed. The sheath is one or two cells thick opposite the strands of xylem, and several cells in thickness opposite the medullary rays (*fig. 8, ph*). Most of the tubes run parallel with the long axis of the stem, but here and there "quergestreckte" examples occur.

This cylinder of metaphloem has a smooth outer surface, but the inner surface is rendered very uneven on account of the wedge-like proliferations of the sieve tissue opposite the leaf gaps. Since this is a phenomenon common to all the species studied, we naturally seek an explanation of this peculiar disposition of the phloem. In his memoir on sieve tubes Janczewski,[17] who could hardly have been prejudiced by any stelar theories, noted that isolated sieve tubes occur occasionally here and there in the medullary rays of *O. regalis*. The writer has found undoubted cases of the same thing in *O. cinnamomea*.

Two such eminent botanists as DeBary and Strasburger have disagreed as to the topographical distribution of the layer of metaphloem sieve tubes in *O. regalis*. The former states[18] that the sheath is continuous, while the latter states[19] that he puts himself in opposition to DeBary on this point, for he considers the phloem to be interrupted opposite the medullary rays. Strasburger does not say for what reason he considers the cells opposite the medullary rays not to be sieve tubes. My own observations on *O. regalis* are precisely in accord with those of DeBary and Janczewski. The cells opposite the medullary rays differ in no way from the sieve tubes opposite the xylem strands. I have found the same to be true of the other species studied, with the additional observation that isolated sieve tubes occur sometimes in the tissues filling the leaf gaps of *O. cinnamomea*.

To this last observation I have two others to add, namely the occurrence of an internal phloem in which the sieve tubes form a more or less continuous ring (*figs. 21*, and *22*), and in rare cases the union of external and internal phloem through a leaf gap. In a certain rich, moist situation about a dozen well nourished plants of *O. cinnamomea* grew, of which, on examination, five showed the phenomenon of a continuous layer of internal phloem. Search in an adjoining locality resulted in finding specimens which showed the same feature. To extend the range of observations, I visited a peat bog some twenty miles distant from Toronto, where I knew the cinnamon fern grew, and secured specimens characterized by the same peculiarity. *Fig. 21* shows a transverse section of a stem found in this last locality.

[17] JANCZEWSKI: *op. cit.* 66.

[18] DEBARY: *op. cit.* 360. [19] STRASBURGER: *op. cit.* 449.

The sieve tubes of this internal phloem are as typical as those of the external, and except for their position not be distinguished from them. They do not always form a continuous ring as do the sieve tubes of the external phloem, but are often in more or less detached groups, embedded in small celled parenchyma. The layer of sieve tubes is from one to three cells thick. It should be added that internal phloem occurs only near where the forking of the stem takes place.

O. cinnamomea shows likewise two other features which are constant throughout every part of the stem, and at once distinguish it from other species: (1) an internal endodermis, and (2) several layers of parenchyma between this and the xylem.

INTERNAL ENDODERMIS.—The internal endodermis possesses the characteristic radial dot, though sometimes not as clearly distinguishable as in the external endodermis (*i. e., fig. 2*). Its cells are usually larger than those of the latter, but are filled with similar contents, most frequently tannin (*fig. 15*). It is further to be noted that it bends outwards opposite the leaf gaps (*figs 10*, etc.), and not infrequently connects through them with the external endodermis. I have examined scores of stems of the cinnamon fern, and in every specimen there was an internal endodermis. On the contrary, it seems to be invariably absent from the other species studied. As the central cylinder of the family Osmundaceae has heretofore been classed as monostelic, the existence of an internal endodermis in one of the species is therefore a matter of considerable moment, especially if it be regarded as a real phloeoterma.

Between the internal endodermis and the xylem there is a cylinder of elongated parenchyma, rich in starch and protoplasm, and from two to seven cells in thickness. This layer is continuous with the medullary rays. In *O. regalis*, *O. Claytoniana*, *T. barbara*, and *T. superba* a similar but thinner layer it found as a rule, and the cells are always smaller and richer in contents than those of the medulla on which they border.

THE MEDULLA.—The medulla is very large in this family, particularly so in *O. Claytoniana*, and consists of large-celled parenchyma. Most of the cells are partly filled with large starch granules, but frequently some of them contain tannin, especially in *T. barbara*. A brownish fluid may occur in inter-

cellular spaces, and in *O. regalis* within the cells themselves. In these regards there is often a striking resemblance between the parenchyma of the medulla and that of the internal cortex in the same plant. But there yet remains to be described a still more significant phenomenon, namely, the occurrence in the pith of brown sclerenchyma of the same kind as is found in the external cortex (*figs. 14* and *20*). This is probably a primitive feature, and in this, as in many other respects, *O. cinnamomea* proves to be most interesting. Out of forty-four pieces of stem, chosen at random, and representing a corresponding number of different plants of this species, twenty-five of the examples showed brown sclerenchyma in one or both ends. It occurs as a central strand, varying in size from a few cells to almost the limits of the pith, or as several small strands irregularly arranged. *Fig. 14* is a photograph of the transverse section of a stem in which there is a large axile strand, and *fig. 15* of one in which the sclerenchyma is entirely absent from the pith. Further it has the peculiar habit of being present at one level, but perhaps not at another; so it is likely to be found in nearly every plant if the stem be sectioned from end to end.

This same habit is characteristic of its appearance in *O. regalis* (*fig. 20*), but more often it is not present at all. That brown sclerenchyma occurred in the pith of *O. regalis* did not escape the observant DeBary,[20] but elsewhere I find no reference to this fact. Strangely enough, however, out of thirty-five or forty plants harvested from one locality there was not a trace of sclerenchyma to be found in the medulla of any of them, while in one region not far distant 25 per cent. showed this phenomenon, and in another a still higher per cent.

Parenchyma is the sole constituent of the medulla of *O. Claytoniana* (*fig. 17*). This is probably true of *T. superba* too. *Fig. 25* is a cross section of *T. barbara* taken too near the growing point to show sclerenchyma, but farther down the medulla was occupied by a large strand of this tissue (*fig. 24*).

Thus medullary brown sclerenchyma is usually present in *O. cinnamomea*, in *O. regalis* not uncommonly, and in *O. Claytoniana* not at all. In *T. barbara* it also occurs, but apparently not in *T. superba*. It is perhaps significant that such series can be

[20] DEBARY: *op. cit.* p. 290.

arranged, but of greater importance is the fact that the occurrence in the Osmundaceae of brown sclerenchymatous tissue, apparently within the cauline central cylinder, has no parallel among existing ferns.

THE FORK.—There yet remains to be described the anatomy of one particular portion of the stem, the part in the region of bifurcation. It has been stated that it is peculiar to the stem of the Osmundaceae to fork once, and that in a horizontal plane. We shall treat first of the phenomenon in *O. cinnamomea*. Tracing the main stem forwards, it is seen to become flattened and then to become constricted in a median vertical plane. Immediately anterior to the point of bifurcation of the vascular axis, there is a wide ramular gap in the central cylinder of each branch (*fig. 10*). Sections of the main axis immediately below the fork show two bands of phloem, one on the upper and one on the lower internal surface of the central cylinder (*fig. 13*). Sections passing through just in front of the region of bifurcation show similar bands of phloem along the inner wall of the central cylinder of each branch (*fig. 11*). Cases have been described above, in which there is a complete cylinder of internal phloem instead of the two isolated bands just referred to (*fig. 21*). The internal and external phloem connect through the ramular gaps (*fig. 11*). Likewise the internal and external endodermis are in textural continuity through these gaps, so that there is free communication between the cortex and the pith (*fig. 10*).

Sometimes the cortex lying between the two branches contains brown sclerenchyma which is continuous through the ramular gaps with strands of the same tissue occurring in the medulla of the branches. Frequently in less vigorous plants a transverse section of the main axis posterior to the point of ramification shows a diamond-shaped piece of cortex surrounded by endodermis (*fig. 12*). Posteriorly this included piece of cortex becomes continuous with the medulla of the main axis (*fig. 13*), and anteriorly with the general cortex (*fig. 11*).

Twenty-five forks of *O. cinnamomea* were selected at random and sectioned. Twelve of them presented the phenomenon of typical wide ramular gaps. Six of them were of the reduced kind just described. In five cases there were gaps in the xylem

only, cortex and medulla never becoming continuous; and in two even the xylem did not open up (*fig. 16*). For reasons to be outlined later, the writer believes the wide gaps to be the most primitive.

O. regalis presents a much degenerated form of ramular gap, for here only the xylem opens (*fig. 19*). In *O. Claytoniana* the degeneration is carried still farther, for as a rule there are no branch-gaps at all (*fig. 18*). In *T. barbara* the xylem alone may open up.

The phenomena of the fork may be thus summarized:

(1) Complete ramular gaps occur only in *O. cinnamomea.*

(2) Internal phloem occurs only in *O. cinnamomea.* It is found in the branches just above, and in the parent axis just below the point of bifurcation of the central cylinder.

(3) The internal phloem may form an entire cylinder.

(4) Where gaps are complete, the cortical and medullary tissues connect through them.

(5) Thus sclerenchyma of the cortex is sometimes continuous with sclerenchyma in the medulla of the main axis, and of the branches.

(6) *O. cinnamomea* presents the following forms of ramular gaps arranged in order of degeneration, (*a*) complete gaps, (*b*), phloem and xylem only open, (*c*) the xylem alone opens, (*d*) no gaps at all.

(7) *O. regalis* and *T. barbara* show gaps in the xylem only, and in *O. Claytoniana* there are usually none at all. *O. Claytoniana*, therefore, presents the extreme case of degeneration.

THE LEAF TRACE.

The leaf traces pass very obliquely up through the external cortex. A section of a leaf trace shortly before it passes into the petiole presents some noteworthy characters. In the first place there is no pith, but a solid horseshoe-shaped mass of xylem with the convex side turned outwards (*fig. 5, x*). The xylem is made up of large scalariform tracheids with a protruding mass of a few small vessels constituting the protoxylem. The protoxylem is situated on the inner face of the single strand of xylem (*px*), and is continuous with that of the stem. In *T. barbara* it frequently breaks into two or three groups.

Surrounding the wood is a layer of parenchyma, which on the concave side of the xylem quite fills the space between the arms of the horseshoe. The phloem consists of a crescentic band of sieve tubes, one to three cells thick on the external side of the leaf trace (*ph*), and a smaller band on the opposite side (*ph*). The protophloem consists of small elements which form a ring, broken only on the concave side of the xylem. Here the ring is completed, however, by the inner band of metaphloem. In *O. cinnamomea* and *T. barbara* isolated protophloem cells have been observed by the writer on the side of the inner band of metaphloem towards the stem axis. On the convex side the protophloem is separated from the metaphloem by parenchyma. There are no "quergestreckte Zellen." The pericycle consists of two or three layers of cells, and is bounded by a well developed endodermis continuous with that of the stem. With reference to the attachment of the leaf trace to the cauline vascular axis Zenetti has given a very careful and accurate description.[21]

Strasburger has held[22] that the stele of the petiole of *O. regalis* is a collateral bundle. He has considered the inner band of metaphloem to be a parenchymatous tissue. However, the cells of this band prove to be characteristic sieve tubes, and are continuous with sieve tubes in the stem opposite the medullary rays. The leaf traces, therefore, are undoubtedly concentric. Several botanists have arrived at the same conclusion for *O. regalis*.[23, 24]

In summary, the most important features of the leaf trace are: (1) the absence of a pith, (2) the endarch xylem strand. (3) the concentric type of stele, (4) the absence of "quergestreckte Zellen," and (5) the cylinder of protophloem completed on the inner face by a band of metaphloem.

THE ROOT.

The roots have a definite relation to the leaves, both in position and in numbers. Two roots invariably originate from the base of every leaf trace, or from the central cylinder immediately below. They come off at the same level, one opposite each arm of the horseshoe-shaped strand of xylem (*fig. 18*) in every case

[21] ZENETTI: *op. cit.* 69.

[22] STRASBURGER: *op. cit.* 448.

[23] SCOTT: *op. cit.* 319.

[24] ZENETTI: *op. cit.* 66.

where there are just two roots to a leaf. They grow almost directly outwards, and so in a transverse section of the stem are cut longitudinally. In such a section it is seen, likewise, that the cortical tissues of the stem and root are entirely independent of each other, and that, therefore, the root is of endogenous origin. This fact is true of the secondary roots also.

The cortex is exceedingly thick, forming by far the main bulk of the root, and consists of large celled sclerenchymatous tissue. The cortical cells diminish in size towards the periphery, and become thicker walled. In *T. barbara*, however, there is a discontinuous ring of exceedingly thick walled brown sclerenchymatous cells immediately surrounding the vascular axis. The endodermis, which is continuous with that of the stem and leaf, is very pronounced in all of the species, and is at once noted by the radial dot, and by the fact that its cells are filled with tannin. In the second particular, exception must be generally made of *O. Claytoniana*.

The stele is comparatively small, and is typically protostelic, since there is no pith. The wood has a narrow elliptical form, consisting mainly of very large scalariform tracheids. At each end of the ellipse there are a few small protoxylem elements, which are especially evident in the young root, and which have no connection with the protoxylem of the stem or leaf. The root, therefore, is diarch. There are likewise two bundles of phloem alternating radially with the bundles of xylem. In all of the Osmundas, however, I have observed triarch steles in the larger roots, which exception is of comparative frequence in *O. cinnamomea*. The phloem consists of two flat bundles or bands. These bands are made up chiefly of thin walled sieve tubes which are of the same kind as occur in the stem. None of them are "quergestreckt." The phloem is separated from the xylem by three or four rows of parenchyma, and from the endodermis by a two rowed parenchymatous pericycle.

DEVELOPMENT OF THE TISSUES FROM THE GROWING POINT.

In discussing this subject there are two points in particular which will receive special consideration: (1) the statements of Strasburger and Zenetti regarding the origin of the endodermis, and (2) the real nature of the "quergestreckte Zellen."

The determination of the relation of the tissues to the apical cells seems of little concern, and moreover in the study of the apical region of the growing point there are serious difficulties. Having described these for *O. regalis*, Professor Bower aptly remarks:[25]

> The meristem being thus at times irregular, and the subdivisions of the segments being variable, it is to be expected that the study of it (the apical region of the growing point) in longitudinal section would present difficulties, and I have not been able to trace any definite and characteristic mode of segmentation. Longitudinal sections cut from a considerable number of stems show that a conical apical cell is present. The relations of the surrounding tissues, and their reference to regularly succeeding segments are difficult to recognize.

To these observations on the extreme apical end of the growing point we have nothing to add, but pass further down the stem.

A short distance from the apex of the stem, the various tissues, though in embryonic form, become apparent. The cylinder of wood, whose thin walled, unlignified cells are still provided with protoplasm and nucleus, can be distinguished from the pith, the parenchyma in the leaf gaps, and the immature phloem. The pericycle is rich in protoplasm, and its cells are radially arranged. At an earlier stage still, even before there is any evident differentiation in the vascular tissues, the leaf traces can be seen coming off from the cauline vascular axis.

When the protoxylem can be first demonstrated by phloroglucin and hydrochloric acid, the endodermis (both internal and external in the case of *O. cinnamomea*) is also demonstrable by the same reagents, though not before. Zenetti has claimed[26] that at the time the protoxylem is formed, the endodermis, pericycle, "quergestreckte Zellen," protophloem cells, and some cortical cells are all in the same radial rows; and that, therefore, all have originated from the same mother layer. Strasburger has asserted[27] that the tissue lying in the stem between the phloem and the endodermis and occupying the position of a pericycle arises by tangential divisions with the endodermis out of the innermost cortical layer. Therefore, not the entire

[25] BOWER: The comparative examination of the meristems of ferns as a phylogenetic study. Ann. Bot. 3: 323. 1889.

[26] ZENETTI: *op. cit.* 64. [27] STRASBURGER: *op. cit.* 449.

phloeoterma, he claims, but the outer division product is that which gives origin to the endodermis.

Now, at the time the protoxylem elements appear, I did not find, in the species examined, the cells of the endodermis corradial with those lying centrad. It is true that in younger stages the cells in this region are in radial rows; but nearer still to the *punctum vegetationis* this is approximately true of all the cells of the stem. At this earliest stage one would hesitate to say, because certain cells were corradial, that they were therefore division products of the same mother cells; so Zenetti's conclusion, based on this sole argument, scarcely seems conclusive, even granting the correctness of his observation. If, too, such a conclusion were correct there would be the curious anomaly of certain phloem and cortical tissues having a common origin.

Evidently the study of transverse sections cannot settle the matter. To attempt to follow these layers upwards is obviously only possible in median longitudinal sections. But in the stems of the Osmundaceae the leaf traces are exceedingly numerous, and at the growing point are closely packed together, and appear before the tissues of the cauline central cylinder become at all differentiated. Hence, no matter what be the plane of section, the endodermis cannot be traced continuously very far anteriorly to the point at which it is differentiated, for a leaf trace is certain to intervene; and I found it quite out of the question to pick out an undifferentiated endodermis on the side of the leaf trace turned towards the apex. Therefore, every attempt failed to refer the endodermis and the rows of cells "occupying the place of the pericycle" to the same initial layer.

The typical protophloem, and the "quergestreckte Zellen" begin to be differentiated simultaneously with the appearance of the protoxylem. They are best examined in tangential sections. Their walls at this time become pitted, and their contents much less granular than those of the surrounding cells. Here, as in the maturer parts of the stem, there appear to be no differences between the typical protophloem and the "quergestreckte Zellen." Their relation to the leaf traces seems to explain their irregularity in orientation. Immediately below the point of origin of a leaf trace they are arranged with their long axes parallel to the long axis of the stem, and there is a gradual transition to

the tangential position. More than this, the laterally placed protophloem cells of the leaf traces can be directly traced into the "quergestreckete Zellen" of the stem. There seems little doubt, therefore, as to their nature.

To summarize observations: (1) The "quergestreckte Zellen" are sieve tubes, as has been demonstrated above; (2) they become differentiated at the same time as the typical protophloem, and (3) occupy the same relative position; (4) they resemble the protophloem cells in form; (5) their orientation is not uniform; (6) they pass imperceptibly into the longitudinally orientated protophloem cells of the leaf traces. Hence there seems no reason to regard them as anything else than protophloem.

CONCLUSIONS.

The question now remains, how to interpret the vascular system of the Osmundaceae. To do this more intelligibly, it will be well to recapitulate the main fibrovascular theories. We shall begin with that of Sachs and DeBary.

These botanists regarded the bundle as the unit, and the vascular system as a more or less simple complex of bundles embedded in ground or fundamental tissue. Developmental studies have shown that this theory is inadequate, for the unit is wrong.

The hypothesis which at present obtains was supplied by Van Tieghem and Strasburger. In this conception[28,29,30] the stele is the unit. The primitive form of stele, the monostele, such as occurs for example in most roots and in the stems of lycopods, is a solid central strand of xylem, surrounded by a sheath of phloem, and marked off from the cortex by the differentiated internal cortical layer, the endodermis. Of this there are many modifications, of which mention is made of the most important. By the repeated bifurcation of the monostele, the polystelic type is presented, as in Primula and Pteris, each segment being in every respect a stele. If these steles fuse laterally, thus forming a ring with internal and external phloem, the gamostele is produced as illustrated by Marsilia. Again, when parenchyma

[28] VAN TIEGHEM: Traité de Botanique 673, 765.

[29] VAN TIEGHEM: Sur la polystélie. Ann. Sci. Nat. Bot. VII. 3: 275.

[30] VAN TIEGHEM: Éléments de Botanique 1: 84, 179.

segregates in the axis of the monostele, and the vascular ring is broken into strands by ectad extensions of this pith (the medullary rays), we have the medullated monostelic type, such as is common in phanerogams. It is to be noted that the medullary and cortical tissues are considered by both these botanists to be of morphologically different value. Now by the bending in of the endodermis of the medullated monostele between the bundles, and the fusion of the ends of adjacent groups on the centrad side of the bundle, so that each bundle has its endodermal sheath, and medulla and cortex become continuous, the schizostelic or astelic type results. Of this phenomenon Ranunculus and Equisetum afford examples. A modification of this type, the gamodesmic-schizostelic, is produced by the lateral fusion of these endodermal sheaths, so that there is a common internal and a common external endodermis. If the internal endodermis degenerates, as it does in *E. arvense*, then there is evidently a simulation of the medullated monostele. It is fair to add that Strasburger dissents[31] from the last two types described, the astelic and the gamodesmic, for he regards the endodermal sheaths about the bundles in the first of these, and the internal endodermis in the second, as not morphologically phloeotermal, but originating from specialized stelar cells.

The researches of Gwynne-Vaughan[32] and Jeffrey[33] have shown that the phenomena said to lead up to polystely do not occur in Primula and Pteris. If the polystelic conception falls, obviously gamostely goes too. Further, astely has been shown, where it occurs in Equisetum and Ranunculus, to be preceded by the gamodesmic appearance. Later the internal and external endodermis may fuse between the bundles, but in no case is there an inward looping of the endodermis. Finally, the stelar origin of the pith of the medullated monostele has been disputed, and the question raised as to whether the medullary and cortical tissues are in reality morphologically different. In other words, is the medullated monostelic type primitive, as its simplicity might indicate, or has it resulted by degeneration from more complex types ?

[31] STRASBURGER: *op. cit.* p. 442.

[32] GWYNNE-VAUGHAN: Polystely and the genus Primula. Ann. Bot. 11: 307. 1897.

[33] JEFFREY: Morphology of the central cylinder of angiosperms. Trans. Canad. Inst. 6: —. (1–40) 1900.

It is interesting to note that Potonié had discussed this last question from the standpoint of fossil botany, and concludes[34] that it seems evident in the case of certain groups, such as the cycads, that the simple results from the complex (for example, the cycads from the Medulloseae). Hence for these groups at least he is inclined to reject this idea of segregation of parenchyma in the center of the protostele to form the medullated monostele, but holds that the medullated monostelic type has probably arisen by degeneration from his "pericaulom." Since this pericaulom was produced, according to his theory, by the lateral fusion of leaf bases in the stem surrounding the originally solid stele, the "urcaulom," the medullated monostele has been derived from a form of central cylinder such as Van Tieghem has described as polystelic, preceded or accompanied by the disappearance of the enclosed urcaulom. The paleontological evidence, however, appears not to be conclusive, for in the very group that Potonié cites, the cycads, so eminent a paleobotanist as Dr. D. H. Scott takes a directly opposite view. He points out[35] that the vascular system of the Medulloseae was typically polystelic, while in the recent cycads there is but one vascular cylinder, and that hence "we should involve ourselves in unnecessary complications if we endeavored to derive the simple, primary structure of the cycadean stem from the more elaborate organization of a Medullosa. It is far more natural to suppose that the monostelic cycads arose from monostelic ancestors."

In 1897, Dr. E. C. Jeffrey put forward another view of the vascular system,[36] based upon a study of the young sporophyte. Here, too, the stele is the unit. According to this conception there are two primitive types of vascular axes; the first the same as Van Tieghem's primitive type, and designated "protostelic;" the second, one in which there is a hollow cylinder, or "siphonostele," whose external wall abuts on the cortex, and whose internal wall encloses the medulla, and which possesses internal as well as external phloem. This is the "amphiphloic siphonostelic" type, called by Van Tieghem the "polystelic." The

[34] POTONIÉ: Die Metamorphose der Pflanzen im Lichte palæontologischer Thatsachen 22.

[35] SCOTT: Studies in fossil botany 395. 1900.

[36] JEFFREY: Trans. Brit. Assn. Toronto. 1897.

commonly called "astelic" modification results from the amphiphloic type by a degeneration of the internal phloem, and the medullated monostelic type of Van Tieghem is derived from the astelic by the loss of the internal phloeoterma or endodermis. A study of development from the seedling is likely to show how these and other modifications in the stellar structure have been derived from the primitive types. Attention is also called to certain portions of the wall of the siphonostele in which the vascular tissues do not develop. These places lie above the points of exit of branch traces, and of leaf traces, and are known as ramular and foliar gaps respectively. Through these gaps the tissues outside and inside connect. In transverse section, the connecting tissues seen constitute the medullary rays, and the segments of the woody cylinder with adjacent phloem and parenchyma the bundles. A fact of great phylogenetic importance in dealing with "gaps" was further pointed out, namely, that in small leaved plants, as in the Lycopodiaceae, Equisetaceae, etc., only ramular gaps occur. These plants are grouped in the division Lycopsida, and their steles are said to be cladosiphonic. In all other vascular plants there is a gap for every leaf. These constitute the large leaved plants, the Pteropsida, and their steles are said to be phyllosiphonic.

As a matter of theory, it is suggested that the siphonostele arose from the protostele for mechanical causes, in the Lycopsida to support the branches, and in the Pteropsida to support the leaves. Potonié also explains the origin of his second primitive type the "pericaulom," the homologue of the siphonostele, on mechanical grounds.

In the light of these theories we can now apply ourselves to an interpretation of the anatomy of the vascular system of the Osmundaceae, and likewise note if the facts already dealt with throw any light on the theories.

First, we are in a better position now to decide whether the internal endodermis of *O. cinnamomea* is phloeotermal or not. It has been noted that in similar cases, that is, in gamodesmic stems, Strasburger has denied the phloeotermal character of the internal endodermis. With regard to the internal endodermis the following facts have been observed:

1. There is present the characteristic cuticularized "radial dot."

2. The structure and contents of the cells are materially the same as of the external endodermis.

3. The sheath is continued into the portions which in some individuals present the phenomenon of internal phloem, just as in any form called by Van Tieghem and Strasburger gamostelic. In the gamostelic type the phloeotermal character of the internal endodermis has been admitted.

4. It generally connects with the external endodermis through ramular gaps, and by no means rarely through foliar gaps. When this occurs, there is no point at which it could be said that the one stops or the other begins.

Having verified these facts in a great many cases, I am therefore of the opinion that the internal and the external endodermis are homologous tissues.

Second, are the medullary tissues morphologically equivalent to the cortical? Again we recapitulate observations.

1. They do not differ in structure or in contents.

2. The medulla very often contains brown sclerenchyma, at least in three species studied, a tissue which, in other ferns, never constitutes a part of the stele.

3. Medulla and cortex connect more frequently than not through the ramular gaps in *O. cinnamomea*, and occasionally through foliar gaps; and neither is there a transition in the nature of the connecting tissues, nor any line at which we can say, the cortical tissues lie externally to this and the medullary tissues internally.

4. The cortical and the medullary brown sclerenchyma sometimes fuse through ramular gaps in *O. cinnamomea*.

5. Portions of stem of *O. cinnamomea* have been found which are of the "gamostelic" type of Van Tieghem. The medulla in gamosteles is granted to be morphologically a cortical tissue.

The conclusion is evident for *O. cinnamomea* at least, and if it be granted that the medullary tissues of this species are morphologically equivalent to the cortical tissues, then biological principles alone would demand a like conclusion for the other species.

Third, of what type is the vascular system of *O. cinnamomea?* Again the facts must form the basis for a decision:

1. The young stem of *O. cinnamomea* possesses an entirely

closed hollow vascular cylinder, sheathed with phloem and broken only immediately above the exit of a leaf trace; and at a level higher up the cylinder is entirely closed again. There is a medulla and an internal endodermis.

2. In older plants the leaves are more frequent, and the gaps extend through several internodes; but yet the cylinder is the unit. The cylinder of phloem is quite rarely broken, except where branching takes place.

3. There is an internal endodermis which is persistent throughout the entire central cylinder of the stem.

4. As a rule the internal endodermis bends out opposite leaf gaps.

5. There is an internal phloem in portions of some plants.

6. Not only does the cylinder of external phloem remain practically unbroken, but opposite leaf gaps there is on the inner side a proliferation of sieve tubes. In *O. regalis* Janczewski found isolated sieve tubes in the parenchyma filling the leaf gap; and the same thing is true of *O. cinnamomea.*

According to Van Tieghem's stelar theory, the last two facts can be explained only by considering the central cylinder of the Osmundaceae to be "gamostelic." The centrad extensions of the phloem opposite the medullary rays could then be explained by assuming that steles had united laterally, with the disappearance of phloem on the medullary side, but with the partial persistence of phloem on the radial planes. This would also explain the occurrence of internal phloem, the union of internal and external endodermis, and the homology of medullary and cortical tissue. But from the study of development there is not a shred of evidence to prove that there has been a union of steles. In fact, such a study shows distinctly that there is but one stele in the stem of *O. cinnamomea* from the very first. Van Tieghem's observations on *O. regalis* have already been quoted (see INTRODUCTION); so we cannot describe the cauline vascular system as "gamostelic," if this name implies a union of steles.

There remains yet another interpretation, namely, that the vascular system of the stem of *O. cinnamomea* is a siphonostele in which some degeneration from the primitive type has taken place. It has been pointed out in a description of the concep-

tion of the vascular system held by Dr. E. C. Jeffrey that the most primitive siphonostele is the amphiphloic siphonostele. In this there is an internal phloem and phloeoterma, and in its phyllosiphonic form there are wide leaf gaps and branch gaps through which internal and external phloem, internal and external phloeterma, and medulla and cortex connect with each other. In *O. cinnamomea* the gaps in this primitive type have closed somewhat, so that medulla and cortex rarely connect except through ramular gaps. Also the phloem forms an almost unbroken cylinder, and the centrad proliferations opposite the medullary rays are the vestigial relics of connection between external and internal phloem. The internal phloem has also disappeared in greater part.

With such a conception of the cauline vascular system of *O. cinnamomea*, the centrad accumulation of sieve tubes opposite the medullary rays, the occasional presence of sieve tubes in the medullary rays, the fact of the internal phloem, the connection of medulla and cortex through ramular and foliar gaps, the presence of sclerenchyma in the medulla, the bending out of the internal endodermis into the leaf gaps, and the facts of development, all become intelligible.

Fourth, which of the species studied possesses the most primitive type of central cylinder?

After a fairly comprehensive study there is one feature that stands out prominently, the great similarity and uniformity of vascular structure in the various species of Osmunda and Todea. According to Solms-Laubach the stems of fossil remains of this family, of which none earlier than the Tertiary have been found, do not present any striking differences from the living representatives. Paleobotany, therefore, offers no solution to the problem. In spite of the conservatism of the central cylinder, there are, however, minor anatomical differences. On the basis of these alone, without referring to the young sporophytes, I think there is sufficient warrant for placing *O. cinnamomea* at one end of the series, possessing as it does an internal endodermis, internal phloem, and wide ramular gaps. It is difficult to say which species is to be placed at the other end of the series. In view of the fact that *O. Claytoniana* never has sclerenchyma in the medulla, that there are small or even no ramular gaps, no internal

sclerenchyma, and even a degenerated external endodermis, we may not be far astray in putting it in the position farthest from *O. cinnamomea*. Now of these two, which retains a central cylinder more nearly primitive? If *O. regalis* has a medullated monostelic central cylinder, as has hitherto been claimed for it, then *O. Claytoniana* has also, and therefore, according to Van Tieghem, a more primitive form than that of *O. cinnamomea*. Assuming the correctness of this for the moment, it will be in order next to see if such phenomena as presented by *O. cinnamomea* could be derived according to Van Tieghem's hypothesis from such a simple medullated monostelic form as that of *O. Claytoniana*.

The phloem sheath must have broken into bundles, and the endodermis must have looped in between the bundles, and connected around them on the centrad side. With the formation of this astelic type some of the cortex would have been included in the medulla, in evidence of which the sclerenchyma in the pith would stand as proof. Then next the bundles must have fused laterally to produce the gamodesmic type in which there is an external and an internal endodermis. Granting that the central cylinder could be so plastic in a single species, there are left yet to be explained the continuous sheath of phloem, the proliferation of sieve tubes opposite the medullary rays, the occurrence of isolated tubes in the medullary rays, the occurrence of internal phloem, and the phenomena of the ramular gaps. Further, there are no facts in development that point to such a series of changes.

Turning now to the other alternative, namely, the possibility that *O. cinnamomea* has the more primitive form of central cylinder, it will be granted that by the degeneration of internal phloem, endodermis, and medullary sclerenchyma, and by the closing of the ramular gaps the central cylinder such as we find in *O. Claytoniana* would result. In proof that such degeneration could have taken place, it is to be noted (1) that in *O. cinnamomea* itself, it has been pointed out that the amphiphloic condition is localized, that the internal endodermis has already begun to degenerate, that medullary sclerenchyma is not a constant feature, and that closed steles above the point of branching are not at all uncommon; and (2) in further proof, analogous cases of

degeneration within the same genus are frequent. Thus within the genus Equisetum two species such as *E. arvense* and *E. hiemale* may be chosen, the first long considered medullated monostelic and more primitive, the second gamodesmic and considerably modified. But a study of development and of nodal portions of the stem has shown that *E. arvense* has a reduced central cylinder, the product of degeneration from a gamodesmic type, and that therefore *E. hiemale* is nearer the primitive. Similar cases of degeneration have been pointed out by Van Tieghem, Poirault, and Jeffrey, in the genera Ophioglossum, Botrychium, Equisetum, Ranunculus, etc. Very lately Boodle,[37,38] has called attention to an interesting series of central cylinders in the family Schizaeaceae. *Aneimia Phyllitidis* has a ring of separate bundles, each with a band of xylem surrounded by a phloem, pericycle, and endodermis of its own; *A. Mexicana* has a complete ring of xylem in the internodes with external and internal cylinders of phloem and endodermis; Schizaea has a ring of xylem surrounding a central pith, but no internal phloem or endodermis. It is likely that here, too, the Schizaea type is derived from the Aneimia type by degeneration. In the Hymenophyllaceae likewise, every grade is found from the case in which the phloem of the solid stele forms a complete ring to that in which it is developed on one side only.

After examining a number of comparatively young specimens of *O. Claytoniana*, I am somewhat doubtful if the study of the development of this species will throw any further light on the subject of morphology; but for *O. regalis* I am more hopeful. Nevertheless, aside from further developmental proofs, I incline to the view that *O. cinnamomea* possesses the most primitive type of central cylinder. I again recapitulate the reasons:

1. The opposite view demands a very plastic central cylinder in one species alone, not differing very greatly in habit from the others.

2. There would still remain phenomena that the opposite view could not explain.

[37] BOODLE: Stem structure in Schizaeaceae, etc. Brit. Assn. Dover, 1899.

[38] BOODLE: On the anatomy of the Hymenophyllaceae. Ann. Bot. 14: 455. 1900.

3. There are no facts of development even in analogous cases to support the opposite opinion.

4. The view adopted here demands only slight changes, and those are of degeneration, to explain all the phenomena.

5. There are precisely similar analogous cases of degeneration.

6. Within the species *O. cinnamomea* itself, every phase of degeneration except the entire disappearance of internal endodermis is observable in suitable specimens.

When we attempt to orient the other species amongst themselves, the task is more difficult, and of little importance. As already indicated, a closer study of development may afford more precise proofs. In the mature stems we have seen that *O. regalis* occasionally has sclerenchyma in the medulla, that there are ramular gaps, though usually small, and that the external endodermis is well developed. In *O. Claytoniana*, on the other hand, sclerenchyma is never found in the medulla, ramular gaps are infrequent, and the external endodermis shows indications of degeneration. In neither of these species is internal endodermis or internal phloem present. The probability, therefore, is that in the genus Osmunda there is a series, *O. cinnamomea* possessing the most primitive type of central cylinder and *O. Claytoniana* the most degenerate, *O. regalis* occupying a middle position, but nearer to the latter. It is merely interesting to note in passing that Professor Campbell concluded[39] from his study of the prothallia of *O. Claytoniana* and *O. cinnamomea*, that the gametophyte of the former was more specialized in many particulars, in other words, was less primitive in type than the latter.

Fifth, does a study of the vascular system help to determine the phylogenetic position of the Osmundaceae?

It was stated at the beginning of this paper that botanists have regarded the Osmundaceae as possessing an anomalous form of central cylinder among the Filicales, their reason being that it seemed to present more of the features of a central cylinder such as is typical for dicotyledons, that is, a medullated monostele in Van Tieghem's terminology. In determining the position of the family, therefore, in any natural system of

[39] CAMPBELL: On the prothallium and embryo of *O. Claytoniana* and *O. cinnamomea*. Ann. Bot. **6**: 49. 1892.

classification, it was hopeless to try to reconcile this single dicotyledonous character with the remaining filicinean characters, and so the vascular system in the family was regarded as anomalous.

It is fair to note that Zenetti dissented[40] from the prevailing view, and evidently for the reason that he attached some value to the nature of the central cylinder from the phylogenetic standpoint. Hence he sought to find the same type amongst the vascular cryptogams. He rejected the ordinary fern type because it is "polystelic," and the lycopod type because there is no pith, obviously overlooking *Selaginella laevigata*, Phylloglossum, etc. So finding no living form with which comparison could be established he turned to paleophytology. Among the Lepidodendraceae he found the prototype sought for, especially in such of these fossils as *L. Harcourtii*, and the Sigillarians, because in these the wood is broken into bundles between which there are medullary rays. But he evidently did not grasp the significance of bundles and medullary rays in relation to leaf traces and branch traces. In *O. regalis*, too, the protoxylem is endarch, while in those ancient lycopods it was exarch. The stele of the Lepidodendraceae, as in all plants bearing palingenetically small leaves, was cladosiphonic, while *O. regalis* is phyllosiphonic, as are all primitively megaphyllous plants. Hence any attempt to establish a relation between the central cylinder of modern ferns and of those ancient horsetails must fail. Indeed, of the early fossil forms preserved, the one with a central cylinder most closely resembling that of the Osmundaceae, as has been pointed out by Dr. Scott,[41] seems to be the cycadofilicinean Lyginodendron (*fig. 26*).

Further, we dissent just as strongly from the view that the family is anomalous in the matter of its vascular system. The typical fern stem possesses an amphiphloic siphonostele, as is especially revealed by a study of development. But degenerated forms of this are to be met with in almost every family, some examples of which have been noted. The Osmundaceae, as has been shown above, all exhibit some degree of degeneration from this type. It is therefore evident that the cauline vascular system of this family is neither primitive nor anomalous among the Filicales.

[40] ZENETTI: *op. cit.* 73. [41] SCOTT: *op. cit.*

SUMMARY OF OBSERVATIONS.

1. An internal endodermis has been demonstrated in *Osmunda cinnamomea*, but in none of the other species examined. This internal endodermis is in textural continuity with the external endodermis through branch gaps, and sometimes through foliar gaps.

2. Internal phloem has been found in *O. cinnamomea* in the region of branching. This is continuous with the external phloem through ramular gaps.

3. The external phloem of the Osmundaceae forms a continuous cylinder, a fact which De Bary has stated for *O. regalis;* and is not broken opposite the medullary rays as Strasburger has affirmed of the same species. Isolated sieve-tubes have been found in the medullary rays of *O. cinnamomea.*

4. The xylem forms a cylinder broken only by foliar and ramular gaps.

5. Brown sclerenchyma has been shown to be usually present in the medulla of *O. cinnamomea*, not uncommonly in *O. regalis*, and not at all in *O. Claytoniana.* It occurs likewise in *Todea barbara*, but has not been observed in *T. superba.*

6. The medullary and cortical tissues of the Osmundaceae are histologically equivalent. Brown sclerenchyma, which is not an intrastelar tissue in other ferns, occurs in both medulla and cortex; and in *O. cinnamomea* the brown sclerenchyma of the medulla is in continuity with that of the cortex.

7. In *O. cinnamomea* the typical ramular gap is one through which internal and external endodermis, internal and external phloem, cortex, and medulla connect. Every stage of degeneration has been observed in *O. cinnamomea*, however, down to the completely closed steles. *O. regalis* has a gap in the wood only, and *O. Claytoniana* usually none.

8. The so-called "quergestreckte Zellen" pointed out by DeBary in *O. regalis*, and more fully described by Zenetti, have been found in all the species studied. They are sieve tubes, possessing all the characteristic features of sieve tubes, even that of callus plugs. Their irregularity of orientation is shared by the other peripheral tissues of the central cylinder, and is apparently due to disturbance caused by the exit of the large leaf traces.

9. Callus plugs have been demonstrated in the sieve tubes.

10. A study of the growing point has further shown that the "quergestreckte Zellen" and the typical protophloem are of the same kind; but it has failed to verify Strasburger's statement that the pericycle and the endodermis arise from a common maternal layer.

11. The phloem forms a continuous sheath in the leaf.

12. The root possesses a protostelic, diarch, occasionally triarch, vascular axis.

SUMMARY OF CONCLUSIONS.

1. The internal endodermis in *O. cinnamomea* is to be regarded as phloeotermal in nature, a fact denied by Strasburger in homologous cases.

2. The medullary and cortical tissues seem to be morphologically equivalent.

3. Observations on the anatomy of the Osmundaceae have been confined heretofore to the cosmopolitan *O. regalis*, and the subtropical Todeas. From these observations it was concluded by Van Tieghem that this family possessed a type of central cylinder anomalous among the vascular cryptogams, a type (the medullated monostelic type) peculiar to the phanerogams. The writer dissents from this view. It appears to be the case that the central cylinder of *O. cinnamomea* is not medullated monostelic, for the medulla is obviously extrastelar. Further, it cannot be regarded as gamodesmic on account of the topographical distribution of the phloem. The most obvious interpretation seems to be that it is a degenerate form of the amphiphloic siphonostelic type of central cylinder (polystelic type of Van Tieghem). *O. cinnamomea*, *O. regalis*, *O. Claytoniana* form a series arranged in order of degeneration of their central cylinders, and the same is true of *T. barbara* and *T. superba*.

