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Structure and Development of the Ovule of Bowenia spectabilis.[1]

BY

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Assistant Lecturer in Botany, Manchester University.

With Plate LXI and sixteen Figures in the Text.

THIS investigation was undertaken in the hope that it might increase our knowledge of Cycads, and also give further information on points of interest in connexion with the study of fossil seeds, more especially with regard to the development and fate of the pollen-chamber. No detailed account of the ovule of *Bowenia* has yet been published, and it seems desirable that every genus of such a limited and ancient family as the Cycads should be investigated.

The greater part of the material was obtained some years ago by Professor Lang from the plants of *Bowenia* growing in the Royal Botanic Gardens, Kew. All this material was unpollinated, but it supplied an almost complete series of stages from an ovule with only the megaspore cell developed to one almost ready for pollination. The rest of the Kew material consisted of aborting ovules of two sizes, the smaller about the size of a wheat grain, the larger the size of a small hazel nut. Although these were abnormal they afforded much useful information. This material was supplemented by a batch of old pollinated ovules (Fig. 9, Pl. LXI, actual size), collected from the Botanic Gardens, Tokyo, by Dr. M. C. Stopes, to whom I am indebted for the material. While the series is thus an incomplete one, a good general idea of the changes in form and structure of the ovule can be obtained from it. Much of the development shows close agreement with that described by various authors[2] for other genera, but the chief stages will be briefly described, since they help to fill in some of the gaps in the various accounts. They also contribute to the data necessary for a comparative review of our knowledge of the ovule of Cycads, which it is hoped

[1] A preliminary account of this investigation was read before Section K of the British Association, Portsmouth, 1911.

[2] Warming: Oversigter d. K. dan. Vidensk. Selsk. Forhandl., 1877 and 1879. Treub: Ann. Jard. Bot. Buitenzorg, vol. ii, 1882; vol. iv, 1884. Lang: Ann. of Bot., vol. xiv, June, 1900.

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to attempt at some future date. The present paper will be mainly descriptive, with an indication of the direction in which the facts for *Bowenia* would seem to point in a discussion of the general phylogenetic problem.

Description of Ovules.

The megasporophylls form a compact strobilus and have a well-defined stalk which expands into a peltate head with a flat or slightly ridged apex (Fig. 9, Pl. LXI). The peltate head is elongated horizontally, and an ovule is borne on the under side of each of the horizontal extensions. The general features of the series of ovules to be described are shown in Text-figs. 1–4 and 14–16, which are outline camera lucida drawings. The main features of the ovules of various ages will be described in order.

(*a*) Unpollinated Ovules.

The youngest ovule obtained (Text-fig. 1) appears as a small swelling of the under side of the sporophyll, with very little constriction at the point of attachment, for no distinct stalk is yet differentiated.

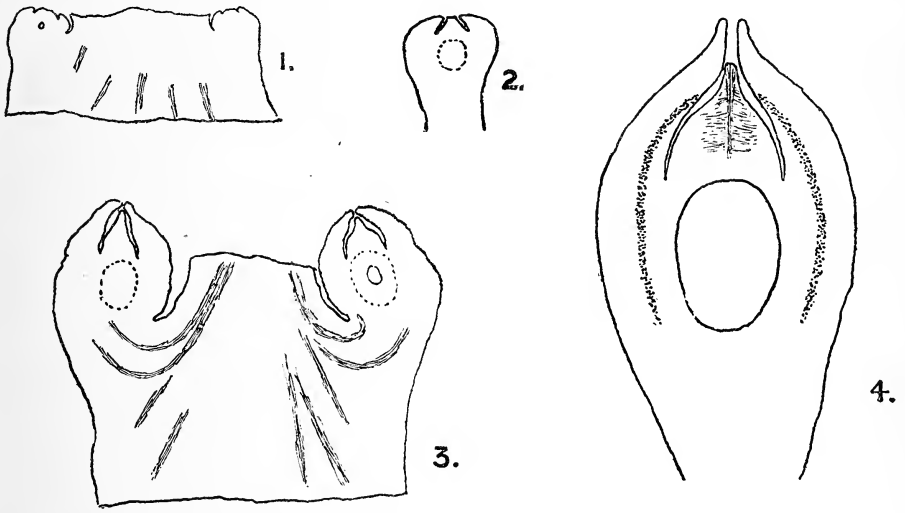
The nucellus is surrounded by the single, differentiated integument a division line being formed by rows of flattened cells which extend from the base of the ovule to the epidermis. A slight cleft in this region of the epidermis indicates the beginning of growth of the free parts of nucellus and integument.[1] The tissue of the nucellus consists of longitudinal rows of cells traceable to the flat apex and evidently produced by periclinal divisions of the epidermis. In the centre of the nucellar tissue there is a rounded mass of cells with rather denser contents than the surrounding cells. Further growth is indicated in this tissue, for many of the cells show signs of a coming division. This is the sporogenous tissue which later becomes more conspicuous and, by analogy with other Cycads[2] in which the earlier development has been worked out, may have formed by the division of the original archesporial cell.