The present research was carried on in the Biological Department of Toronto University under the direction of Dr. E. C. Jeffrey, to whom I wish here to express my obligations for his advice throughout. My thanks are due to Professor R. Ramsay Wright for the facilities afforded in the department. For some of the material used I am indebted to Mr. Oakes Ames, Assistant Director of the Botanical Gardens, Harvard University; Sir

William Thistleton Dyer, Director of the Royal Gardens, Kew; Dr. Brodie, Toronto; and Mr. R. B. Thomson, B. A.

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EXPLANATION OF PLATES XIV–XVII.

Abbreviations used.

cp, callus plugs.
c, cortex.
e. c., external cortex.
i. c., internal cortex.
e, endodermis.
e. e., external endodermis.
i. e., internal endodermis.
lt, leaf trace.
m, medulla.
m. r., medullary ray.
p, pericycle.
ph, phloem.
p. ph., protophloem.
px, protoxylem.
qu, "quergestreckte Zellen."
r, root.
s. s. s., strands.
sc, sclerenchyma.
x, xylem.

PLATE XIV.

FIG. 1. Transverse section of the stem of *Osmunda cinnamomea.*

FIG. 2. Transverse section of part of central cylinder of *O. cinnamomea.*

FIG. 3. Tangential section of *O. regalis.*

FIG. 4. "Quergestreckte Zelle" of *T. barbara*, showing sieve plates and callus plugs.

FIG. 5. Transverse section of leaf trace of *O. Claytoniana* near the growing point.

FIG. 6. Transverse section of part of central cylinder of *O. cinnamomea.*

FIG. 7. Sieve tubes of *T. barbara*, showing sieve plates, "globules brillants," and callus plugs.

FIG. 8. Transverse section of part of central cylinder of *O. Claytoniana.*

PLATE XV.

FIG. 9. Tangential section of *Todea barbara*, showing "quergestreckte Zellen."

FIG. 10. Transverse section of *O. cinnamomea*, immediately above point of ramification, showing open branch gaps.

FIG. 11. Transverse section of *O. cinnamomea* through nearly the same region in another plant.

FIG. 12. Transverse section of same plant as in *fig. 11*, but lower down.

FIG. 13. Transverse section of same plant as in *fig. 12*, but lower down.

FIG. 14. Transverse section of the central cylinder of *O. cinnamomea*, showing internal endodermis and brown sclerenchyma in the medulla.

PLATE XVI.

FIG. 15. Transverse section of central cylinder of *O. cinnamomea*, showing internal endodermis and an absence of brown sclerenchyma in the medulla.

FIG. 16. Transverse section of the stem of *O. cinnamomea* in the region of forking, showing absence of ramular gaps.

FIG. 17. Transverse section of the stem of *O. Claytoniana*.

FIG. 18. Transverse section of the stem of *O. Claytoniana* in the region of forking.

FIG. 19. Transverse section of the stem of *O. regalis* in the region of forking.

FIG. 20. Transverse section of the central cylinder of *O. regalis*, showing brown sclerenchyma in the medulla.

PLATE XVII.

FIG. 21. Transverse section of the central cylinder of *O. cinnamomea*, showing internal phloem.

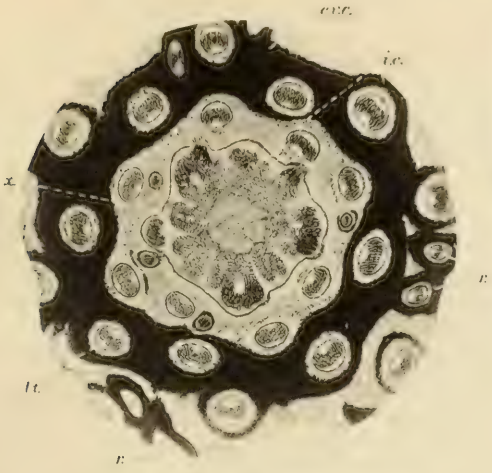
FIG. 22. A part of the central cylinder of *O. cinnamomea* shown in *fig. 21* more highly magnified.

FIG. 23. A transverse section of the young sporophyte of *O. Claytoniana*, showing one foliar gap, und the corresponding leaf trace opposite.

FIG. 24. Transverse section of the stem of *T. barbara*, showing brown sclerenchyma in the medulla.

FIG. 25. Transverse section of a part of the stem of *T. barbara* nearer the growing point.

FIG. 26. Transverse section of *Lyginodendron Oldhamium*, showing a leaf gap, a leaf trace opposite, strands of sclerenchyma in the medulla, and strands of primary xylem centrad of the cylinder of secondary xylem.



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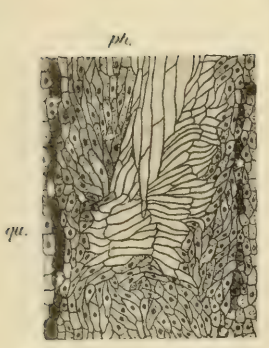

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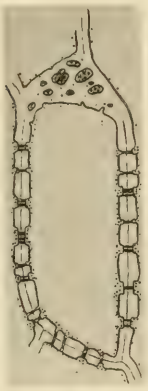

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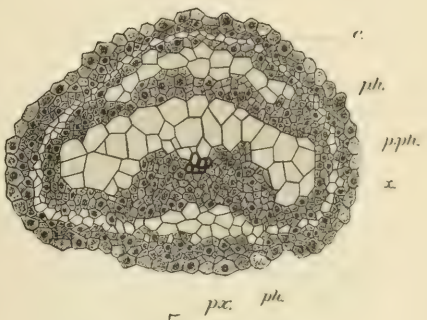

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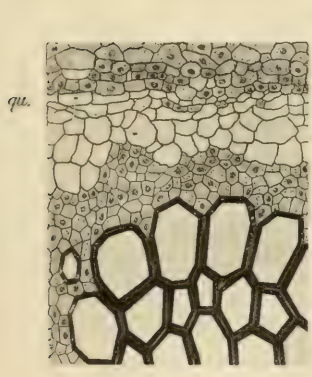

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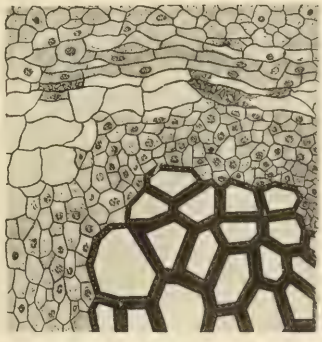

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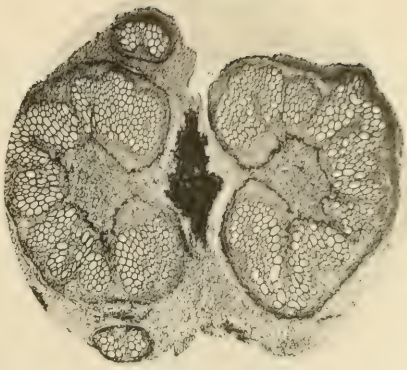

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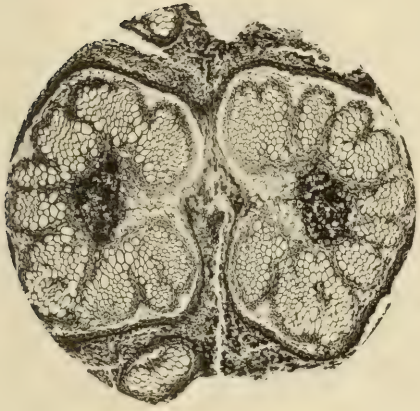

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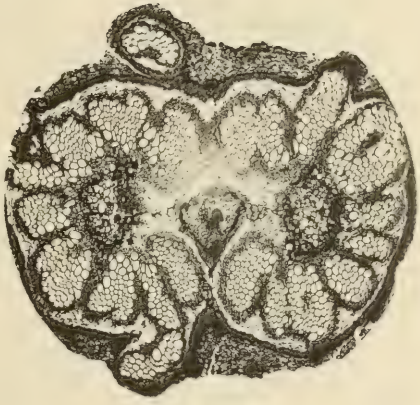

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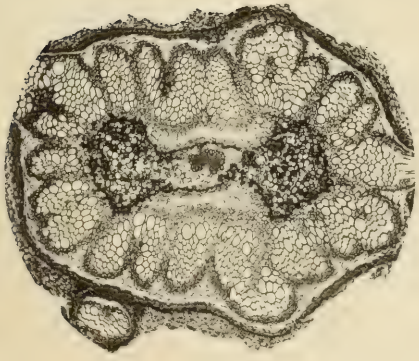

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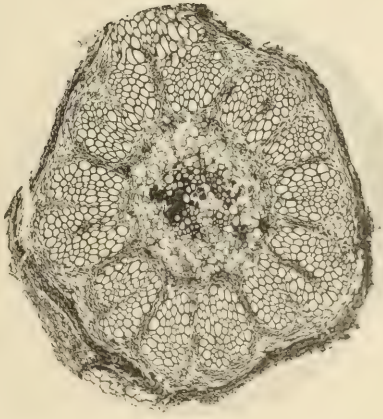

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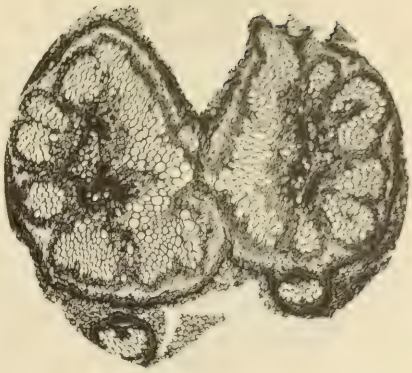

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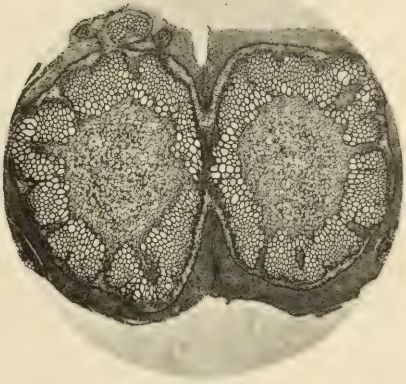

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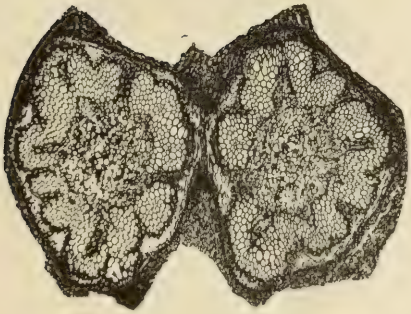

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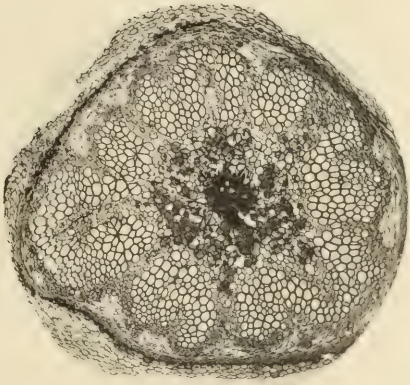

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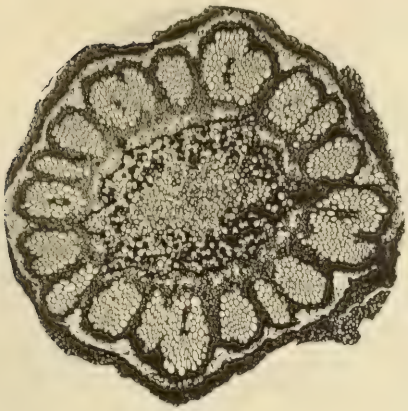

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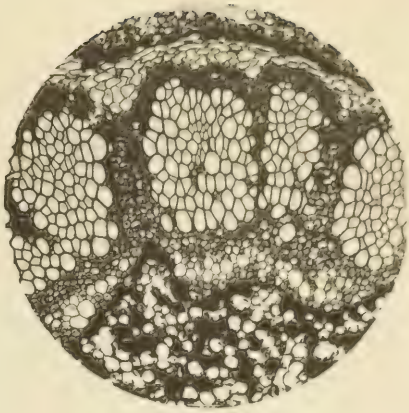

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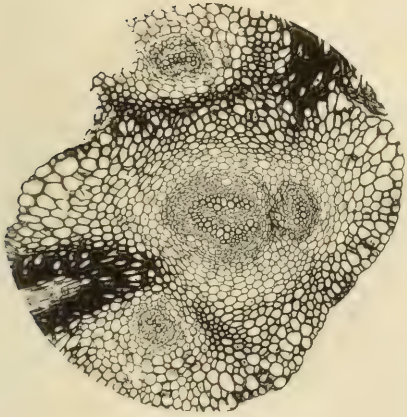

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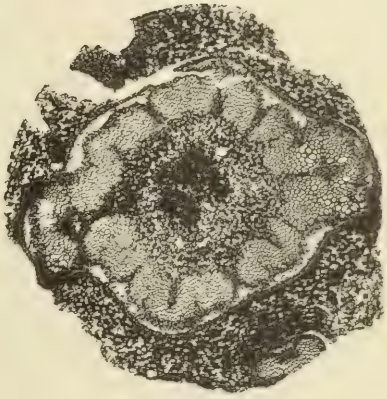

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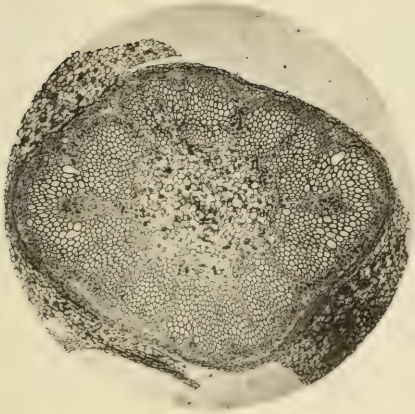

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ON THE IDENTIFICATION OF MECKELIAN AND MYLOHYOID GROOVES IN THE JAWS OF MESOZOIC AND RECENT MAMMALIA,

BY B. ARTHUR BENSLEY.

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ON THE IDENTIFICATION OF MECKELIAN AND MYLOHYOID GROOVES IN THE JAWS OF MESOZOIC AND RECENT MAMMALIA

BY

B. ARTHUR BENSLEY, B.A.

LECTURER ON ZOOLOGY IN THE UNIVERSITY OF TORONTO

ON THE IDENTIFICATION OF MECKELIAN AND MYLOHYOID GROOVES IN THE JAWS OF MESOZOIC AND RECENT MAMMALIA

Owen, in his well-known Monograph of the Mesozoic Mammalia, noted the common occurrence of a linear furrow on the inner surface of the jaw in Jurassic mammals which he designated as a "mylohyoid groove," and Marsh ('87) has described a similar groove for several species from the Jurassic deposits of America. The possible significance of this structure was first commented upon in the discussion which took place during the years 1838-39 as to the mammalian or non-mammalian nature of the original specimens of *Amphitherium* and *Phascolotherium* from the Stonesfield Slate, De Blainville ('38, p. 733) having called attention to an inferior marginal groove, in *Amphitherium*, which he regarded as a suture and as indicative of the composite structure of the jaw in question. De Blainville's opinion was criticized at the time by Duméril, but accepted by Grant ('39), the latter considering the composite structure of the jaws to be obvious "from the distinct deep fissure extending along their base between the dental and opercular pieces." Owen ('38), on the other hand, believed the grooves to be due to the pressure of a nerve or vessel, although he appears to have been, at the time, a little uncertain of his interpretation, partly, perhaps, on account of the presence of a second upper groove in the specimen of *Phascolotherium*, and partly, doubtless, on account of the criticisms of Ogilby ('38) who, while having no explanation of his own to offer as to the significance of the grooves, objected to their being regarded as of vascular origin. In his "British Fossil Mammals," published some years later ('46), however, Owen replied to the opinions of Ogilby and Grant, and showed that the groove in the specimens of *Amphitherium* possessed an entire surface, and was therefore not referable to a suture. In his subsequent monograph ('71) he repeated his opinion, referring to the groove as "mylohyoid." Following Owen, Osborn ('88) has compared the structure with the true mylohyoid groove in the human jaw, at the same time considering it to be of little taxonomic value on account of its variable presence in recent mammals. In the ninth edition of the Encyclopædia Britannica we find the following statement by

Flower ('83, p. 376)—"the mylohyoid groove [is] persistent [in *Amphitherium*], as in some of the existing Marsupials and the Whalebone Whales. This groove, a remnant of that which originally lodges Meckel's cartilage, mistaken for a suture, was once considered evidence of the reptilian nature of these jaws." It thus appears that two somewhat similar, but fundamentally distinct structures have been confused under the designation "mylohyoid groove," and it is chiefly towards pointing out their distinction and distribution, and the taxonomic importance of that occurring in the mesozoic Mammalia, that the following remarks[1] are directed.

The true mylohyoid groove appears to be typically developed only in the primates and artiodactyl Ungulata, although it is by no means always present in the former, as, for example, in *Loris*, *Lagothrix*, *Tarsius*, and *Galago demidoffi*. It is fairly frequent in the Edentata (*Tatusia*, *Bradypus*, *Cholœpus*). It is present in *Lepus* among the Rodentia, and possibly also in other forms, but is difficult to identify in this group on account of the presence of other mandibular grooves. As to its general character it is usually a broad superficial furrow (*cf.* Plate, fig. 3, my.), beginning near and below the dental foramen and terminating abruptly a short distance forwards. It is frequently double, and its extent of development varies somewhat in different individuals. In the human jaw this groove is stated by Quain ('82, p. 56) to lodge the mylohyoid nerve with its accompanying artery and vein.

The relations of the groove which occurs in the mesozoic Mammalia have been amply illustrated by Owen ('71), Marsh ('87), Osborn ('88), and Goodrich ('94). Two of its modifications are here represented by figs. 1 and 2 in the plate (*Amblotherium*, *Spalacotherium*), copied from Osborn's memoir. Apart from its regular linear outline its more important features are as follows: (a) its close relation with the dental foramen; in many forms (*Amphitherium*, *Amblotherium*, *Amphilestes*, *Spalacotherium*, *Tinodon*) it appears to be simply an anterior continuation of the latter; (b) the fact that while sometimes confined to the posterior part of the jaw (*Spalacotherium*, *Phascolotherium*, *Amphilestes*) it frequently traverses the whole length from the dental foramen to the symphysis (*Amblotherium*, *Achyrodon*, *Dryolestes*, *Docodon*). As regards the distribution of this groove it is typically

[1] Where not otherwise noted these are based on the osteological collection of the British Museum (Natural History), London.

developed only in the presumably higher mesozoic forms, being absent as far as known in the Multituberculata.

There can be no doubt that Flower's opinion as to the relation of this structure with Meckel's cartilage is the correct one. An exactly similar groove lodging Meckel's cartilage may be seen in embryos of existing mammals, and its somewhat close resemblance to the true mylohyoid groove may be easily shown to be the result of coincidence. In the plate (figs. 10*a-d*) will be found illustrations of four transverse sections through the lower jaw of a 6cm. pouch-fœtus of *Macropus*[1]. Section *a*, taken immediately behind the symphysis, passes through what is at this stage the anterior limit of Meckel's cartilage. The symphysial portion of the latter has already been reduced or the anterior part of the jaw has grown beyond it. This section and section *b*, which is taken further back, show Meckel's cartilage lodged in a groove on the inner surface of the bony mandible, and separated by a bony strand from the dental nerve and artery in its interior. Section *d*, taken immediately behind the dental foramen, shows the dental nerve and artery in close relation with Meckel's cartilage, while section *c* shows the condition at the foramen where the nerve and artery become separated from the cartilage, the two former passing into the body of the jaw while the latter enters the groove on its inner surface. A short distance posterior to section *d* in this series the nerve and artery are seen to give off mylohyoid branches.

A comparison of these sections and of the dissection of a fœtal jaw represented in the plate (fig. 5) will suffice to show the identity of the groove lodging Meckel's cartilage in the embryo with that in the mesozoic Mammalia. The cause of its resemblance to the true mylohyoid groove will also be apparent, since in the embryo we find Meckel's cartilage leaving the dental nerve and artery at the dental foramen and passing into a groove on the inner surface of the jaw, in much the same way that, at a later stage, the mylohyoid branches leave the inferior dental trunks at the foramen and pass into the mylohyoid groove. It is probable that the condition described above for *Macropus*, namely, the lodging of Meckel's cartilage in a groove, represents the general one in the Mammalia. Parker ('85) has described and figured it for several of the Edentata and Insectivora, and

[1] For this specimen, with many others, the writer is indebted to Professor Bashford Dean, of Columbia University, New York.

the writer has observed it in the case of several genera of marsupials, including *Myrmecobius*, *Phascogale*, *Trichosurus*, *Phalanger*, *Dasyurus*, *Perameles*, and *Thylacomys*[1].

It is obvious that in many cases the mylohyoid groove must, during development, become superposed to the Meckelian groove. Magitot and Robin ('62) have described what is apparently that condition for man. But that such is not always the case may be seen from the forms represented in figs. 8[2] and 9 of the plate, in which both grooves are present with similar relations to the dental foramen but with different positions in the jaw.

Considering the nature of the groove represented in the mesozoic Mammalia we can scarcely expect to find it fully developed in adults of recent mammals. Owen ('38, '71) described and figured a groove in the jaw of *Myrmecobius*, which he regarded as equivalent to that in the mesozoic forms, but Osborn ('88) was unable to recognize this structure in two specimens belonging to the Yale University collection, and he has further stated, on the authority of Mr. Thomas, that it is absent in the British Museum specimens. Leche ('91) also failed to find it in three of his specimens, but has mentioned its presence in a fourth immature one. The fact of the matter is that a short broad furrow does occur in *Myrmecobius* exactly as Owen has described and figured, but its great width almost precludes its being spoken of as a groove, and it has obviously nothing to do either with the mylohyoid or the Meckelian groove. Its presence is due simply to the elevation of the internal alveolar edge. A much more definite groove, due to the same cause, is frequently present in recent mammals (*cf.* Plate, fig. 4).

Owen also mentioned a similar groove for *Phascolomys*. This structure, which is amply illustrated in the British Museum specimens, appears to represent a mylohyoid groove. In adult jaws it is frequently found to be branched. In a young animal of which the writer dissected this region, the posterior portion of the groove was alone developed, and it lodged the mylohyoid nerve. In the young wombat the groove is placed just at the point where the anterior portion of the inflected angle joins the body of the jaw. A similar structure is frequently present in other marsupials. Its somewhat

[1] The specimens representing these genera were kindly lent by the late Mr. Martin F. Woodward, of the Royal College of Science, London.

[2] For the loan of this specimen—a foetal jaw of *Propithecus*—the writer is indebted to Dr. Forsyth Major, of the British Museum, London.

different appearance as compared with the mylohyoid groove of placentals is due to the presence of the angular inflection.

Undoubted traces of the Meckelian groove are, however, to be seen in adults of recent mammals, although in most cases only as variations. Fig. 4 of the plate shows the appearance of it in an aged specimen of *Didelphys*. This may be compared with fig. 9 which shows the normal condition in a young animal. Figs. 6 and 7 show the condition in two other specimens representing *Tatusia* and *Chrysochloris*; the former is not fully adult. Similar conditions are observable in some specimens of the following forms,—*Trichosurus*, *Phalanger*, *Perameles*, and *Petauroides*, among the marsupials, *Xenurus* and *Dasypus* among the Edentata, *Hemicentetes* and *Echinops*, among the Insectivora. The groove is doubtless frequently present in many other forms, but such a reduced and variable structure almost defies recognition. As stated by Eschricht and Reinhardt ('66) it is present in the adult of *Balæna mysticetus*.

It is an interesting question why the Meckelian groove is not present in the Multituberculata. Following Cope's suggestion ('88) as to the monotreme affinities of these animals, the writer examined the condition in three specimens of *Echidna* of 2, 9, and 16cm. head and body lengths. The relations in this form, however, proved disappointing. In the 2cm. egg-embryo there were only a few traces of bone formation in the lower jaw, while in the two larger animals the dentary element was well formed but was not in relation with Meckel's cartilage posteriorly. In both of the later stages the symphysial portion of the cartilage was seen to be very much elongated, and for a short distance behind the symphysis the cartilage was lodged in a concavity of the dentary bone. Immediately posterior to this point, however, the cartilage was found to leave the jaw, and to pass backwards independently of it. In the 9cm. embryo its position was internal and ventral with reference to the jaw, and in the older animal its separation from the jaw and its internal position were still more marked.

The condition in *Echidna* is apparently the result of the great reduction or degeneration of the jaw characteristic of this form. It is possible that the conditions in *Ornithorhynchus* might throw some light on the question, but no embryos of this form were available. It seems most unlikely that in the Multituberculata the Meckelian cartilage could have had the same relations as in existing mammals,

and was absent in the adult stage. There is a possibility that in the mammalian prototype the cartilage was either completely enclosed in the dentary bone or co-ossified with it. It is interesting to note that Parker (*op. cit.*) has described a partial ossification of the cartilage in the young of *Centetes* and *Talpa*, and a partial enclosure of it in the latter form and in *Erinaceus*. The frequent exposure of Meckel's cartilage in the jaws of lower Vertebrata, however, warns against the adoption of such an explanation before the acquisition of more definite evidence.

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EXPLANATION OF PLATE

Fig. 1. *Amblotherium soricinum.* Right mandibular ramus, showing the groove for Meckel's cartilage. (After Osborn.)

" 2. *Spalacotherium tricuspidens.* Left ramus reversed, showing the relation of the groove for Meckel's cartilage with the dental foramen. (After Osborn.)

" 3. *Mycetes ursinus.* Right ramus, showing the true mylohyoid groove.

" 4. *Didelphys marsupialis.* Right ramus of an old individual, in which traces of the groove for Meckel's cartilage are present as a variation.

" 5. *Macropus sp.* Moist preparation of the left ramus of a 7cm. pouch-fœtus, showing Meckel's cartilage lodged in its groove. The ear-bones are schematically represented.

" 6. *Tatusia novemcincta.* Right ramus of an immature individual, showing the groove for Meckel's cartilage and the mylohyoid groove below it.

" 7. *Chrysochoris trevelyanus.* Left ramus, showing traces of the groove for Meckel's cartilage in the adult.

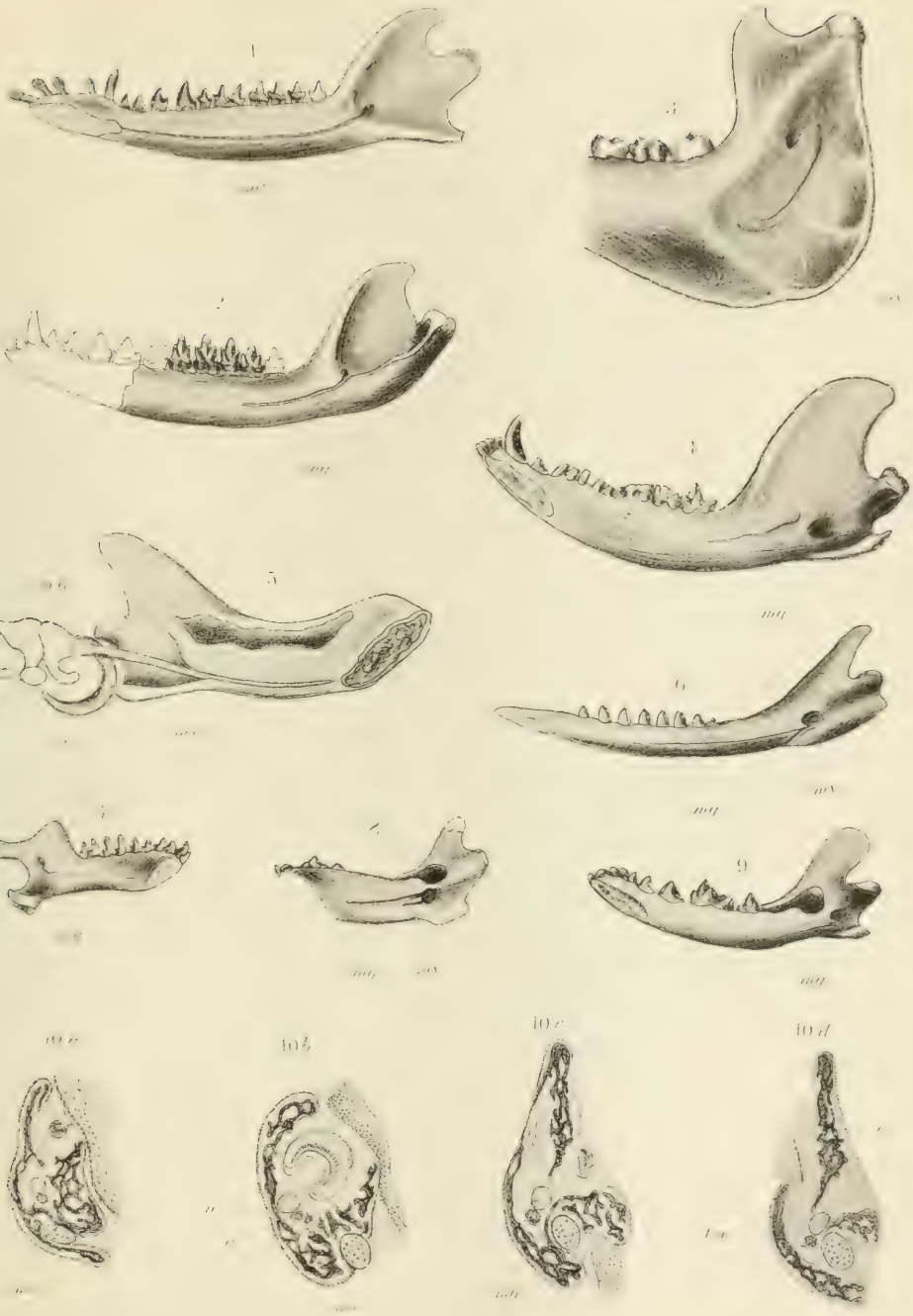
" 8. *Propithecus sp.* Right ramus of fœtus; the mylohyoid groove is here formed below that lodging Meckel's cartilage.

" 9. *Didelphys marsupialis.* Right ramus of an young individual showing the normal appearance of the groove for Meckel's cartilage in the later stages of its reduction.

" 10. a-d. *Macropus sp.* Transverse sections through the right ramus of a 6cm pouch-fœtus, showing the relations of Meckel's cartilage and the dental nerve and artery to the jaw. For explanation see text.

Abbreviations

mg—groove for Meckel's cartilage.
my—mylohyoid groove.
mc—Meckel's cartilage.
ml—malleus.
i—incus.
st—stapes.
ty—tympanic annulus.
n—dental nerve.
a—dental artery,
c—coronoid process of mandible.
fm—masseteric foramen.



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No. 4: THE MEGASPORE-MEMBRANE OF THE GYMNOSPERMS, By R. B. THOMSON

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THE MEGASPORE-MEMBRANE OF THE GYMNOSPERMS

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THE MEGASPORE-MEMBRANE* OF THE GYMNOSPERMS

Hofmeister, in his classic comparative researches of 1851, indicated the fundamental connection between the ovule of the gymnosperms and the sporangium of the higher cryptogams. He showed the homology of the embryo-sac of the former with the free spore of the latter in words which deserve prominence in connection with the present work (Hofmeister,[1] p. 141): "Der Embryosack der Coniferen lässt sich betrachten als eine Spore, welche von ihrem Sporangium umschlossen bleibt." Mettenius,[2] Solms-Laubach,[3] Scott[4] and Worsdell[5] have directed attention to the cryptogamic nature of the foliar and peduncular bundles of the gymnosperms, thus carrying out in another direction the generalization which Hofmeister reached in the case of the ovule. The occurrence in palaeozoic strata of a group of forms,designated by Petonié[6] the "Cycadofilices," forms which combine pteridophyte and gymnosperm features, and some of which have been recently shown to be seed-bearing (the so-called Pteridospermae[7]), indicates the close connection that existed in past times between these two phyla. The discovery within the last few years of antherozoids in the cycads and *Ginkgo* by Hirasé,[8] Ikeno,[9] Webber,[10] and Lang,[11] affords more evidence of the relationship of the gymnosperms to the vascular cryptogams.

The investigation with which the present account is concerned deals with another of the cryptogamic features of the gymnosperms. A megaspore-membrane has for a long time been somewhat generally recognized as occurring in certain forms of this group of primitive seed-plants. The extent of its occurrence and the character of the coat in the whole group have, however, not as yet received the attention they deserve, in view of the comparison made by Hofmeister in 1851, as indicative of the free-sporing ancestry of the gymnosperms,

* The term megaspore-membrane or megaspore-coat is applied ordinarily only to the coat of the uninucleate spore. It is here used to designate as well the investing coat of the prothallium, undoubtedly the representative of the former which has been delayed in development.

and from the phylogenetic importance which attaches to the relative state of development of the membrane in the different groups and subgroups of this division of the spermatophytes. Indeed at the present time, information which will help to indicate the phylogenetic positions, especially of the subgroups of the conifers, is very desirable on account of the balanced state of conflicting testimony from various sources, especially of such as is based on the interpretation of the female "flower," and also because of the lack of historical evidence, a lack which Dr. Scott clearly states in his recent *Fossil Botany* (p. 483): "On the whole, it is impossible, in the present state of knowledge, to say which tribe or family of the Coniferae is the most ancient." It is then chiefly with the object of affording evidence of the relative antiquity of the forms of the Coniferales that this study of the megaspore-membrane is concerned.

In addition to the investigation of the megaspore-membrane, the tapetum has received considerable attention, since a relationship between the two structures was observed during the progress of the work.

Material for the purpose of the investigation was secured during the last two years. Many of the forms required not being native, I could not personally collect material of them and am indebted to the kindness of the following persons for rendering it available : Sir W. T. Thiselton-Dyer, Director of the Royal Gardens, Kew; D. H. Scott, M.A., Ph.D., Honorary Keeper of the Jodrell Laboratory, Royal Gardens, Kew; W. H. Lang, D.Sc., Lecturer in Botany, University of Glasgow; Wm. Trelease, Director of the Missouri Botanical Gardens, St. Louis; J. M. Coulter, Ph.D., Head Professor of Botany, University of Chicago; C. J. Chamberlain, Ph.D., Instructor in Botany, University of Chicago; M. A. Chrysler, Ph.D., Fellow in Botany, University of Chicago; A. A. Lawson, Ph.D., Lecturer in Botany, Stanford University; B. L. Robinson, Ph.D., Asa Gray Professor of Systematic Botany and Curator of the Herbarium, Harvard University; E. W. Oliver, A.M., Instructor in Botany, Harvard University; W. C. Coker,

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In the following account the order in which the forms are taken up is that indicated in Engler and Prantl's "Die natürlichen Pflanzenfamilien."

CYCADALES.

I. *Cycadeae :* Ovules of *Cycas revoluta* at a comparatively early stage of development have a well marked megaspore-membrane. The coat is 4.5 μ thick at the stage when the prothallium consists of a parietal stratum of protoplasm with a single row of numerous nuclei* embedded in it. It consists of two layers (photo. 1, pl. 3),the endosporium† and the exosporium, which are approximately equal in thickness at this stage. The double nature of the coat is, however, usually not apparent in sections from ordinarily prepared paraffin material. The coat withstands the infiltration of paraffin much more than the tissues of the ovule and in consequence is often "dragged" in sectioning. In the "dragged" condition it appears as a single layer with fine granules slightly projecting from its surface. It is variable in thickness but ordinarily about twice as thick as the norm. The true structure of the coat is always apparent in sections from ovules which have

* About 100 nuclei are present in a 5 μ section through the megaspore, and cell formation is beginning in the basal region.

† The endosporium is always towards the left in the figures, next the prothallium.

been subjected to prolonged infiltration of paraffin (m.p. 56°C.) and cut 2 to 5 μ thick at a low temperature. In such preparations the endosporium and the exosporium are quite distinct from each other, a clearly defined line of demarcation occurring between them. Along this line in the sections a separation of the layers sometimes occurs for a short distance (middle of photo. 1, pl. 1). Moreover, the two layers of the coat are different from each other in structure, and hence the double nature of the coat is the more apparent (fig. 1, pl. 1). The exosporium is granular towards its somewhat irregular external border and a fine radial striation can be made out in this part, while towards the inside it becomes gradually more homogeneous. The endosporium appears in section to be sub-divided into two longitudinal strata, by a granular area (fig. 1, pl. 1). The outer of these strata is very finely granular and yellow in colour in the unstained condition, as is the exosporium, while the inner is quite homogeneous, translucent and almost colourless. The zone separating the two sublayers is indistinctly defined, the area in question presenting the appearance of being radially and somewhat coarsely granular at its centre, gradually merging into the strata on either side of it. In very rare instances I have observed that, in the sections of the coat, the two strata of the endosporium are torn from one another at places along the granular area. The endosporium besides being quite different from the exosporium in structure, expands much more than the latter in certain fluids. A tension is thus set up between the layers which, in sections, results either in their separation, or in a "coiling" of the whole coat. The former occurs in cases where the sections of the coat remain attached to the slide (middle of photo. 1, pl. 1), the latter where the sections are detached and float freely in the fluid. In addition, it would seem that the endosporium is more impenetrable to paraffin than the exosporium, since in sectioning the former is often "dragged" and its structure obscured, while the latter is clearly cut.

The double nature of the coat and the structural as well as the chemical differences in its layers are clearly brought

out by the action of stains and reagents. A number of stains were experimented with, but the best results were obtained from the use of a very dilute solution of Ehrlich's acid haematoxylin, with a small amount of alum solution added, followed by a dilute aqueous solution of safranin. The exosporium stains cherry red, the endosporium a variety of colours passing into one another gradually. From dark red along the outer border the endosporium becomes reddish-violet, then dark violet along the median granular area, and finally dark and then lighter yellow towards the inner side. Sometimes a tint of orange is seen in the yellow area. The action of the stains on the layers of the coat gives an intimation at least of their chemical composition. A more definite idea of this is obtained by the use of certain standard reagents. Most reliance has been placed on the action of chlorzinc iodine. When thin sections of the young membrane (photo. 1, pl. 1) are treated with this fluid the exosporium becomes yellowish-brown with a tint of red in it, while the endosporium exhibits a variety of colours, one shading into another. The outer stratum of this layer is somewhat darker than the exosporium but very similar to it in colour, while the inner is slightly violet becoming less so towards the inner side. It is usually more pink-violet than the walls of the ordinary parenchyma cells of the ovule. Between the two differently coloured strata of the endosporium is a narrow greenish-yellow area which shades gradually into those on either side. The boundary line between the endosporium and the exosporium is dark and distinct after treatment with chlorzinc iodine, and the coat is somewhat swollen by the fluid and appears less granular. After the action of iodine and sulphuric acid upon sections of the membrane, the outer layer becomes dark yellowish-brown, while the inner has a yellowish-brown outer stratum which entad passes into light yellow, greenish-yellow, and finally into blue. Within the blue can often be seen a homogeneous inner greenish-yellow margin into which the blue shades. From the well known action of the stains and reagents used it is evident that we have to do with a coat whose exosporium is suberized but whose

endosporium is of very complex chemical composition. The inner part of this layer contains a substance which seems closely related to pectin.* Towards the median area of the endosporium this substance is largely replaced by cellulose. The cellulose in turn, in the outer stratum of the endosporium, gives place gradually to suberin, the outer border of the layer being constituted chiefly of the latter.

In older ovules of *Cycas revoluta* the megaspore-membrane is still distinctly double but it has increased considerably in thickness. At the stage of prothallial development when cells are forming (two layers thick in this case) the membrane is of quite uniform distribution around the prothallium and slightly more than 5 μ thick. The increase in thickness does not affect the two layers equally but is chiefly confined to the endosporium which at this stage is about one and one-half times as thick as the exosporium. The latter layer appears more distinctly radially striated in cross-section than in the case of the younger membrane. Sections of it cut parallel to the free surface of the coat are very coarsely granular. The granules are dark yellow in colour but differ from one another both in size and in form. They appear to be surrounded by irregular spaces. The exosporium would thus seem to be formed of little columns or fibrillae which are radially arranged and so responsible for the appearance of radial striation in cross-sections of the coat. This interpretation of the structure of the exosporium is verified by the character of the cleavage of the layer. When sections are broken across the cleavage plane is always at right angles to the free surface of the coat and along the line of the striation. Sometimes two breaks in the exosporium occur near one another and a small rectangular mass is torn out of the layer. This cleavage feature of the coat is characteristic of the younger membrane of *Cycas* as well, though the latter is not so frequently found broken. The inner layer of the megaspore-coat, as stated above, is much thicker at this stage than at the previous one. It pre-

* This part, as was stated, does not colour a decidedly orange-yellow in safranin (Strasburger [12], p. 135), but a rather dark yellow. At a later stage, however, to be described next, the orange-yellow is more distinct.

sents clearly the appearance of being subdivided into two strata. These, however, vary considerably in relative thickness at different places in the sections, but the outer portion is always thicker than the inner, sometimes appearing as thick as the exosporium. The boundary between the two strata of the endosporium is, as in the young condition, a somewhat radially granular part. Moreover, a slight difference in the chemical composition of the endosporium is to be noted at this stage. A greater portion of the outer stratum of the layer is completely suberized, while its inner part gives a more decided indication (orange yellow in safranin) of the presence of pectin, the cellulose being more definitely confined to the median part of the layer at this than at the previous stage. The coat "coils" more readily also in the older condition, "coiling" being an especially marked feature at this stage in sections which have been treated with potash solution. The endosporium swells very much in this fluid and the coat may attain a thickness of 8 μ.