Situated usually slightly above the centre in the sporogenous tissue is a conspicuous cell, 0·04 mm. long, larger and with a rather thicker wall than the surrounding cells (Fig. 1, Pl. LXI). This is the mother-cell of the embryo-sac. The protoplasm of the cell is unvacuolated and the large central nucleus has a distinct nucleolus. Ovules of approximately the same age show a rather later condition of the nucleus of the megaspore mother-cell (Fig. 2, Pl. LXI). This seems very like the synapsis condition in preparation for the coming reduction division. The chromatin matter is contracted in a mass to one side and the rest of the enlarged nuclear area is clear. Since, however, the material was only roughly fixed in 70 per cent. spirit it was not suitable for cytological work. The vascular bundles which

[1] Cf. Lang, loc. cit., Fig. 2, Pl. XVII.

[2] F. G. Smith : Development of Strobilus of *Zamia*. Bot. Gaz., vol. l, 1910.

are to supply the ovule can be seen as procambial strands in the sporophyll. The changes which take place in the ovule as growth proceeds can easily be followed in the slightly older ovules. All the parts become larger and more differentiated, but the most marked change is that which occurs at the apex of the ovule in the free parts of nucellus and integument. In the ovule just described the nucellus had a broad flat top, almost on a level with the slightly rounded top of the integument and only separated from it by a slight cleft (Text-fig. 1). From this time onwards the parts of the integument and nucellus above the cleft show marked growth. The epidermal cells actively divide by periclinal walls and gradually build up the free areas of nucellus and integument. As the nucellus grows its sides gradually



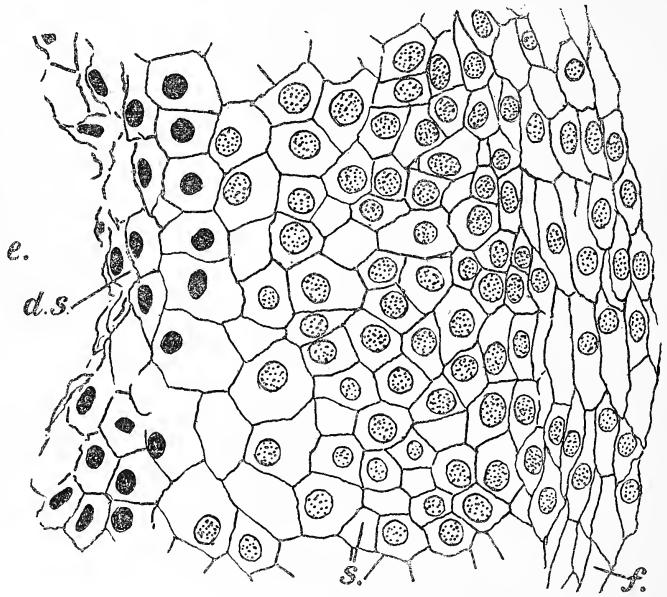
TEXT-FIGS. 1–4. Series of median longitudinal sections of ovules of various ages. × 12.

become sloping, while the top remains flat, so that the whole of the free part resembles a truncated cone (Text-fig. 2). The integument keeps pace in the growth and follows closely the sloping surface of the nucellus, the apex of which gradually becomes more pointed (Text-fig. 3). The nucellus for some time grows at the same rate as the integument, and the two are therefore of about the same height, but at the stage seen in Text-fig. 3 the integument has begun to outgrow it. From this time onwards the free ends of the integument grow more rapidly than the nucellus, and the micropyle between becomes gradually narrower.

A very similar method of growth of nucellus and integument was described in *Stangeria*, and photographs 2, 7, and 12, Pl. XVII [1] compare very closely with Text-figs. 1–3 of this paper.

[1] Lang, loc. cit.