In addition, there is present on the inner side of the coat a thin layer which is in very intimate association with it, so intimate in fact that it is readily mistaken for a part of the coat. It is, however, formed of the outer walls of the superficial prothallial cells. This apparent "reinforcement" of the coat may be very considerable in older ovules. Indeed in ripe seeds of many forms a *suberized* band is present over the free surface of the prothallial cells which is as thick or even thicker than the coat itself and is difficult to distinguish from it, since the two structures are alike in chemical composition and often closely in contact with one another.

The chief change in the megaspore-membrane of *Cycas revoluta* at the stage when the archegonia are formed is in the relative thickness of its two layers. The exosporium is fully double as thick as the endosporium, though the thickness of the whole coat is but slightly increased. The exosporium is more plainly radially striated than at previous stages.

I obtained only a single ovule of *Cycas Rumphii* for examination. It was at a stage of development similar to the

earliest examined of *C. revoluta*, i.e., when the prothallium is about to begin cell formation. At this stage the coat of *C. Rumphii* is so similar in structure, in thickness, and in chemical composition to that of *C. revoluta* as to be indistinguishable from it. In fact had not the shape of the ovule been different in the two species I should have been unable to distinguish the sections of them.

In order to determine whether the structure and chemical composition of the coat of the megaspore of *Cycas* has been affected by the retention of the spore in the megasporangium it seemed desirable to study the coat of the microspore for comparison. Material of *Cycas* was not available, but a study of the coat of the mature microspore of *Pinus resinosa* was made instead. The coat in this form is about 5 μ thick in the region above the prothallial cells, its exine and intine being about equally thick and separated by a dark line. The outer layer of the coat is roughly radially striated and the inner homogeneous. When thin sections (3 to 5 μ thick) have been treated with chlorzinc iodine the exosporium is yellowish-brown and the endosporium shades from light violet on the inside to darker violet about the middle, then into greenish-yellow, and finally into light brownish-yellow along the outside. In safranin the intine is somewhat orange yellow on the inside and pink towards the middle and outer parts. The exine is cherry red. In haematoxylin the inner and outer borders of the intine stain less intensely than the median part. It is thus seen that the strata of the intine of the microspore of *Pinus* react similarly with stains and reagents to those of the endosporium of *Cycas*, though they are not so distinct from one another as are those of the latter. The enclosed and the free spore coats are thus in structure and in chemical composition fundamentally alike.

For the purpose of comparison of the process of suberization in spore-coats and in cell-walls an examination was made of the suberized walls of the epidermal cells on the upper surface of the megasporangium of *Zamia integrifolia*, a form whose megaspore-coat will be described later. When sections

are treated with chlorzinc iodine, around the protoplasm of these epidermal cells is a uniformly thick homogeneous layer which is violet in colour. Over the free surface of the cells is a dark brown band from which characteristic wedge-like projections pass inwards between the outer parts of the violet walls of contiguous cells. The dark brown of this band and its projections never comes into intimate contact with the violet of the inner layer but is always separated from it by a greenish-yellow area just as in the inner layer of the spore-coats described above. This colour is no doubt produced from a blending of the colours of the adjoining strata, so that it would appear that we have here to do with an area of composite chemical character, a transitional area between strata of different chemical composition. The suberized walls of epidermal cells are undoubtedly formed from within, and since the endosporium of the coat of *Cycas* consists of similar chemical strata and has a like transitional area between the cellulose and the suberized layers, it seems probable that it, too, is formed from the inside. Confirmation of this view is indicated in the action of chlorzinc iodine on the two suberized parts of the coat. The outer stratum of the endosporium is darker brown in colour than the exosporium and so according to the well established colour reaction of this reagent is of more recent formation.

The present work on the Cycadeae has demonstrated the occurrence of a megaspore-coat in the subgroup and has determined its structure, thickness, and chemical composition. The coat is double, its outer layer suberized and composed of radially arranged fibrillae, while its inner is fairly homogeneous and formed of strata which, though they differ fundamentally in chemical composition, are yet not distinct but pass by gradual change of chemical components insensibly into one another.

The tapetum of *Cycas* at the youngest stage of prothallial development described above consists usually of a single layer of large somewhat cubical cells, on the inside of which there may often be seen here and there some more or less

collapsed ones (fig. 1, pl. 1). The distribution of the tapetum is uniform around the prothallium at this stage just as is the megaspore-coat. The large cells have one to several nuclei in each, two being not uncommon in a section. In the cells are irregular-shaped grains which in stained preparations and in those treated with chlorzinc iodine, etc., appear as vacuoles (fig. 1, pl. 1). These grains in iodine solution* colour at first a brownish-pink or purple, some of them even a red brown. On heating the colour fades, and on cooling and applying fresh fluid the brown colour does not return but is replaced by pink to bluish-purple. These grains are then not glycogenous as at first seemed probable but consist largely of amylodextrin (Strasburger[12], pp. 82 and 83). They are quite different from the starch grains of the nucellar and integumental tissues, the latter being much smaller and giving the characteristic starch reaction. The tapetal cells at the later stage of prothallial development when cell formation has begun are devoid of amylodextrin and their protoplasm appears to be fairly uniformly distributed. The most interesting point, however, with regard to the tapetum in the present connection is apparent when sections of it and of the nucellar tissue are treated with chlorzinc iodine. All the megasporangial cells, even the very thin-walled ones of the more or less collapsed tissue immediately adjoining the tapetum, have violet-coloured walls, while those of the contiguous large tapetal cells are dark yellowish-brown. The walls of the tapetal cells are thus suberized and so afford a striking contrast between this tissue and that of the nucellus. On the other hand the suberization gives indications of an association between the tapetal cells and the megaspore itself, which, to say the least, suggests very forcibly a common origin, and is thus almost in the nature of a demonstration of the sporogenous character of the tapetum.

II (a). *Zamieae-Stangerieae.* In some very young ovules of *Stangeria paradoxa*, the only form included in the Stangerieae

* The solution used was made up as follows: 5 c.g. iodine and 20 c.g. potassium iodide were dissolved in 15 c.c. of water.

the megaspore-nucleus had already divided several times, probably about eight free nuclei being present in the somewhat parietal protoplasm of the young prothallium. Around the protoplasm at this early stage there is present an undifferentiated, glassy-looking thickening which represents the young megaspore-membrane.

At a later stage, when a cellular prothallium has been formed and the ovules are about ready for pollination* the megaspore-coat is a very conspicuous feature. It is about 4.5 μ thick and is very distinctly double (photo. 2, pl. 3), in fact it is very similar to that of *Cycas revoluta* at the stage when the prothallial cells are forming. The exosporium is fairly granular and very deeply and distinctly striated. The endosporium consists of two homogeneous bands, the outer of which is thicker than the inner. These have a narrow granular area between them just as in the case of *Cycas*. The coat, too, is "reinforced" from within by the outer walls of the superficial layer of prothallial cells, so that it appears to consist of four layers, a pair of inner narrow and a pair of outer relatively broad bands. Haematoxylin stains both layers of the endosporium, the outer less than the inner but still to a considerable extent. Safranin stains the outer stratum also and colours as well the exosporium. The outer part of the endosporium is intensely stained as a result of the double staining, and a good contrast is obtained between the layers as is shown in photograph 2, plate 3. The thickness of the inner part of the endosporium appears less in the photograph than its actual thickness, because of the over-staining of the outer part of this layer and its consequent encroachment on the inner. There is also but little indication in the illustration that the inner lighter part is of a composite character, since the "reinforcement" from the cell-walls is very closely associated with the inner part of the membrane. In sections treated with chlorzinc iodine, however, this is evident, since the cell-walls are coloured yellow while part of the endosporium is blue-violet. The transition

* For the material of *Stangeria* at this stage I here express my especial indebtedness to Dr. Lang of Glasgow University, who was kind enough to send me several embedded ovules.

area from the violet to the yellowish-brown (tinted red) of the outer part of the endosporium is greenish-yellow as in *Cycas*. This outer part of the endosporium is homogeneous and coloured similarly to but darker than the finely granular and radially striated exosporium. When sections of the coat are treated with iodine and sulphuric acid the inner part of the endosporium is greenish-yellow to blue in colour and shades as in *Cycas* into the dark yellowish-brown of the outer parts of the coat.

Dr. Lang has represented the coat of *Stangeria*, in his work on the ovule of this form, as a single, thick, somewhat granular band (Lang[11], fig. 16, pl. 17), at a stage of prothallial development similar to that at which I have described it above. I find that the coat of *Stangeria*, like that of *Cycas*, "drags" very frequently in sectioning, and that in this condition it appears as an unstratified, fairly homogeneous layer, much thicker than in normal section, fully as thick as Dr. Lang has represented it. Dr. Lang was primarily interested in other important features and possibly his sections, like many of mine, while showing the structure of the tissues perfectly, gave but a false impression of the coat.

The megaspore-membrane of *Stangeria* thus is similar in every respect to that of *Cycas*, It is double, its endosporium subdivided into two more or less homogeneous strata. The exosporium and the outer stratum of the endosporium are suberized, the latter containing a little cellulose, while the inner part consists largely of this substance with which some pectin is probably associated. Between the two strata of the endosporium is a transitional area, transitional both in structure and in chemical composition, just as in the case of *Cycas*. In thickness, however, the coat of *Stangeria* is somewhat less than that of *Cycas* even when the latter is at a younger stage of prothallial development.

Dr. Lang has described the tapetum of *Stangeria* in several stages of development. His account of it when the prothallium has become cellular is as follows (Lang[11], p. 287) : "Around the megaspore a layer of cells was present which is

clearly to be traced to the sporogenous group. The thick zone of sporogenous tissue present in earlier stages has, however, become reduced to a single layer. . . . The cells of this persistent layer (fig. 16) are very large, and stand with the longer axis at right angles to the surface of the megaspore. Sometimes a single nucleus is present; often two are found in each cell." This layer is said ultimately to disappear in the seed. In infertile ovules Dr. Lang has further observed that the tapetum is well developed. My own observations on the tapetum in *Stangeria* agree, so far as they go, with Dr. Lang's description. In the young condition when the nucleus of the megaspore has undergone probably three successive divisions the tapetal tissue is of considerable thickness. The walls of its cells have not, however, as yet differentiated chemically, colouring greenish-yellow in chlorzinc iodine. At the older stage at which the megaspore-membrane has been described the tapetum consists of a single, or at rare intervals of a double, layer of cells. These have thin but yet distinctly suberized walls, just as in the case of *Cycas* at a similar stage of development. In sections of infertile ovules of the same age as the fertile ones described above the tapetum is a very prominent structure, being four to five layers of cells in thickness. Its cells, too, are large and have very abundant protoplasmic contents, while those of the surrounding tissue are in a more or less collapsed condition. Further, the walls of the tapetal cells are thick and suberized while those of the adjoining nucellar tissue are thin and composed of cellulose. The greater development of the suberized tapetum in ovules which develop no prothallium would seem to be in keeping with Dr. Lang's view that the single-layered tapetum, which is characteristic of the older stages of fertile ovules of *Stangeria*, results from the gradual disintegration and absorption by the growing prothallium of the inner cells of the originally many-layered "sporogenous group" surrounding the uninucleate spore. The sharp line of chemical distinction between the walls of the large exterior tapetal cells and those of the adjacent collapsed cells of the nucellar tissue affords evidence of a

similar character, while in addition, by laying emphasis upon the definiteness of the boundary between these tissues, it brings into more intimate association the tapetal cells and the megaspore, affording thus clearer evidence of the sporogenous nature of the tapetum.

II (b). *Zamieae-Euzamieae. Dioon imbricata* at a stage of prothallial development previous to the appearance of archegonia has a well developed coat about 3.8 μ thick and very distinctly double. The exosporium is about one and one-half times as thick as the endosporium, and the fibrillae of which it is composed are quite regularly radially arranged and coarse. The endosporium presents the appearance of being subdivided into two strata, the outer thicker than the inner, but both quite homogeneous. The inner stratum of the endosporium stains with haematoxylin, while the outer as well as the exosporium are coloured by safranin. In chlorzinc iodine the exosporium and the outer part of the endosporium are yellowish-brown, the former a little lighter than the latter. The inner part of the endosporium is pinkish-violet and passes by a darker coloured, somewhat granular narrow portion into the yellowish-brown of the outer part of the layer. There does not seem to be any shade of red in the suberized part of the coat when it is treated with chlorzinc iodine, as has been noted in the case of *Cycas* and *Stangeria*, but this may be due to the, unfortunately, poorly preserved material examined.

Warming in 1879[14] gave an illustrated description of the mature coat of the same species of *Dioon* that I examined. He states that the coat is layered like that of *Ceratozamia robusta*, whose megaspore-membrane he described and figured two years previously. There is, however, a great difference in his figures of these forms, two layers more being represented in *Ceratozamia* than in *Dioon*. (Compare Warming,[13] fig. 23, pl. 3, and Warming,[14] fig. 7, pl. 6.) The statements, too, in the layering of the coat in the résumé and in the original (Danish) of Dr. Warming's article do not agree (Warming[13], résumé p. 18, and the Danish p. 100). Yet it seems probable that the coat of both forms is considered double since this is

the statement of its structure in the Danish and since, also, Warming's statement on the cycads in general (*Handbuch der Botanik*, p. 168) would lead us to so regard it. Further, Dr. Warming states that the outer part of the coat of *Dioon* appears to be made up of a large number of small "prismatic bodies," structures which are apparently considered the homologues of certain "fusiform crystal-like structures," stated to be common in other tissues of *Dioon*. It is in this way that Dr. Warming accounts for the radial striation of the outer portion of the coat. Undoubtedly the "prismatic bodies" correspond to the little columns or fibrillae which I found present in the coat of *Dioon* and other forms. The structures I have observed are, however, too irregular to be termed prismatic though they have something of a hexagonal outline in cross-section, and in this respect correspond somewhat to the representation of them that Dr. Warming has given (Warming,[14] fig. 6, pl. 6). The coats of *Dioon* and *Ceratozamia* are considered as completely suberized, colouring dark yellow in chlorzinc iodine. I find that the inner stratum of the endosporium contains no suberin. In fact in chemical composition the coat of *Dioon* is very similar to that of *Cycas* and *Stangeria*, the inner stratum of the endosporium giving a decided reaction for the presence of cellulose, while its outer stratum and the exosporium are suberized.

Dr. Warming has figured a younger stage of *Dioon* in which the membrane is represented as a single homogeneous layer. I have not examined any material of *Dioon* at a similar stage but it seems probable that the coat does not differ from that of other cycads that I did examine (See *Cycas*, photo. 1, pl. 1, and *Zamia integrifolia*, pp. 17-18).

Several stages in the development of the megaspore-coat of *Zamia integrifolia* were examined. When the prothallium is in a comparatively early stage of free nuclear* division a distinct coat about 3 μ thick is present around the parietally placed cytoplasm. The coat "drags" very readily in section-

* I counted as many as 16 nuclei in one section through the centre of the prothallium. This section was not more than 5 μ thick.

ing but appears distinctly double in well prepared sections. Its inner half in unstained sections is transparent and homogeneous, while the outer is yellow, opaque and somewhat granular especially towards the exterior. Moreover, there is present a very dark and granular line about the middle of the coat. Though the general appearance of layering is evident in sections of the coat yet the boundaries of its strata and substrata are less clearly defined than in the case of any other cycad coat that I have examined. In sections stained in haematoxylin and safranin the inner and somewhat irregular border of the endosporium is light pink in colour and exterior to this is a homogeneous violet band which externally becomes darker, its outer boundary being formed by an intensely dark granular line which separates the coat into two equal parts. Exterior to this central line is a violet-pink band slightly granular in appearance and stained more intensely than any other part of the coat except the line above referred to. Apparently continuous with this deeply stained part is one stained a lighter pink. It is more granular than the latter and indications of a radial arrangement of the granules can be detected. This part probably represents a poorly differentiated exosporium about one-third the thickness of the whole coat. In chlorzinc iodine the inner margin of the coat is light greenish-yellow and after several days' treatment separates from the coat as a thin film. The greenish-yellow is succeeded externally by a yellowish-violet which in turn is replaced by dark green when the median granular area is reached. Exterior to the middle line of the coat is a dark yellowish-brown band, neither border of which is clearly defined. It passes on the outside into the lighter yellowish-brown of the exosporium.

At a stage when about two layers of cells are present in the prothallium, the megaspore-coat of *Zamia integrifolia* has increased somewhat in thickness and the layers are more differentiated. The exosporium constitutes about one-half of the coat and is more distinctly granular and radially striated than it is in the younger condition. The endosporium is composed of two strata and has an addition to its thickness

from the walls of the outer prothallial cells. The so-called "reinforcement" is similar in colour when the sections of the coat are treated with chlorzinc iodine to the inner light yellow border of the younger coat. Like the latter, too, after prolonged treatment it separates from the coat. The inner stratum of the endosporium is violet in chlorzinc iodine and the outer, which is about double as thick, is brownish-yellow, as is the exosporium. The boundary line between the exosporium and the outer part of the endosporium is more clearly marked than in the younger coat.

At a later stage, when cell-formation is well advanced in the prothallium the megaspore-coat of *Zamia integrifolia* is 3.8 to 4 μ thick. The exosporium at this stage is thicker than the endosporium even with its "reinforcement" which has increased considerably. The outer layer of the coat, too, is more distinctly radially granular.

In mature seeds of *Zamia integrifolia* I found that the coat is quite thick, 4.5 μ at least, without the addition from the cell-walls. The latter has differentiated into an inner "cellulose" and an outer "suberous" layer so that when the coat is in contact with the prothallial cells it is difficult to make out the inner cellulose layer of the endosporium even when sections are treated with chlorzinc iodine. The whole endosporium at this stage is very much reduced in thickness constituting only between one-fourth and one-sixth of the whole coat. The exosporium is plainly radially striated, the fibrillae of which it is composed being quite fine and somewhat irregularly radially arranged.

The tapetum in the mature seed is represented merely by some suberized débris from collapsed cells. In the younger stages it is very similar to that of *Cycas* and *Stangeria*, being formed of a jacket of cells tolerably uniform in thickness, investing the prothallium. The cells have suberized walls which are more or less spongy-looking, as if they were irregularly radially perforated. Usually they are uninucleate and contain some small amylodextrous grains. The number of layers of cells constituting the tapetum varies at different times.

The material of *Zamia floridana*, at a stage when fertilization is about to take place, showed a megaspore-membrane which in section is very similar to that of the other species and about 4.2 μ thick. The endosporium and the exosporium were not so unequal in thickness as in the case of *Z. integrifolia*.

At a stage when the archegonia have been fully formed, *Ceratozamia longifolia* has a megaspore-membrane which is 4.5 μ thick in the lateral and basal parts of the ovule. In the archegonial region, however, it thins out fully one-third. The exosporium is coarsely radially striated and fully three times as thick as the endosporium. It has a very thin homogeneous inner portion and the boundary line between it and the endosporium is distinct.

Warming,[13] as already stated, has described the coat of *Ceratozamia robusta*. His figure indicates that it is in appearance very like that of *C. longifolia*. The coat he describes is mature, however, and is represented as much thicker (by my computation from his figure) than the younger coat of the latter species that I examined. The coat of *C. robusta*, too, is said to be suberized, while I found the one of *C. longifolia* not different in this respect from that of the other cycads.

The megaspore-membrane of the Cycadales in all the forms examined shows the same fundamental characteristics of structure and chemical composition. The coat is double, its outer layer, especially in the later stages of development, being composed of distinct, radially arranged fibrillae. This layer is suberized in all cases. The endosporium presents the appearance in cross-section of being composed of two more or less equal and homogeneous longitudinal strata. These differ in chemical composition, suberin, cellulose, and pectin replacing one another gradually from the outside to the inside of this layer. The character of the succession of the chemical strata of the endosporium and the transitional nature of the areas between them have led to the conclusion that the coat is formed from within—that the endosporium is in fact the formative layer of the coat. This conclusion gains support from the fact that as the exosporium increases in thickness,

the endosporium decreases, the former apparently at the expense of the latter. With regard to the increasing distinctness of the striation of the exosporium which keeps pace with its increase in thickness, it may be possible to consider it as an indication of a progressive degeneration resulting from physiological activity. The development of the striation is at least associated with the growth of the prothallium and the accompanying transfer of food-material from the surrounding cells to it. No matter what view is taken of the mode of formation and differentiation of the coat, its uniformity in structure and in chemical composition in the Cycadales as a group has been demonstrated. Moreover, the coat is thickest and best developed in the Cycadeae, and thinnest in the Euzamieae, while in the Stangerieae it is of an intermediate character.

The tapetum is uniformly well developed in the Cycadales judging from the representatives of its subgroups that I have examined. It is evenly distributed around the prothallium and in certain cases is as much as four to five layers of cells in thickness. In all forms it probably thins out in the later stages of ovular development, being practically eliminated from the mature seed, though even here its pre-existence is indicated by certain suberized remains. In all the forms that I have examined the tapetal cells have suberized walls. The suberization it would seem possible at first sight to regard as a cenogenetic feature developed in relationship to the function of the tapetal cells, whose office it is to supply nutrition to the growing megaspore. The study of various tapetal layers, however, shows that the cells constituting such layers may have cellulose walls (*infra* pp. 25-26). Again if such were the case it might reasonably be expected that the suberization of the tapetal cells would not be so marked in infertile ovules where no megaspore is developed. As has been observed in the case of *Stangeria* suberization is even more marked in such cases. Thus suberization of the walls of tapetal cells would appear to be a palingenetic feature and to afford almost a demonstration of the sporogenous nature of this tissue. The plurinucleate condition of some of the cells

constituting the tapetum in the various forms and the course of development of this tissue as indicated by Dr. Lang's work on *Stangeria* affords further evidence of a similar nature.

GINKGOALES

As early as 1855 there is a record of the occurrence of a megaspore-membrane in *Ginkgo*. Hooker and Binney[15] state that "an extremely delicate membrane (fig. 16) surrounds the albumen of Salisburia." It is represented in their figure by a line. Williamson,[16] in 1876, referred to the coat in the same connection but with no more detail of its structure. Recent investigators seem to have overlooked it entirely. In fact in Seward and Gowan's[17] résumé of the knowledge of *Ginkgo* (up to 1900) there is an indirect negation of its presence. They say: "The upper part of the endosperm is covered by a thin papery membrane which represents the crushed remains of the nucellus."

On embedding and sectioning some mature seeds I found that this "thin papery membrane" is of a composite character and represents not only the "crushed remains of the nucellus" but tapetal débris and the megaspore-membrane as well. The megaspore-coat in fact is in a fair state of preservation in the mature seed (fig. 2,* pl. 1). It is very distinctly double and 4.5 to 5 μ thick, thinning out somewhat in the archegonial region. The exosporium is from four to six times as thick as the endosporium and composed of quite loose fibrillae which are very irregularly radially arranged, so much so that in sections the outer layer often presents the appearance of a net-work, though the reticulation is more apparent in oblique sections than in accurately transverse ones. Staining differentiates the inner part of the endosporium from the other parts of the coat. The former takes the haematoxylin stain while the rest of the coat stains in safranin. Under the action of chlorzinc iodine this inner part becomes violet and the outer part of the endosporium yellowish brown. There is present in *Ginkgo* as in the cycads a greenish yellow but very narrow

* Because of technical difficulties I have not represented the stratification of the endosporium in *Ginkgo* and many other forms.

transitional area between the strata of the endosporium. The exosporium is yellowish brown, the reticulation being very distinct in this fluid. The outer walls of the superficial prothallial cells are very thick and consist of a suberized outer layer and a cellulose inner one, the former about one-half as thick as the latter. Where the coat abuts directly on the suberized part of the cell wall it is difficult to distinguish between the "reinforcement" and the coat itself.

When the megaspore of *Ginkgo* is in the parietal nucleated condition, at about the same stage* as that of the younger ovule of *Cycas* (photo. 1, pl. 1) described before, its coat is quite thin, about 3 μ thick. It is double, as in the mature seed, but the exosporium is not more than one and one-half times as thick as the endosporium. The former is plainly striated at its outer border but becomes less and less so towards the endosporium, and finally blends almost imperceptibly with the outer stratum of the latter. The endosporium is formed of two chemically different strata, as the staining and treatment with chlorzinc iodine indicates. Haematoxylin lays hold of the immature coat more strongly than of the mature one and more readily obscures the layering of the coat, especially of the endosporium. The action of chlorzinc iodine on the membrane at this stage is similar also to that on the mature coat. A thin film, of the same colour as the protoplasm in chlorzinc iodine, extends along the inner side of the coat sometimes in contact with it but usually separated from it in the sections. It may be a part of the megaspore-coat or the beginning of the outer walls of the first row of prothallial cells which will shortly be formed. It is, however, characteristically present, and adds about .3 μ to .4 μ to the thickness of the coat.

At a very early stage of nuclear division in the megaspore of *Gingko*, when possibly sixteen free nuclei have been formed, there is a slight thickening around the protoplasm in which

* In a section about 5 μ thick through the centre of the megaspore I counted over 80 nuclei, while not more than 100 are present in *Cycas* at the stage indicated in photo. 1, pl. 1.

the nuclei are embedded. In chlorzinc iodine this young membrane is coloured light yellow, possibly with a shade more of green in it than the protoplasm to which it adheres.

The megaspore-coat of *Gingko* is thus very similar to that of the Cycadales. It is quite uniformly distributed around the prothallium in both groups, and in structure and chemical composition presents essentially the same features, though the endosporium of *Ginkgo* at the stages I examined does not seem to be so thick as that of the cycads.

The tapetum in *Ginkgo* at the youngest stage described above is four to five layers of cells in thickness. The inner cells are square to oblong in longitudinal sections of the ovule and have less dense contents than the outer ones, which are somewhat elongated tangentially. When the megaspore has many nuclei in a parietal stratum of protoplasm (at the intermediate stage described above) the tapetum consists of a jacket one to several cells in thickness. The cells have fairly dense contents and the walls though not well developed are suberized. Some of the cells, too, are plurinucleate. When the seed is matured the tapetum is represented merely by some suberized débris around the prothallium (fig. 2, pl. 1). The tapetum of *Ginkgo* like its megaspore-membrane is not so well developed as it is in the case of the cycads but undoubtedly it is an homologous structure, originating from the sporogenous tissue.

CONIFERALES.

I (a). *Pinoideae-Abietineae-Araucariinae* : In the posthumous account of *Gnetum* by William Griffith,[18] which appeared in 1859, there is a reference to the megaspore-membrane of *Agathis*, one of the two genera constituting the Araucariinae. We are told simply that this structure, which he termed the "amnios" and of which he probably did not recognize the homology, forms a very distinct lining to the cavity in the "nucleus" (nucellus) of *Agathis*. This is at a comparatively early stage of prothallial development.

In the youngest stage of *Agathis Australis** that I have examined, the megaspore has about 16 nuclei in a 10 μ longi-

* Material of this form has become available recently through the kindness of Professor Treub and Dr. Valeton.

tudinal axial section. At this stage the megaspore-coat is very variable in thickness, averaging possibly 4.5 μ. It has a somewhat granular inner stratum and an outer one about equally thick but hyaline in appearance. There is no sharp line of distinction between the strata of the coat though the inner stains in haematoxylin and the outer in safranin. In chlorzinc iodine the inner stratum is violet, light yellowish towards the inside and darker towards the outside. The latter portion passes by a greenish-yellow area into the slightly brownish-yellow outer part of the coat. Around the coat there are the remains of a suberized tissue, in intimate contact with it. Possibly its presence may indicate that a suberized tapetum was present at an earlier stage. Numerous pollen-tubes (6–10), entering the apical region of the nucellus, converge irregularly around the megaspore, some passing even deeper than the latter which occupies the middle half of the longitudinal axis of the sporangium. The pollen-tubes and the megaspore resemble one another very closely at this stage, so closely that in cross-sections it is difficult to distinguish them unless a number of consecutive sections are examined. They are alike in size, similar in contour and irregularly arranged in the axial portion of the sporangium. Their walls, too, are closely associated with the surrounding tissue and in structure and in chemical composition are very similar. The similarity amounts almost to an identity in certain of the cross-sections, since numerous nuclei and much protoplasm are present in the pollen-tubes at this stage.*

Around the megaspore at the young stage described above and filling up more or less the areas between the pollen-tubes are groups of cells very full of starch grains and protoplasmic contents. This tissue undoubtedly supplies nourishment to

* Six or seven nuclei are not uncommon in a tube at this stage, while in one I counted as many as thirteen. This recalls the interesting condition recently described by Juel[19] in *Cupressus Goverriana*, where, however, not a nuclear but a cell-complex is present in the pollen-tube, derived in this case from the body cell. A detailed account of the pollen-tube in *Agathis*, and of certain other peculiar features to which my attention was directed in the course of the present work will appear in a subsequent paper.

the prothallium and must be considered as a tapetum. It differs very markedly, however, from the tapetum in the cycads and *Ginkgo*. Its cells are uninucleate and their walls composed of cellulose while the tissue itself has no sharp external boundary but passes by easy transition stages into the ordinary nucellar tissue, being merely a differentiated inner part of the latter.

At a later stage just after fertilization has been effected, the coat does not present even so definite structural features as it does in the younger condition. It is much thinner and colours more yellow in chlorzinc iodine. The prothallium is relatively very large at this stage, much of the surrounding nucellar tissues having been absorbed. A few of the inner layers of the latter are starch-bearing but more collapsed than in the younger condition. Remains of pollen-tubes are apparent especially in the apical region of the prothallium. In the ripe seed of *Agathis* the coat is still present. It is fairly thick in some places but usually thin and much disorganized. The prothallial cells of the mature seed have very thick cellulose walls and over the superficial ones a suberized band extends, which is, however, clearly distinct from the coat. In infertile ovules collected at the same time as the ripe and fertile ones, the prothallium has collapsed, though the integument and nucellus are nearly as fully developed as in the fertile ones. The shrunken prothallium is surrounded by the megaspore-coat which closely invests it. This structure is undifferentiated so far as layering is concerned, but fairly uniform in character. In chlorzinc iodine the bulk of the coat is yellow to brownish-yellow in colour, the inner border only being violet.

The coat of the megaspore of *Agathis* is thus poorly differentiated structurally at all stages. In the young and better developed condition it resembles very closely the wall of the pollen-tube, though it is slightly thicker than the latter. Indeed so very close is the resemblance that the numerous pollen-tubes surrounding it are readily mistaken for infertile megaspores. The resemblance of the megaspore-coat of *Agathis* to the intine of the microspore of *Pinus*, both in struc-

ture and in chemical composition, is very intimate. This becomes even greater when the microspore has shed its exine and grown down into the tissues of the nucellus, for the wall of the pollen-tube (intine) of *Pinus* is then of increased but more variable thickness. The megaspore-coat of *Agathis* never differentiates any farther, but in the later stages becomes even less definite in structure and finally is much disorganized in the mature seed. As it becomes older the composition changes gradually, the cellulose being almost completely replaced in the later stages by a substance resembling suberin. The latter gives, however, not so dark a brownish-yellow in chlorzinc iodine as ordinary suberized layers.

The functioning tapetum of *Agathis* differs very decidedly, as has been noted, from that of the cycads and *Ginkgo*. It is clearly of nucellar origin and in this respect may be distinguished as a secondary tapetum, that of the cycads and *Ginkgo*, which is regarded as sporogenous, being considered as of a primary nature. The latter is represented in *Agathis* only by suberized remains.

The coat of *Araucaria imbricata* is very variable in thickness at the stage indicated in photograph 3, plate 3, and is closely applied to the surrounding tissue. In this young condition it is in appearance very like that of *Agathis* at about the same or possibly a little later stage of development. It is much thinner, however, and in composition has a larger proportion of cellulose in it. A careful examination of mature seeds of *Araucaria Braziliensis* and of *A. imbricata*, as well as of infertile ovules of the former, failed to reveal the presence of even a trace of the coat. The coat is then much less developed than that of *Agathis* though undoubtedly similar in character to it. In both the characteristic outer suberized layer which is found in the cycads and *Ginkgo* is absent and the coat resembles the wall of the pollen-tube both in structure and in chemical composition. It is suggestive, in this connection, that the wall of the megaspore of the Araucariinae grows in intimate association with the surrounding tissues, just as that of the pollen-tube does.

Around the megaspore of *Araucaria* in the younger stages (see photo. 3, pl. 3) there is a mass of tissue of elliptical outline which represents the tapetum. Its cells contain many starch (amylodextrous) grains and their walls are suberized. The tissue is chiefly aggregated around the base of the megaspore, being very thin along the sides and fairly so at the apex. At places its suberized cells shade gradually into the surrounding cellulose-walled cells of the nucellus, while at other parts they are distinctly marked out from them. The tapetum of *Araucaria* is very different from that of *Agathis*. In the latter it is of fairly uniform distribution while in the former it is massed in the basal region. In *Agathis*, too, the only suberized part consists of collapsed cells within the functional tapetum, while nearly all the tapetum of *Araucaria* has suberized cell walls. The tapetum, however, in each form looks as if it were an integral part of the nucellus, merely a differentiated inner portion of its tissue.

I (b). *Pinoideae-Abietineae-Abietinae* : An idea of the distribution and prominence of the megaspore-membrane in this subgroup may be gained by a glance at photographs 4 to 10 (pls. 3 and 4). The coat is thick in the chalazal region and thins out gradually towards the micropylar portion of the prothallium, being not more than one-third as thick at the apex as at the base of the megaspore.

The coat of *Pinus resinosa* (fig. 3, pl. 1) at the stage indicated in photograph 4, plate 3, just prior to fertilization, is about 4.2 μ thick at the lateral basal region of the prothallium from which part the drawings have usually been made. It is distinctly double,the endosporium being about one-third as thick as the exosporium (fig. 3, pl. 1). The former is homogeneous and appears as in the cycads to be subdivided into two longitudinal strata, the inner, in the case of *Pinus*, only about one-half as thick as the outer. The former stains in haemotoxylin while the rest of the coat takes the safranin stain. In chlorzinc iodine the inner stratum of the endosporium is violet while the outer is yellowish-brown, considerably darker than the exosporium. The fibrillae of the latter are more or less

collapsed and irregularly radially arranged, as indicated in the figure. The tapetum of *P. resinosa* at this stage is almost confined to the basal part of the ovule and consists of uni- or pluri-nucleate cells which are more or less broken down. Their protoplasmic contents are sparse and their suberized walls irregular and somewhat porous, like those of the cycads but yet much thinner. It is rather a striking feature with regard to this primary tapetum that it thins out with the megaspore-membrane, thickest at the base and almost disappearing around the apical region of the prothallium. *P. strobus* differs from *P. resinosa* in having a more delicate megaspore-coat and one which is more evenly distributed around the prothallium. The coat at the stage just subsequent to fertilization is not more than 3.8 μ thick. Its endosporium is nearly as thick as its exosporium. The latter is so irregular and " ragged " along its outer border as to give one the impression that the decrease in thickness may be due to the destruction of the outer part of this layer. The tapetum like the megaspore-coat is fairly evenly distributed around the prothallium, disappearing only above the archegonia. It consists of a jacket of partially collapsed cells with suberized walls which lie away from the megaspore-coat at a considerable distance. In the intervening space some granular material is found. Outside the tapetum is a layer of very loose open cells which are not compressed as in most other cases. *Pinus sylvestris* and *P. Austriaca* have coats and tapeta which at the stage when the archegonia have been fully developed are similar to those of *P. resinosa.*

The coat of the larches differs from that of the pines in its distribution. In both *Larix Europaea* (photo. 5, pl. 3) and *L. Americana* there is scarcely a trace of the megaspore-coat in the archegonial region. In the former the thinning out process is somewhat abrupt while in *L. Americana* which has a longer prothallium it is more gradual, the latter occupying an intermediate position in this respect between the European species and the pines. The coat of these two forms is somewhat thicker and coarser than that of *Pinus.* Its endosporium

does not constitute more than one-sixth to one-fifth of the whole coat (figs. 4 and 5, pl. 1) and consists of two substrata which are more nearly equal in thickness than in *Pinus*. The exosporium is very regularly formed and the fibrillae composing it are coarse, especially in the case of *L. Americana* (fig. 5, pl. 1). The distribution of the tapetal cells agrees with that of the megaspore-coat. In the chalazal region of the prothallium there are a few cells with suberized walls while around the rest of the gametophytic tissue there are only scattered traces of the tapetum.

The megaspore-membrane of the spruces examined is quite similar to that of the larches at the same stage of development. It is slightly thicker, however, and is not so attenuate in the micropylar region. Photographs 6 and 7, plate 3, are of *Picea nigra* and *Picea excelsa* respectively and indicate the distribution of the coat around the prothallium. Figures 6 and 7, plate 1, illustrate the structure of the membrane in the lateral region of *P. excelsa* and in the basal region of *P. nigra*. The thickness of the membrane in our black spruce is not quite so great as that of the European species. Both are quite thick, however, though the endosporium is extremely thin in each. *Picea alba* has a coat which is possibly a little thinner than that of *P. nigra* but otherwise like those of the other species.

The megaspore-membrane of *Tsuga Canadensis* looks very thick, as photograph 8, plate 4, shows. It is, however, not so thick as it appears. Some granular material is massed against it and makes its outer layer appear thicker at a low magnification than it really is. In figure 8, plate 1, this material is represented as slightly removed from the coat, which in thickness and general character resembles that of *Pinus resinosa* very much (cf. figs. 3 and 8, pl. 1). The hemlock, however, has a thinner endosporium than *P. resinosa* and its exosporium is somewhat more regular.

Abies balsamea (photo. 9, pl. 4) has a well formed megaspore-membrane which is of about the same thickness as that of *Picea nigra*. Its endosporium is very thin and its exosporium thick and coarse (fig. 9, pl. 2). All the membranes of the Abietinae so

far described have been from ovules at approximately the same stage of development, about the time of fertilization. They are all quite similar to one another as is indicated in figures 3 to 9 (pls. 1 and 2). The membranes are "reinforced" on the side next the prothallium by the adjacent cellulose walls of its superficial cells. Two areas are recognized in the endosporium of all forms, an inner one which stains with haemotoxylin and becomes violet in chlorzinc iodine, and an outer one which is stained by the safranin and colours dark brownish-yellow in chlorzinc iodine. The exosporium is finely or coarsely fibrillar in all. It stains in the safranin and is coloured a lighter yellowish-brown in chlorzinc iodine than the outer part of the endosporium. The tapetum at this stage is distributed much as is the megaspore-coat. It consists of at least a single layer of loose cells at the base of the prothallium and thins out towards the apical region. The cells have suberized walls and some of them at the base are plurinucleate as well. In these respects they resemble the tapetal cells of the cycads and *Ginkgo*.

In the mature seed of *Pinus ponderosa*, which is very large, the megaspore-membrane is 4.5 to 5 μ thick. The exosporium is five to six times as thick as the endosporium and consists of fibrillae which are fine but which look at places as if they were more or less disorganized. They are always much pressed together and seem almost to have lost the radial arrangement which characterized them at an earlier stage. In chlorzinc iodine there is a violet inner border to the coat but the body of it is yellowish brown. The mature coat of *Pinus Banksiana*, whose seed is small, is quite thick, 4.2 μ. Its endosporium is thin and homogeneous and the exosporium is coarsely and irregularly granular. In chlorzinc iodine the coat is yellowish-brown with a violet inner border. *Pinus insignis* is very similar to the former in the size of the seed and in the character of the megaspore-coat. The exosporium is, however, somewhat finer and retains more of the radial arrangement of its fibrillae. Ripe seeds of *Cedrus Atlantica* have a coat which in structure, thickness, and in chemical composition is almost

identical with that of *Pinus ponderosa*. The size of its seed is, however, much less. The megaspore-coat in the ripe seed of the European larch is slightly thinner than that of *Cedrus*, but otherwise similar to it. From the comparative uniformity of the thickness of the megaspore-membrane of the above mentioned forms it would seem that there is no direct relationship between the size of the ovule and the thickness of the coat, though possibly the larger seed has slightly the thicker coat. The tapetum in the mature seeds of all the forms examined is entirely disorganized.

At a young stage in *Pinus resinosa* when the prothallial cells are forming (three cells in depth) the megaspore-coat is about 3 μ thick and appears quite similar to those of *P. pumilio* and *P. sylvestris* as Mlle. Sokolowa[20] has represented them, incidentally, in her work on endosperm-formation, as a means of indicating the orientation of the cells. I find that this is true also of *P. sylvestris* at a slightly younger stage (fig. 10, pl. 2). The endosporium is homogeneous and composed of two substrata as in the more mature condition described before. The exosporium is finely and indistinctly radially granular, but only about one and one-half times as thick as the endosporium. The exosporium and the outer layer of the endosporium are suberized. The tapetum is better formed and more evenly distributed at this than at the later stage. Its cells are irregularly shaped and one to several nucleated. Their walls are not differentiated but the walls of the cells immediately adjoining them (to the right in fig. 10, pl. 2) are of cellulose. The coat, too, is of uniform distribution around the prothallium at this stage (photo. 10, pl. 4). In very young ovules of *Pinus resinosa*, when about six nuclei are present, in the parietal protoplasm of a section 4 to 5 μ thick through the young prothallium, the only trace of a megaspore-coat is a more homogeneous outer border around the protoplasm. The tapetum is thick but its cells have not acquired differentiated walls. The outer ones are elongated tangentially and quite narrow radially while the inner ones are nearly equiaxial. These cells have a large

granular nucleus. The whole layer is four to five cells in thickness and the one form of cell gives place gradually to the other. At a later stage when about twelve nuclei are present in a section of the prothallium the tapetum is differentiated into two layers of oblong cells with large open nuclei. The long axes of these cells are directed radially. Similar stages in the development of the megaspore-coat and of the tapetum have been observed in *Pinus sylvestris* and in *P. strobus* and *Larix Europaea.*

The Abietinae have a megaspore-membrane and a tapetum which are very uniform in structure and in the character of their distribution. With respect to distribution both the tapetum and the megaspore-membrane are peculiar and strikingly different from the cycads. The reduction of both in the Abietinae towards the micropylar region about the time of fertilization suggests that in these forms this part is the seat of the activities which are adverse to the retention of these structures. Probably the reproductive processes are concerned in this matter since the coat and the tapetum develop quite uniformly around the prothallium until shortly before the time when reproductive activity is at its maximum. The coat, too, in the Abietinae is not so thick even in the basal region as it is in the cycads but in structure and in chemical composition the membranes in the two groups are similar. The tapetum, though very much less developed and more quickly disorganized than in the cycads, is of the same nature, and in the course of its development passes through similar stages to that of *Stangeria* (*supra*, pp. 14-15). The cells, too, composing it are sometimes several nucleate and their walls are suberized. The tapetum of the Abietinae is thus a primary one, derived as in the cycads and *Ginkgo* from the sporogenous tissue.

I (c). *Pinoideae-Abietineae-Taxodinae* : Photograph 4, plate 4, is of *Sciadopitys verticillata.* The gametophytic tissues have shrunk considerably and are widely separated from the tapetum. The latter consists of from four to five layers of more or less collapsed cells which have as yet thin walls and are very full of granular material. The

tapetum in turn has separated from the nucellar tissue. The megaspore-membrane adheres to the prothallium and in sections is a very prominent structure. It is double and 4.2 μ to 4.5 μ in thickness, the endosporium being from one-half to one-third as thick as the exosporium (fig. 11, pl. 2). At places in the sections these two layers are torn from one another. The endosporium consists of two homogeneous bands with a narrow dark and granular-looking area between them, the inner hyaline and the outer light yellow and less homogeneous. The exosporium is light yellow also. It is, however, indistinctly radially striated and finely granular. When breaks occur in the sections of this layer they are always transverse just as in the case of *Cycas*. In fact the coat is very similar to that of *Cycas revoluta* at nearly the same stage of prothallial development. The whole coat is somewhat thinner, however, while its exosporium is if anything thicker. In staining the coat shows a tendency to take up haematoxylin readily and the structure of the endosporium especially is quickly obscured. In chlorzinc iodine the layering comes out very distinctly. The inner part of the endosporium is bluish-violet, lighter towards the interior and dark along the narrow granular area between the strata. The outer stratum is brownish-yellow as is the granular exosporium. When "dragged" the coat in chlorzinc iodine appears as an irregular thick single layer consisting of a mass of dark brown granules in a homogeneous yellow matrix. I have not observed any "reinforcement"* of the coat in *Sciadopitys* though this may be so closely associated with the coat as to escape observation. In distribution the coat is uniform around the prothallium, just as it is in the Abietinae at about the same stage of development (cf. photos. 10 and 11, pl. 4). But it is thicker than the latter at this stage by fully one-half (cf. figs. 10 and 11, pl. 2), and in this respect, and in distribution as well, approaches *Cycas*.

The tapetum in *Sciadopitys*, though thick all around the prothallium, is especially thick in the basal region (photo. 11,

* The inner border of the endosporium is certainly yellowish, but it was not observed to separate from the coat as is the case in *Cycas*.

pl. 4) and thus is suggestive of the much reduced tapetum of the Abietinae at about the time of fertilization when the tapetum in the latter is practically non-existent except in the basal region of the prothallium. (Compare photo. 4, pl. 3, and photo. 11, pl. 4). The walls of the tapetal cells turn yellow in chlorzinc iodine.

A figure of a young ovule of *Cunninghamia* appears in Dr. Arnoldi's paper on the Sequoiaceae (Arnoldi,[21] fig. 2, pl. 7). The prothallium is represented as in the parietal multinucleate condition and the tapetal tissue ("archesporial tissue" of Arnoldi) is four to five cells in thickness in the basal region, almost as thick, but more disorganized than I found it in the case of *Sciadopitys* even at a later stage. The megaspore-coat is not represented in this nor in any of Arnoldi's figures, nor referred to even in such forms as *Sciadopitys* where I have found that it is very thick. It seems probable then that, since the tapetum, which is evidently of a primary nature, is so well developed in *Cunninghamia*, a fairly thick coat is present, since I have found that there is a correspondence in the state of development of these two structures. Material of *Cunninghamia*, however, was not available for examination.

Considerable attention has been recently devoted to the study of the Sequoias. In young ovules of *Sequoia sempervirens* numerous megaspores, ten to twelve according to Lawson,[22] are found. These acquire thick walls and begin germination. Only two or three develop very far (beyond the first division), and but one of these, growing at the expense of the others, develops a cellular prothallium and later bears the archegonia. This one, previous to the formation of cells, is shown in photograph 12, plate 4, with some abortive megaspores around it. The walls of the megaspores are quite prominent in sections and their close association with the tissues of the nucellus apparent. The large megaspore encroaches irregularly on the nucellar tissue, there being little or no suberized remains present, to indicate the presence of a primary tapetum. Shaw[23] states, however, that a well developed tapetum is present at earlier stages but if such is the case it must be disorganized quickly.

The coat at the stage indicated in the photograph "drags" very readily in sectioning and its thickness and structure could not be as definitely determined as is desirable. It is, however, about 2.5 μ thick and consists of two layers (fig. 12, pl. 2). In chlorzinc iodine the inner border of the coat is violet and exterior to this is a yellowish-brown homogeneous stratum, the outer part of the endosporium. The exosporium is yellowish-brown also but finely and indistinctly radially granular. It is about one and one-half times as thick as the endosporium. The coats of the infertile megaspores seem to consist almost completely of cellulose though they are apparently quite thick.

In *Sequoia gigantea* there is but a single megaspore present. This has no coat in the material I examined, when the prothallium is in an advanced stage of free nuclear division, probably about 128 nuclei being present in the peripheral protoplasmic layer. The outer border of this layer is more homogeneous than the rest of it, and this is the only indication of the presence of a megaspore-coat. In the mature seed of *S. gigantea* the megaspore-coat is thin, about 1.5 to 2 μ in thickness. It consists of a homogeneous inner layer and a coarsely granular outer one. The coat comes into intimate contact with the suberized layer of the superficial prothallial cells and is about equal to it in thickness. Within the latter there is a slightly thicker cellulose layer, the "reinforcement" of the coat being thus much thicker than the coat itself at this stage. When "dragged" the coat appears very thick. It consists of a uniform ground-substance which stains considerably like the cellulose walls of the endosperm cells and has large granules more or less rectangular in outline embedded in it or partly projecting from it. These granules stain like the suberized layer of the cell walls but appear when the high power of the microscope is focussed on the matrix as dark, somewhat rectangular areas in the latter. The tapetum in the younger stage consists of a more or less broken row of cells scattered along the inner border of the nucellar tissue. The walls of these cells are not differentiated chemically, but the tissue probably represents a poorly developed primary tapetum.

With regard then to the difference between the Sequoias which has been shown to exist, at least in the *time* of development of the megaspore coat, it may be said that a certain amount of variability is to be expected in vestigial structures. The difference in the present instance is, however, associated with others, which seemed of enough importance to Arnoldi[23] to warrant a separation of the two species by the revival of an old genus, *Wellingtonia*, for the reception of one of them, though Karsten,[24] who has reviewed Arnoldi's work, thinks that there is not justification for the separation of the two species. I have merely referred to the matter here because of the additional point of diversity afforded by the study of the megaspore-membrane.

Cryptomeria Japonica at a younger stage (photo. 13, pl. 4) than that of the young ovules of *Sequoia gigantea* examined has no trace of a megaspore-coat. A poorly developed tapetum is present, however, but the walls of its cells are not differentiated at this stage. In the mature seed of *Cryptomeria* the megaspore-coat is not so thick as it is in *S. gigantea*, but otherwise is very similar to that of the latter.

In *Taxodium distichum* at a comparatively early stage of free nuclear division a megaspore-coat is present, as the figures in Coker's recent work on this form indicates (Coker,[25] figs. 50, 51, and 54, pl. 4). Dr. Coker has also stated that at a later stage, about the time of cell-formation in the prothallium, "the wall of the spore" is "furnished with pits" (Coker,[25] p. 20, and figs. 97, 98, and 99, pl. 7). From his figure of them in surface view they are small perforations of the wall of rectangular to roundish outline. The only material of *Taxodium* that I examined was of mature seeds. The megaspore-coat in these, though much collapsed, is about 2.5 μ thick. It consists of a homogeneous inner layer and an outer coarsely and irregularly radially granular one, about double as thick as the inner. The whole coat, except its inner border which is slightly violet in chlorzinc iodine, is suberized. When "dragged" the coat appears as a somewhat thick band, very much like that of *Sequoia gigantea*. With regard to Dr. Coker's statement

(Coker,[25] p. 20) that the coat is pitted, I find that this is very true of the exosporium of the ripe seed, but not of the endosporium. The pits are really not pits but irregular spaces around the fibrillae of this layer, as is the case in all the forms that I have examined. The "dragged" coat does, however, look very much as if it were pitted when examined at a low focus.

Dr. Coker has very fully described the tapetum in *Taxodium* (Coker,[25] pp. 17-20). It consists of large starch-bearing cells in the young condition (megaspore mother-cell stage) which not until later become differentiated from the surrounding cells. When the megaspore is in an early stage of free nuclear division the tapetum is two to three cells thick in the apical and lateral regions, and four to five in the basal part (Coker,[25] fig. 47, pl. 4). At a later stage, but before the formation of cells in the prothallium, the tapetum consists of a single definite layer of well formed cells (Coker,[25] p. 18, fig. 51). This layer consists of collapsed cells when the prothallium has become cellular (fig. 53) and is said to become ultimately disorganized, when the prothallium is mature.

Dr. Arnoldi has described in *Sequoia gigantea*, *Cryptomeria Japonica* and in *Taxodium distichum* tapeta which are similar to the singly layered tapetum that Dr. Coker has figured for *Taxodium*. My own observations verify the presence of such a tapetum in the first two species. I also found some suberized material between the gametophytic and nucellar tissues in the mature seed of *Taxodium*. It thus seems probable that a poorly differentiated *primary* tapetum is characteristic of them all. This is in keeping with the relatively poor state of development of the megaspore-coat in these forms. Reference has already been made to Arnoldi's description of the tapetum in *Cunninghamia*. In *Sciadopitys* both tapetum and megaspore-membrane are thick and very different from those of other members of this subgroup which have been examined. Reference has already been made to Arnoldi's proposed separation of the two species of *Sequoia*, and to the added feature of difference between them which the study of the megaspore-membrane has

brought out. Dr. Arnoldi has also found that *Sciadopitys* is so different from the other Taxodinae as in his opinion to be better removed from them. The great difference in development of the megaspore-membrane and the tapetum in *Sciadopitys* from that which was found to be characteristic of the other Taxodinae examined lends support to Arnoldi's view.

II (2). *Pinoideae-Cupressineae* : In *Biota* (*Thuja*) *orientalis*, at a stage (photo. 14, pl. 5) when the young embryos are forming, the megaspore-coat appears quite thick. It is not so thick as it appears, however, since the outer walls of the superficial endosperm cells add much (about one-half) to its apparent thickness (fig. 13, pl. 2). At thickest it is not more than 2 μ at this stage. It consists of two layers, the inner homogeneous and the outer granular (fig. 13, pl. 2), the latter perhaps slightly thicker than the former. In haematoxylin and safranin the bulk of the endosporium stains a very intense pink, its inner border violet. The exosporium is coloured light pink. In chlorzinc iodine the two layers of the coat are yellowish-brown, the inner darker and with a slightly violet inner border. Scarcely a trace of the tapetum is present. The coat is of quite uniform distribution around the prothallium at this stage as the photograph shows. *Thuja occidentalis* (photo. 15, pl. 5) thins out normally,* perhaps a little more than *Biota* in the archegonial region. The coat, too, is not so uniform in thickness at other parts as is the case in the former, nor is its thickness ever so great (usually about 1.5 μ). Even the "reinforcement" of the coat from the prothallial cells is less than in *Biota*. Very little trace of a tapetum is found at this stage, and the megaspore-coat comes directly into contact with the cellulose-walled cells of the nucellus.

In the mature seed of *Biota* the coat is about 2 μ thick, not so thick as is the suberized layer of the superficial endosperm cells. The cellulose part of the latter is thicker again than the suberized zone, so that the "reinforcement" in the mature seed is more than double the thickness of the coat. In *Thuja* the

* In one case the coat appeared thicker in the archegonial region than at any other part. Fertilization had not been effected in this case.

coat is very thin, almost disorganized, when the seeds have matured.

Two species of *Cupressus*, *C. sempervirens* and *C. thujoides*, have a coat which in ripe seeds is very similar to that of *Biota orientalis* but intermediate in thickness between this form and *Thuja occidentalis.*

Some young material of *Chamaecyparis* sp. (?), when about 16 nuclei are present in a 10 μ axial section of the megaspore, showed only a trace of a coat, very much like that of *Sequoia gigantea* at the young stage described before. The tapetum, too, is but poorly differentiated.

The prothallium of *Juniperus sabina*, about the time of fertilization, has a moderately thick (3 μ ±) megaspore-coat (photo. 16, pl. 5). It is fairly uniform in distribution up to the archegonial region where it thins out about one-third. The coat "drags" very readily in sectioning and in the photograph appears much thicker than it really is. When cleanly cut it is seen to consist of two nearly equal layers, the outer granular and the inner homogeneous (fig. 14, pl. 2). The exosporium stains light pink in the safranin and the outer part of the endosporium dark pink, while the inner border of the latter is blue in haematoxylin. In chlorzinc iodine the coat appears dark yellowish-brown, the inner part darker than the outer and with a distinct violet inner border. At a younger stage, when the endosperm cells are forming, the coat of *Juniperus* is somewhat thinner (2.5-3 μ) but uniformly distributed around the prothallium, and otherwise much as it is in the older stage described above. The tapetum at this stage consists of a somewhat loose layer of cells. These are more or less equiaxial but somewhat irregular in outline. They contain from one to several nuclei. In chlorzinc iodine their walls, which are thin, are yellow, while the walls of the cells immediately adjoining them externally are violet.

In the mature seed of *Juniperus Virginiana* the coat is not more than 2 μ thick. As in the younger stages it has a homogeneous inner and a granular outer layer. It is intimately associated with the thick suberized layer of the prothallial

cells. This together with the cellulose layer of their walls is fully two and a half times as thick as the megaspore-coat. When "dragged" the coat appears as a fairly thick band light in colour and with yellow granules partly embedded in it, very similar both structurally and chemically to that of *Sequoia gigantea*, described above.

The megaspore-coat of the Cupressineae in contrast to that of the Abietinae does not thin out gradually towards the micropylar region of the prothallium, but is of more or less uniform thickness up to the immediate neighbourhood of the archegonia. The difference in distribution in the two groups is associated with a difference in the arrangement of the archegonia and possibly to be accounted for on this basis, since, as was referred to in the discussion of the distribution of the coat in the *Abietinae*, the reproductive processes which centre around the archegonia may have to do with the partial destruction of this portion of the coat. The tapetum (in *Juniperus* at least) approximates in distribution to the megaspore-coat. Both structures are less fully developed than in the Abietinae. The coat in the young stages as well as in the mature seeds is much thinner and not so well differentiated and the tapetum disappears earlier in the Cupressineae. The tapetal cells also, so far as I have observed, do not acquire such thick nor such clearly suberized walls as they do in the Abietinae.

II (3). *Taxoideae - Podocarpeae*: In *Podocarpus coriacea** at a stage when the suspensorial cells have elongated and forced the embryo-cells somewhat into the prothallial tissues I could find no trace of a megaspore-membrane, though the material was in a good state of preservation. In chlorzinc iodine the thick outer wall of the superficial prothallial cell is deep blue. There is only a trace if any of a light yellow border to the walls, nor is there present between the gametophyte and the nucellus more than a vestige of suberized substance and no indication whatever of a megaspore-coat. The ovules

* The species is the one from Darlington, S.C., whose gametophytes and embryo Dr. Coker[26] investigated, and with material of which he was kind enough to supply me.

of the other species of *Podocarpus* examined, *P. Makoyi*, were in the mature condition, some having viviparous embryos (Lloyd[27]) projecting from the seeds. In this species as in the last I could find no trace of a megaspore-membrane. It is of course possible that a megaspore-coat is present in the younger stages. Since, however, no reference is made to such a structure in the literature on this genus and since I have found, with but few exceptions, some trace of the coat in the later stages of development and even in the ripe seed, where it occurs in the developing ovule, I consider that the coat must be either absent in *Podocarpus* or at least very poorly developed. Again, Dr. Coker refers to the absence in *Podocarpus coriacea* of the tapetum or "spongy tissue" which, he states, is characteristic of so many conifers and which I have found to be correlated in its state of development with the megaspore-membrane. This form then must be considered to have either none or a very poorly developed megaspore-coat and tapetum.

In *Dacrydium laxifolium*, on the other hand, the megaspore-membrane is well developed at the stage indicated in photograph 17, plate 5, a similar stage to that of *Podocarpus coriacea* at which I found no megaspore-membrane present. The coat is 4.2 μ thick and very distinctly double, the endosporium being about one-third as thick as the exosporium. The former is homogeneous while the latter is very irregularly and coarsely striated (fig. 15, pl. 2). When stained there is a dark blue inner border to the endosporium while the rest of the coat is pink. Under the action of chlorzinc iodine this inner border of the endosporium is violet to bluish while the rest of the coat is yellowish-brown, the inner part darker than the outer. The exosporium appears at places to consist of little globules of suberin and to be disorganizing. Some of the appearance is no doubt due to the fact that the material was in the herbarium for four years before it was "revived" and fixed. The "reviving" process, however, seemed to be very successful in this case.

I do not pretend to be able to explain fully the difference between *Podocarpus* and *Dacrydium* with respect to the

megaspore-coat, but wish to point out in the former certain correlated specialized features and in the latter corresponding primitive ones. In *Podocarpus* no "spongy" tissue is present around the prothallium, while in *Dacrydium* the remains of such a tissue are evident in sections of prothallia with the megasporangium intact (fig. 15, pl. 2). That this is a mere coincidence cannot be granted, since in the forms which are known to be primitive, e.g. the Cycadales, and Ginkgoales, there is an association in the state of development of the tapetum or "spongy" tissue and the megaspore-membrane. In addition, in other forms which have no megaspore-membrane the primary tapetum has also been found to be absent or poorly developed, as will be seen in the case of forms to be described next. In certain morphological features of the female "flower" these genera are very different from one another. In *Podocarpus* the ovule is anatropous and has two well differentiated and fused integuments, an inner woody and an outer fleshy one. The fertile scales, also, are united in some species to form a "receptaculum" which becomes berry-like at maturity. In *Dacrydium* on the contrary the fertile scales are not fused and differ but little from the ordinary vegetative leaves even when the fruits are mature. The ovules are never anatropous (orthotropous in *D. laxifolium*) and the outer integument is represented by an "arillus"-like structure which may only partly enclose the inner one but is never united to it, except at the base. The absence of the megaspore-membrane and of the tapetum in *Podocarpus*, the fusion of the parts of the "flower" and the resultant complexity of its structure contrast very strikingly with the more primitive state of affairs in *Dacrydium* where the megaspore-membrane and tapetum are present and the "flower" maintains the distinct individuality of its parts.

II (4). *Taxoideae-Taxeae* : In *Cephalotaxus Mannii* at nearly the same stage as that of the *Taxus* indicated in photograph 18, plate 5, there does not seem to be even a vestige of a megaspore-membrane. The outer prothallial cells in this case have a superficial layer which is yellow in chlorzinc

iodine. This is the only indication of suberized material between the latter and the cellulose-walled cells of the nucellus. Another species of *Cephalotaxus* (an undetermined one) presents features similar to those of the one described above. Mlle. Sokolowa has observed that the megaspore-membrane of *Cephalotaxus* is single and thinner than in the case of any other gymnosperm except *Ephedra*, with which she associates it. Her figure of the megaspore-coat of *Cephalotaxus Fortunei* represents it as a single finely granular layer about 1 μ thick, but with indistinct borders. This is at a stage when cells are forming in the prothallium, as we learn from her figures (Sokolowa,[20] figs. 14 and 15, pl. 11).

The material of *Torreya nucifera** that I examined is at an early stage of embryo development. In sections it appears on first sight that a well developed megaspore-membrane is present. Marking out the tissues of the gametophyte from those of the nucellus is a rather uniform band which on treatment with chlorzinc iodine is seen to be suberized. In thin sections which were made to determine the structure of this supposed megaspore-coat, it is evident that the layer consists of an aggregation of fibres. The prothallium encroaches irregularly on the surrounding tissues, and no doubt the band referred to is formed of the walls of cells pressed closely together by the growing prothallium which has absorbed their contents. The cells of the inner layer surrounding the gametophyte are here and there apparently empty though most of them are very densely packed with granular substance and have large nuclei. Two or three layers of cells similar to the latter form with the inner layer a quite distinct but irregular jacket around the prothallium. The outer of these cells contain usually a considerable number of starch grains. This tissue undoubtedly supplies nourishment to the prothallium and is of a tapetal nature. Its cells are, however, uninucleate and their walls are composed of cellulose. They are not fundamentally different from the ordinary cells of the nucellus, though they have more

* Dr. Coker, who kindly supplied me with the material, says that it was obtained in the Botanic Gardens of Pisa, Italy, and that the species seems to be *T. nucifera*.

dense contents than the latter, into which at places they pass over very gradually. The tapetum here is thus of secondary or nucellar origin as was found to be the case with the functional tapetum of *Agathis*.

In *Taxus Canadensis* at the stage indicated in photograph 18, plate 5, it is possible that there is a trace of a megaspore-membrane in the basal region of the prothallium. If so, the structure is exceedingly thin. The superficial prothallial cells have their free walls covered by a partly suberized layer which is about .75 μ thick. Beneath it and of about equal thickness is the cellulose part of the wall. The pollen-tube, which is much expanded when it has reached the prothallium, contrasts (see photograph) very strikingly with the megaspore in the development of its wall. The tube wall varies in thickness at different parts from 3.3 to 6 μ. In one quite young ovule of *Taxus* examined two embryo-sacs were present, one of these comparatively large with archegonia developed on it, and the other small with its cytoplasm in a parietal layer and having several nuclei (16 probably). Around the smaller one which would probably remain infertile there is a slight but distinct thickening, while around the other no membrane could be distinguished. This is in keeping with Dr. Scott's recent observation of the difference in thickness between the walls of the fertile and infertile megaspores of *Lepidocarpon* (Scott, [28] p. 299). The later stages of *Taxus* up to maturity of the seed gave no indication of the presence of a megaspore-coat, nor was any trace of a tapetum observed at any stage.

The chief genera of the Taxeae thus present a striking uniformity in the absence or poorly developed state of the megaspore-coat and of the primary tapetum. In this respect they contrast very strikingly with the Podocarpeae, in which group there is much difference in the state of development of these structures. The Taxeae from the present standpoint are to be regarded as a specialized subgroup, while the Podocarpeae contain some specialized and some primitive forms.

GNETALES.

In ovules of *Ephedra vulgaris* Mlle. Sokolowa represents a megaspore-membrane, at a stage when the endosperm-

cells are beginning to form, as a single and finely granular layer about 1 μ in thickness (Sokolowa,[20] fig. 23, pl. 12). In older ovules I found the membrane still present (photo. 20, pl. 5). It is difficult to determine its structure, however, since the coat is very closely associated with the suberized walls of the surrounding collapsed and compressed cells of the nucellus. Still in very thin sections it can be made out as an attenuate double layer around the base of the prothallium, while around the upper part it appears more as if it were single, the exosporium not being distinct. The outer part of the coat is suberized and its inner border contains some cellulose, the whole coat in the apical region being largely composed of this substance. No tapetum is present at the stage I examined, but there is a band of the compressed inner cells of the nucellus which is thick in the basal region and thins out gradually towards the apex of the nucellar cavity, being in distribution similar to the tapetum of *Araucaria*, and like the latter also having suberized walls.

Hooker[29] in his paper on *Welwitschia* (1863) states that in this form "the embryo-sac is a delicate membrane" which, when the nucellus has elongated, "is found to have disappeared over the summit of the endosperm." Sir Joseph Hooker compares *Welwitschia* and *Gnetum* with regard to this characteristic of the distribution of the coat. This investigator also states that "the membranous remains of the embryo-sac may often be found on the surface of the nearly mature endosperm." In photograph 19, plate 5, the character of the distribution of the coat is indicated, at a stage soon after fertilization has taken place. The coat is very thin, about 1.3 μ, at the stage examined, but still distinctly double, the outer part granular and the inner homogeneous (fig. 16, pl. 2). No trace of a tapetum is present. The nucellar tissue in the basal region is differentiated, however, into an inner more or less collapsed part (see photo. 19, pl. 5), which if compressed would appear thinner but probably very much like the band that has been described in *Ephedra*.

In Griffith's[18] paper on *Gnetum* (1859) there is a statement that a megaspore-coat is present at a young stage of ovular development but that it disappears in the later stages. Hooker's[29] (1863) statement that the coat of *Gnetum* is similar in distribution to that of *Welwitschia* gives us further information with regard to it. Reference is made in Karsten's[24] paper (1892) which deals with this genus to the presence of a megaspore-membrane around the young spore. Lotzy[30] (1899) has referred to the coats of the megaspores of *Gnetum Gnemon*, "which make them appear much more cryptogamous than the embryo-sacs of most higher plants." The megaspore-membranes are represented as single and finely granular layers. Those of the prothallia which will become fertile are, I have estimated from his figures, about 1.2 μ thick when free-nuclear division is well advanced (Lotzy,[34] fig. 27, pl. 4). Those of the infertile prothallia are thicker, some of them at certain places being represented as fully 2.3 μ thick.

The coat in the three genera of the Gnetales is thus thin and the tapetum poorly developed. In distribution the coat is of an accentuated Abietinae-type, scarcely a trace of it being present in the apical region of the prothallium. The coat is double in the basal region in two of the forms about the time of fertilization, and will probably be found to be similar at a like stage in *Gnetum*. Material of this form was not examined, however, and the point remains in doubt.

FOSSIL GYMNOSPERMOUS SEEDS.

Numerous seeds of a primitive gymnospermous character occur in the palaeozoic rocks. Many of these with the structure well preserved are found in the Carboniferous and Permian strata. Hooker and Binney[15] were the first to study their internal features. Later Brongniart[31] and Williamson[16] worked out in detail their intimate structure. A glance over the illustrations of these investigators gives one an idea of the prominence of the megaspore-membrane in the early seed-plants. Brongniart whose specimens were exceptionally well preserved refers to the megaspore-coat as follows, (Brongniart,

31, p. 242) : "La membrane intérieure ou périspérmique est très différente de celle qui limite le nucelle ; elle est extrêmement mince et ne paraît pas cellulaire, mais marquée d'aréoles dûes à l'application des cellules qu'elle enveloppait et dont il ne reste généralement plus de trace." Williamson's specimens were not in so good a state of preservation but the megaspore-membrane is represented in many of the figures in his paper. In the text also he often refers to the coat, and compares it with the similar structure described by Brongniart. These Palaeozoic seeds belong to a great variety of forms, some related to the cycads, some to the conifers, and some, probably very many, to the Cordaitales, the dominant gymnosperm group of this era. Others again are seed-bearing Cycadofilices, the Pteriodospermae, a group recently established by Oliver and Scott.[7] Still others are lycopods with seed-like fructifications which Dr. Scott has described under the generic name of *Lepidocarpon*. The latter have an especially prominent megaspore-coat, which in some cases is plainly double as its separation into two layers in the chalazal region indicates (Scott,[28] figs. 27 and 28).

In the mesozoic rocks the abundant remains of gymnospermous plants belong chiefly to the Bennettitales, a group with strong cycadean affinities. Carruthers[32] in 1870 described for the first time the female strobilus in *Bennettites*, the type genus of this group. Neither he, Solms-Laubach, nor Scott, who have studied the European forms, have observed that a megaspore-coat is present. In fact, for the only structure which might be interpreted as such Solms-Laubach distinctly claims a nucellar origin (Solms-Laubach,[33] pp. 441 and 442). The American representatives of the Bennettitales are being worked over by Dr. Wieland who has already made very important additions to our knowledge of the reproductive organs of the group. In correspondence in regard to the presence of a megaspore-membrane Dr. Wieland states that he is unable to affirm directly that such a structure is present in the material he has examined. With regard to other mesozoic gymnosperms, the forms that are related to the modern ones, the

ancestral Ginkgoales, Coniferales, etc., I have been unable to get any information on the presence of a megaspore-membrane that is of value.

It is thus evident that in these fossil seeds the more primitive, palaeozoic forms have a much more prominent megaspore-coat than the specialized and more recent mesozoic ones, in which even the occurrence of a coat does not seem to have been demonstrated with certainty.

GENERAL CONSIDERATIONS.

The present work has determined the extent of the occurrence of a megaspore-membrane in the gymnosperms, as well as the structure of the coat and its chemical composition. The megaspore-membrane is present in all the groups and subgroups of these seed-plants, except the Taxeae of the Coniferales, from the ovule of whose forms it is entirely or almost entirely eliminated. The coat is strikingly uniform in structure and in chemical composition throughout this division of the spermatophytes with the exception of one subgroup, the Araucariinae. It is double, its exosporium, in the later stages of development, composed of radially arranged fibrillae, and its endosporium presenting an appearance, in section, of being subdivided into two more or less equal, homogeneous strata. The exosporium is suberized while the endosporium is of composite chemical character. The outer stratum of the latter is suberized but contains cellulose towards its inner border, while the inner stratum consists chiefly of cellulose with which entad is associated, a substance resembling pectin. The megaspore-coat in fact closely resembles that of a microspore (e.g. that of *Pinus*) both in its structure and in its chemical composition, and thus affords additional evidence of the free-sporing nature of the ancestral forms of the gymnosperms.

In the forms where the normal type of membrane occurs there is present a more or less well-developed tapetum. This tapetum is derived from the sporogenous tissue as is shown by the course of its development, the plurinucleate condition of its cells, and by the suberization of their walls. It is quite

distinct from that which is derived from the nucellar tissues and has for convenience been designated a "primary" tapetum.

The abnormal type of megaspore-membrane present in the Araucariinae is comparable to the wall of the pollen-tube both in structure and in chemical composition, a typical suberized exosporium not being present. This group in many other respects occupies a somewhat isolated position among the subgroups of the Coniferales. The tapetum is of a peculiar character in both *Agathis* and *Araucaria*,—just as abnormal as is the megaspore-membrane. The "female flower," too, is difficult to homologize with that of any of the other forms. Some consider that the seminiferous scale is a very reduced structure being composed of the almost completely fused fertile and infertile bracts, while others regard the ovule-bearing structure as a simple sporophyll, and on this ground consider the Araucariinae as the most primitive of conifers. In support of this view reference is often made to the evidence afforded by early occurrence of fossil Araucaria-like wood. Dr. Scott[34] (p. 483), however, finds the case in this respect "emphatically ‚not proven' on existing evidence". The character of the coat and of the tapetum is in keeping with his finding and indicates in addition that the Araucariinae are to be regarded as a specialized subgroup of the Coniferales.

Leaving out of consideration the Araucariinae whose megaspore-membrane and tapetum cannot at present be satisfactorily associated with the other gymnosperms, certain general features which are important from the phylogenetic standpoint have been demonstrated. The coat is thick, well developed, and of fairly uniform distribution around the prothallium in the Cycadales, the group which is recognized as the most primitive of the modern gymnosperms. In the Ginkgoales it is thinner than in the Cycadales but similar in distribution to that of the latter. The group is a recently established one for the reception of the single form *Ginkgo biloba*, which was previously included in the Taxeae of the Coniferales but which is now considered "as the one surviving

member of an ancient stock, derived from the same cycle of affinities as the palaeozoic Cordaiteae" (Scott,[34] p. 485), and has been given a phylogenetic position below the Coniferales. In the last mentioned group, which comprises the much diversified forms of the present-day gymnosperms, the coat is very varied in thickness and peculiar in its distribution around the prothallium. It is, however, though thinner than in *Ginkgo*, on the whole much thicker and more fully developed than in the Gnetales, the group of gymnosperms which is recognized as having the greatest affinity to the angiosperms. Thus in the living forms it is seen that there is a direct relationship between the thickness and state of development of the megaspore-coat, and the primitive character of the group, a progressive destruction of the coat having gone on as the forms become more specialized. The development of the suberized primary tapetum in the different groups parallels that of the megaspore-coat and affords confirmation of the evidence derived from the state of development of the latter. Again in the fossil forms the primitive palaeozoic representatives have a much thicker megaspore-coat than the higher and more specialized mesozoic ones, since, as was seen, the coat in the former is described as a well preserved structure, while in the latter it is so poorly developed as to have escaped observation, or, at least, description. Thus in both the great modern and fossil groups of primitive seed-plants we have evidence that the megaspore-coat varies in thickness according to the primitive or specialized nature of the forms. That this is true of the subgroups as well as of the large divisions is indicated by the study of the megaspore-membrane of the Cycadales, the Cycadeae having the thickest and the Euzamieae the thinnest coat.

The interpretation of the inter-relationships of the subgroups of the Coniferales in the light of the above generalization is the chief object of the present work, and in connection with the statement of the results obtained reference will be made to certain other features of general phylogenetic importance which are in keeping with the evidence afforded by the state of development of the megaspore-coat.

All the subgroups of the Pinoideae have a megaspore-membrane and a tapetum. Those of the Araucariinae are of a specialized nature and have been referred to separately. Of the other subgroups of the Pinoideae, the Taxodinae, which Eichler in his classification has placed between the Abietinae and the Cupressineae, show affinity to each of these subgroups in the character of the megaspore-coat and the tapetum. Reference has already been made to Arnoldi's proposed separation of the two species of the Sequoias (p. 37). In the same paper he gives it as his opinion that all the Taxodinae (Sequoiaceae) except *Sciadopitys* would be better associated with the Cupressineae. The evidence afforded by the study of the megaspore-coat of these forms is in favour of Arnoldi's view and indicates that the Taxodinae is a composite group, all the forms examined, except *Sciadopitys*, being best associated with the Cupressineae to which their megaspore-coat approximates in its state of development. The associated forms of the Taxodinae and the Cupressineae have a very thin membrane and a poorly developed tapetum, being in this respect highly specialized, the true Cupressineae on the whole somewhat more so than the Taxodinae. The forms of the combined groups resemble one another in certain other features, such as the absence of male prothallial cells, the grouped arrangement of the archegonia and the degenerate nature of the brachyblast, all which point to their specialized character. The cyclic arrangement of the leaves and sporophylls in the Cupressineae proper is evidence of a similar nature.

The megaspore-coat of *Sciadopitys* is in distribution and in structure similar to that of the Abietinae at the same stage of prothallial development (see p. 34). It is, however, fully one-half thicker, being almost or quite as thick as the coat of *Cycas* at a similar stage. The tapetum of *Sciadopitys*, too, is thick, especially so in the basal region, and its distribution thus suggests the state of affairs at a later stage in the Abietinae, when the tapetum consists of only a few cells in the basal region. The megaspore-coat and the tapetum of *Sciadopitys* would thus seem to be of a primitive abietineous type.

Other features as well indicate its affinity to the Abietinae. Arnoldi has referred to the distribution of the archegonia as being similar to that in the Abietinae. Again, in the vegetative parts the development of long shoots and short shoots is characteristic of both. The so-called "leaves" of *Sciadopitys* are peculiar and lend confirmation to the brachyblastic theory of the seminiferous scale, which upon anatomical grounds is considered to hold good for all the Coniferales. This interpretation of the character of the ovule-bearing structure of the Coniferales gains support from teratological evidence, and is "in favour of the view that the Abietinae, and the Taxodineae as well, are somewhat primitive orders" (Jeffrey[35], p. 456) since in these subgroups alone do proliferous cones occur. In this connection attention is directed to the very common occurrence of proliferating female cones in *Sciadopitys*, (see Sir W. T. Thiselton-Dyer's reference to Master's work, *Ann. Bot.*, 1903, pp. 779-787), and to the additional evidence which is thus afforded of the primitive nature of this form. The presence of two prothallial cells in the microspore, and the lack of differentiation in the male cells themselves, only one of which functions in fertilization, are features which point in the same direction and corroborate the testimony afforded by the state of development of the megaspore-coat and the tapetum.

The Taxoideae have one order, the Taxeae, which must be regarded as very specialized from the standpoint of the development of the megaspore-coat and the primary tapetum, since no such structures, or only traces of them, are present in the three of its genera examined. In keeping with this condition of affairs the female "flower" is of a very specialized type. The axillary buds (brachyblasts) are reduced in number and degenerate in organization. Moreover, no prothallial cells are present in the microspore, and the single functional male cell (in *Taxus* at least) is relatively very large and specialized. In addition *Taxus* is the only one of the Coniferales in which no resin ducts are developed, a feature in which it resembles the Gnetales. Again, in *Torreya* a *secondary, nucellar*, but no

primary tapetum is developed. In the other suborder of the Taxoideae, the *Podocarpeae*, there is a great difference in the stage of development of the megaspore-coat and the tapetum in its two chief genera. Reference (see p. 43) has already been made to certain associated differences which are in keeping with the former and from which it would appear that *Dacrydium* is a much more primitive genus than *Podocarpus*. Certain resemblances of the whole group to the Abietinae have been recently pointed out by Dr. Coker, such as the occurrence of winged pollen grains, the arrangement of the archegonia, the presence of two prothallial cells in the microspore (*Podocarpus* and perhaps others) and certain other features which have led him to conclude "that in the Podocarpeae are to be found the nearest living relatives of the Abieteae" (Coker,[26] p. 103).

From the standpoint, then, of the relative state of development of the megaspore-coat and the tapetum we are to regard the Abietinae as the most ancient group of the Coniferales; the Taxeae as the most recent; the Taxodinae and the Podocarpeae as complex groups, with some forms as ancient as, or even more ancient than the Abietinae, and other forms quite recent,—while the Cupressineae are considered as occupying a somewhat intermediate position in the phylogenetic series.

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EXPLANATION OF PLATES.

Plates 1 and 2 consist of drawings which were outlined by the aid of a camera lucida. The drawings were made from the lateral basal region of the prothallium except where otherwise stated, since the membrane in this region is more uniform and assumes about its average thickness. The figures are oriented so that the prothallial tissue is to the extreme left of each. An attempt has been made both by the use of the camera lucida and by actual measurement to reproduce the relative thickness of the coats in the various forms. For accurate comparison however, the measurements must be taken as the basis. Only in a few cases has any attempt been made to indicate the stratification of the endosporium.

Plates 3-5 are reproductions of photomicrographs, and for details of structure should be examined with a lens.

Plate 1.

Fig. 1. *Cycas revoluta.* (4. 5 μ) The endosporium and the exosporium are about equal in thickness. The former presents the appearance of being subdivided by a broad and coarsely granular area ; the latter is finely striated. The tapetal cells are large and contain many amylodextrous starch grains. These appear in the unstained condition as vacuoles.

Fig. 2. *Ginkgo biloba.* (4.5-5 μ) Membrane of adult seed, separated slightly from the endosperm cells, the outer walls of which are especially thick and have an outer suberized layer. The exosporium is relatively very thick and is finely and irregularly striated. The tapetal and nucellar tissues consist of collapsed strands.

Fig. 3. *Pinus resinosa.* (4.2 μ) For the stage see photo. 4, pl. 3. The endosporium is about one-third as thick as the exosporium. There is but a slight "reinforcement" from the endosperm cell walls. To the right of the membrane the tapetal tissue is represented and the inner edge of the nucellus as well.

Fig. 4. *Larix Europaea.* (4.4 μ) The walls of the endosperm cells are just forming. The tapetum consists of a single layer of rather collapsed cells.

Fig. 5. *Larix Americana.* (4.7 μ) The drawing is from the basal part of the megaspore. The membrane is like that of *L. Europaea*, but of heavier structure and slightly "reinforced" by the outer walls of the endosperm-cells. The tapetal cells are large in this region.

Fig. 6. *Picea excelsa.* (4.7 μ) The stage of development is indicated in photo. 7, pl. 3. The exoporium is relatively very thick. It is finely and very regularly striated in structure. The reinforcement is slight.

Fig. 7. *Picea nigra.* (4.6 μ) Basal region of megaspore-membrane—very similar to that of *P. excelsa.* Collapsed tapetal cells are present.

Fig. 8. *Tsuga Canadensis.* (4.5 μ) Membrane as in last two but thinner. To the right of the exosporium is a layer of tapetal débris which is really closer to the membrane than it has been represented and makes the membrane appear quite thick (See photo. 8, pl. 4).

Plate 2.

Fig. 9. *Abies balsamea.* (4.6 μ) The stage of the ovule from which drawing was made is seen in photo. 9. pl. 4. The membrane is slightly "reinforced." The endosporium is homogeneous and about one-fourth as thick as the exosporium which is very distinctly and coarsely striated.

Fig. 10. *Pinus sylvestris.* (3 μ) Membrane of an ovule at a slightly earlier stage than that indicated in photo. 10, pl. 4, in the parietal nucleated condition. The endoporium is homogeneous while the exosporium is finely granular and shows signs of an indistinct radial striation. To the right of the megaspore the tapetal cells appear as quite a distinct layer.