Shortly after the beginning of active independent growth of nucellus and integument, the megaspore mother-cell begins to increase in size, and the reduction divisions occur. These have not been observed, but the result, a row of three cells, is shown in Fig. 3, Pl. LXI, which is taken from an ovule slightly younger than the one represented in Text-fig. 2. The upper two cells are smaller and their nuclei less dense than the lowest one, which becomes the embryo-sac. The same development of the embryo-sac is described in *Ceratozamia*[1] and in *Stangeria*.[2] From this time a marked increase in size of the embryo-sac is noticed, and the sister cells show signs of disorganization (Fig. 4, Pl. LXI). The nucleus of the embryo-sac divides



TEXT-FIG. 5. Portion of longitudinal section of ovule represented in Text-fig. 3, showing sporogenous tissue surrounding the embryo-sac. *e.* = embryo-sac; *d.s.* = degenerating sporogenous cells; *s.* = active sporogenous tissue; *f.* = flattened cells of nucellus, bordering on the integument. × 300.

into two, which are shown in Fig. 5, Pl. LXI, with the disorganized sister cells above. Other sections obtained show the result of a second division, an embryo-sac with four free nuclei.

Free nuclear division proceeds and the embryo-sac gradually enlarges, but the protoplasmic contents do not increase at an equal rate, and hence it appears for a long time as a hollow sac with a thin lining of protoplasm in which lie the free dividing nuclei (Fig. 6, Pl. LXI).

The growth of the embryo-sac is accompanied by growth of the surrounding sporogenous tissue. Many of these cells are actively dividing

[1] Treub, loc. cit., 1885.

[2] Lang, loc. cit.

and appear more densely granular than the surrounding nucellar cells. This tissue appears to act as a nutritive tissue or tapetum. All the evidence points to the tapetum being a modification of the sporogenous tissue as in *Stangeria.*

In rather later stages of the ovule, crushed and disorganized cells of the tissue lie immediately around the embryo-sac (Text-fig. 5). The nutriment which they contained has evidently been absorbed by its encroachment. In this same ovule, which is of the size indicated in Text-fig. 3, the outermost cells of the sporogenous tissue show signs of active division. It is evident that for some time the increase by division of the outer sporogenous cells keeps pace with the destruction of the innermost cells by the embryo-sac, and the result is that the embryo-sac is surrounded until a much later stage by a layer of nutritive tissue. Cf. Text-fig. 5, and Fig. 5, Pl. LXI.

The last of the series of normal, unpollinated ovules is much larger than the previous stages described, and shows marked advances in the structure and differentiation of the various parts. One of these ovules is seen in median longitudinal section in Text-fig. 4, which shows the general relation of parts. The free part of the nucellus is a large dome-shaped mass (Fig. 7, Pl. LXI), surrounded by the integument, which is 0·6 mm. thick, and shows signs of differentiation into the three characteristic layers. The micropylar tube is wide and contains mucilage, the function of which is probably to assist later in catching the pollen-grains. The embryo-sac is so large that it almost fills the lower part of the nucellus.

The chief interest of an ovule of this age is that it shows the first indication of the formation of the pollen-chamber (Fig. 7, Pl. LXI). Running down the centre of the nucellus from the tip to a short distance above the level at which it fuses with the integument, a length of 1 mm., is a strand of cells. These are conspicuous since they are elongated in the vertical direction, while the cells immediately around them are slightly elongated in the horizontal direction and thus appear to radiate from the central strand. Also the surrounding cells have denser contents, larger nuclei, and appear in a more active state of growth than the central cells. Warming[1] figured cells of this nature in the nucellus of *Zamia*, and described them as a kind of conducting tissue. A similar tissue was also figured by Brongniart[2] in *Cardiocarpus augustodensis*, also by Stopes[3] in *Cycas Rumphii.* Although there is at this stage no sign of breaking down of tissue, it is evident that this central strand indicates the area which will ultimately dissolve in the formation of the pollen-chamber. The embryo-sac has grown with the rest of the ovule, being 1·8 mm. long by 1·2 mm. wide. It still appears essentially as in the last stage, a hollow sac with a thin lining of protoplasm containing nuclei, some of which are undergoing division, but with no sign of

[1] Warming : Rés. du Bull. de l'Acad. Roy. Dan. des Sciences et des Lettres, 1877.

[2] Brongniart : Recherches sur les graines silicifiées. Paris, 1881.