Fig. 11. *Sciadopitys verticillata.* (4.2 μ) The stage of the ovule is indicated in photo 11, pl. 4. Cell formation is proceeding in the megaspore. The exosporium is very finely granular and radially striated and about one and one-half times as thick as the endosporium, whose two substrata are fairly distinct.

Fig. 12. *Sequoia sempervirens.* (2-7 μ). The drawing was made from a part of an ovule (at about the stage indicated in photo. 12, pl. 4) where the membrane was free from the nucellar tissues. Usually it is more closely pressed up against the latter than the figure would indicate, no tapetum occuring between them.

Fig. 13. *Biota orientalis.* (1.7 μ) The drawing shows that the apparently thick megaspore coat indicated in photo. 14, pl. 5 is not really a true megaspore-membrane but that a great part of the thickness of the investing layer is made up of the thickened outer walls of the peripheral endosperm cells. The endosporium is homogeneous, the exosporium granular.

Fig. 14. *Juniperus Sabina.* (2.7 μ) The stage of development of the ovule is indicated in photo. 16, pl. 5. The membrane seems to vary somewhat in thickness in different parts of the section. The two layers of the coat are about equally thick. The endosperm cells "reinforce" the membrane considerably. To the right the collapsed tapetal cells are evident and the inner border of the nucellar tissues.

Fig. 15. *Dacrydium laxifolium.* (4.3 μ) Membrane at the stage indicated in photo. 17, pl. 5. It invests closely the endosperm and is "reinforced" by the outer wall of the peripheral endosperm cells, the thickness of which is about equal to that of the inner layer of the membrane proper. The exosporium is coarsely and irregularly striated. The tapetal tissue has collapsed.

Fig 16. *Welwitschia mirabilis.* (1.3 μ) At the stage indicated in photo. 19, pl. 5, the megaspore-coat is thin even in the lateral basal part of the ovule. The inner border of the nucellar tissue consists of very much collapsed cells.

Plate 3.

Photo. 1. *Cycas revoluta.* (Meg-memb. 4.5 μ thick.) The ovule is at a stage just previous to cell-formation in the prothallium. The coat is double, its two layers about equally thick. (For details see fig. 1, pl. 1.) An artificial separation of the layers is indicated about the middle of the photograph. The staining is such as to bring out the layering of the coat but not the structure of the prothallial and other tissues.

Photo. 2. *Stangeria paradoxa.* (Meg-memb. 4.2 μ thick.) The endosporium appears subdivided in this case. Overstaining for contrast effect has obscured the structural details of the layers. The large cells of the tapetum are indicated to the right and the gametophyte to the left.

Photo. 3. *Araucaria imbricata.* (x 50) Archegonial initials have developed in the lower lateral parts of the prothallium. The distribution of the tapteum is fairly well indicated, though usually much more is seen in the apical region.

Photo. 4. *Pinus resinosa.* (x 20) The megaspore-membrane closely invests the prothallium, becoming much thinner in the archegonial region. The archegonium has not been fertilized. Remains of the tapetum are apparent in the basal region.

Photo. 5. *Larix Europaea.* (x 25) Several archegonia are present, two with neck and ventral canal cells. The megaspore-membrane is scarcely perceptible in the archegonial region and very thin for some distance below this, while in the chalazal region it is thick. The basal portion of the tapetum and of the nucellus have been torn away.

Photo. 6. *Picea nigra.* (x 50) Just prior to fertilization. Megaspore-membrane somewhat broken but closely investing the prothallium while the tapetal remains are sparse but distinct in the basal region.

Photo. 7. *Picea excelsa.* (x 25) Some of the archegonia have been fertilized and the first nuclear divisions have taken place. Others have not been fertilized. The megaspore-membrane thins out uniformly towards the archegonial region. The tapetum is apparent in the basal region.

Plate 4.

Photo. 8. *Tsuga Canadensis.* (x 40) The megaspore-coat thins out somewhat in the archegonial region, but not so much as in *Larix,* etc. The tapetum is pretty well destroyed but can be detected in the basal region. Granular material adds somewhat to the apparent thickness of the coat.

Photo. 9. *Abies balsamea.* (x 20) The ovule has been fertilized and the megaspore-coat is very thick in the basal region thinning out gradually towards the micropylar part. Tapetal remains are evident in the basal region.

Photo. 10. *Pinus sylvestris.* (x 80) Ovule in longitudinal axial section. The coat is double and evenly distributed around the prothallium, which is loosely invested by tapetal cells.

Photo. 11. *Sciadopitys verticillata.* (x 40) "Revived" material. Longitudinal axial section of an ovule at the stage when archegonial initials are appearing. The megaspore-membrane is of uniform thickness all around the prothallium. The tapetum is thick, especially in in the basal region.

Photo. 12. *Sequoia sempervirens.* (x 50). The megaspore-membrane is attached more or less to the nucellar tissue. The endosperm is becoming cellular and collected towards the chalazal region. The section passes through several megaspores towards the micropylar end.

Photo. 13. *Cryptomeria Japonica.* (x 100) No membrane is to be seen at this stage. The tapetum consists of a single rather loose layer of cells.

Plate 5.

Photo. 14. *Biota orientalis.* (x 10) Apparently shows quite a thick megaspore-membrane at the stage when numerous rudimentary embryos are developing. The explanation is given in fig. 13, pl. 2, where the "reinforcement" from the cell walls is seen to be very thick.

Photo. 15. *Thuja occidentalis.* (x 20) A young embryo is seen in the upper portion of the prothallium. The megaspore-membrane is thin even in the basal region, and the tapetum not apparent.

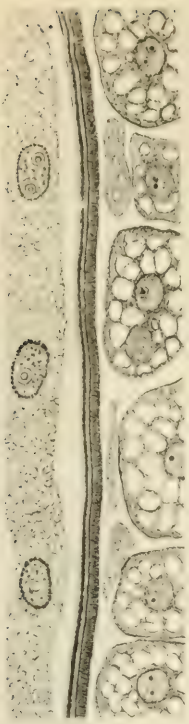
Photo. 16. *Juniperus Sabina.* (x 20) The megaspore-coat appears very thick, much thicker than it really is since it is "dragged". The tapetum can be seen in the basal region. In the archegonial region the contents of several pollen-tubes are apparent.

Photo. 17. *Dacrydium laxifolium.* (x 20) Sections of dry herbarium material "revived" 4 years after collection. The integument and nucellus have been removed. The megaspore-membrane is thus the thick coat enclosing the prothallium in whose axis embryonic and suspensorial cells are visible.

Photo. 18. *Taxus Canadensis.* (x 50) The nucellus and contained structures only are present. Above the embryonic and suspensorial cells a pollen-tube without any contents is apparent.

Photo. 19. *Welwitschia mirabilis.* (x 25) The megaspore-membrane lies free, midway between the endosperm and the nucellus. It thins out very perceptibly towards the micropyle. Suspensorial cells with a triangular mass of embryonic tissue at the base are visible in the longitudinal axis of the prothallium about one-third of the distance from its apex.

Photo. 20. *Ephedra distachya.* (x 12) The megaspore-membrane is fused with a felt-work from the adjoining nucellar issue. Numerous free nuclei are present in the egg-cells.



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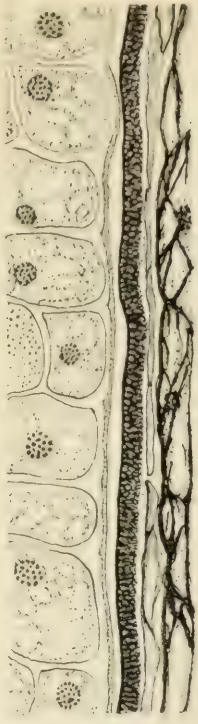

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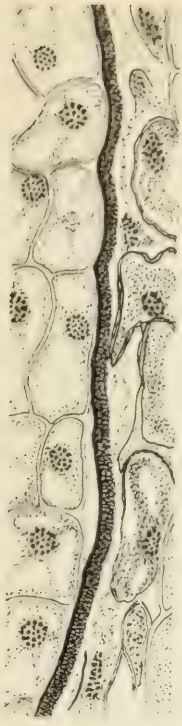

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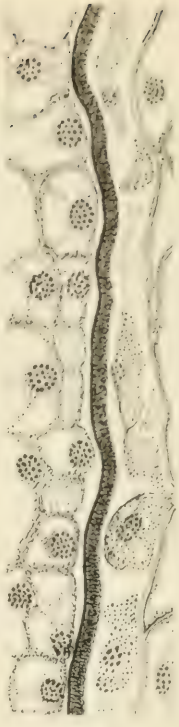

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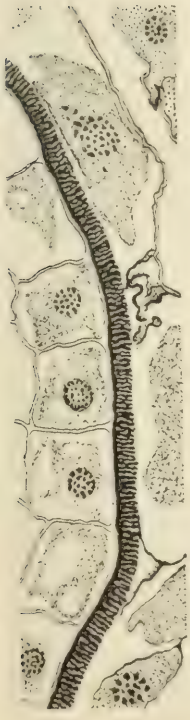

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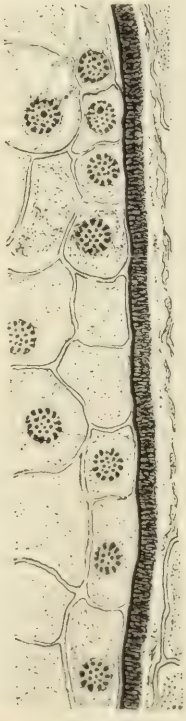

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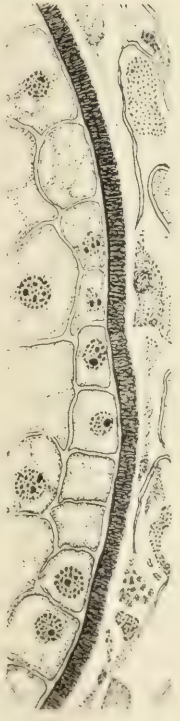

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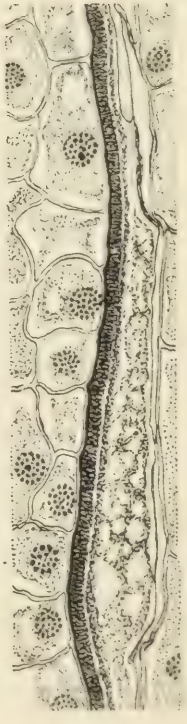

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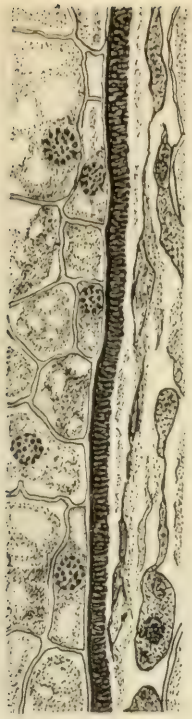

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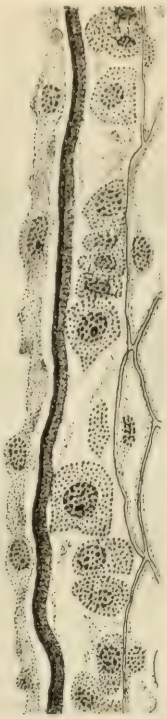

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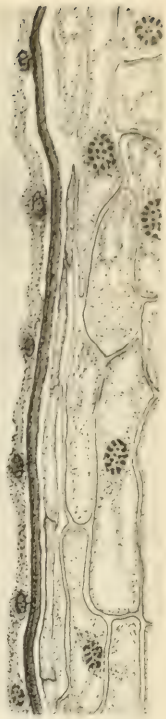

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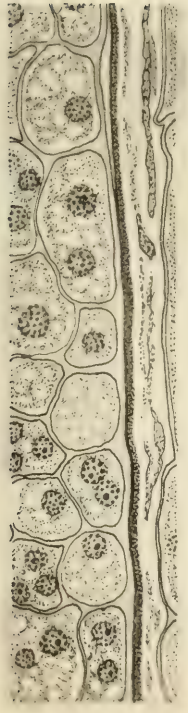

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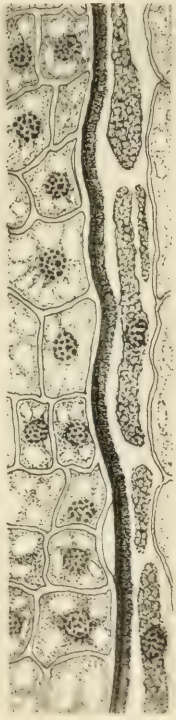

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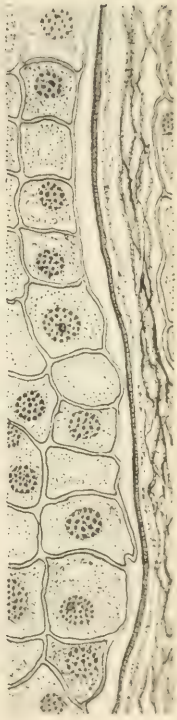

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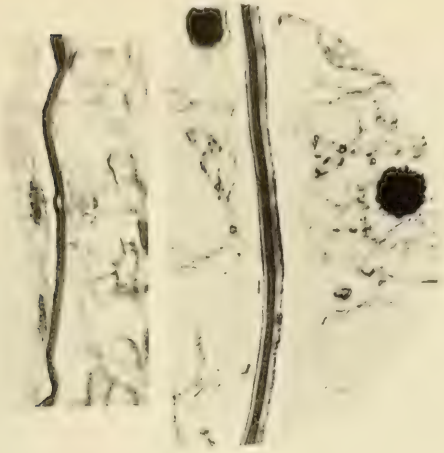

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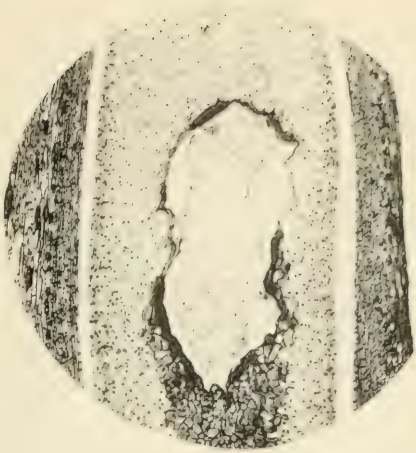

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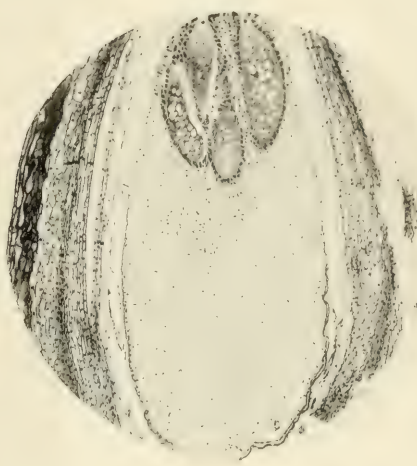

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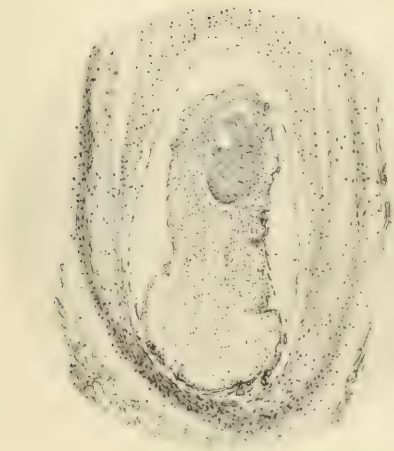

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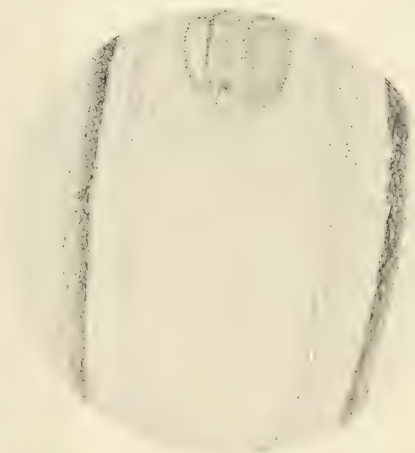

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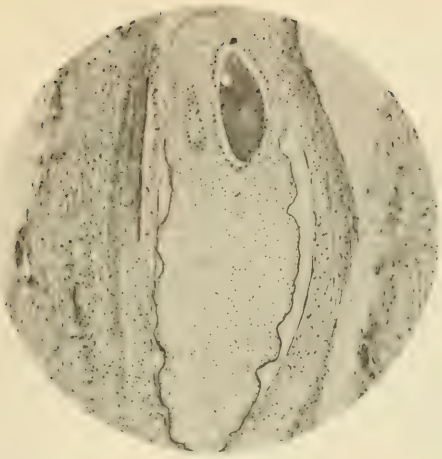

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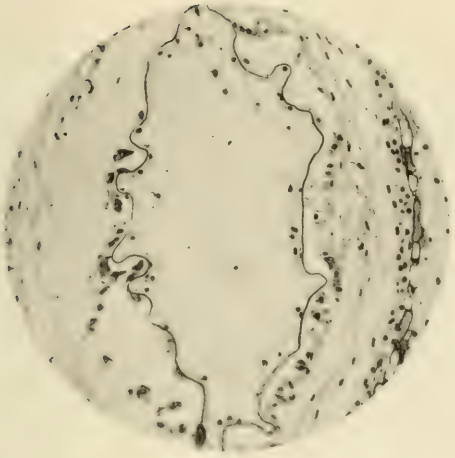

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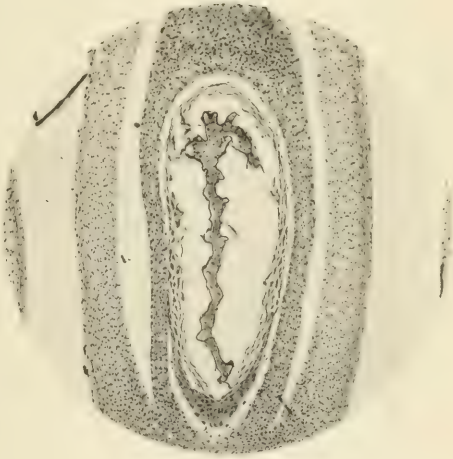

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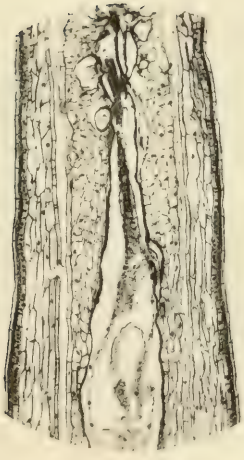

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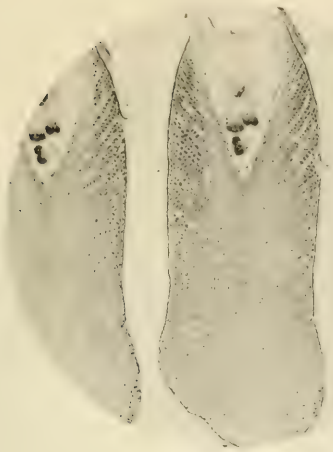

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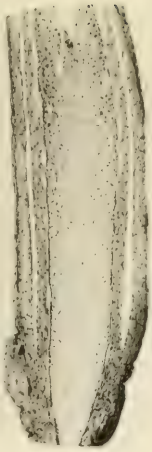

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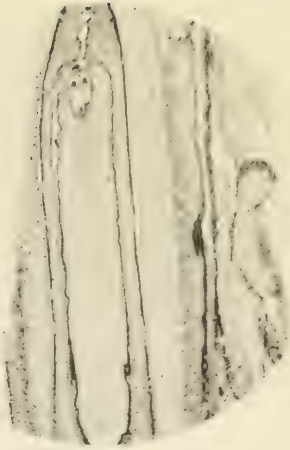

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No. 5: THE HOMOLOGIES OF THE STYLAR CUSPS IN THE UPPER MOLARS OF THE DIDELPHYIDAE

BY B. ARTHUR BENSLEY

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THE HOMOLOGIES OF THE STYLAR CUSPS IN THE UPPER MOLARS OF THE DIDELPHYIDAE

BY

B. ARTHUR BENSLEY, PH.D.

LECTURER IN ZOOLOGY IN THE UNIVERSITY OF TORONTO.

THE HOMOLOGIES OF THE STYLAR CUSPS OF THE UPPER MOLARS OF THE DIDELPHYIDAE*

Introduction

In the course of my studies on the relationships of the Australian Marsupialia I had occasion to examine the extensive series of skulls of modern Didelphyidae preserved in the British Museum, with the object of defining the ancestral characters present in the dentition. My notes on this subject included a thorough survey of the stylar elements in the upper molars; but, since the main changes in secondary evolution are played upon the main cusps of the molar crown, the modifications of these elements were not referred to in the general results as published,† except where points of sequence seemed to demand their consideration. In fact, the detailed study which I devoted to these structures was undertaken largely through interest in Winge's theory of dental evolution, according to which they are considered as normally three in number and as forming the original elements of the molar crown.‡ It has since appeared to me probable, however, that an account of the modifications of the stylar cusps in such a primitive group as the Didelphyidae would afford a basis for their comparison as molar elements in marsupials with similar structures in placentals, and that possibly their characters might afford a better means of discriminating the molar patterns of early Tertiary or Pretertiary representatives of the two groups than those of the main cusps of the molar crowns, in view of the fact that both are of trituberculate origin. The identification of stem forms depends on the distinction of the primitive characters of marsupials and placentals from one another, and from those which may be common to both; and no adequate conception can be formed concerning them until the habit of denoting the characters of early placentals as "mar-

* Read at the meeting of the Society of Vertebrate Palaeontologists, New York, December 28th, 1905.

† Bensley, B. A., *On the Evolution of the Australian Marsupialia, etc.* (Trans. Linn. Soc., London; Ser. 2, vol. 9, pp. 83-217.)

‡ Winge, H., *Om Pattedyrenes Tandskifte, saer med Hensyn til Taendernes Firmer.* (Vid. Medd. f. d. Naturh. Foren., Copenhagen, 1882.)

supial" rather than as "primitive," or "marsupioplacental" has been finally abandoned.

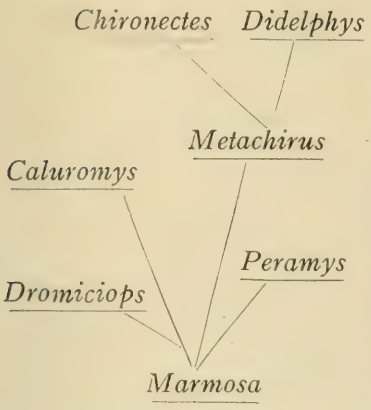
GENERAL CHARACTER AND DISTRIBUTION OF STYLAR CUSPS IN THE EXISTING MARSUPIALS

The stylar elements are accessory structures in the molar crown. They are serially arranged and represent processes of an external ridge or cingulum which passes along the outer faces of the paracone and metacone. They are particularly characteristic of the Didelphyidae, but are found among the Australian marsupials in the Dasyuridae, Peramelidae, and in the phascolarctine division of the Phalangeridae. It is apparent from their associations that they are primitive and more or less conservative elements belonging to the insectivorous stage of evolution. Although reduced in number they are retained in the carnivorous development of the Dasyuridae, two of them being associated with the paracone and metacone in the production of a double shearing edge. In the incipient omnivorous development, as indicated in the Peramelidae, they are retained as in more primitive forms. This is true also, although to a less extent, of the herbivorous and selenodont development as seen in the Phascolarctinae. In the omnivorous and herbivorous developments of the Phalangerinae, in which there is a bunodont modification of the molars, the stylar elements disappear and even in the primitive forms the cingulum is barely indicated. Their function in the insectivorous stage is apparently that of preventing the food from slipping off the smooth concave outer faces of the paracone and metacone, although they are doubtless accessory piercing agents as well. They are associated particularly with the paracone and with the piercing tip of the metacone. The trenchant spur of the metacone tends to be free of these elements on its outer side apparently in order that the shearing action may not be hampered.

THE RELATIONSHIPS OF THE EXISTING DIDELPHYIDAE

In considering the arrangement of the stylar elements in the Didelphyidae it is advisable to bear in mind the probable

relationships of the various representatives of the family. These relationships as deduced from a study of the dentition and foot-structure and of other characters are indicated in the appended plan, the details of which are more fully explained in the paper already mentioned (loc. cit. pp. 182-185). The two subgenera *Marmosa* and *Peramys* include the smallest and most primitive forms of the family. They show the closest correspondence in dentition, *Peramys* being if anything the more primitive, as seen in the greater development of the posterior premolar, a character which belongs also to the Oligocene *Peratherium*, judging from the examples which have come to my notice. The subgenera *Chironectes* and *Didelphys*, together with their prototype *Metachirus*, are to be considered apart from *Caluromys* and *Dromiciops*. The former are larger but, in dentition, conservative forms retaining the general conditions of *Marmosa* and *Peramys*, while the latter show special characters indicating the beginnings of omnivorous specialization.



The Stylar Elements in Peratherium

In the estimation of primitive conditions in the stylar formula the question naturally arises—what was the condition of these structures in *Peratherium?* Although through the kindness of Dr. Smith Woodward I was able to examine in detail the British Museum specimens, I was unable to decide this question to my satisfaction. The majority of the specimens represent mandibular rami.* Of the few fragments of upper jaws only one shows the characteristics of the external

*Lydekker, R., *British Museum Catalogue of Fossil Mammalia*, pt. 5, pp. 283-288. 1887.

styles. In this specimen (No. 27807), however, they appear in the molars of the right side in a beautifully preserved, unworn and unbroken condition. They are moderately developed, and present the same condition in m1 and m2. They tend to be reduced in m3, and in m4 they are absent, this tooth being reduced just as in modern Didelphyidae. In m1 and m2 the external cingulum bears in all six elevations, the arrangement of which is shown in the appended diagram of the external profile of m2 of the right side reversed. Three elements, a, b, and c, are conspicuous, and three others, b1, c1, and c2, are subsidiary. Style a is situated at the tip of the anterior spur of the paracone, b opposite its concave face; c is situated opposite the small anterior spur of the metacone. Of the subsidiary styles b1 and c1 are accommodated in the space between styles b and c and appear to be related respectively to these elements. Style c2 is placed on the outer edge of the enlarged metacone spur. This element is so small as to be scarcely recognizable, and its recognition is still more difficult in this specimen on account of the dark colouration assumed in fossilization.

Fig. 1. Stylar Cusps in Peratherium

Apparently the stylar cusps are as well developed in American specimens. Cope* remarks of *Peratherium* that "the superior molars, excepting the last, present two median V's which would be termed external but for the fact that the external basal cingulum is so developed as to constitute an external crest."

THE STYLAR ELEMENTS IN EXISTING DIDELPHYIDAE

Peramys†

P. dimidiata. Two specimens in the British Museum collection show the stylar cusps in an unworn condition, and both

*Cope, E. D., *Tertiary Vertebrata*, pp. 789 et seq. 1884.

†In this and the succeeding subgenera the descriptions refer only to young specimens or those in which the external styles are quite unworn.

present the same pattern (Fig. 2A—97.1.1.4*). In m1 the elements representing b and c of *Peratherium* are conspicuous. Style a is indicated as a slight projection, and in one of the specimens there is a faint indication of c1. In m2 five projections are shown on the cingular ridge, and these are identical in size and arrangement with those in *Peratherium*, the sole difference being in the absence of c2. In m3 we find again the same condition except that the whole ridge tends to be reduced.

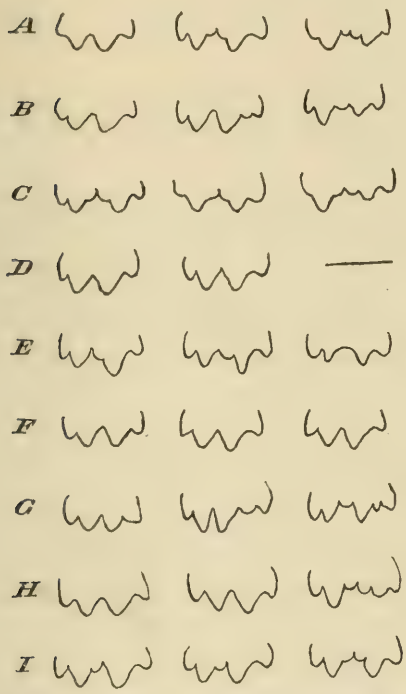


Fig. 2. Stylar Cusps in Peramys

P. scalops. In two specimens m1 and m2 show the absence of the intermediate styles b1 and c1. In m2 the element c2 is present. In m3 style b1 is present in association with c, which is reduced (fig. 2B).

P. iheringi. Two specimens show in all three molars the predominance of b and c, and the presence of both intermediate styles b1 and c1. Style a tends to be reduced, and style c2 is absent; otherwise the pattern is much as in the specimen of *Peratherium* (figs. 1, 2C—61.12.2.9). In m3 the same reduction of the posterior styles is shown as in the specimens of *Peramys scalops*.

P. sorex. In one young specimen displaying the first two molars the number of styles represents the minimum, only a, b, and c being developed (fig. 2D).

P. americana. In one specimen four elements are present in m1 and m2. Style c1 is seen in association with c, style b1

*The numbers indicated are those of the British Museum Catalogue.

being absent (fig. 2E). In m3 is shown a reduction of all elements, those present being a, b and c.

P. domestica. Two specimens show in all three molars the minimum of three styles as described for the first two molars of *P. sorex* (fig. 2F—52.2.22.10). A third specimen shows a conspicuous difference. In m2 styles b and c are enlarged and approximated, as generally in the more specialized *Chironectes*, *Didelphys*, and *Metachirus*. Style c2 is present also as in the latter. In m3 styles b and c are separated by an element probably representing c1, but style c2 is still present as a small element (fig. 2G).

P. brevicaudata. Two specimens show the reduced formula in m1 and m2, as in the specimens of *P. sorex* and *P. domestica*. In m3 five elements are present and the posterior ones are reduced, as in the specimens of *P. iheringi* (fig. 2H—0.5.16.60). A third specimen shows practically the same condition except that styles b1 and c1 are only faintly indicated in m3, and style c2 is developed on the posterior face of c. In a fourth specimen m1 and m2 show the presence of style b1 (fig. 2I—67.4.12.540).

Marmosa

M. simonsi. In four specimens the cingular ridge is moderately developed and bears five projections giving a pattern much like the specimen of *Peratherium*, except that the intermediate elements are relatively larger in m1 and m2, and that in m3 style c1 tends to be divided (fig. 3A—99.8.1.23).

M. elegans. In two specimens five elements are again indicated in m2 and m3. In m1 styles a and c1 are more moderately developed and b1 is absent (fig. 3B—98.8.2.12). In five other specimens the intermediate styles b1 and c1 are absent, while in two others the same condition obtains except that c1 is indicated in m3 (fig. 3C).

M. sinaloe. In two specimens only four styles are developed. Of these a, b, and c show their usual relations, while style c1 is in comparison enlarged (fig. 3D). This is a feature which becomes prominent in the species *M. murina* and *M. cinerea*.

M. marica and **M. dryus.** In three specimens of each species the same conditions are seen as in *M. sinaloe* except that in *M. dryus* style c1 is still better developed and c reduced.

M. microtarsus. One specimen shows the condition of *M. sinaloe* except that in m3 the intermediate styles b1 and c1 are both present as in *M. simonsi.*

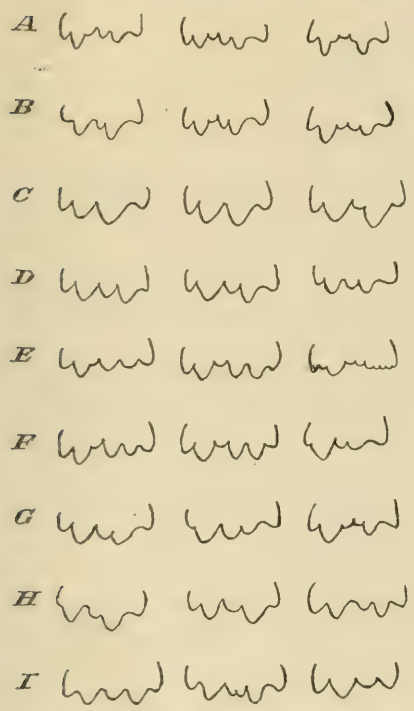


Fig. 3. Stylar Cusps in Marmosa

M. murina. Four specimens show the presence in m1 and m2 of five styles, of which a, b, and c are moderately developed. Style c1 is more definitely enlarged and compares in size with c. Style b1 is barely indicated (fig. 3E, F—97.4.7.12,0.5.1.59). In m3 a different pattern is shown in each specimen. In three of them a, b, and c are indicated, and in two c1 is present (fig. 3F). The fourth specimen shows a more significant arrangement, the cingulum ridge being poorly developed and occupied by a number of low crenulations (eight) prophetic of the condition in the next genus *Caluromys* (figs. 3E, 4B). A fifth specimen shows the increased development of c1 in comparison with c, especially in m2. In m3 the relation is primitive, b1 and c1 being present together and of moderate size (fig. 3G—97.6.7.52). In a sixth specimen a more primitive and moderate development is seen in all three molars (fig. 3H—97.6.7.26).

M. cinerea. The general condition is much as in *M. murina.* In m1 and m2 of one specimen the main stylar

elements are b, c1, and c, and these are developed to about an equal extent. Style b1 tends to be slightly cleft. The same three elements are seen in m3, but they decrease from before backwards from b (fig. 31). In four other specimens the styles are less modified, five elements being typically present, of which b and c show their normal predominance, while b1 and c1 are present together, but of small size. There is a slight reduction of style c in m2.

The relations in *M. murina* and *M. cinerea* are of interest as showing among the variations tendencies on the one hand towards the more primitive condition of the styles, as seen for example in *M. simonsi*, and on the other towards the reduction of the cingulum, as seen in *Caluromys*. The apparent increase of style c1 signifies not an increase in development of the cingulum but a general levelling off of the stylar projections. In the omnivorous and bunodont development of the molars, as seen in the phalangerine division of the Australian Phalangeridae, the cingulum is obliterated, being only seen as a faint ridge in primitive forms. *Caluromys* among the Didelphyidae shows indications of embarking upon this modification, and the two species of *M. murina* and *M. cinerea* which are in many respects prototypal to *Caluromys* show in association with the latter the very first stages in this reduction.

Caluromys

C. laniger. In one specimen the transitional characters between this genus and *M. murina* and *M. cinerea* are well exemplified. In m1 four elements are present projecting to almost the same extent. Particularly noticeable is the increased size of c1 in comparison with c and the absence of b1. In m2 the condition is slightly changed by a peculiar reduction of style b. In m3 the

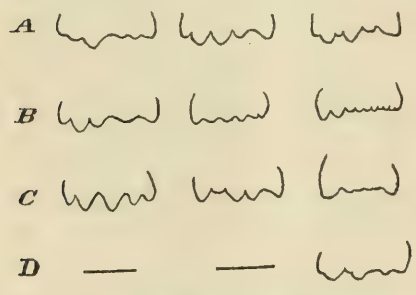


Fig. 4. Stylar Cusps in Caluromys

cingulum is simply crenulated and the individual elements are not apparent (fig. 4C—44.12.18.28). In a second specimen showing the same characters in m1 and m2 the individual elements are apparent, although greatly reduced, and the ridge would still be described as crenulated (fig. 4D—80.5.6.88). In the variety *C. laniger pallida* two specimens show distinctly the reduction of the cingulum (fig. 4A, B—0.7.11.82, 0.7.11.35).

C. trinitatis. Two specimens show minute crenulations only.

Metachirus

The general conditions in this genus and in *Chironectes* and *Didelphys* appear as modifications of those of the primitive species of *Marmosa* and *Peramys*, and it is seen that the development in *Caluromys* is a special one ending as far as the modern Didelphyidae are concerned with that genus. The general arrangement is seen in the approximation of styles b and c, development of style c2, an element scarcely recognizable in the smaller forms, through the shifting forwards of style c, the variable indications of the intermediate styles b1 and c1.

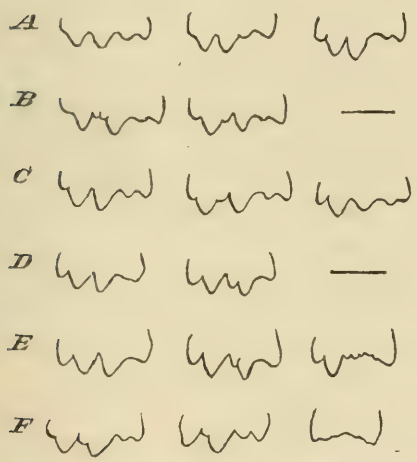


Fig. 5. Stylar Cusps in Metachirus

M. opossum. In one specimen four elements make up the series, namely, a, b, c and c2 (fig. 5A—46.2.2.4). This specimen is abnormal as seen in the development of m4 like the remaining teeth. In m4 styles b and c are present though of small size. A young specimen of *M. opossum var. melanurus* shows the characteristic form of styles b, c, and c2, but the

original elements b1 and c1 are present together in m1, definitely showing the homologies (fig. 5B—97.11.7.60). Style b1 is shown in m2 of this specimen and also in a second specimen (fig. 5C—0.7.11.79). In a third specimen it is seen in all three molars. A fourth shows exactly the condition described in the specimen of *M. opossum* in which the intermediate elements are absent in all three molars.

M. crassicaudata. Two specimens show the conditions represented in fig. 5E, F (85.11.26.11, 79.5.1.13), which are easily referable to the general type in *M. opossum*. The cingulum is greatly reduced in m3 in this species.

Chironectes

C. minimus. One specimen shows the approximation and extra development of styles b and c, and style c2 is evident in m1 and m2, so that the type corresponds with that of *Metachirus*. Intermediate styles are absent in this specimen (fig. 6A—849.a), but in a second young specimen they are indicated in m2 (fig. 6B—849.f).

Didelphys

D. marsupialis azarae. The predominance of styles b and c is indicated in two specimens in all three molars, but especially in m1 and m2. In m2 and m3 is shown the presence of two styles posterior to c, probably indicating a division of style c2. In m3 the intermediate b1 is indicated (fig. 6C—84.2.8.25). This element was identified in several specimens. It is sometimes present in the deciduous premolar, which has a molariform pattern.

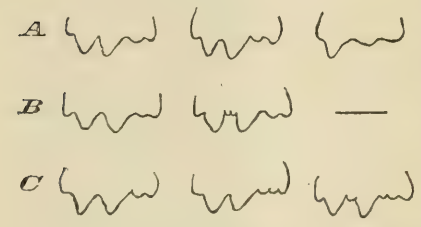


Fig. 6. Stylar Cusps in Chironectes and Didelphys

General Summary

In comparing the characters of the stylar cusps in an extended series of specimens such as indicated above, three features become apparent. In the first place, as compara-

tively small and subsidiary structures in the molar crown are certain to exhibit signs of variation, they are surprisingly constant in their relations. Secondly, they show throughout the family indications of a general type, best seen in *Peramys* and *Marmosa*. Thirdly, even in such a poorly differentiated radiation as that represented by the modern Didelphyidae, they show signs of adaptive change. The variations which appear seem to be for the most part significant. The general type indicated is one in which there are three main elements and three more subsidiary ones which I have designated respectively as a, b, c, and b1, c1, c2. If the modern Didelphyidae reflect in the upper molar patterns the primary trituberculate type these main elements are the ones for which homologies must be sought. The general type indicated seems to conform with that described for *Peratherium*, but whether this correspondence would be confirmed on examination of other specimens of *Peratherium* it is not possible to say. That the stylar elements are worthy of consideration in estimating adaptive changes in the molars, or relative specialization, is indicated by Sinclair's* studies on the Santa Cruz marsupials, and my own on the Australian forms. Finally, considering the stylar elements of the modern Didelphyidae as structures not wholly conservative but showing signs of adaptive change and in comparing the family with other, supposedly primitive, forms, the characters presented by *Marmosa* and *Peramys* should be consulted rather than those of the larger specialized forms such as *Didelphys*.

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* Sinclair, W. J., *The Marsupial Fauna of the Santa Cruz Beds.* (Proc. Amer. Phil. Soc., vol. 49, pp. 73-81, 1905.)

UNIVERSITY OF TORONTO STUDIES

BIOLOGICAL SERIES

No. 6: ON POLYSTELY IN ROOTS OF ORCHIDACEAE, BY J. H. WHITE

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COMMITTEE OF MANAGEMENT

ON POLYSTELY IN ROOTS OF ORCHIDACEAE

BY

J. H. WHITE, B.A.

PREFATORY NOTE.

The work embodied in this article was done by Mr. J. H. White, while pursuing graduate studies in Botany in the University of Toronto, during the year 1906-07.

J. H. Faull
Lecturer in Botany.

ON POLYSTELY IN ROOTS OF ORCHIDACEAE.

In his essay on polystely Van Tieghem ('86) defined polystely as follows: "Les faisceaux conducteurs peuvent être groupés en plusieurs cercles autour d'autant d'axes diversement

BIOLOGICAL SERIES No. 6

ERRATA

Page 7 [169] line 23 should read:
"Split off from the large stele, itself underwent division. The"

Page 10 [172] line 9, for "monostele" read "stele."

Page 11 [173] line 2, after "root" insert "in *H. hyperborea.*"

Page 12 [174] line 14, after "*H. orbiculata*" insert "and *H. blephariglottis.*"

Page 14 [176] line 10, after "*H. orbiculata*" insert "and *H. blephariglottis.*"

Page 15 [177] line 3-4, omit the word "extra-stelar," and line 21, after "placed" insert "by these botanists."

Page 18 [180], third reference from the bottom (Strasburger, Lehrbuch der Botanik) for '03 read '04.

Plates I & II, heading, for "Biological Series No. 7" read "Biological Series No. 6."

gators since Van Tieghem's time, with the result that it has been disproved. Leclerc du Sablon ('90) has shown that the numerous strands in the stem of *Pteris* do not originate by division of a monostele. Likewise, Gwynne-Vaughan ('97) has disproved Van Tieghem's statement in regard to *Primula.* Jeffrey (:00, :02), by an exhaustive study, has shown that in none of the so-called polystelic Pteridophyta, nor in the few

ON POLYSTELY IN ROOTS OF ORCHIDACEAE.

In his essay on polystely Van Tieghem ('86) defined polystely as follows: "Les faisceaux conducteurs peuvent être groupés en plusieurs cercles autour d'autant d'axes diversement disposés, de manière à constituer tout autant de cylindres centraux distincts, ayant chacun sa moelle, ses rayons médullaires, son pericycle et son endoderme, tous reliés et enveloppés par une écorce commune. . . . La disposition de l'appareil conducteur est polystélique." This condition he conceived to be derived by repeated division of the monostele. He based this view on his conception of the anatomy of the stems of *Primula, Gunnera,* etc., and extended his conclusions to the stems of most vascular cryptogams. As regards polystely in roots, he described but one example—some of the roots in certain Lycopodineæ.

Van Tieghem mentioned the "tubercules" of *Orchis, Ophrys,* etc., which show numerous steles, only to state that they merely simulate polystely. "Les tubercules des *Orchis, Ophrys,* etc., possèdent, comme on sait, un plus ou moins grand nombre de stêles distinctes dans une écorce commune, mais toutes ces stêles s'attachent independamment, quoique en des points très voisins, sur le rameau qui porte le tubercule; elles ne dérivent pas l'une de l'autre par voie de division. Ce tubercule est donc constitué par un faisceau de racines concrescentes et non par une racine polystélique. C'est un des exemples qui montrent le mieux combien il est nécessaire de dégager la polystélique vraie des illusions produites par la concrescence."

Polystely in stems has received the attention of several investigators since Van Tieghem's time, with the result that it has been disproved. Leclerc du Sablon ('90) has shown that the numerous strands in the stem of *Pteris* do not originate by division of a monostele. Likewise, Gwynne-Vaughan ('97) has disproved Van Tieghem's statement in regard to *Primula.* Jeffrey (:00, :02), by an exhaustive study, has shown that in none of the so-called polystelic Pteridophyta, nor in the few

cases among the Angiosperms, is this condition due to bifurcation of a single stele. Instead, it has been proved that the central cylinder is always a monostele, and that the gaps caused by the departure of the foliar and ramular traces from a medullated monostele furnish the explanation of the so-called polystelic type.

Polystely in roots has likewise received some attention, and it is said to exist in the tuberous or abnormal roots of the Leguminosæ, Cycadaceæ, and Palmaceæ. Of these, the roots of the Palmaceæ have been most carefully examined, notably by Cormack ('96) and Drabble (:04). Cormack discovered what he considered to be polystely in this group, and his observations have been corroborated by Drabble, and summarized as follows: "Cormack grouped the arrangements met with under several headings, commencing with the normal type possessing a complete endodermis surrounding a central fibrous or sclerenchymatous ring in which lie the xylem and phloem elements. Passing through the condition in which the cylinder is still complete, but longitudinally lobed, the endodermis dipping into the depressions, but being quite continuous, he came to those roots in which the central cylinder is composed of a series of independently running strands of fibrous tissue presenting the form of arcs of circles in transverse section; between these the endodermis dips in and becomes discontinuous; and, finally, he described the case of *Areca,* where one or more of these independent strands presents a complete radially symmetrical cylinder or 'stele,' as he terms it, round which the endodermis is complete." Drabble points out that the "polystelic" condition is restricted to the proximal portion of the roots; the distal is always that of a normal monostelic root. Whatever interpretation may be placed upon these observations, it will be seen that the phenomena to be described in the roots of the Orchidaceæ differ in some respects from those in the Palmaceæ.

Since Van Tieghem's time no comparative investigations of the multistelic roots of the Orchidaceæ have been made to determine the homologies of their stelar system, and all later references agree with his views.

Strasburger (:04), in his text-book of botany, says: "Einen eigenen morphologischen Aufbau zeigen die Knollen der Orchideen. Sie werden der Hauptmasse nach aus fleischig

angeschwollenen, nicht von einander gesonderten Wurzeln gebildet, oben schliessen sie aber mit einer Stammknospen ab" (p. 40, also p. 435). Schoute (:02) says: "Wenn man den Namen Polystelie dennoch beibehalten will, so kann man diesen anwenden auf diejenigen Fälle wie die Orchideenwurzelknollen, wo durch Verwachsen mehrerer Wurzeln ein einheitlicher Körper entsteht, der wirklich mehrere Stelen führt. Bekanntlich bezeichnet aber gerade Van Tieghem diesen Fall nicht als Polystelie, weil er hier durch Verwachsen entstanden ist" (p. 156).

These views, however, have not seemed conclusive, and the present investigation was begun with a view to determine, first, the structure of the roots of terrestrial Orchidaceæ, and secondly, the interpretation of their structure in terms of modern stelar hypotheses.

The roots of these Orchids are outgrowths of an adventitious shoot—a fact due to the habit of vegetative reproduction characteristic of these plants. Each year one or more stolons are developed, each of which produces a new bud. The old plant dies away, and the adventitious shoot perpetuates the species the following season. In connection with this method of reproduction many of these Orchidaceæ show a differentiation of the root system into a primary tuberous root—part of the so-called "tuber" and having the characters of a storage root—and somewhat slender lateral roots. This is especially true of the Ophrydinæ. It is in the roots of this class of Orchidaceæ that there is an appearance of polystely, and it is with the anatomy of these alone that we are here concerned.

THE LATERAL ROOTS

Among the lateral roots there are two types, the monostelic and the polystelic. The monostelic type is characteristic of *Habenaria bracteata*, R. Br., *H. repens*, Nutt, *Orchis spectabilis*, L., and some of the roots of *H. orbiculata*, Torr. In these forms there is a single stele, consisting of bast and wood elements arranged fairly radially around a central pith, and surrounded by an endodermis. As a rule the bast is not very well developed. The strands composing this stele come from different parts of the vascular supply of the stem,

just as in the case of such monostelic genera as *Goodyera, Epipactis,* etc. Plate I, figure 4, represents a transverse section of the stele of *H. bracteata.* In the apical region there is a well-formed root-cap, and just back of it the meristem from which are differentiated the three embryonic regions, namely dermatogen, periblem and plerome.

The polystelic (pseudo-polystelic, according to Van Tieghem) type is not mentioned by Van Tieghem as occurring in the lateral roots, but has been described by Holm (:04) in several forms. *Habenaria orbiculata* furnishes examples of distelic roots, and there are indications of distely in *Orchis spectabilis.* In the latter species many cases were met with in which the stele showed a distinct flattening, and while no case of two steles was seen, it is very probable that this condition sometimes occurs. The lateral roots of *H. orbiculata* are always monostelic at their base. Farther out, the stele in some of them flattens, and still farther out, assumes a horse-shoe shape. Plate I, figure 6, represents the stele of *H. orbiculata* when just beginning to flatten, and Plate I, figure 7, the latter condition. Farther out still, the stele is constricted into two equal parts (Plate I, figure 8), or in other words, by an act of bifurcation, the monostele gives rise to two steles, a phenomenon that Van Tieghem apparently could not have observed, standing as it does in direct opposition to his statement on this subject (Van Tieghem and Douliot ('86). These strands continue side by side throughout the greater part of the root. At the tip of the root a typical root-cap is present, and just behind it a single meristem giving rise to a single dermatogen, periblem, and plerome. At some distance back the primary plerome strand gives rise to two secondary plerome strands corresponding to the two steles and to the ground tissue separating them. Each of these steles is a typical root stele, possessing alternate bast and wood elements, and each is enclosed by an endodermis. As already stated, the roots of *H. orbiculata* are not always distelic, those of the earlier generations and the smaller ones being monostelic throughout.

Of the polystelic type none is of greater interest than the roots of *H. blephariglottis,* Hook. In these the strands composing the root stele bear the same relation to the vascular supply of the stem as in the case of any monostelic form, such as

H. bracteata. Each of the lateral roots in most of the specimens that were examined was found to be monostelic at its base as in *H. orbiculata.* It is in many cases protostelic for some distance out, and then becomes siphonostelic, owing to the appearance of a pith and an internal endodermis (Plate I, figure 1), a condition that is unique in the structure of root steles. Farther out from the stem a gap appears in the stele, through which the pith and cortex connect, and the internal and external endodermes become continuous (Plate I, figure 2, *a*). As the series is followed on in the same direction the gap enlarges, giving the stele a horse-shoe shape. Successive acts of segmentation then take place, steles being pinched off from the ends of the shoe, more or less alternately (Plate I, figure 3), till finally in place of the monostele there is a number of small steles of approximately the same size regularly arranged in a circle. Each of these small steles has the bast and wood elements arranged in an irregularly radial manner (Plate II, figure 10).

The roots of this species that were examined showed only minor points of difference among themselves, such as a smaller or a larger number of steles, according to the size of the root, or an at first scattered, irregular, instead of circular arrangement of the individual steles. In some cases a stele, after being roots of *H. hyperborea,* R. Br. (Plate II, figure 9). The main fact held for most of them, namely, that the individual steles were derived by a process of segmentation from a monostele. Occasionally, however, in robust roots, the conditions at the base approach those in *H. hyperborea.*

In more specialized forms the fibro-vascular supply comes off from the stem in a small number of steles, as, for example, in the roots of *H. hyperborea,* R. Br. (Plate II, figure 9). The origin of the strands composing these steles is in no way different, however, from what obtains in the three species already described. The small number of steles seems to mean that the division of the monostele is carried back to the stem connection. Farther out these steles are to be found undergoing division, resulting in a large number of small steles of approximately the same size, just as in *H. blephariglottis* (Plate II, figure 10).

The tip in all these polystelic roots is clothed by a single root-cap, just as in *H. orbiculata,* behind which is a small-celled meristem from which all of the organs of the root originate.

It is worthy of note from a phylogenetic standpoint that in the roots of plants of younger generations the number of steles is very much smaller than in those of older generations. For example, in the roots of plants of *H. orbiculata* belonging to very early generations never more than one stele was present; in *H. hyperborea* as few as two were found, and in *H. blephariglottis* the number was likewise small. The increase in the number of steles in the roots of plants of each succeeding generation from the seed till the flowering plant is reached without a proportionate increase in the number of steles entering these roots, and without any indication of a disturbance of the conditions described in the *punctum vegetationis* of each, seems out of accord with the theory of concrescence.

Examination of very young plants in a number of species, where the roots were just beginning to grow out from the stem, showed that but one meristematic mass was present, and in no case was there any indication of a fusion of two or more roots. In accordance with the view that the phylogeny of the race is recapitulated in the ontogeny of the individual, one might have expected to find, in some cases at least, discrete meristematic areas corresponding to component roots, if the concrescence theory were correct; but one for each root is the invariable rule.

The other polystelic species examined were *H. clavellata,* R. Br., *H. psycodes,* Gray, *H. virescens,* Spreng, *H. lacera,* R. Br., *H. obtusata,* Rich., *H. leucophaea,* Gray, and *H. Hookeriana,* A. Gray. The structure of the roots of these species agrees very closely with that just described for *H. hyperborea.*

THE PRIMARY ROOT OF THE "TUBER"

As already stated, vegetative reproduction is characteristic of terrestrial Orchidaceæ, and in many of them the adventitious shoot is tuberous. A typical "tuber" consists of a stolon or rhizome bearing a lateral bud, from which in turn there is developed an axial tuberous root, occupying the position of a primary root. Among the "tubers" there is much variation in the length of the stolon, and the direction of the axis of the root. Thus in *H. bracteata* and *H. orbiculata,* for example, the stolon is very short, whereas in *H. clavellata, H. blephariglottis* and *Orchis spectabilis* it is comparatively long. In most of

these Orchids the primary root is vertical, but there are all gradations to the horizontal position assumed in the case of *H. dilatata,* Gray. There may be even some variation in the same species. Thus in *H. hyperborea* there is considerable variation in the length of the stolon, and it is noticeable that an increase in the length of the stolon is accompanied by a greater departure of the root from its usual vertical position.

The stolon possesses a single stele of cauline structure. There is a continuous ring of xylem around a central pith, with the bast externally placed, and the whole is surrounded by an endodermis, that is, there is an ectophloic siphonostele (Plate I, figure 5). This seems to agree with the primitive monocotyledonous type of cauline stele as defined by Chrysler (:04), and Plowman (:06). This siphonostele goes to supply the young shoot and its root.

Characteristically the primary axial root is polystelic, the number of steles varying from as few as two in *Orchis spectabilis* to twenty or more in *H. blephariglottis.* It remains now to be determined whether these are steles of a fundamentally unit root, as has been shown to be the case in the lateral roots, or monosteles of concrescent roots. Among the lateral roots, as has been seen, there are both monostelic and polystelic forms, with instances of distely occurring in *H. orbiculata* as a connecting link. All of the lateral roots, except the most specialized forms, are monostelic at their base, the root tips of all show but one small-celled meristem (Plate II, figure 15), and the young roots originate each from a single meristematic area. A careful examination should reveal the extent to which these conditions prevail in the axial roots.

In the transitional region between stem and axial root, in a form such as *Orchis spectabilis,* the stolon siphonostele gives off the vascular axis of the adventitious bud, and a pair of steles which turn down into the tuberous root. Almost immediately in some plants a third root stele is given off. After supplying the bud the siphonostele at once becomes much attenuated, curves down, and ends in a minute projection of meristematic tissue on the surface, on the way giving off a single trace to a leaf which sheathes the new shoot. The meristematic projection is the tip of the stolon, and hence the adventitious bud is distinctly lateral. The three root steles do not form a mono-

stele at their base, though they leave the stolon at practically the same level. They recall the condition of affairs in the base of the lateral roots of *H. hyperborea* (Diagram 1). Much the same phenomena obtain in the case of *H. orbiculata* (Diagrams 2-13). Here a pair of root steles is given off just as in *Orchis,* but the third stele is given off at a lower plane. Usually this third stele soon bifurcates, so that of the four steles present in the axial root two, at least, are obviously derived from a pre-existing monostele by an act of bifurcation.

In those forms which have acquired a diageotropic habit, as, for example, *H. clavellata,* the origin of the root steles is very diffuse—a phenomenon on which Van Tieghem based his main argument in support of the concrescence theory. The stolon has the same mode of origin as in the preceding forms, and possesses a similar siphonostele. Serial sections show that this siphonostele soon begins to give off steles. These are very small and are usually given off right and left alternately, while the remainder of the siphonostele continues on in the same relative position. They slant downward and run along close to the ventral surface, the whole of them forming an arch when viewed in transverse section. These small steles are usually all derived from the main stele; only very rarely does one of them bifurcate after being given off. A marked difference is observable in the character of the cortex in the dorsal and ventral regions, in that the cells of the dorsal region are smaller and more compact than those of the ventral. From the dorsal portion of the siphonostele the stele to the new shoot is given off. As in the forms already described, the stele of the stolon then becomes abruptly attenuated, gives off one or two more root steles, curves up, and ends in the stolon tip on the dorsal surface, on its way giving off the leaf trace. It is important to note that in spite of their very diffuse origin all of the steles given off along the stolon proceed to the tip of the tuberous root, and end in one meristematic area in the growing point, just as in all the other species examined (Diagram 14).

On comparing this form with *Orchis* and *H. orbiculata* it is noteworthy that there is no difference other than that the root steles have a more diffuse origin—a phenomenon that is probably to be accounted for by the unusual position of the root. That this is the case is well shown by *H. hyperborea.* As

already stated there is much variation in the length of the stolon and the position of the root, the two being associated more or less. In those cases where the stolon is extremely short and the axial root accordingly perpendicular, no steles are given off throughout the length of the stolon proper, and the origin of the root steles is no more diffuse than in *Orchis* or *H. orbiculata.* But in those instances where the plant puts forth a long stolon and the axial root takes on a slanting position, steles are given off as in *H. clavellata* (Diagrams 15-26). Hence, it seems evident that the diffuse origin of the root steles in forms like *H. clavellata* is to be ascribed to the diageotropism of the root. It might be stated here that in *H. blephariglottis,* where the position of the root comes nearest, of the species examined, to that of *H. clavellata,* the degree of diffuseness of the root steles is intermediate between the condition in *H. hyperborea* and that in *H. clavellata.*

Plants of *H. hyperborea* of a very early generation possess another feature, however, of even greater import. For several sections below the region of the stolon stele, that is, at the upper end of the axial root, all of the vascular elements are enclosed within one endodermis. This single root stele then simultaneously breaks up into the two or three steles which are present throughout the remainder of the axial root to its tip (Diagram 27), a fact that amounts almost to a demonstration that the axial root is a unit, and not the result of concrescence.

Thus there is a fairly complete series, in the case of the axial or tuberous root, from the monostelic to the most extreme polystelic type, which parallels closely what has been already described for the lateral roots.

It is further to be noted that the tip of the axial root has a single root-cap, and behind it the meristem, which is differentiated as in the lateral roots. Figures 11, 12, 13, of Plate II, are from serial sections of the tip of the axial tuberous root of a plant of *H. hyperborea* of an early generation, in which there is a primary plerome strand that is continuous proximally with secondary plerome strands. Occasionally the tip is forked, as, for example, in *H. bracteata.* This forking, however, occurs only in the older roots, and seems to be of the nature of a splitting or dichotomy, and bears no relation to the number of steles. Apparently the root tip is first flattened, and a meri-

stematic area in the centre of the tip ceases growing, while the isolated areas on either side continue their growth, which results in the forking just mentioned. Drabble (:04) has figured a similar forking in *Kentia,* Sp.

The same fact has been observed with regard to the tuberous roots as was seen in the lateral roots, namely, that in those of the earlier generations the number of steles is much smaller than in those of the later generations. Thus it is not uncommon in *H. bracteata* to find three steles in the axial root of one generation, and six steles in the corresponding root of the next generation. Again, in the tuberous root of *H. hyperborea* of a young generation (Diagram 27) two steles were found, while the next generation showed very many more, and in early generations of *H. orbiculata* there is but one. It is altogether probable that in the earliest generations of all of them there is but a single stele.

THE "TUBER"

Van Tieghem looked upon the "tuber" as a branch carrying a "tubercule" with a greater or smaller number of distinct roots, the steles of which attach themselves independently, though at points very close together, on the branch. According to him, the "tubercule" is a bundle of concrescent monostelic roots, and not a single polystelic root. Others have gone further and included more in the concrescence. Thus, Germain de St. Pierre ('55) includes in it stem and leaf portions as well as roots, and he has lately been followed by Holm (:04). But according to the foregoing observations on the primary root of the "tuber" taken in conjunction with those on the lateral roots, the "tubercule" is simply a swollen root which is usually polystelic. One other fact has been overlooked in previous accounts of the "tuber," namely, that the stolon continues beyond the origin of the adventitious shoot, and that the leaf ensheathing the upper part of the "tuber" derives its vascular supply from the distal portion of the stolon.

CONCLUSIONS

The "concrescence" theory has been stated already. The grounds on which this view is based are the diffuse origin of the steles of a tuberous root, and the forking of the root tips in

forms such as *H. bracteata* and *Orchis latifolia.* But in the light of the foregoing observations these grounds are scarcely tenable.

The forking of tuberous roots is apparently a matter of accident. There is no indication of it in young roots prior to the differentiation of the steles. Moreover, it bears no relation to the steles. Forking is not characteristic of the more slender roots, and it is never present in lateral roots, nor even in the markedly multistelic roots of *H. clavellata,* though not of infrequent occurrence in swollen roots of several other species. The forking is probably caused by purely mechanical forces, and in view of the facts recorded of the earlier stages of development can be regarded in no way as indicating concrescence.

It is apparent from former accounts that the theory of concrescence has been based on an examination of the tuberous roots only. To understand the structure of these, however, in regard to the origin of the steles, the conditions obtaining in the lateral roots are of material assistance. Thus it has been seen that the lateral roots of *H. bracteata* exhibit typical monostely, and the other species may be looked upon as presenting deviations from this type. Of these, *H. orbiculata* shows the least deviation, only some of the roots being distelic, and these for the central portion only of their length. The roots of *H. blephariglottis* are characterized by a still greater deviation, in that they possess more than two steles, but although this is the case they are characteristically monostelic at the base, and hence can only be looked upon as single roots. *H. hyperborea* departs from the type more widely still, in that the roots are never monostelic at the base. However, the fact that they possess but a few steles in this region, and that the larger number farther out is derived from these by segmentation, shows the tendency towards the condition in *H. blephariglottis,* and may be taken to indicate that the division of the monostele has been carried back into the stem. To cite an analogous case, the carrying back of the cleavage of a fibro-vascular strand to its origin is seen in the leaf traces of certain ferns. Primitively the foliar fibro-vascular supply in the *Filicales* comprises a single strand, but in many of the more specialized ferns this strand is divided, and commonly the segments are attached separately to the stelar axis of the stem. Indeed, the facts relative to the origin of the steles

in multistelic lateral roots point to the conclusion that they are the result of the segmentation of a single stele, and are not indicative of concrescence of several monostelic roots.

The steles of the multistelic axial or tuberous roots are much more frequently attached separately to the cauline stele than is the case in the lateral roots, but this phenomenon is not as universal as Van Tieghem thought. In fact, segmentation of a root stele is by no means infrequent, and sometimes monosteles exist at the base of axial roots in earlier generations. Thus, the tuberous roots of early generations of *H. orbiculata* are monostelic throughout, and what may be considered of greater significance, corresponding monosteles in *H. hyperborea* divide after leaving the stem, plainly fulfilling the conditions expressed by Van Tieghem in his definition of polystely. Moreover, the most striking examples of a diffuse origin of the steles occur in species like *H. clavellata,* in which the axial roots are characterized by an extreme diageotropic habit, and, as has been shown, the degree of diffuseness is measured by the extent of the defection of the root from the vertical position. Hence, a phylogenetic series can be constructed, beginning with the condition noted in early generations of *H. orbiculata* and *H. hyperborea,* and terminating with the condition in *H. clavellata* or *H. dilatata.* Such a series harmonizes the phenomena in axial roots with those in the lateral, rendering it quite certain that polystely is the true explanation in the one as it is in the other.

But there yet remain other facts that are incompatible with any theory of concrescence. A single root-cap clothes the tips of all roots, and behind this root-cap there is a single small-celled meristem, consisting of dermatogen, periblem, and plerome initials. Moreover, the first indication of a root is the appearance of a single meristematic area, and from this alone, without concrescence with any other, the root develops.

The interpretation of the phenomena in polystelic roots in terms of the modern stelar hypotheses, especially of those that attach importance to the differentiated meristematic areas at the growing point is a matter of some interest. The view of Hanstein that the three meristematic areas, dermatogen, periblem and plerome, in the growing point of vascular plants corresponded to the epidermis, cortex, and stele of the mature organ is well known. But it is very evident in these roots that a

primary plerome strand is not wholly young stelar tissue, for on tracing it back in *Habenaria orbiculata, hyperborea,* etc., we have found that it passes into secondary plerome strands and extrastelar ground tissue of the same kind, continuous with, and indistinguishable from the cortical tissues. The secondary plerome strands are transformed into steles, each, it may be, with its pith and endodermis, while the ground tissue undergoes no further change. That the plerome is not necessarily the undifferentiated stele has already been proved for certain forms by Schoute (:02), Campbell (:05), and more recently by Conard (:06). But nowhere is this so strikingly the case as in the examples we have described.

In this connection the homologies of the pith and the tissues occupying the axis in polystelic Orchid roots are particularly interesting. There are two theories in regard to the character of pith. One of these regards the pith as always stelar, while, according to the other, the pith in many cases is an extrastelar tissue. The first position has been maintained by Van Tieghem ('86) (except in the case of "gamostely"), Strasburger ('91), Boodle (:01), Tansley and Lulham (:05), and others. Considerable stress has been placed on the correspondence of Hanstein's meristematic areas in the growing point with the areas in the mature portions of the plant. The other view, first proposed by Jeffrey, that the pith is cortical in its homologies in some cases, has for its support the similarity of the cells of the cortex and medulla, their continuity at the gaps, the "dipping in" of the endodermis, certain facts of degeneration, and the incompatibility in certain cases of the Hanstein conception, as noted in the last paragraph. Jeffrey (:00; 02) described the "intrusion" of the cortex into the stele above the point of departure of the leaf traces in seedlings of the Pteropsida, and above the branch traces in the Lycopsida. Faull (:01) described the continuity of the cortex and pith through the ramular gaps in his account of the Osmundaceæ. Chandler (:05) has added further corroborative proof drawn from a study of an extensive series of sporelings.

Further corroboration of the view that the pith may be extrastelar in some cases has been found by the author in his study of the lateral roots of *H. blephariglottis*—a proof amounting to a demonstration. In the majority of Orchid root steles in which a "pith" occurs there is no connection between the pith and the

extrastelar tissues. But in *H. blephariglottis* (Plate I, figure 1) there is an internal endodermis surrounding a pith of quite a different character. On tracing the pith forward from the base towards the root tip (Plate II, figures 2, 3) it has been seen that it is continuous with the extrastelar tissue separating the steles into which the monostele breaks up—a tissue that is unquestionably homologous with the tissue in a similar position in lateral roots of *H. hyperborea, psycodes,* etc. (which are polystelic at their base), and with the tissue separating the two steles in the lateral roots of *H. orbiculata.* The extrastelic character of this tissue is especially evident in the last, for the monostele flattens, bends into a horse-shoe, incompletely enclosing a part of the cortex, and then breaks into two steles of equal size by a constriction or "intrusion" of the cortex from opposite sides (Plate I, figure 6; Plate II, figures 7, 8).

In conclusion, polystely adds another specialized feature to the many possessed by various members of this remarkably specialized family. It is difficult to say how this habit may have arisen, but there is evidently some connection between polystely and the tuberous character of the roots in which it occurs, for it is to be noted that with the increasing size of polystelic roots in succeeding generations there is an increased number of steles in each. The phenomenon, as it exists here, plainly answers to Van Tieghem's definition of polystely. Finally, the conditions existing in these roots must be reckoned with in any stelar hypothesis, for it has been established according to the observations recorded in this paper that the middle and certain more or less radially placed cells belonging to the primary plerome or to the plerome initials directly give rise to extrastelar elements.

SUMMARY

I. There are two types of roots among the terrestrial Orchidaceæ—the monostelic and the polystelic, in Van Tieghem's sense of the terms. In reference to the latter, the term "concrescence" is inapplicable.

II. Polystely has been found in both the lateral and the tuberous roots of *H. orbiculata, H. blephariglottis, H. hyperborea, H. clavellata, H. lacera, H. psycodes, H. virescens, H.*

obtusata, H. leucophaea, H. Hookeriana; and in the tuberous roots of *H. bracteata* and *Orchis spectabilis.*

III. In the basal portion of the monostele which is ordinarily present in the lateral roots of *H. blephariglottis* a pith and an internal endodermis may occur. The pith in such cases is plainly extrastelar in its homologies.

IV. The plerome initial cells in all polystelic roots are differentiated into stelar and extrastelar tissues.

The above investigation was carried on in the Biological Laboratory of the University of Toronto under the direction of Dr. J. H. Faull, by whom the subject was suggested, and to whom I wish to express my deep obligations for much patient criticism and advice throughout, as also for some of the material. My thanks are due to Professor R. Ramsay Wright for the facilities afforded in the laboratory.

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EXPLANATION OF PLATES.

Plate I.

Fig. 1. *H. blephariglottis.* Transverse section through base of lateral root, showing siphonostele with internal endodermis.

Fig. 2. Same stele farther out.

Fig. 3. Same stele still nearer the root tip.

Fig. 4. *H. bracteata.* Transverse section of lateral root, showing typical monostele.

Fig. 5. *H. clavellata.* Transverse section of stolon, showing ectophloic siphonostele.

Fig. 6. *H. orbiculata.* Transverse section of lateral root, showing monostele beginning to flatten.

Plate II.

Fig. 7. The same farther out, stele horse-shoe-shaped.

Fig. 8. The same still farther out, after bifurcation.

Fig. 9. *H. hyperborea.* Transverse section through base of lateral root, showing the few large steles.

Fig. 10. *H. hyperborea.* Transverse section of lateral root, showing typical polystelic condition.

Figs. 11, 12, 13. *H. hyperborea.* Transverse serial sections of tip of axia root of plant of an early generation, showing differentiation into secondary plerome areas.

Fig. 14. *H. blephariglottis.* Transverse section of axial root near tip.

Fig. 15. *H. hyperborea.* Longitudinal section of tip of lateral root.

Diagram 1.

Orchis spectabilis. Diagrammatic representation of vascular system, showing stolon stele supplying young shoot and its axial root; it ends in the meristematic stolon tip which is sheathed by a leaf containing a single stelar trace. There are three root steles: *sh. s.*, stele of adventitious shoot; *s. s.*, stele of stolon; r_1, stele of axial root (r_2, on the opposite side and corresponding to, is not figured); r_3, stele of axial root; *s. l. t.*, stele of sheathing leaf; *s. t.*, tip of stolon; *a. r.*, axial root; *s.*, stolon; *s. l.*, sheathing leaf; *sh.*, adventitious shoot.

Diagrams 2-13.

H. orbiculata. Regional sections of the transition region between shoot and root of a "tuber." A reconstruction would give a figure like Diagram 1, except that the stolon tip is relatively higher up, and there are four root steles instead of three. *Sh. s.*, stele of adventitious shoot; *s. s.*, stele of stolon; r_1 r_2, r_3, r_4, steles of axial root; *s. l. t.*, stele of sheathing leaf; *s. t.*, tip of stolon; *a. r.*, axial root.

DIAGRAM 14.

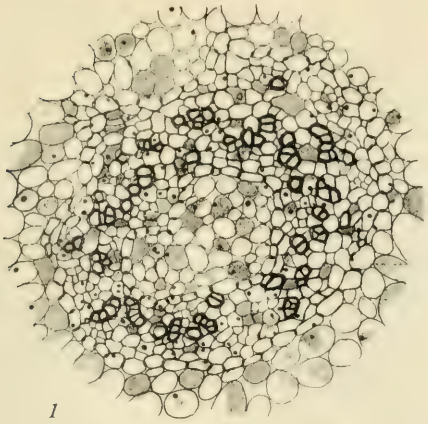
H. clavellata. Diagrammatic representation of vascular system. Note the diffuse origin of the root steles due to the diageotropism of the root. *Sh. s.*, stele of adventitious shoot; *s. s.*, stele of stolon; r_1, r_2, r_3, r_4, r_5, steles of axial root; *s. l. t.*, stele of sheathing leaf; *s. t.*, tip of stolon; *a. r.*, axial root; *s.*, stolon; *s. l.*, sheathing leaf; *sh.*, adventitious shoot.

DIAGRAMS 15-26.

H. hyperborea. Regional sections of the transition region between shoot and root of a "tuber." Note that the stolon stele gives off four steles along its length before the region of the root proper is reached. The diagram represents a condition intermediate between Diagrams 2-13 and Diagram 14. *Sh. s.*, stele of adventitious shoot; *s. s.*, stele of stolon; *l. r.*, lateral root; r_1, r_2, r_3, r_4, r_5, r_6, steles of axial root; *s. l. t.*, stele of sheathing leaf; *s. t.*, tip of stolon; *a. r.*, axial root corresponding to shoot.

DIAGRAM 27.

H. hyperborea—plant of an early generation. Diagrammatic representation of vascular system, showing stolon stele supplying young shoot and its axial root; it ends in the meristematic stolon tip which is sheathed by a leaf containing a single stelar trace. Note that the root stele is monostelic at its base, and later divides into two steles. *Sh. s.*, stele of adventitious shoot; *s. s.*, stele of stolon; r_1, r_2 steles of axial root; *s. l. t.*, stele of sheathing leaf; *s. t.*, tip of stolon; *a. r.*, axial root; *s.*, stolon; *s. l.*, sheathing leaf; *sh.*, adventitious shoot; *r.*, monostelic axial root, which lower down bifurcates into r_1, and r_2.



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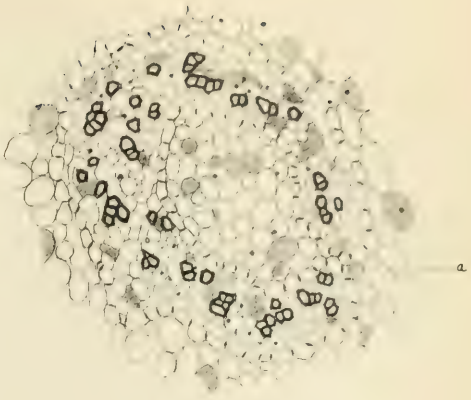

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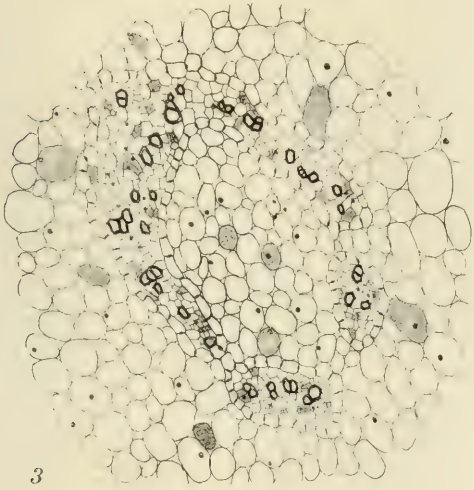

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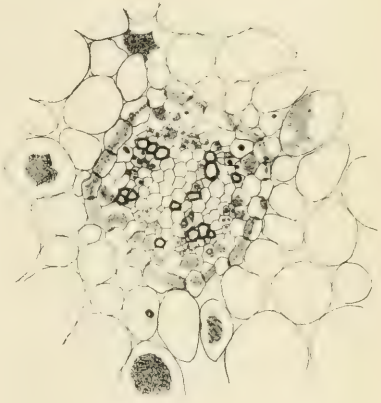

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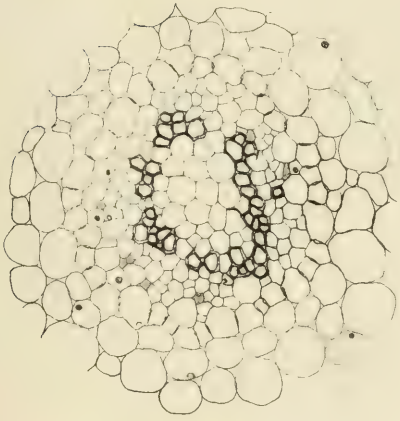

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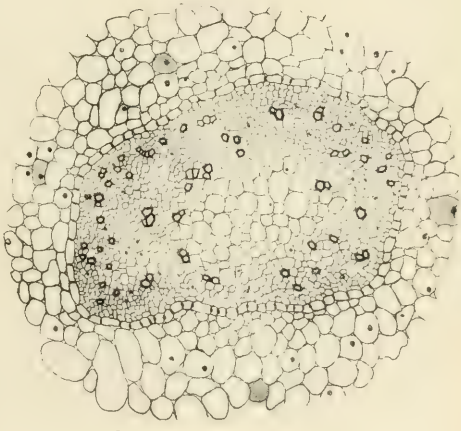

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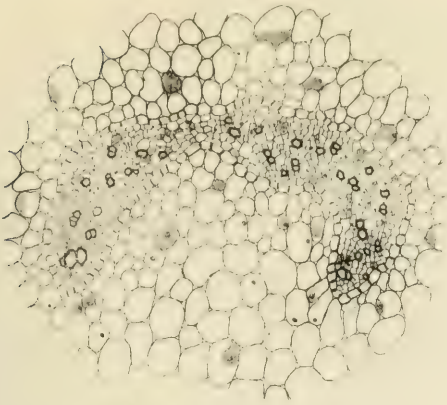

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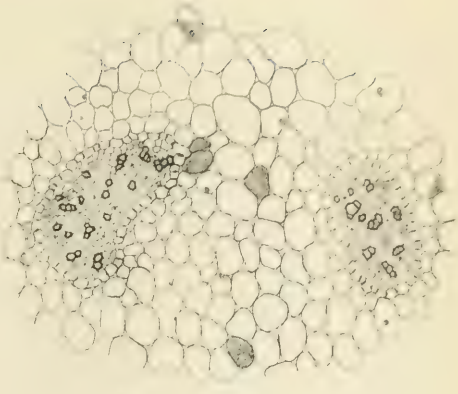

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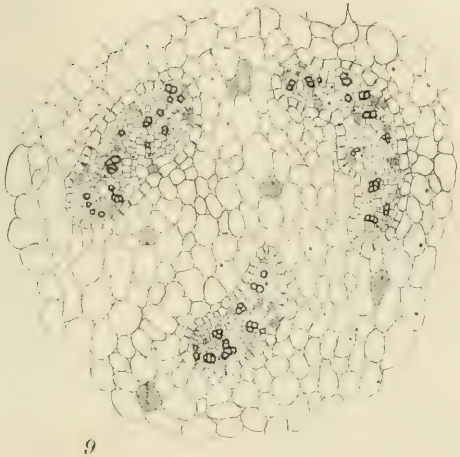

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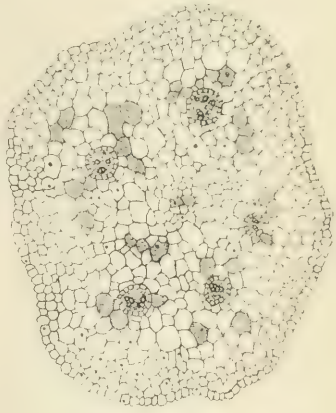

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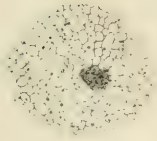

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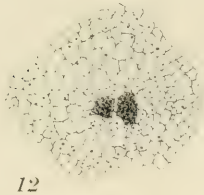

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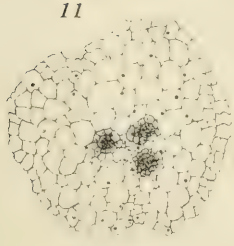

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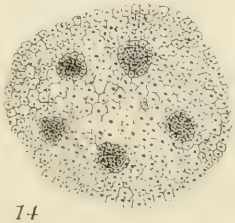

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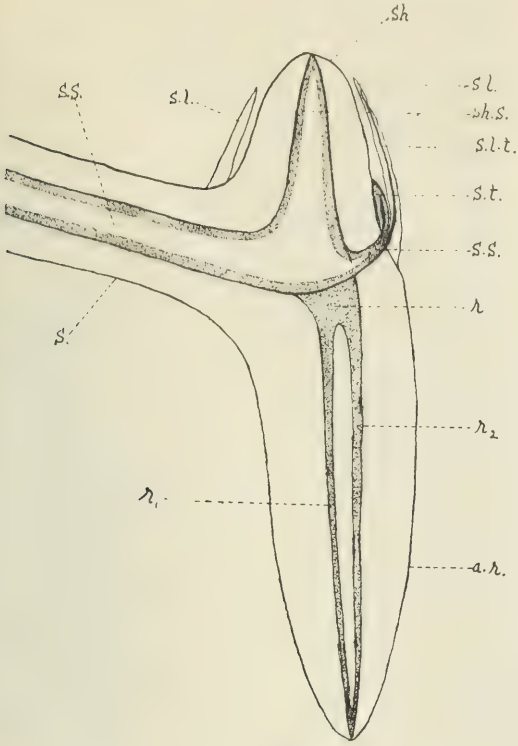

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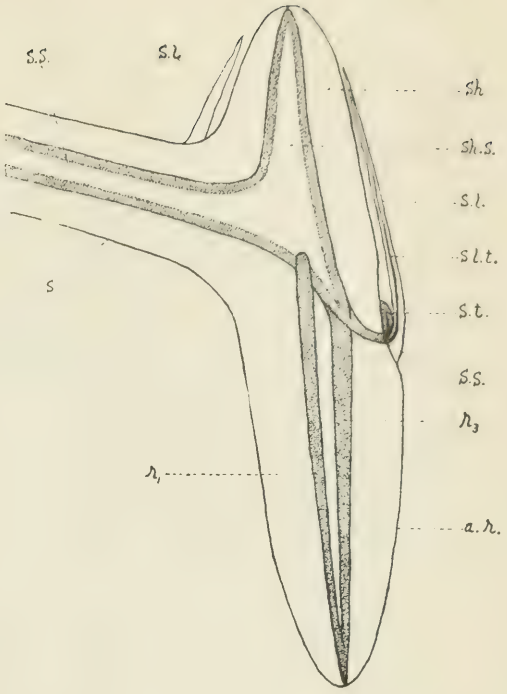