[3] Stopes : Beiträge zur Kenntnis der Fortpflanzungsorgane der Cycadeen. Flora, vol. xciii, 1904.

walls between them. The megaspore membrane forms a conspicuous covering, but is thin in comparison with that of other Cycads, and shows no differentiation into layers as described for some genera, e. g. *Cycas revoluta.*[1] In the oldest ovule examined the megaspore membrane is thin and undifferentiated. If, as Thomson suggests, a thick megaspore membrane is to be regarded as a sign of primitiveness, then in that respect *Bowenia* as compared with other Cycads must be regarded as well advanced. The sporogenous tissue which formed so large a mass round the embryo sac of the younger ovule has almost entirely gone. It has evidently failed to keep pace with the advancing embryo-sac, and appears as one or two layers of granular, compact cells at the outside and a few broken and disorganized cells lying loosely around the megaspore membrane (Fig. 8, Pl. LXI). There is no sign of any active formation of new cells. Evidently at a slightly earlier stage the formation of new cells ceased, then, as is shown in Fig. 8, Pl. LXI, the cells of the tissue separated from one another, gradually dissolved, and were absorbed by the encroaching embryo-sac ; cf. *Zamia.*[2] In rather later stages, when the prothallus tissue fills the embryo-sac, the whole of the nutritive tissue has disappeared. The length of time which this tissue persists and its appearance during the last stages varies in different genera. In an ovule of *Stangeria*, with the pollen-chamber beginning to form and the embryo-sac completely filled by the prothallus tissue, the sporogenous tissue is shown [3] to still persist in the form of a single layer of much enlarged cells with smaller crushed cells on their inner side. These are the remains of a tissue which earlier appeared as a compact surrounding mass very similar to that in the young stages of *Bowenia.* The embryo-sac gradually absorbed the sporogenous cells around it, except the outermost layer, which enlarged to form the definite layer which Lang termed a tapetum. How long this layer persists cannot at present be stated. Lang was unable to follow it, for in the fertilized seeds which he had all traces had disappeared. A further supply of *Stangeria* material which I have examined confirms Lang's description, but does not help in this point. In an ovule of *Dioon* [4] with prothallus and pollen-chamber development a definite 'jacket layer' is present round the prothallus, and this acts as a tapetum. It thus appears from the present facts that the sporogenous tissue of *Stangeria* and *Dioon* persists much longer than in either *Bowenia* or *Zamia.*[5]

The integument of the ovule at this stage shows indications of the three layers characteristic of Cycads. The cells destined to form the stone layer are smaller than those forming the fleshy parts, though they

[1] Thomson, R. B.: The Megaspore Membrane of the Gymnosperms. Univ. Toronto Biol. Series, No. 4, 1905. [2] Smith, F. G., loc. cit. [3] Lang, loc. cit.
[4] Chamberlain: The Ovule and Female Gametophyte of *Dioon.* Bot. Gaz., vol. xlii, 1906.
[5] Smith, loc. cit.

have yet unthickened walls. They extend from the base of the ovule up into the micropylar region (Text-fig. 4). There is a definite and regular epidermis of small rectangular cells limiting the outer fleshy layer in which tannin sacs and mucilage ducts are beginning to appear. The inner fleshy layer is composed of rectangular parenchymatous cells which are indistinguishable from the adjoining cells of the nucellus.

The Vascular Supply of the Ovule.

In an ovule of this age it is easy to trace the vascular supply. Longitudinal and transverse sections of the ovule on the sporophyll can be cut before the sclerization of the stone layer. The bundles are, however, not fully developed, and the detailed description of them will be given later in connexion with the oldest ovule. Two series of vascular bundles are present in the ovule. The outer series consists of from 7 to 9 bundles which extend from the base into the micropylar region and only rarely branch. The inner series consists of 12–14 bundles at the base of the ovules. They branch frequently on their way up the ovule and number about 40 at their termination, which is usually just below the point at which nucellus and integument become free (Text-fig. 6). These bundles lie apparently on the border line between nucellus and integument. In some cases, however, which will be described later, there is reason to think that this inner vascular supply is nucellar. The whole of this vascular system is supplied by branches from one of the foliar bundles of the sporophyll (Text-fig. 7). The bundles of the sporophyll which serve the sporangia are markedly large, with a few elements of centripetal xylem ; cf. Worsdell.[1] The position and branching of the bundles can be made out by comparing Text-fig. 7 and the series 8–12. In the stalk of the sporophyll the foliar bundle *A* divided into two branches, *a* and *b*—the former being the outermost. The branch *a* divides into a^1 and a^2 (dotted line) long before reaching the ovule, and these two branches serve mainly the part of the ovule nearest to the sporophyll. One branch supplies the anterior and the other the posterior region of this part, and each sends branches to both inner and outer series. The branch *b*, after dividing from *a*, runs down for some distance in the sporophyll before turning in the direction of the ovule. It then forks and one branch, fb^1, remains in the sporophyll, while *b* turns towards the base of the ovule. Before actually entering the ovule it gives off a second foliar branch, fb^2. The branch *b* supplies the part of the ovule distal to the stalk of the sporophyll, and sends branches to both the inner and outer series.

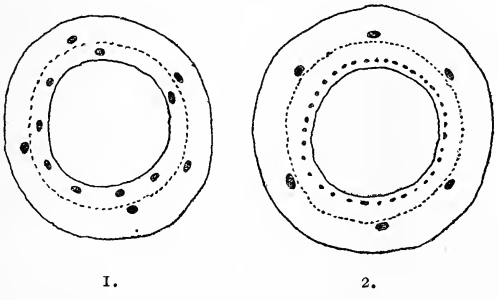
(*b*) Old Pollinated Ovules.

The last of the normal ovules investigated were much larger than those previously described, and were in addition pollinated. The material con-

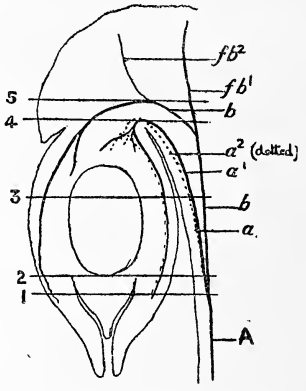
[1] Worsdell: Vascular Structure of Sporophylls of Cycadaceae. Ann. of Bot., vol. xii, 1898.

sisted of about a dozen sporophylls, each with a pair of ovules, one of which is represented in natural size in Fig. 9. Very frequently one of the pair of ovules was malformed and had a very twisted, shrunken appearance, but was otherwise quite healthy. The prothallus and archegonia of such an ovule were normal. Possibly the distortion was the beginning of a late abortion, caused by the great pressure exerted on the ovules during the growth of the cone. The normal ovules are about 2 cm. long and 1·8 cm. across. They are distinctly oval in shape with a prominently pointed micropylar tube. The surface of the ovule is smooth and covered with brown flecks due to the tannin sacs beneath the epidermis.

The general relation of parts of such an ovule is seen in diagrammatic view in Text-fig. 13. The three layers of the integument are differentiated, the stone layer being represented by the closely dotted layer.



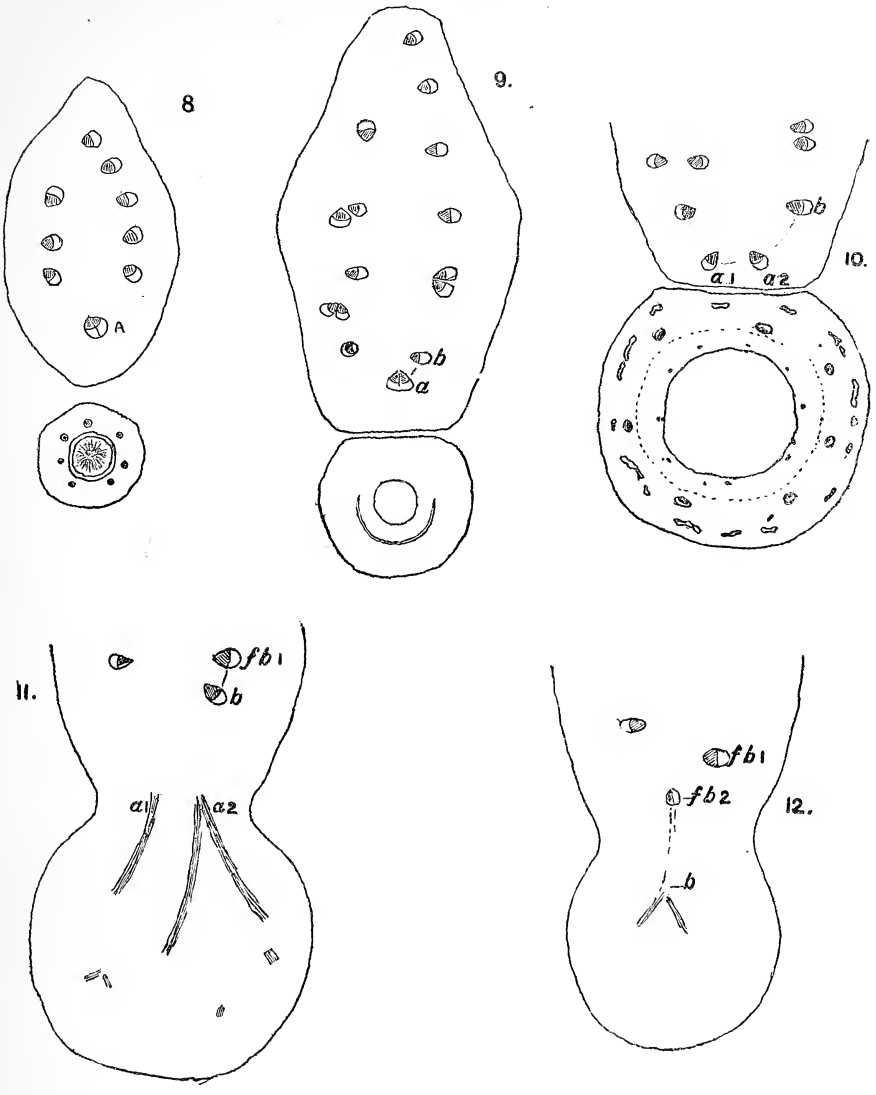
TEXT-FIG. 6. Diagrammatic transverse sections of the ovule, showing the vascular supply. (1) The base of the ovule. (2) Just below the point at which nucellus and integument become free.