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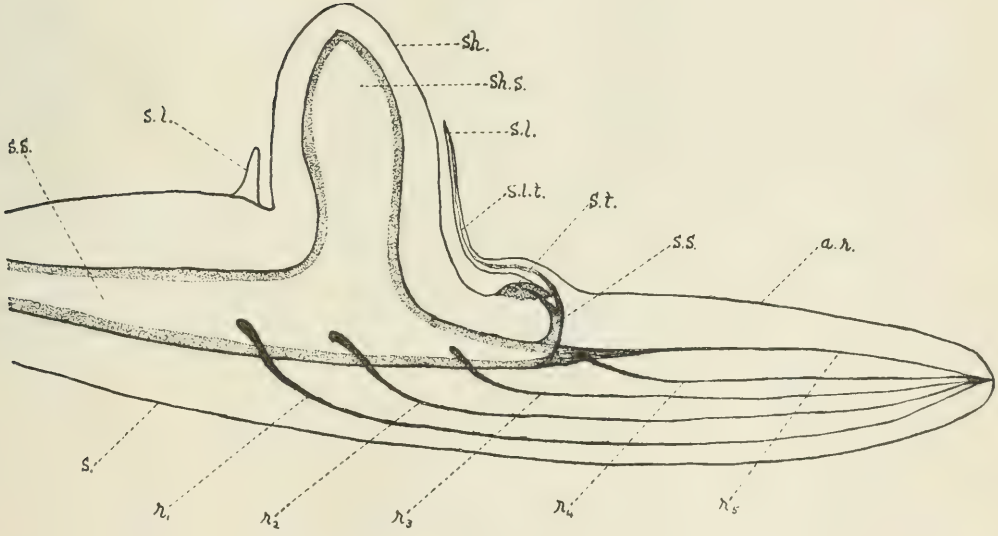

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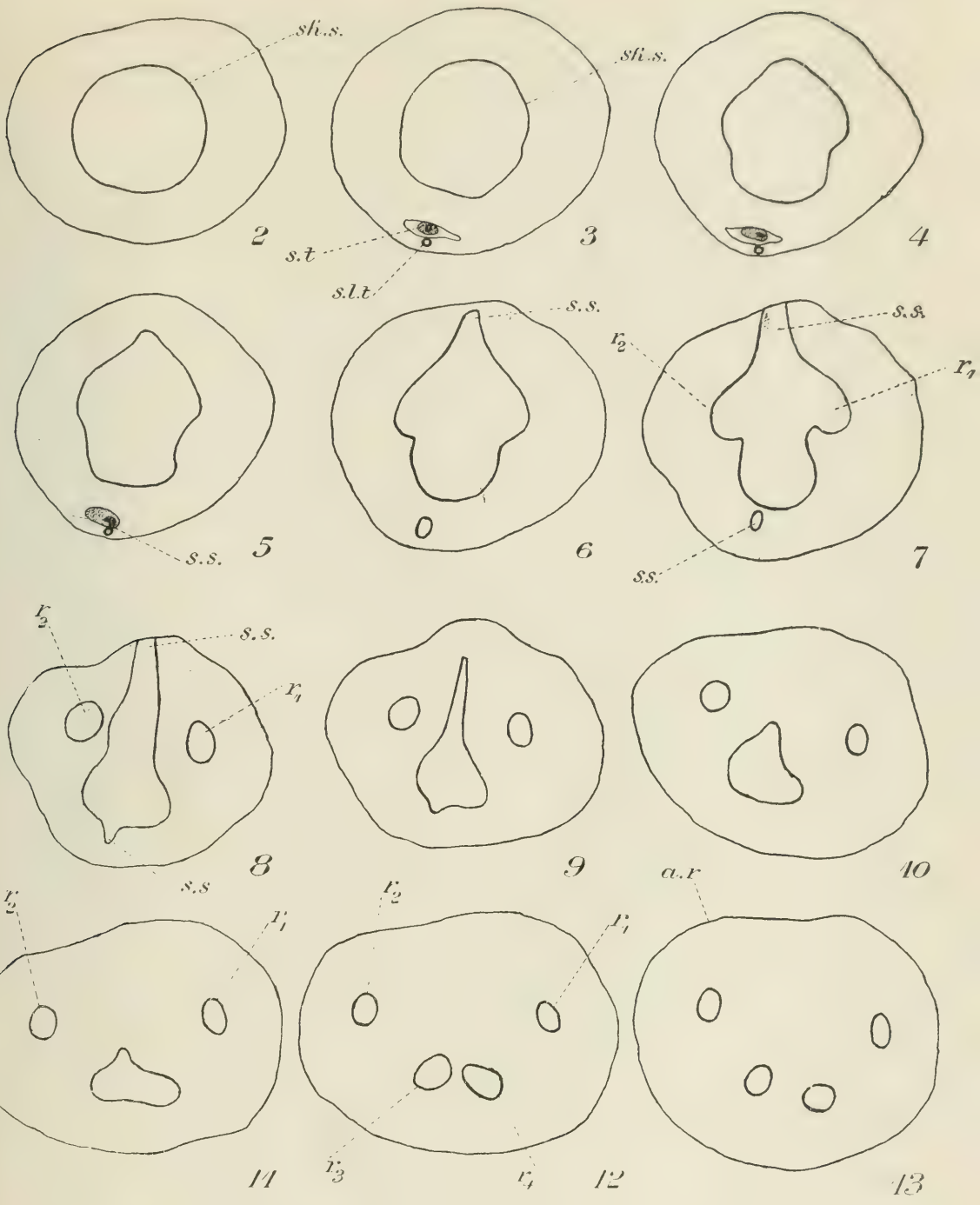

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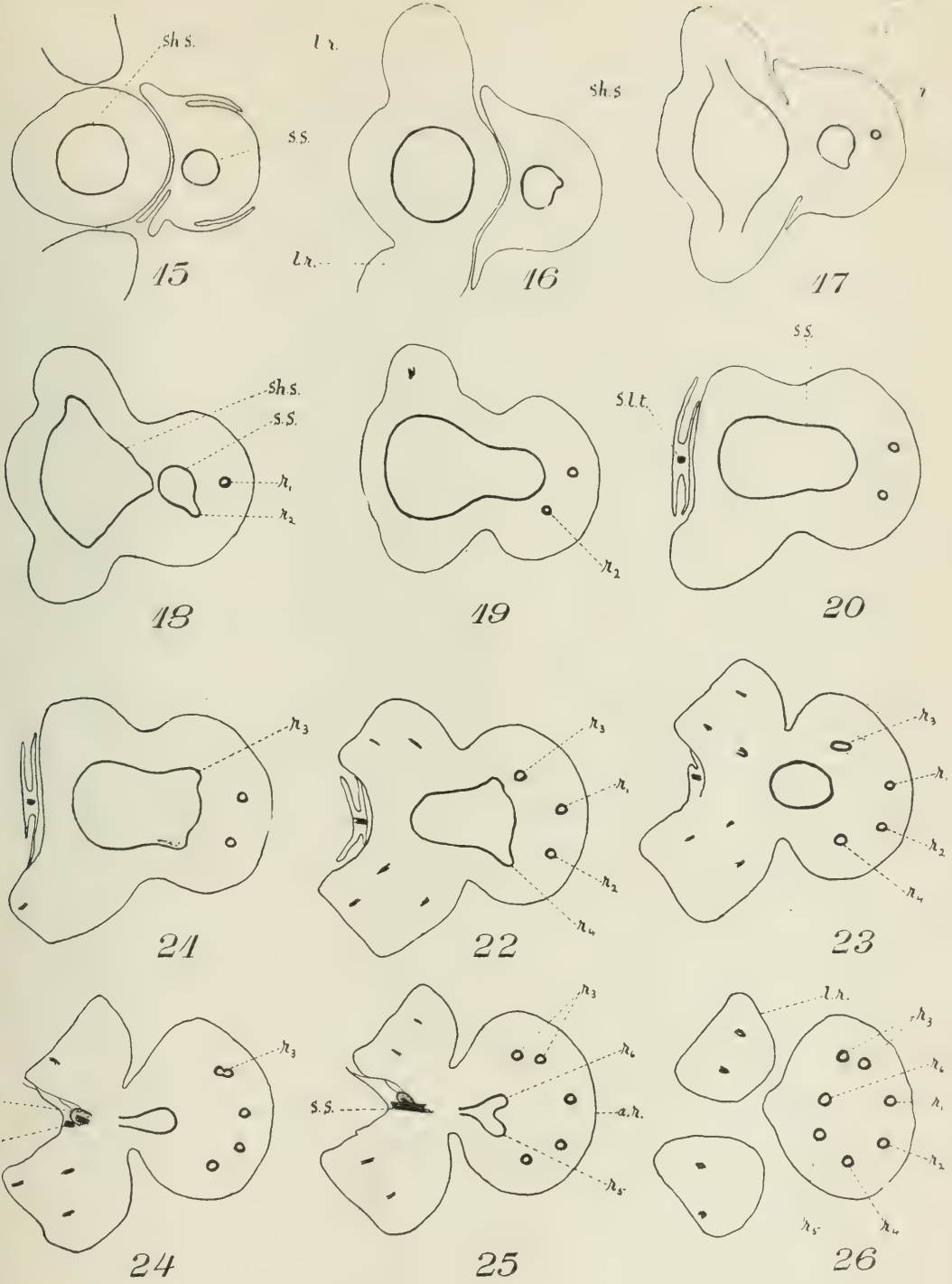

DIAGRAMS 15–26.

UNIVERSITY OF TORONTO
STUDIES

BIOLOGICAL SERIES

No. 7: AN EARLY ANADIDYMUS OF THE CHICK

BY R. RAMSAY WRIGHT

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III.—*An Early Anadidymus of the Chick.*

By Professor Ramsay Wright,

Biological Department, University of Toronto.

Read May 23rd, 1906.

The embryo which is described in the following pages was prepared and sectioned in June, 1905, for class purposes but its abnormality did not attract attention until it was brought into the laboratory. I am, therefore, unable to figure the surface view, and so far have not had leisure to model out its most interesting features.

The series contains 200 sections of 15 microns in thickness, corresponding to a length of 3 mm. in the hardened condition. The egg had been in the incubator for 24 hours, but, 10 somites having been observed, it was marked as practically equivalent in age to Duval's embryo of 29 hours (No. 1, Fig. 89 and Pl. XVI).

It was noted that the incubator was running at a temperature somewhat higher than the normal, which may account not only for its more rapid development, but also for its abnormality, as may be inferred from Dareste (No. 2, page 121).

Hertwig (No. 3:—Vol. I, p. 993) and others have remarked on the rarity of cases of Anadidymus in Sauropsida in comparison with the Ichthyopsida. This case is of particular interest, because, unlike Hoffmann's (No. 4, page 40) there appears to be no indication of a double primitive streak, and, therefore, it is to be placed in the same category with Dareste's embryo (No. 2, Plate 16, Figs. 5 and 6), and possibly that of Mitrophanow (whose paper I have not been able to consult) cited by Kaestner (No. 5, page 88). The occurrence of such a case does not, in my opinion, invalidate the argument of Kaestner that all such cases are primitively double (No. 6, page 141), because it depends entirely upon the degree, locality and method of the interference of the two components, whether an organ shall appear double or single. My figure of section 131 (Fig. 13) would not be suspected to come from an embryo otherwise than normal, while the inspection of section 126 (Fig. 12) at once shows that each half of it in reality belongs to a different embryo. From this point, the interference caudad has been more complete than cephalad, so that in the backward growth of the primitive streak region (cf. Hertwig, No. 2, pp. 895 and 896) the embryo appears to be single.

Attention must be called to the contrast in the method of interference in the head-region of my embryo and that in Kaestner's (No. 6, Taf. VII) where the ventral surfaces have interfered more than the dorsal, the result being a single heart and a double brain, instead of a double heart and a single brain (cf. my figure 9). The plane of interference becomes caudad more and more truly sagittal, so that the chordæ, at first widely divergent (Fig. 10), eventually fuse, (Fig. 13).

I now proceed to the description of the various systems of organs.

Nervous System.

As a starting-point, I select section 12 (Fig. 5) through the region of the optic vesicles. It is easy to understand how the condition here pictured is arrived at if we proceed from the normal state as seen in Duval's Figs. 253 and 254. The two embryos have been inclined with their dorsal surfaces towards each other, and have interfered in such a way that the right and left lips of the neural groove of the one, have fused with the right and left lips of that of the other. In this way, no room is left for the complete development of the "median" optic vesicles which, consequently, are very minute (ov'). The points of fusion are still noticeable and it is obvious that that of the left and right lips of the right and left components respectively (which now form the floor of the composite neural canal), is less complete, in such a way that some mesoderm cells have intruded into the neural canal at this point. The double character of the neural canal is brought strongly out by the two infundibula which diverge laterally towards the two blind foregut ends (ph.) beneath which the slightly thickened patches of ectoderm already indicate the hypophyses.

It is less easy to interpret the preceding sections (Figs. 1 to 4), but if two components such as are represented in Duval's Fig. 252 have interfered in such a way as materially to reduce in size the contiguous halves, then it becomes apparent that the convex floor of the composite neural canal in figure 4 is formed of the left and right brain-halves of the right and left components which have fused in the region of their dorsal neural sutures, while their ventral sutures are still widely separated. Still further forward (Fig. 3) these brain-halves are fused so that the most anterior end of the neural canal (Figs 1 and 2) is formed of the lateral brain-halves only of the two components. It is noticeable that the separation of the brain from the ectoderm has apparently taken place sooner than is normal (No. 3, Vol. 2, page 252).

In the diencephalic region (Fig. 6) the brain is much compressed from side to side, but it soon widens out into the mid-brain (Fig. 7).

In the trigeminal region of the hind-brain the neural canal is open for some thirteen sections, but before the auditory region is reached it is again closed as far as section 84, near which point (Fig. 11) there is again a failure to close for a few sections; thereafter, however, the canal is closed as far as section 126, Fig. 12, behind which point the groove is, at first narrowly, and then widely, open.

In section 160 (Fig. 16) the fusion of the ventral wall of the neural groove and the notochord begins and is continued in the following sections (Figs. 17-20), the complete fusion of the ectoderm, chorda, mesoderm and entoderm being attained at the 175th section (Fig. 20). Beyond this point we can hardly speak of a neural groove; the 181st section (Fig. 21), indeed, shows an unsymmetrical fissure which is not uncommon in the primitive groove of normal embryos, and by section 190 all traces of the primitive streak have disappeared and the germinal area presents a normal appearance (Fig. 23). The comparison of my Figures 15-22 with those of Hertwig (1 c., Figs. 536-545, page 891) shows that there is little difference except in the less amount of closure of the neural canal, and without an inspection of sections further forward, it would be impossible to detect any symptom of "duplicitas."

Notochord.

The conduct of the two notochords has already been sufficiently referred to in the hinder region; it only remains to call attention to their gradual increase in size from their first appearance in section 9 (immediately behind figure 5) till their fusion in section 131, also to their gradual convergence to this point.

Mesoderm.

As already remarked there are ten somites, and this is the case with the "median" series of fused somites which lie exactly in the same plane as the lateral ones. Of the "median" series, the seven posterior are better demarcated than those further forward, and are sometimes notched on their ventral surface. The rudiments of the Wolffian body may be seen in the region represented in Figs. 12 and 13.

Vascular System.

A convenient starting-point for the description of the vascular system is the region depicted in Fig. 10 (section 67), where the vitelline veins are perfectly normal, and the only thing that arrests attention is the "median" descending aorta. Fig. 9 shows that the vitelline veins have not become fused into a single heart as in a normal

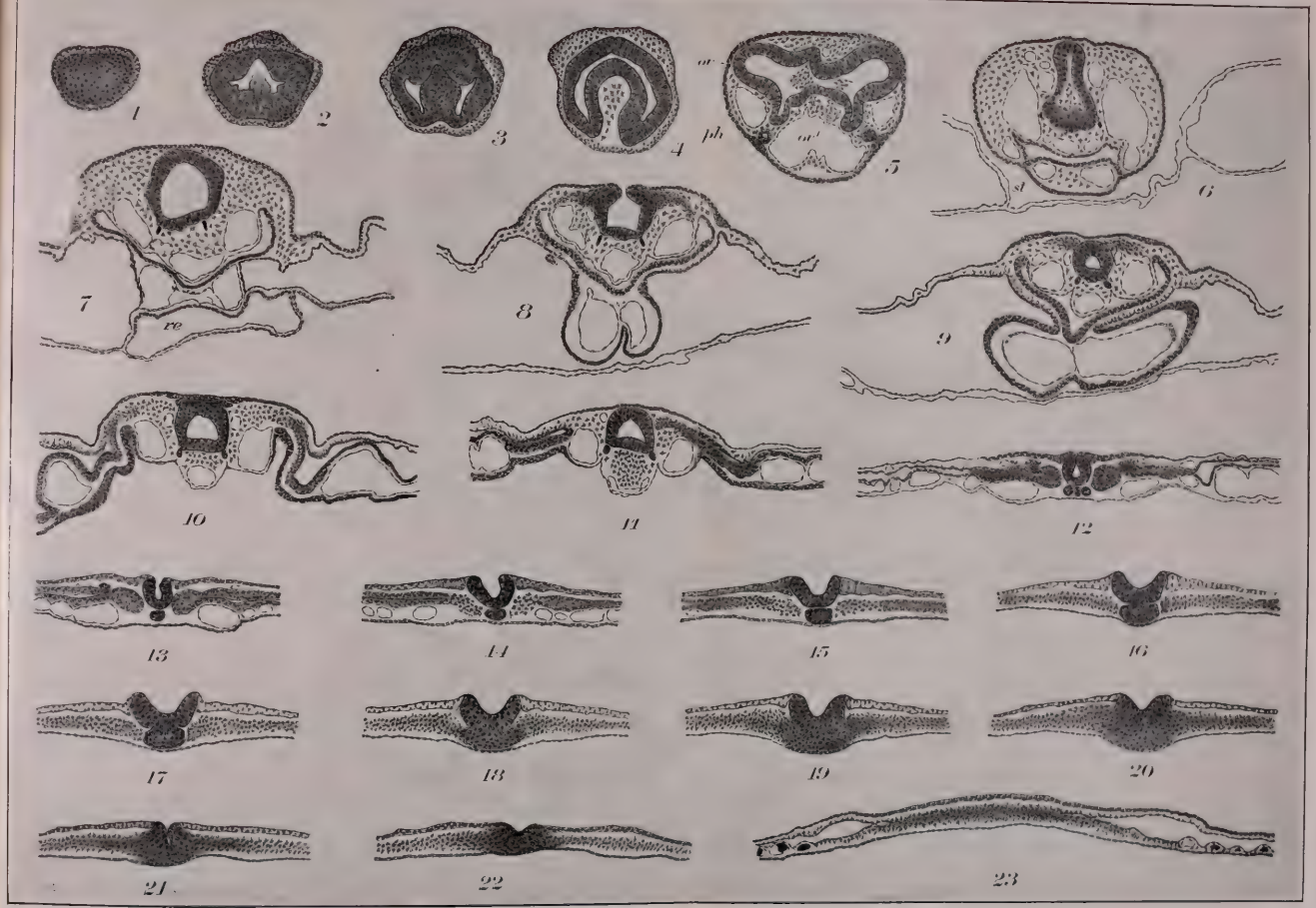
embryo. Their endothelial tubes remain independent throughout, but the splanchnic mesoderm [1] does not at first dip in very far dorsad so as to furnish an independent wall for each heart. Further forward, however, it does so (Fig. 8), and eventually the two bulbs of the heart are widely separated and enclose between them a portion of the common cœlome (Fig. 7). But the two heart-tubes as seen in Fig. 9 do not contract gradually into the condition seen in Fig. 8; on the contrary, there is a marked constriction at the opening of each heart into its bulbus, beyond which a ventricular *cul-de-sac* extends cephalad for a few sections on each side.

The picture presented by Fig. 6 is best calculated to show the anterior duplicity of the vascular system, because when each bulbus approaches the stomatodæum it divides into two ventral aortæ. Of these the lateral aortæ alone form arches up the sides of the pharynx, for the median ones first anastomose below the pharynx, then subdivide into four small vessels which bend round its anterior surface, and finally open into the large vascular space represented in Fig. 5, situated between its anterior diverticula. Tracing this space backwards dorsad of the composite pharynx, we first find four vessels similar to those referred to above, which soon, however, fuse into the "median" dorsal aorta. This retains its size until we reach the segmented region of the embryo, in which it tends to be obliterated opposite the somites and to expand again intersomitically. The "lateral" dorsal aortæ conduct themselves as in a normal embryo, and the same may be said of the veins as far as they are developed.

Entodermic Tract.

Proceeding cephalad from Fig. 11 in which the median ridge formed of the median row of somites alone distinguishes this from the entoderm of a normal embryo we find nothing remarkable until about midway between Figs. 8 and 9, there the lateral pouches of the pharynx reach a little nearer the ectoderm in the region of the first gill-clefts, but a few sections further forward (Figs. 6 and 5) the two stamatodæa at once arrest attention, as do the two anterior diverticula corresponding to the pouches of Seesel of normal embryos.

I venture to enter a mild protest against Professor Kaestner's note (No. 6, p. 128) on the usage of the words somatopleure and spanchnopleure. Surely, if it is desirable to have mononyms for "somatic mesoblast," and "splanchnic mesoblast," it would be easy enough to form them instead of using terms which were invented and are constantly used to designate something else. If the language of anatomists knows only one meaning for *πλευρα'* that of zoologists is not so restricted. A Pleuronectid does not swim on its "pleura!"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In conclusion, in spite of the apparent posterior simplicity of this embryo I am of the opinion that it can best be explained by assuming a double gastrulation at points very close to each other on the surface of the embryonic area.

Literature Cited.

I have thought it unnecessary to cite all the papers consulted. Hertwig (No. 3) and Kaestner (No. 6) give a full list of papers to some of which, unfortunately, I have not had access.

No. 1. Duval—Atlas d'Embryologie.
No. 2. Dareste—Production des Monstruosités.
No. 3. Hertwig—Handbuch der Entwickelungslehre.
No. 4. Hoffmann—Arch. mikr. Anat. XLI.
No. 5. Kaestner, Arch. Anat. Phys., '98.
No. 6. Kaestner, Arch. Anat. Phys., '02.

EXPLANATIONS OF THE FIGURES ON PLATE.

The sections were projected and carefully outlined on the drawing paper by means of the Zeiss Epidiascope and 20 mm. micro-planar, at such distances as to give an enlargement of 102 for figures 1 to 9, and 116 for figures 10 to 23.

Subsequently, the drawings, which were made by Mr. J. R. G. Murray, student in biology, University of Toronto, were reduced rather more than one-third, so that the magnification is respectively 63 and 72.

Figs. 1-4,— Nos. 4, 5, 6, and 8, of the series, through the forebrain.

Fig. 5,— No. 12, through the anterior blind ends—ph.— of the pharynx. Ov. and ov' the right and left optic vesicles of the right component.

Fig. 6,— No. 19, through the stomatodæa of both components and the diencephalic region; round the composite pharynx are grouped eight arteries; two ventral, and two dorsal aortæ on each side.

Fig. 7.—No. 33, through the mesencephalon. Ventrad of the pharynx are the two aortic bulbs; dorsad, the median dorsal aortæ have united into a single vessel; *re*, ectodermic recess under the head.

Figs. 8, 9, and 10.—Nos. 47, 55, and 67, respectively, through the fifth, seventh and eighth, and ninth nerves.

Fig. 11,— No. 80, through the second intersomite. The median dorsal aortæ have given place to a mass of mesoderm.

Fig. 12,— No. 126, behind the last somite. The chordæ are gaining in size, and the mesodermic mass diminishing. The rudiment of the Wolffian body is seen in this and in Fig. 13.

Fig. 13,— No. 131, the chordæ have fused.

Figs. 14 and 15,— Nos. 150 and 154, the chorda and the wall of the neural groove gain in size.

Fig. 16,— No. 160, the beginning of the fusion between the floor of the neural groove and chorda.

Figs. 17, 18, 19 and 20,— Nos. 164, 168, 171 and 175, respectively, show the progressive fusion of the neural wall, chorda, mesoderm and entoderm.

Figs. 21 and 22,— Nos. 181 and 186, are through the hinder end of the primitive streak. The former shows traces of an oblique fissure.

Fig. 23,— No. 196, shows the nature of the mesoderm behind the primitive streak.

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No. 8: THE HABITS AND LARVAL STATE OF PLETHODON CINEREUS ERYTHRONOTUS BY W. H. PIERSOL

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THE HABITS AND LARVAL STATE OF *PLETHODON CINEREUS ERYTHRONOTUS.*

By W. H. Piersol, B.A., M.B.

THE HABITS AND LARVAL STATE OF *PLETHODON CINEREUS ERYTHRONOTUS.*

By W. H. Piersol, B.A., M.B.

Although *Plethodon cinereus* is considered by Cope ('89) "the most abundant salamander in the northern and central United States" its habits and development have received but little attention. Cope ('89) gives a brief outline and Wilder ('94) a scant mention, Montgomery ('01) adds a few points and describes the larvæ of one bunch of eggs. Reed ('08) discusses the coloration of adults. Kingsbury ('95) touches on the questions of the transference of sperm and the season of egg-laying but without coming to any definite conclusions. Sherwood ('95) gives a date on which eggs were found. As regards *Plethodon oregonensis* the behavior in captivity of a female found with her eggs is described by van Denbrugh ('98), and Hubbard ('03) deals with some protective devices. There are several other papers in which Plethodon is mentioned but without reference to habits or development. The observations recorded below have been made partly in the field, partly in the laboratory, and on specimens from several localities, all however within a radius of fifteen miles from Toronto. Unless recorded as single occurrences, all observations have been verified in at least one subsequent season. Cope ('89) divides *P. cinereus* into three sub-species of which *P. cin. cinereus* and *P. cin. erythronotus* only are common. For the sake of ensuring uniformity the latter variety alone forms the subject of this paper. Many larvæ of *P. cin. cin.* have also been found and comparisons made with similar stages of *P. cin. eryth.* show that in the larva as in the adult the sole distinction between the two varieties is the coloration. The difference in geographical distribution which Cope mentions is not invariable, one bit of woodland may yield the two varieties in about equal numbers, another quite similar but a few miles away may contain *P. cin. eryth.* in abundance and but few *P. cin. cin.* No locality yielding either variety alone, or a majority of *P. cin. cin.* has been met with.

The typical coloration of the two sub-species is closely adhered to, the intermediates noted by Reed ('08) are very rare and even then approach closely the types; and only one specimen with much more than the normal amount of red was found. About 250 adults have been under examination, a number not so great as that used by Reed but sufficient to show that in

this region there is less variation in colour than in the one where his studies were made.

Plethodon cinereus may be found in almost any wood in which the underbrush has not been entirely cleared away. They seek shelter under logs, fallen branches, flakes of bark separating from old stumps, or even masses of decaying leaves; the cracks of rotting logs and stumps are also favorite places. In the latter situation the variety *erythronotus* receives some protection from the red in its coloration, the shade of red is often that of the decaying wood and the dark edging is a fair reproduction of the dark and narrow cracks that run through the log. Occasional specimens may be found in the latter part of April; during the next three weeks they become abundant and by the middle of May can be found as plentifully as at any time in the year. Just where they pass the winter has not been determined though search has been made both early in the spring and late in the autumn; digging quite through stumps and logs embedded in the soil will not expose them, though it will probably result in finding the wood-frog, *Rana sylvatica*. A few trifling things have suggested that the animals follow down the cracks in the roots of old stumps to a considerable depth, but this has not been verified. Farther south Montgomery ('01) finds them in the same situations both winter and summer.

Plethodon is strictly nocturnal, so far at least as regards life above ground; however, a specimen that is suddenly uncovered is by no means dazzled by daylight; it may remain motionless for a minute or two or may at once crawl beneath the nearest shelter. Nor does it stop when under cover but keeping out of sight crawls rapidly to a considerable distance, so that if not captured as soon as exposed it runs a very good chance of escaping entirely. This sensitiveness to light has rendered disappointing the results from keeping specimens in a terrarium, an amount of light sufficient to reveal them at all either arrests all movement or sends them under shelter.

An examination of the stomach contents shows the food to consist of a variety of small insects; in one case the remains of a small spider were found.

In handling living specimens it will frequently happen that the end of the tail will apply itself closely to a finger in a semi-circular loop, and hold thus for a few seconds. This power may perhaps be regarded as the first step in the development of a prehensile tail such as is described for Autodax (Ritter & Miller, '99).

Two habits of Plethodon deserve notice as being of rare occurrence among Urodeles. When excited it occasionally aids its progress by leaping. In such cases as have been observed under conditions that admitted measuring the length of the leap it has been found about equal to the length of the animal's body. If the animal is running on a rather even surface it lands on its feet and continues the run having gained by its leap; but it will just as readily leap into difficulties. Held in the open hand it will frequently leap off, no matter what may be the height of the hand above the ground. In jumping the back is slightly arched and the front limbs with most of the trunk are raised in the air to about the height of one centimetre; then with a snap the tail is slapped against the surface over which the animal is moving and the body sharply straightens and shoots forward. The whole movement is so rapid that it cannot be distinguished with certainty whether the limbs aid in the leap. Two things suggest that they do; it is difficult to imagine a force to raise the anterior part of the body to the height it attains if it does not chiefly lie in a spring given by the anterior limbs. The posterior limbs are even stouter and are in a good position to aid in the forward propulsion. The young as well as adults possess this power of leaping, indeed the only specimen observed to give a succession of leaps, three in fact, was one of 24 mm. The explanation of the greater development of the posterior limbs in the later larval stages, noted by Montgomery ('01), may lie in this habit. In this connection Cope ('89), says: "It frequently climbs to the summit of low vegetation, from which it springs by a sudden straightening or curvature of the body, as the case may be, in the manner of a caterpillar." The curvature and straightening in leaping are evident; the climbing of low vegetation to leap from it has not come under observation.

The second noteworthy habit is also connected with escaping enemies. Plethodon will sometimes break off a portion of its tail. Two things suggested the existence of this habit before it was actually observed. In searching for Plethodons in decaying logs not infrequently as the covering is lifted the animal will be found crawling stealthily away from the bit of tail that is making itself very conspicuous by its violent movements. This will occur at times when so little force has been used in picking apart the log that it is difficult to conceive how the piece could have been broken or pinched off. Again about 10% of all specimens found show the end of the tail in the process of regeneration, raising the suspicion that it is a mutilation out of the ordinary. On one occasion when a young Plethodon had been repeatedly touched by a small rod it suddenly gave a jump breaking off at the same time the terminal third of its tail. Two

adults while being held lightly by the tail cast off a portion of it, the separation occurring in the part grasped and leaving in the one case a stump 1 cm. and in the other 1.75 cm. long, measuring from anus to end of stump. In no case observed has the length of tail retained been less than .5 cm.; it was usually more. There does not seem to be any one place where separation greatly tends to occur. Incomplete separation has also been noted, the vertebrae and muscles separating but the skin failing to break; this causes a slight grooving of the tail as though an invisible thread were tied around, compressing it. Longitudinal sectioning was used to examine both proximal and distal pieces in those cases that occurred under observation, and the relation of parts in the wound was found to be as follows, (Figure 1.) Separation of the vertebral column occurs between vertebrae; this is the condition also in *P. oregonensis* (Hubbard, '03). In the muscle the myomeres are not broken but separation occurs in the myocomma opposite the middle of the last vertebra retained. The skin however does not break here but at the myocomma opposite the middle of the first vertebrae of the piece cast off. Two things are accomplished by this; first the wound that now terminates the animal's tail is protected by the extra length of skin which collapses laterally upon it and almost covers it in. Second, on the piece cast off considerable raw surface is left exposed, the irritation from which is doubtless largely responsible for the rapid contortions that occur. This piece must play an important part in the protective device for by its violent movements it would draw the attention and invite the first attack of an enemy.

A similar habit has been noted in *P. oregonensis* by Hubbard ('03) but there are distinct points of difference. Briefly in *P. oregonensis* on moderate stimulation the glands on the dorsum of the tail swell greatly and pour out an abundant secretion. Only the most powerful stimulation—the act of being swallowed by a snake, or being plunged into a fixing fluid without previously being anæsthetized—would rouse the animal to the point of sacrificing its tail. Separation always occurs at a constriction just behind the anus. No mention is made of the behaviour of the piece separated and, the subject of the paper being correlated protective devices, it is perhaps fair to assume that it presents no striking peculiarity. In *P. cin. eryth.* the tail shows neither constriction nor swelling nor does the great development of dorsal glands occur, the thickness of the dorsal skin being one-twelfth to one-eighth of the diameter of the tail, while it constitutes one-fourth of the swollen tail of *P. oregonensis*.

Owing to the extreme aversion to light already mentioned it has been impossible to ascertain in a terrarium the manner of fertilization of the

eggs. That fertilization is internal, and that it precedes egg-laying by a considerable time may be inferred from the following. The female is provided with spermathecæ which are already filled with spermatozoa at a time when the eggs are yet in the ovaries and have not attained a greater diameter than two mm. Quite likely the spermathecæ are filled long before this because at this time no spermatozoa are to be found in the genital tract of the male. The testes are filled with spermatocytes which as Mongomery ('03) describes undergo their maturation during the summer; spermatozoa may be found after the middle of August. Further observations are necessary to determine whether transference occurs in autumn or in spring.

Although there is a total lack of secondary sexual characters it is usually possible to distinguish between the sexes owing to the thinness and translucency of the muscle and skin. Because of the dark colour of the testis and the light colour of the eggs the posterior part of the abdomen when viewed from beneath is dark or light according as the specimen is male or female. This distinction is most evident in the spring, the eggs being largest at that time but in most cases it can be made at any season. In addition, the cloacal glands of the male cause a whiteness and a swelling at the sides of the body just behind the posterior limbs.

There is considerable latitude for a Urodele in the time at which egg-laying occurs. The earliest date on which eggs have been found was June 16th—3 clusters, the eggs in early stages of segmentation; the latest is July 3rd—1 cluster, in the same condition. The difference is not due entirely to different seasons being early or late, nor to one bit of woodland being better sheltered and warmer than another, for clusters of eggs with the females on guard and other females with the season's eggs yet in the ovaries have been found in the same log on the same day. The date, Oct. 25th, given by Sherwood ('95) for the finding of eggs of Plethodon is not accompanied by any description of them ; and its exceptional lateness leaves the meaning of his observation doubtful.

The eggs of the one individual seem to be deposited all at the one time. No female was found guarding a cluster of eggs having also any in the oviduct or even in the ovary that were nearly full size. It is however not at all uncommon to find an egg in one ovary about half-size; this should be associated with the small egg of some bunches described below and considered as one that should have been laid this season but which, having failed to develop its proper amount of yolk, is retained in the ovary.

As a situation for the eggs marked preference is shown for logs almost entirely embedded in the humus, logs that have decayed to a point where the substance is friable with large cracks running lengthwise. In one

of these cracks the eggs are hung from the roof like a little bunch of grapes (Figures 2 and 3). Rarely a similar cavity within a decaying stump is selected. All the localities in which Plethodon has been found contain conifers and almost without exception it is in coniferous wood that the eggs have been found; though the adults may be found plentifully in wood of all kinds. Usually the eggs are placed from three to five inches beneath the surface of the log, at which depth its substance is constantly moist, and it can well be imagined that the air is saturated with water vapour. They are always accompanied by the female and if in exposing them she has not been alarmed she will be found holding the dorsal surface of her head and neck against the under side of the bunch. That it is the female that remains on guard was determined by the dissection of over twenty specimens. In only one case was a male found by eggs and then in company with a female; the eggs were several days advanced in development and the presence of the male was perhaps merely an accident. Cope ('89) and Mongomery ('01) speak of finding the animals and their eggs under stones but if the above mentioned shelters are available they are always preferred. Wilder ('94) notes that the adults are seldom to be found under stones. Sherwood ('95) gives the habitat as beneath logs and stones, while the eggs are to be found in damp moss and beneath the bark of decaying stumps.

Among Urodeles that do not lay their eggs in water contact between the body of the female and the eggs seems to occur in all cases; Amphiuma (Hay '88) and Autodax (Ritter and Miller '99, and Ritter '03) coil round them, Desmognathus (Wilder '99) inserts herself among the eggs wearing them like a necklace or belt. *Plethodon oregonensis* (van Denbrugh '98) is described as holding the bunch in a loop of her tail and moving them from place to place. But as this was after removal from their natural surroundings it is quite possible that the eggs had been torn from their original support and that part of the mother's uneasiness arose from their unattached condition. The Cæcilians (Gadow '01) also when not viviparous coil round their eggs.

The number of eggs in a cluster varies from three to twelve. The number of ovarian eggs in advanced condition, in specimens taken just before the egg-laying season is also within these limits and the length of the incubation precludes a second brood in the one year. Consequently the preservation of the species must depend upon the larval stages being so perfectly adjusted to surrounding conditions that the mortality is very small, rather than—as is the case with most Urodeles—upon the production of large numbers of young. The slender and almost cylin-

drical form of body (the proportion of length to greatest breadth being fourteen to one) in an animal so small, along with the necessity for producing eggs containing a large amount of yolk are doubtless factors determining the small number of eggs; another will be mentioned later.

The necessity for the large amount of yolk in the egg arises from the purely terrestrial development of the larva. Aquatic larvæ have at command the minute and abundant fresh-water plankton as food supply and are thus at an early age rendered independent of the nourishment provided in the yolk. The insect life that constitutes the early food of the terrestrial Plethodon is of larger size than much of the plankton and much less abundant. Consequently the animal on leaving the egg must be able to wait for food through comparatively long intervals and also to capture food of larger size than an aquatic larva need do. Both of these things demand an advanced development that can only occur when a considerable quantity of yolk is provided.

Each egg is surrounded by a series of mucous spheres as is customary in Urodeles. In their natural condition the number of these is rather difficult to determine but after soaking a few minutes in water they swell somewhat and the following is plainly seen:—an innermost sphere very close to the surface of the egg; a second enclosing this but separated from it by a greater interval than that between the innermost sphere and the egg; occasionally this sphere is represented by two, one of them fitted very closely around the other. The outermost sphere—usually the third—fuses with the outermost spheres of neighboring eggs at all points of contact. On its surface are threads and bands of a milky white mucus which seems tougher than the rest, which is transparent; these are especially numerous between eggs and at the upper part of the bunch where several uniting form the stalk by which the cluster is suspended. This mode of attachment is probably derived from one originally like that of Desmognathus (Wilder '99) in which each egg of the cluster is independent of all the rest and has its own cord joining it to the common stalk. The tension of the envelopes, especially the innermost, is needed to preserve the spherical shape of the egg. These envelopes are very tough; a weak hypochlorite solution will soften them so that they can be removed but when their support is gone the egg, even in water, flattens until the vertical diameter is little more than half the transverse. In the younger stages it is therefore necessary to fix and harden the egg first and remove the membranes later, they should be removed as soon as possible for if left around the egg the latter will in time disintegrate. The method devised by Morgan ('91) has proved the most satisfactory.

As development proceeds the amount of fluid between the egg and

the innermost sphere increases markedly, thus relieving the growing embryo from pressure.

The eggs vary from 3.5 to 4.5 mm. in diameter. In many of those bunches which contain several eggs there is one egg considerably smaller than the others; with this exception there is great uniformity in the size of the eggs of the one cluster. The considerable variation noted above exists between the eggs of different clusters.

The colour of the egg is a very pale yellow, a tint due to the yolk for no pigment is present. The upper part of the egg being the more free from yolk is almost white. Variation exists in the tint of the eggs of different clusters, the range being between very pale cream and light orange-yellow.

The usual rotation of amphibian embryos in their earlier stages occurs also in Plethodon but is modified by the large amount of yolk present. In the usual type of amphibian embryo, such as Amblystoma or Rana, the amount of yolk is not so great as to render it impossible for the embryo to turn over when the course along which the ciliary movement urges it so demands. Until the Plethodon embryo has reached a length of 6 mm. it lies along a meridian of the yolk mass and a true forward movement would require the yolk at times to be uppermost. Whether because the ciliary movement lacks force to drive it into this position or for some other reason less obvious the rotation that occurs is around the vertical axis. There is no uniformity of movement even in eggs of the same bunch; an occasional one will be motionless and the others about evenly divided between motion to right and motion to left. A little later when the embryo twists itself into the horizontal plane the appearance in rotation is that of the ordinary amphibian embryo but the same lack of uniformity of direction obtains, some few embryos will not be moving at all, of the rest the head will be preceding in some cases but the tail in quite as many others. This latter direction was not expected and caused an examination of Amblystoma eggs to be made subsequently to determine if anything comparable was to be found there. Eycleshymer ('95) notes an early irregularity of direction but this is not the impression derived from a brief examination of larvæ in later stages; nor is it to be expected from the account of the ciliation of Rana and Triton larvæ given by Assheton ('96). The stage of developement of Amblystoma equivalent to the one of Plethodon in which the backward movement is so marked is that in which the gills are beginning to appear and the larva is curved laterally in a semicircle to accommodate itself to its spherical envelope. At this stage nearly all embryos examined will be

found moving slowly forward very nearly in the horizontal plane of the body; some few will be motionless and about 1% will be moving backward. It is not difficult to mark these latter by thrusting small splinters of wood through the jelly until their points almost touch the sphere surrounding the larva; a few hours later these individuals will be found moving forward and another set moving backward. Reversal of direction has also been noted in some Plethodon larvæ. Evidently the condition in Plethodon is nothing peculiar to the genus but an exaggeration of one found elsewhere among Urodeles. How widely spread it may be and whether it means a reversing of the usual movements of the cilia are points left for future determination.

The rate of rotation varies greatly, extremes of one minute and three and one half minutes having been noted under similar conditions.

The service of the rotation to the embryo probably is that it prevents adhesion between the egg and the envelopes. Smith ('06) found this occurring in eggs of Cryptobranchus lying in dishes in the laboratory; interference with development resulted. He makes no mention of rotation in the eggs but suggests that gentle rocking due to the current of the stream in which the eggs are laid prevents adhesion under natural conditions. Apparently in eggs laid in waters that have no current—as Amblystoma or Rana—or in other equally quiet situation—as Plethodon—the rotation suffices to prevent adhesions until the embryo beginning muscular movements is past all danger of their occurrence. Besides rotation other movements in embryos of 6 to 7 mm. are performed at intervals and consist of elevating the head from the yolk and waving it from side to side. As development proceeds these movements pass into occasional wrigglings of the tightly coiled larva whereby the whole position within the envelope often becomes altered.

Development and growth are very rapid for a few days, an embryo of 5 mm. will increase in length to 9 mm. in six days; but at this point the processes become much slower and to increase from 9 mm. to 11 mm. requires fourteen days. This change in the rate of growth is shown very plainly in sections by a sudden diminution in the number of mitotic figures which from being very numerous in the smaller embryos become rare after the length of 9 mm. is passed. In its later stages development is retarded in the central eggs of a cluster where the embryos are smaller and show but little reduction of the gills while the outer ones are larger and have the gills almost entirely absorbed (compare Figures 13 and 14). Measurements of a typical case gave central embryos 17.5 mm. with gills 1.75 mm. long and outer embryos 20 mm. with gills reduced to

.25 mm. A similar condition is found in Amblystoma, Rana and other amphibia where the eggs are laid in clusters; the cause being the difficulty with which respiration is carried on in the central eggs.

Escape from the mucous envelope occurs when the larva has reached the length of 20 to 25 mm.; this is about the first week of September. The outer larvæ of a cluster escape first but remain with the mother beneath their less developed brethren. Respiration is now more perfect in these; in a few days their gills have dissappeared and they also escape. However the interval does not suffice to bring them so far on in development as were the outer ones when they escaped; they have absorbed less yolk and are shorter. They also seem unfit to come in competition with their more favored brethren of the outside of the bunch of eggs, their movements being comparatively feeble and ineffective; many of them probably die. Thus is supplied another factor tending toward the production of small numbers of large eggs; for those young will be best fitted for life that come from broods where all the nourishment available as yolk is divided among but few eggs, so few that none are crowded into the centre of the cluster.

The family does not scatter at once, an unusual thing among Urodeles that have attained their adult form, but accompanied by the mother, perhaps led by her, the brood leaves the interior of the log to live beneath stones, fragments of wood or bark, lying on the surface of the ground, or even among layers of mouldering leaves. If not disturbed when uncovered the young will be found in contact with the body of the mother, probably to obtain moisture; for the localities in which they now are contain little moisture at this season of the year. The families do not hold together for long. In the latter part of September a few solitary young will be found and early in October all the broods seem to have broken up. The rate of growth in Plethodon must vary enormously in different individuals, for at this season it is easy to collect a series beginning with young accompanying the mother and ending with full grown specimens, the increase in size being so gradual that it is impossible to draw with certainty a line between this year's and last year's broods. Apparently the ability to withstand the winter is independent of size for early in May an occasional specimen will be found that is little larger than the young at the time they escape from the egg—that is less than 30 mm. in length.

The following description of the external appearance during development is based upon the examination of over 170 embryos that had been fixed in Zenker's fluid and preserved in 80% alcohol, the members of each

bunch being kept together and separate from other bunches. In the series twenty different stages of development are represented, though only the most important of them will be described. As the eggs vary somewhat in size so do the individuals of the same stage of development; the descriptions given are those of average specimens, extremes in any direction (very few in number) being disregarded.

The embryo may be considered as well defined upon the yolk when the medullary folds are complete and approximated through a little more than the posterior half of their extent. (Figure 4). Together they give this part of the embryo a width of .75 mm. except at the posterior end where they separate slightly before becoming continuous giving here a width of 1 mm. In their anterior part the folds are widely separated giving to that part of the head an extreme breadth of 1.5 mm. In front of this part an almost transverse piece connects them while behind it, they converge, meeting at a point a little anterior to the middle of the body. In living specimens placed in a strong light a delicate scalloping of the inner margins of the folds in the cranial region indicates clearly the neuromeres, but no method yet used has succeeded in preserving them through fixation. In a corresponding stage in the eggs of some Amphibia the areas of brain substance that will form the optic vesicles are indicated by depressions, sometimes pigmented (Eycleshymer '93), but in Plethodon no trace of these can be found. The length of the figure defined by the medullary folds is three millimetres.

Larva 5 mm. Figure 5.

The form of the body is still determined by the yolk and the central nervous system. The medullary folds are in apposition throughout. The head is raised off the yolk as far back as the midbrain; eyes and ears are indicated externally. Behind the head the mesoblast makes a border on each side of the spinal cord extending but a short distance laterally over the yolk. The anterior three-fifths of it is divided into ten somites opposite the third and fourth of which it is thickened forming the beginning of the anterior limb. The posterior two-fifths of the mesoblast is not yet divided into somites. The medullary folds flatten and a little in front of the well marked blastopore disappear.

Larva 5.5 mm. Figure 6.

The pharyngeal region is now distinct and shows four gill arches; sections, however, show that the gill slits are not yet perforate. Posteriorly a crescent shaped furrow, the horns pointing forward, defines the termination of the body and marks its tendency to rise off the yolk.

Though thus hidden from view by the beginning of the tail, sections show the blastopore opening into the bottom of this furrow. At this time the increase of fluid around the egg begins. The whole body is assuming a cylindrical form and standing out from the yolk which is slightly flattened near it, and markedly flattened in the region against which lies that part of the body from the anterior limbs forward.

Larva 6 mm. Figure 7.

During the growth from 5.5 mm. a twisting of the anterior part of the body through 90 degrees has occurred so that one side of the head is now turned toward the yolk; of eleven specimens this was the right side in nine, the left side in two. That portion of the body attached to the yolk, *i.e.*, all posterior to the pharyngeal region, shares in the twisting and becomes curved laterally upon the yolk in such a fashion that the anterior three-fourths of the dorsal surface is brought into the one plane. Opposite and external to the last three somites now distinguishable is, on each side of the body, a low mound of thickened tissue—the beginnings of the posterior limbs. The terminal millimetre of the tail is quite cylindrical and free from the yolk. The first traces of pigmentation now appear on the dorsal surface of the anterior part of the body.

Larva 9 mm. Figure 8.

The anterior part of the body has more than doubled in thickness and has grown so far off the yolk that the anterior limbs are now free from contact with it. This gradual freeing of the body from its attachment to the yolk is brought about by growth of the central part of the body crowding off the extremities, accompanied by but little pinching off of the connection between body and yolk; for the length of body attached to the yolk has remained, and will for some time yet remain, constant at 3.5 to 4 mm. As the body is thus projected forward those parts of it that were spread out laterally on the yolk move ventrally and unite in the middle line. This is well shown in the anterior limbs which from projecting dorsally as at first are compelled by this movement at last to point outwardly. They are now .35 mm. long. Similar growth backward has brought the posterior limbs to the verge of the attachment of body and yolk posteriorly. These limbs are yet represented by rounded thickenings only. All traces of the gill arches have disappeared from the surface while the external gills have appeared as three points .25 mm. in length. The costal grooves are visible and pigment in a broad band extends over the dorsal surface of the neck and anterior half of the trunk.

Larva 9.5 mm. Figure 9.

The eye is now pigmented. The mouth is well defined and the gular fold projects a little over the base of the gills; these now appear as three points on a common base. The anterior limbs have a length of .5 mm. the posterior of .25 mm.; the latter are now attached on the line between yolk and body, consequently the anus lies in the free part of the body posterior to the yolk.

The accumulation of fluid between the egg and the innermost envelope has increased the diameter of the egg and envelopes together from 4.5 or 5.5 to 6.5 or 7 mm. This is not due to a thickening of the walls of the spheres but to such a distension of the inner one that yolk and larva together occupy only the lower two-thirds of the cavity. No further enlargement of the cavity occurs, consequently the larva becomes more and more closely coiled as its length increases, for the diminution of yolk that soon becomes noticeable does not compensate for the increased bulk of the larva.

The rotation ascribed to cilia on the ectoderm has been noticed in some larvæ of this stage of development, but in none later.

Although the blood is not yet red a hand lens will reveal the following plan in the circulation over the yolk mass, the vessels appearing as colourless lines against the white background of the yolk; the direction of flow can be followed as the corpuscles are easily visible. Small arteries metamerically arranged run laterally over the yolk; they branch and anastomose in such fashion that a network two or three meshes in width is formed between the margin of the body above and the vein in which they terminate ventrally. (Figure 9). Usually there are two such veins at first, a right and a left; at a variable time in later development one disappears, as is usual in Urodeles. Sometimes the anterior part of the network on one or both sides collects into a separate vein, making for a time three or four separate trunks that unite just posterior to the heart. Sections show these vessels all lying, as might be expected, in the splanchnic mesoblast which has ere this completely surrounded the yolk.

Larva 10.5 mm.

At about this length sections show for the first time perforation of the gill pouches, two only—the first and second—ever become perforate. The anterior limbs show the first indications of digits in that their extremities are flattened and show a terminal notch and a shallow groove leading to it on each side. The posterior limb is not appreciably flattened.

Larva 11 mm.

Each division of the anterior limb above noted is showing two tubercles, the four digits being thus indicated. The posterior limb has exchanged its rounded contour for an angular one but no notches or grooves appear on it.

Larva 12 mm. Figures 10 and 11.

The digits of the posterior limbs appear as five tubercles, the second and the third of which are the most prominent.

Larva 16 mm. Figure 12.

The posterior limbs are growing the more rapidly; they are now as long as the anterior ones—2.5 mm.—and much stouter. Both pairs are provided with well developed toes. The gills have developed greatly each being two to three mm. long and consisting of a central stem with six or eight side branches. Considerable variation in the length of the gills is found in larvæ from different broods in what is otherwise the same stage of development, the shorter gills having also the shorter side branches. Some diminution of the yolk mass is for the first time noticeable, its vertical depth having lessened, though the attachment to the body is still along 3.5 mm. of the ventral surface. Sections show that in larvae from 9 mm. onwards the body walls are actually complete, but the more ventral part of the mesoblast is so much thinner than the dorsal and the line along which the two parts meet is so sharp as to produce in the entire larva the appearance of having body walls developed only over the upper part of the yolk mass. At this stage rather more than the dorsal half of the yolk is covered by this thickened part of the body wall. The pigmentation typical of the adult is complete.

Larva 17.5 mm.

The posterior limb has outgrown the anterior, the lengths being 3 mm. and 2.75 mm. respectively. The length of the gills is reduced to 1.75 mm. or less. The yolk mass has so diminished as to be almost covered in by the thicker parts of the body wall, only a portion 2.5 mm. long and .5 mm. broad projecting slightly in the mid-ventral line.

Larva 20 mm. to 25 mm. Figure 13.
(just on the point of escaping from the mucous envelopes.)

The anterior and posterior limbs are 3 mm. and 3.5 mm. long respectively. The yolk mass is almost entirely covered in. The gills are

reduced to a few small points not over .25 mm. long. Sections of several larvæ of this general stage but differing slightly in development show that both the gill slits become closed before escape from the envelopes occurs, the second one being the first to close.

In their development the digits of the posterior limb but partly bear out the expectations of Cope ('89). He would regard Hemidactylium with its posterior foot possessing only the first four digits as having for permanent form that which is larval in Plethodon. Dealing with *P. cinereus* he finds the adult has the outer digit longer than the inner, but in younger specimens it is shorter and in his youngest (18 mm. only but having already lost its gills) it is but a minute tubercle, "and in a little earlier stage cannot but be wanting though this I have not seen." On this point fifty-one larvæ were examined covering twelve stages, beginning with larvæ of 11 mm. long and ending with the smallest found living alone, its length being 23 mm. In larvæ of 12 mm. the whole five digits appear at once and the fifth is no less prominent than the first. There follows a brief period—*viz.* until the larva attains a length of 16 mm.—in which the rate of growth of the fifth digit as compared with that of the first, varies; of twenty-nine specimens within these limits the first exceeded the fifth in sixteen, the fifth exceeded the first in three, and in ten they were equal. (Seven broods are represented in this, in four of them only were all the larvæ alike on this point). This period alone is in accord with the argument of Cope. In all larvæ over 16 mm. (thirteen in number representing four stages) the external toe was longer than the internal.

Effects of Terrestrial Development.

The influence of a purely terrestrial development is seen chiefly in the following points.

The large amount of yolk in the egg in proportion to the size of the animal, a point already dealt with.

The yolk mass retains its globular form until late in development (larvæ of 13 to 15 mm.), when the absorption of its substance causes it to become fusiform. In aquatic larvæ the mass early elongates to produce a slender body capable of rapid darting movements, a necessity not laid upon the yolk in the inactive larva of Plethodon.

The development of limbs occurs early. Traces of the anterior limbs are distinct in larvæ of 5 mm. and of the posterior limbs in those of 6 mm. In Amblystoma these traces appear in larvæ of 7.5 mm. and 13 mm. respectively. From the large size of the posterior limbs

in his larvæ Montgomery ('01) was led to suggest the possibility of their development before the anterior limbs, contrary to the usual condition in Urodeles. This proves not to be the case, though they do appear earlier than usual and grow more rapidly.

The development of gills is retarded. No trace of them as separate points is found until the larva reaches a length of 8 or 9 mm.; in Amblystoma the same occurs at a length of 6 mm. Looking at limbs and gills together the contrasts are marked; when Plethodon first shows external gills its posterior limbs have been for some time distinct; when Amblystoma first shows posterior limbs its gills are 1.25 to 1.5 mm. long and plentifully branched.

It is of general occurrence that the gills of such amphibian larvæ as have no free aquatic life are proportionately longer than those of larvæ that do. The condition in Salamandra atra as described by Chauvin ('77), shorter and stouter gills being assumed when aquatic life was forced on the larva, is typical. This obtains also in Plethodon, the filaments as well as the main trunks of the gills being much longer but much less numerous than in aquatic larvæ of the same size, *e.g.* those of Amblystoma. Such marked reduction in the number of filaments does not occur among all Urodele larvæ of non-aquatic development. In aquatic larvæ the gills are largely directed backward to afford as little resistance as possible to passage through the water; in Plethodon they spread out as widely as possible, the direction being a matter of indifference. The point of importance is that they apply themselves to as much of the mucous envelope as possible and so place themselves where they can best obtain a supply of oxygen.

Plethodon has neither the balancers nor the adhesive discs common among other amphibian larvæ.

The body of a Plethodon larva is from the first that of a terrestrial animal, cylindrical and without trace of median fins. Like the larva of Autodax (Ritter and Miller '99) it has lost the swimming instinct; when placed in water it sinks to the bottom and falls on one side; often indeed it twists its body and writhes violently but such movements never result in any progression or even in temporarily regaining balance. They seem to be only the wriggling that occurs periodically within the egg envelopes; it can always be induced by any stimulation of the larva.

The complete darkness in which the development of Plethodon takes place is partly responsible for peculiarities in colouring. Pigment is entirely lacking in the egg nor does it appear in the larva until a length

of about 6 mm. is reached. When pigmentation does begin it rapidly assumes the pattern and colours of the adult; this is evidently related to the fact that when the larva issues from the egg envelopes it at once assumes the habits and habitat of the adult. The lack of a free larval life differing from that of the adult renders unnecessary the distinctive larval colouration so common among Amphibia. At the same time the surrounding darkness renders it safe for the larva to receive the colouration of the adult. The reason why the larva of Autodax (Ritter and Miller '09; Ritter '03) should under somewhat similar circumstances show a darker colour than the adult may lie partly at least in the fact that its development occurs in dimly lighted cavities and not in absolute darkness as does that of Plethodon.

Amblystoma (or Spelerpes), Desmognathus, Plethodon, and Autodax form an interesting series the members of which, taking larval and adult life together, show an increasing adaptation to terrestrial life. The first is terrestrial only in adult life and returns to the water to lay its eggs. Desmognathus (Wilder '99) begins its development on land like Plethodon but near the water; it leaves the egg while yet a larva completing its development in the water and accordingly stands as an intermediate between Amblystoma and Plethodon. The habitat of the adult Desmognathus, never far from a stream, also shows a less degree of adaptation to terrestrial life than that attained by Plethodon whose habitat bears no relation to bodies of water. On the other hand Autodax is less dependent on moisture in its surroundings even than Plethodon. I have had adult Plethodons die in confinement from conditions of no greater dryness than those described as supported by Autodax (Ritter and Miller '99). Moreover the prehensile tail, the greater ability in leaping and more intelligent use of the power would also indicate more perfect adaptation to terrestrial conditions than has been attained by Plethodon. As larva, Autodax has entirely lost the fringed condition of the gills; Plethodon still retains these fringes but in such varying degrees in different individuals as to indicate their decadence as organs. The degree of parental care of eggs and larvæ increases regularly in the series Amblystoma, Desmognathus, Plethodon and Autodax.

Experiments.

To determine what part the mother may play in the incubation various plans of development under artificial conditions were tried. If removed from their natural surroundings and suspended in air the eggs show signs of rapid loss of moisture and the larvæ soon die. To obtain an atmosphere as moist as that in which they naturally hang, clusters

of different ages were suspended over water, each cluster in a wide-mouthed bottle the cork of which had two shallow grooves cut down the sides to admit small quantities of air. The bottles were kept in a cellar where the temperature was practically the same as that of the natural situation of the eggs. In all the clusters development proceeded without interruption, in some cases for as much as twenty-five days when an impending absence from the laboratory rendered it necessary that the experiments be terminated and the larvæ were fixed. About eight per cent. of the larvæ always die under this artificial incubation, in some bunches no deaths at all occurring, in others several. This is quite striking, for no unfertilized eggs or dead larvæ were ever found under natural conditions. Three things may account for this; injury in conveying to the laboratory, the growth of mould during incubation, and lack of the normal increase of fluid between the egg and the inner envelope. Mould on the eggs although never encountered under natural conditions sooner or later makes its appearance on all eggs reared as above described. It does not however always have an early fatal effect, for all the larvæ in a bunch have been found alive after having been quite obscured for two days by a growth of mould. The increase of fluid between egg and envelope can hardly be said to occur under these artificial conditions and presumably some pressure is exerted upon the larva. These things suggest that the female may in some way prevent the growth of mould on the eggs and also supply them with moisture and an endeavour was made to test this in the following way. In a wide-mouthed jar pieces of the log in which the eggs were found were arranged to form a little chamber in which the female was placed and the piece suspending the eggs then added as a roof; more fragments were placed upon this and the surface covered with a little humus and moss, a few drops of water were occasionally sprinkled on the surface. Jars so prepared were kept under the same conditions as the bottles previously mentioned for three weeks, by which time mould would certainly have appeared on eggs kept in bottles, but none was found, and the amount of fluid surrounding the larva was as great as natural. In a subsequent set of experiments it was found that even thoroughly wetting the cluster two or three times a day, in addition to keeping it over water as before, would not suffice to bring about the normal accumulation of fluid. This was only obtained by allowing the lower end of the cluster to remain in contact with the surface of the water in the bottle for about twelve hours out of each twenty-four.

No final statement should be based on such scanty experimental evidence but such weight as it has is entirely in support of the supposition that the mother in this case—and presumably in similar cases reported

among Urodeles—does along the lines mentioned actively promote the welfare of her brood.

In those last mentioned and in other experiments in which the clusters of eggs were entirely immersed in running water it was noticeable that the mucous envelopes would imbibe readily a small quantity of water and swell slightly in consequence, but the limit to this process was usually reached within the first half hour, nor did any second period of imbibition occur. No softening of the envelopes was to be noted even after constant immersion for three weeks. These qualities of the mucus mark it as different from that of most amphibian spawn which continues to absorb water and to soften until the larvæ escape. They probably are to be considered adaptations to prevent rains of even most unusual duration from softening the mucus and turning loose the larvæ prematurely.

The bottle method before described was used to test the possible effect of light upon development. Several bunches were exposed to daylight, which however never became direct sunlight, while others of the same stage of development were kept in entire darkness as controls, the temperature being in each case, within limits of variation of three or four degrees, the same. At the end of twenty-five days no difference in the degree of development of the two lots was to be detected. The lack of pigment in the egg might be put forward but the whole explanation cannot rest on this for early in the life of the larva pigment appears in the skin and rapidly increases in amount; and in the experiment this pigment was present for the last twenty days. In the experiments of various investigators to determine the effect of light upon growth Amphibian larvæ in aquaria have frequently been used; in which case the oxygen and part of the food have been derived from the water. Since both these things would be more abundant in the better lighted aquaria, factors must be allowed for, whose exact influence is unknown. In the present case the food and oxygen factors are the same for both sets of larvæ and the uncertainty of result due to several factors varying simultaneously is lacking.

Points Arising Out of the Lungless Condition.

In Amphibia generally the function of the skin as an organ of respiration accessory to the lungs has long been recognized. Later Maurer ('98) drew attention to the advantageous position for purposes of respiration held by the capillaries of the bucco-pharynx, many of them being situated in the epithelium itself. To these localities therefore the attention of investigators was naturally directed in seeking the means which

would compensate for the lack of lungs. The conclusions reached were not uniform, some investigators accepting both skin and bucco-pharynx with more or less of the œsophagus as sharing with something like equal importance in the respiration, others concluding that the skin is no more efficient in respiration in lungless salamanders than in those with lungs. Some examinations of Plethodon along these lines was in progress but ceased when the paper of Seelye ('06) on the Circulatory System of Desmognathus came to hand for the points already dealt with showed that the conditions in Plethodon would be but a repetition of those in Desmognathus: and would lead to the same conclusion, namely that as an organ of respiration the skin is much more important in lungless than in lunged salamanders. The same paper also gives a sufficient review of the question and its literature so all that will be attempted here is to bring forward three additional pieces of evidence in support of the above conclusion.

First, as noted in the paper itself the value of the cutaneous capillary network for respiration will depend upon the permeability of the membrane through which diffusion must take place. The fact that this membrane is the epidermis and not the entire skin renders exact experimentation impossible. Nevertheless the experiments performed by Seelye indicate that the entire skin of lungless forms is much more permeable than that of those with lungs and it would be strange to urge that the difference in the cutis accounts for this for it is the lungless forms that have the thicker cutis. This point of structure is, according to Seelye, the only one of general distinction between the skins of the two types in question; a conclusion that it is hard to understand unless it is due to the presence of two European forms with lungs among those examined. A more trustworthy comparison would be one between forms that live in the same environment and in the case of Plethodon this is possible, for small specimens of *Amblystoma punctatum* and of *Diemyctylus viridescens* in its terrestrial stage of life are occasionally found along with *Plethodon cinereus*. The skins of specimens so found and of adult Plethodons of about the same length were examined all being submitted to the same procedure, *viz.* the entire animal was fixed in Zenker's fluid with the usual after treatment, then from similar regions of head, trunk, and tail, pieces from dorsal, ventral, and lateral aspects were sectioned perpendicularly to the surface. The only considerable and constant difference in the epidermis is one of thickness. To estimate this correctly several measurements in micra were made from each piece of skin and these were averaged. Finally the figures thus obtained for each of the areas investigated were averaged to obtain a figure that would fairly

represent the average thickness of the epidermis over the whole body. These averages were 22.4 micra for Plethodon, 46 micra for Amblystoma, and 44 micra for Diemyctylus. Consequently as regards this factor unless we assume a most improbable thing, namely that the epidermis of Plethodon is of a material less permeable than that of Amblystoma and Diemyctylus, we must conclude that of the three the skin of the lungless form Plethodon is fitted to be by far the best respiratory organ.

Second. The bucco-pharyngeal respiration is indeed established very soon after escape from the egg yet even in the adult it may be suspended for a considerable time without serious inconvenience. When confined in a glass vessel the animal will occasionally rest the ventral surface of head, neck, and more or less of the trunk against the glass; the adhesion between the glass and the moist and sticky skin is sufficient to prevent the respiratory movements. I have frequently seen this continue for two or three hours and have found the position of the body apparently unchanged after even much longer intervals, but in these cases observation not being continuous it is possible that the animal may have moved for a time and then resumed its exact original position, but such a thing is unlikely.

Third. There is a period of a few days in the life of a Plethodon during which such respiration as occurs at all must take place through the skin. This is the period just prior to the escape from the egg envelopes. The gills attain their maximum of development—a length of 3 mm.—in larvæ of about 15 mm.; after this they decrease in size and for some time before the escape of the larva are reduced to small points not over .25 mm. in length. No movements of the ventral pharyngeal wall are to be observed at this time so it cannot be that there exists a mode of respiration similar to the aquatic pharyngeal respiration noted in *Diemyctylus*, both *viridescens* (Gage '01) and *torosus* (Ritter '97), the fluid within the egg envelope playing for Plethodon the part of the surrounding water for Diemyctylus. Consequently whatever oxygen is used by the larva must be absorbed through the skin. The amount of oxygen required by the larva may well be less than that required by the free-living animal yet it is by no means an inconsiderable fraction of it. The muscular activity in the beating of the heart and the wriggling of the larva within the envelopes that occurs not infrequently is probably little less than that of the free-living animal, which though capable of active movement rarely indulges in it, forming in this respect a marked contrast to Diemyctylus. If the larva can carry on its respiration through its skin alone, hampered as it is by the surrounding fluid and envelopes,

it is difficult to escape the conclusion that in the free-living stage as well the skin must be an important factor in respiration.

The development of gills in the first place is governed by the same necessity as exists in other Amphibia; sections show that the beginning of their degeneration coincides with the skin becoming sufficiently developed for the circulation in it to become somewhat extensive; and that their diminution keeps pace with the elaboration of the cutaneous circulation, just as in most Amphibia it accompanies the increasing activity of internal gills or lungs.

EARLY DEVELOPMENT AND DEVELOPMENT OF INTERNAL ORGANS.

Studies in these fields have revealed several points of interest which it is proposed to consider in a future paper.

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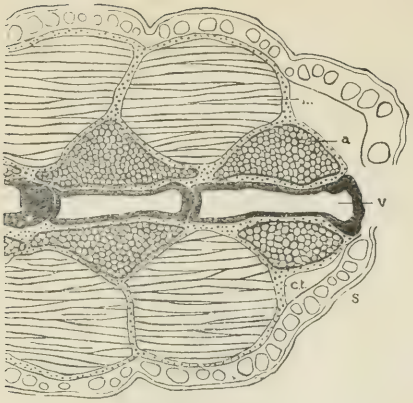
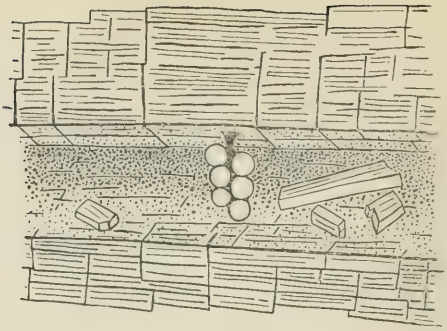


Fig 1



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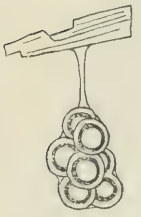

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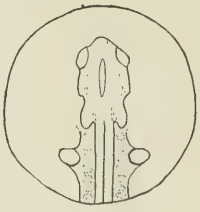

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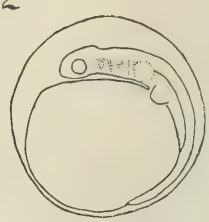

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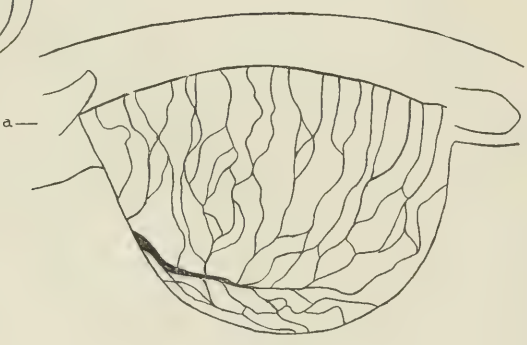

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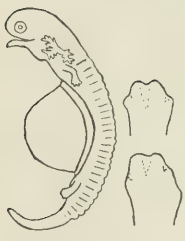

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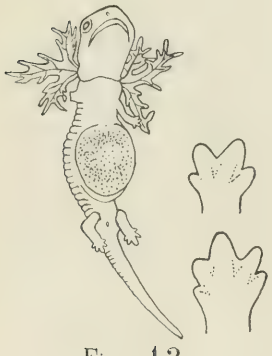

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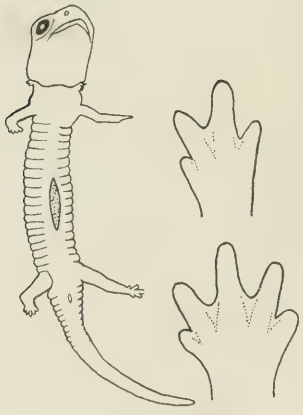

Fig. 13

Fig. 14

ILLUSTRATIONS.

Figures 1 and 9 are camera lucida drawings, 2 and 3 from photographs, 4-8 and 10-14 from projections by Zeiss epidiascope. Figures 4-8 are enlarged to the same extent, which is twice as great as that of figures 10-14. Figures 4 and 5 from eggs of maximum size, 6 and 7 from eggs of minimum size. The outlines of the feet, Figs. 10, 12 and 13, are camera lucida drawings; they are right feet viewed from the dorsal surface under low magnification.

Figure 1. Horizontal section of tail through the proximal wound of an autotomy.
v—centrum of vertebra.
a—perivertebral adipose tissue.
m—myotome.
c. t.—connective tissue.
s—skin.

Figure 2. Cluster of eggs suspended in a large crack in a decaying log.

Figure 3. Cluster of eggs.

Figure 4 Stage 3 mm. Egg viewed from side, the dotted line being the equator of the egg when the latter is in its natural position.

Figure 5. Stage 5 mm Egg viewed from above.

Figure 6. Stage 5.5 mm. Egg viewed from the side. The outline of the mucous sphere was added from a living specimen.

Figure 7 Stage 6 mm Egg viewed from above.

Figure 8. Stage 9 mm. Viewed from above, the head and tip of the tail are much bent towards the ventral surface.

Figure 9. Vitelline circulation in larva 10 mm. long.
a—anterior

Figures 10 and 11. Stage 12 mm.

Figure 12 Stage 16 mm. Taken from a small larva, actual size, 14.5 mm.

Figure 13. Stage 20-25 mm Actual size of larva 20 mm.

Figure 14 Specimen from the centre of the same bunch of eggs as that of Figure 13, which is one of the outer larvæ

UNIVERSITY OF TORONTO STUDIES

BIOLOGICAL SERIES

No. 9: SPAWN AND LARVA OF AMBYSTOMA JEFFERSONIANUM, BY W. H. PIERSOL

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SPAWN AND LARVA OF AMBYSTOMA JEFFERSONIANUM

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Spawn

Among the various accounts of the habits and spawn of *Ambystoma punctatum* occasional mention may be found of *Ambystoma jeffersonianum* but always in such connections as to suggest that *A. jeffersonianum* is by far the less common species in the locality. This along with the considerable similarity existing between the spawn of the two species may explain why no account of the spawn of *A. jeffersonianum* has as yet appeared. Descriptions of the spawn of *A. tigrinum* sufficient for distinguishing it from that of the other two species is given by B. G. Smith (1907).

In most localities near Toronto *A. punctatum* is a much more common species than *A. jeffersonianum,* however in one piece of woodland that is quite isolated from all the others examined, the former species is rarely to be found, while the latter is very abundant. This woodland contains four pools that last throughout the year, although they become heavily choked by vegetation during the late summer and autumn. The value of these pools as a collecting ground for spawn, *Branchippus,* etc., was discovered some years ago by my colleague, Dr. Huntsman and his observations on the *Ambystoma* spawn suggested to him the possibility of distinguishing in it two kinds. Later the writer also became familiar with this woodland in connection with observations on *Plethodon* and with the consent of Dr. Huntsman undertook also the investigation of the *Ambystoma* spawn of the pools.

The writer first visited these pools in spawning time

three years ago and found a small amount of spawn of a type already familiar to him for some years from its abundance in pools in other localities. But the greater amount was of a type that differed from this in the points detailed below. These two types have proved to be the *punctatum* type and the *jeffersonianum* type, respectively. The predominance of the latter subsequently found its explanation in the fact that 31 of the 33 individuals captured in the woodland since then have been of the latter species. It is impossible to determine accurately the proportions in which the two types of spawn occur, but estimating roughly, the *jeffersonianum* type is at least ten times as abundant as the other.

As will appear below, a small percentage of the eggs of *A. punctatum* will approach in size, or color, or mode of deposition—but rarely in more than one of these points at a time—the eggs of *A. jeffersonianum*. Consequently, the separation of the latter as a type when found in a pool where the *punctatum* spawn greatly predominates, is not an obvious thing. But when the proportions are reversed, as in the special pools mentioned, the distinction is most easily made. Observations in the field have agreed in all four seasons and have been supplemented by the capture of females just previous to egg-laying and comparison of mature ovarian eggs and eggs laid by them in the laboratory, with those obtained in the pools; and finally by the rearing in the laboratory of larvæ from the two types of spawn.

The points of difference in order of constancy are as follows:

1. *Size.*—The eggs of *A. jeffersonianum* are distinctly the smaller, the usual diameter being 2–2.25 mm.

2. *Color.*—The eggs of *A. jeffersonianum* are much the darker, the pigment being but little removed from a true black and covering a much larger proportion of the surface of the egg than in *A. punctatum;* even the lower surface is usually as dark as the upper surface of many of the eggs of the latter species.

3. *Time of Laying.*—The deposition of most of the

spawn by *A. jeffersonianum* precedes that by *A. punctatum* by a few days. It has been impossible to visit daily the pools where the spawn of *A. jeffersonianum* is most abundant, owing to their distance from the university; one pool much nearer has yielded a small amount of it and has provided more accurate although more scanty data. In general the deposition of the bulk of the *jeffersonianum* spawn coincides with that of the first *punctatum* spawn. Variations from this occur—for instance, this year the spawn in the single pool just mentioned followed the above rule, while in the group of four pools nearly all the *jeffersonianum* spawn had been deposited three days before any *punctatum* spawn appeared; and to complete the irregularity the last spawn of all to be deposited was that of *A. jeffersonianum*. It was in small quantity and probably all from one female. (These eggs and the larvæ from them were unusually small, the larvæ seemed vigorous, but could not be kept alive many days after their own supply of yolk was exhausted.) Another check on the time is furnished by the spawning of *Rana sylvatica*. This year—an unusually early season—the writer observed the first deposition of spawn in these pools by the wood-frog. It began at 10.30 A.M., March 31. Spawn of *A. jeffersonianum* had appeared seven days previously.

4. *Spawn-masses.*—The typical spawn mass of *A. jeffersonianum* is a small one, the number of eggs being usually about twenty; the extremes encountered have been small masses of jelly without any eggs and a mass containing forty-one. *A. punctatum* does indeed deposit masses of spawn containing as few eggs as this, but the number is usually much larger. The complement of ripe ovarian eggs carried by two females of average size was 128 and 161. These are probably representative numbers and indicate a rather smaller complement than that possessed by *A. punctatum*—130 to 225—(Wright and Allen, 1908) which in turn is much smaller than that of *A. tigrinum*—1,000 or more (Powers, 1907).

5. Hardly less characteristic than the small masses is

the manner in which they are frequently to be found attached in succession to long slender twigs, each mass being usually in contact with its neighbors. A sentence in one paper on *A. punctatum* (Wright, 1908), "one stem—had within a length of one and a half feet 14 bunches of eggs, 15–20 eggs to the bunch," reads very much like a description of spawn of *A. jeffersonianum*. Many stems so laden have been found each year in the special pools mentioned. The largest piece in Fig. 1 is a portion of one of them. The twigs selected by *A. jeffersonianum* are, as a rule, very slender. *A. punctatum* will make use of both stout and slender twigs indifferently, and no small quantity has been found attached to the margins of leaves and to grass, even in the presence of such twigs as are generally preferred. Eggs of *A. jeffersonianum* have not been found except attached to twigs or stems of water plants.

The low vitality of much of the spawn of *A. jeffersonianum* is a feature that has been noticed in each year. No accurate estimate of the proportion that dies has been made, but judged roughly by the conditions found in the pools it is probably not overstating the loss to say that three fourths of the eggs do not live to begin gastrulation. The same proportion of loss has occurred in spawn reared in the laboratory, while spawn of *A. punctatum* brought from the same pools a little later and kept under the same conditions has suffered practically no loss. The egg does not die, as a whole, but cells here and there precede, the others going on dividing as usual one or more times, only to die at last. The surface view of such an egg when death is complete shows an irregular mingling of minute cells with many others two or three times as great, and at intervals others even up to eight or ten times as great, in diameter. These dead eggs imbibe considerable water, and become very much larger than the living ones and under natural conditions are soon infected by fungi; but in the laboratory they have been kept for weeks and have remained free from it; showing that death has not been caused by a fungus that

only later becomes visible. All the eggs of a mass either die or develop properly; one or two of the eggs may prove exceptions to this, but whatever the defect may be it involves practically all the eggs of a bunch. Whether it may extend to all the eggs of a female it has not been possible to determine. This loss has also been observed in spawn of *A. jeffersonianum* from a second locality and is not likely to be due to any quality of the water, for in the pools of each locality spawn of *A. punctatum* has been found developing with very little loss, and that apparently due to infection by fungus. Neither can it be ascribed to low temperatures from early deposition, for the earliest is no more liable to die than that which comes later along with or after the spawn of *A. punctatum*.

Larva

Spawn of *A. jeffersonianum* brought to the laboratory has been allowed to develop and the larvæ fed until the larger specimens had attained a length of 30–40 mm. In these it has been possible to detect a peculiarity of marking not present in similar larvæ of *A. punctatum*. This peculiarity consists of a massing of dark chromatophores into three or four spots placed in a row along each side of the mid-dorsal line, giving the animal, when viewed from above, the appearance of being banded (Fig. 2). Viewed from the side the same can be detected, but is less conspicuous (Fig. 3). Incipient banding is often indicated as soon as the chromatospheres are well differentiated (Fig. 4).

In looking over a large number of larvæ all gradations will be found between individuals in which the above shows distinctly and those in which it is impossible to detect it. For example, in 115 laboratory-reared larvæ examined at one time, 80 (69 per cent.) showed the distinctive marking. Of the balance, some individuals under different conditions showed it also (either extreme expansion or contraction of the chromatophores obscures the pattern), but some never did. Exact numbers for this division of the 31 per cent. are not available.

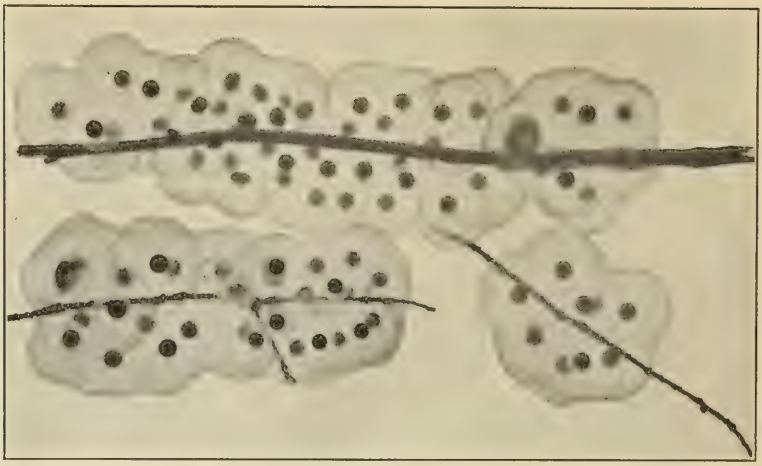

FIG. 1. Spawn of *A. jeffersonianum*. Natural size. The eggs of some of the masses are dead, others are in various stages from blastopore to medullary groove formation.

FIG. 2. Larvæ of *A. jeffersonianum*. Enlarged three diameters. Chromatophores considerably but not extremely contracted.

FIG. 3. Larva of *A. jeffersonianum*. Enlarged three diameters. Chromatophores considerably but not extremely expanded. The viscera and right side of the trunk have been dissected away and the photograph taken by both direct and transmitted light.

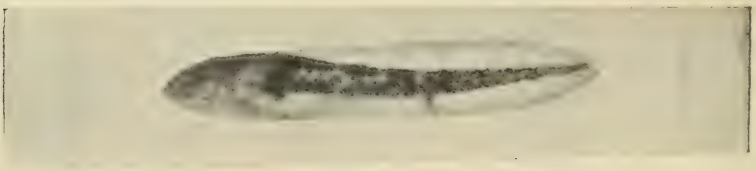

FIG. 4. Larva of *A. jeffersonianum*. Enlarged four diameters.

This year an attempt was made to remove all the spawn of *A. punctatum* from the special pools. In the middle of June larvæ 30–35 mm. long were collected from them and examined as to this pattern; it was found in but 35 per cent. Two causes may have contributed to this—the abundance of brush in the pools may have caused some spawn of *A. punctatum* to be overlooked, and the great expansion of the chromatophores—much greater than ever attained in the laboratory, the pools being very dark, probably disguised it in some cases. It was found impossible to put these larvæ under observation in the laboratory to test this point, for owing to the long journey or to the change of water they invariably died within a few hours.

Little importance would have been attached to a point of coloration so variable as this had it not been found to be uniformly lacking in similar larvæ of *A. punctatum*, whether raised in the laboratory or taken from the pools known to contain little, if any, spawn of *A. jeffersonianum*. In view of the range of coloration for *A. tigrinum* as larvæ (indicated by Powers), the degree of constancy noted is perhaps the most that could be expected.

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