TEXT-FIG. 7. Longitudinal section of sporophyll, showing vascular supply to the attached ovule.—Explanation in Text-figs. 8–12.

The outer and inner series of vascular bundles are well developed. The free part of nucellus and integument is only a small portion of the whole ovule. In the pollen-chamber there are developing pollen-grains whose rooting ends have penetrated the tissues of the nucellus. The prothallus is of large size, usually about 1·8 cm. in length, and has a ring of 5–7 archegonia at the apex.

An examination of the integument shows it to be thin compared with that of other Cycadean ovules of a similar age. The outer flesh forms more than half of the whole thickness, 1·4 mm. Indeed at the end of the micropyle almost the whole of the tissue is outer flesh. This layer of the integument is bounded by a regular layer of small epidermal cells with strongly cuticularized outer walls. The rest of the tissue consists



TEXT-FIGS. 8–12. A series of transverse sections through the sporophyll, showing the vascular supply to the attached ovule.

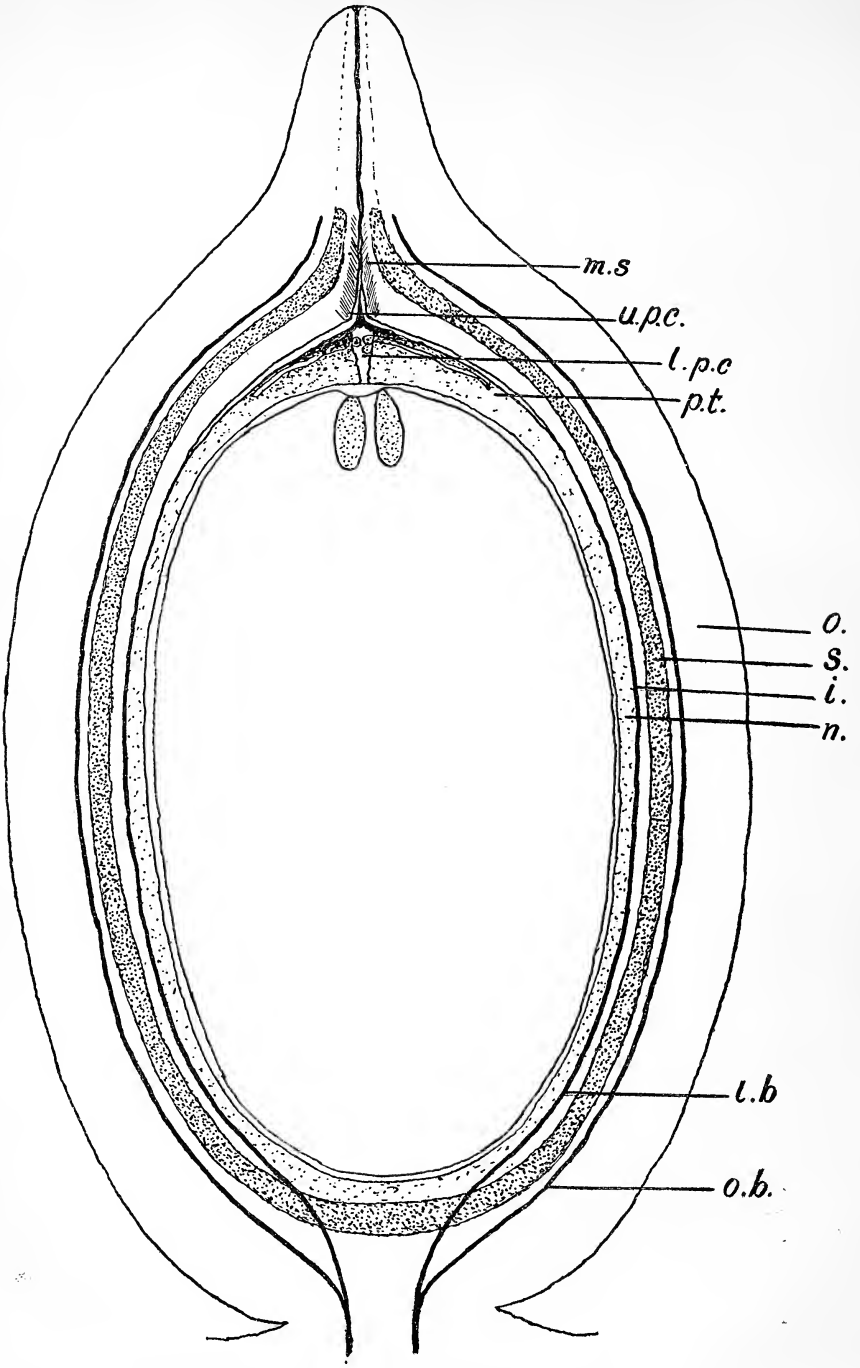
TEXT-FIG. 8. A section low down in the stalk of the sporophyll with the tip of the pendant ovule—at level 1 in the longitudinal section of the ovule (Text-fig. 7). The main bundle A is showing signs of division.

TEXT-FIG. 9. At level 2. The main bundle A has divided into *a* and *b*, and *a* shows signs of division.

TEXT-FIG. 10. At level 3. *a* has now divided into a^1 and a^2 (dotted line), which are bundles of equal size, supplying the anterior and posterior sides of the part of the ovule next to the sporophyll.

TEXT-FIG. 11. At level 4. The bundles a^1 and a^2 are entering the ovule. The branch *b* has divided into a foliar branch, fb^1, and the ovular branch.

TEXT-FIG. 12. At level 5. The branch *b* is entering the ovule, and also gives off a foliar branch, fb^2.



TEXT-FIG. 13. Longitudinal section of an ovule (diagrammatic). *o.* = outer fleshy layer of integument; *s.* = stone layer; *i.* = inner flesh; *n.* = nucellus; *i.b.* = inner series of vascular bundles; *o.b.* = outer series; *m.s.* = sclerenchyma round micropyle; *u.p.c.* = upper pollen-chamber; *l.p.c.* = lower pollen-chamber; *p.t.* = pollen-tube.

of large parenchymatous cells containing starch. The innermost cells are completely filled with large starch grains, while in the cells further out the number and size of the starch grains gradually diminish. Those near the epidermis contain only a few small grains. Amongst the outermost cells of this layer there are scattered tannin sacs, which begin to form in a single cell; then frequently an aggregation of such cells leads to the formation of one large sac. Deeper seated in this layer is a ring of large mucilage ducts, about 1·5 mm. in diameter. The main ducts are vertically placed, and from them there are branches and anastomoses in a lateral direction. The outer series of vascular bundles lie among the innermost cells of this layer, separated from the stone layer by about two rows of cells.

The bundles of this series are collateral and have a large amount of centripetal xylem (Fig. 10, Pl. LXI). The centrifugal xylem consists of fairly regular rows of tracheides proceeding from the protoxylem; the phloem elements follow on in equally regular rows towards the outside, where there are a few crushed protophloem elements. The centripetal xylem is about equal in amount to the centrifugal, but the elements are rather larger in size. They lead directly from the protoxylem, and the elements spread towards the inner part of the integument. There is a definite sheath of parenchymatous cells surrounding the bundle. This structure is similar to that described in *Encephalartos* by Stopes,[1] and is a much simpler type of bundle than that which is described by the same author in some species of *Cycas*.

The stone layer, which is yet only partially sclerized, extends from the base of the ovule, where it stretches completely across (Text-fig. 13),[2] and is penetrated by the inner series of vascular bundles, to about half-way up the micropylar beak. It is of almost uniform thickness, 0·3 mm., except in the basal region, where there is a slight increase. There is no indication of a differentiation of the stone layer into distinct parts as is the case in many Cycads.[3] The tissue consists of cells slightly elongated vertically, which in the basal region interlace with each other, forming what would be a very resistent tissue when the cell-wall had hardened. Sclerification has as yet only taken place in the micropylar region and about half-way down the free part of the integument (Fig. 11, Pl. LXI). The process apparently begins in the innermost cells of the layer, which have very thick walls with slit-like pits, and gradually spreads outwards. There is a sharp line of demarcation between the outermost sclerized cells and the adjoining cells of the outer flesh, which contain starch. An interesting feature of the micropylar region of the ovule is the occurrence of a second ring of sclerized cells almost bordering on the micropylar tube and extending up about half its length (Fig. 11, Pl. LXI).

[1] Stopes, loc. cit.

[2] Cf. *Dioon*, where there is a gap in the basal region of the stone layer.

[3] *Dioon*—Chamberlain, loc. cit. *Cycas Beddomii*—Stopes, loc. cit.

The cells forming this ring are long and narrow and obliquely placed, making an acute angle with the micropylar tube. The position of these cells shows them to be part of the inner fleshy layer of the integument, but they are very closely related to the stone layer. In some parts they are actually in contact with the sclerized cells of the stone layer, and in other parts the separating parenchymatous cells themselves show signs of sclerification beginning. It seems probable that in an older ovule the whole of this basal region of the micropyle would be one sclerized mass of cells. There seems to be no exact parallel to this condition among living Cycads or fossil seeds. The upper part ot the micropylar tube is now closed, and can scarcely be seen in a transverse section. Lower down the tube becomes slightly wider, and the long pointed beak of the nucellus extends up into it. The separating line between inner flesh and stone layer is difficult to make out in the parts where the latter is not sclerized, also there is no obvious line of division between nucellus and inner flesh. The cells of both consist of rather elongated parenchymatous cells with scattered tannin sacs. The inner series of vascular bundles lie in this region and follows an imaginary line dividing nucellus and integument, and obtained by continuing the slit between their free portions towards the base. In many of the ovules the bundles terminate just below the division into free nucellus and integument. In several of those examined, however, the bundles of this inner series were found to extend for a short distance into the free part of the nucellus (Fig. 12, Pl. LXI). From the earliest stages of the ovule where the bundles were beginning to develop it could be seen that they were not integumental, as has been recently generally assumed for Cycads. From the later stages examined it seems that one would be justified in stating that in the case of *Bowenia* there are strong reasons for assuming the inner vascular supply to be nucellar. A transverse section of a bundle of the inner series in the lower part of the ovule is seen in Fig. 13, Pl. LXI. The bundles differed much from those of the outer series. The protoxylem is mesarch, and the greatest development of wood is in the centripetal direction with only a few tracheides of centrifugal direction. The phloem is small in amount and difficult to identify. The possible elements seem to be arranged all round the xylem. As compared with the outer collateral bundles, the inner ones have an almost concentric appearance. As the bundles branch and pass upward through the integument they gradually lose their mesarch appearance. The protoxylem is no longer recognizable, and the bundle consists of a flattened mass of short thick tracheides without phloem. These bundles are very numerous and close together in the upper part of the ovule (Text-fig. 6).

The free area of the nucellus is a comparatively small part 1·4 mm. in length which caps the top of the prothallus. It is limited by a regular epidermis with cuticularized outer walls and terminates in a long beak-like

part 0·4 mm. long, fitting closely into the micropylar tube (Fig. 11, Pl. LXI). This part is shrivelled and black in colour, as are also a few of the outer cells of the lower, expanded part of the nucellus. Below the beak-like portion the tissue of the nucellus is hollowed out and forms a large chamber, which in some ovules extended completely through the nucellus, and so was in direct communication with the prothallus. In others the lower part of the nucellus was intact and no such communication had been established (Fig. 14, Pl. LXI). In this large lower cavity numerous pollen-grains were growing, sometimes as many as twenty in one ovule, and all at approximately the same stage of development (Fig. 15, Pl. LXI). In *Zamia*, on the contrary, pollen-grains at almost all stages of development may be found in one nucellus. The pollen-grains have produced long, unbranched, rooting ends which have penetrated the wall of the cavity and into the nucellar tissue (Fig. 14, Pl. LXI). They run out horizontally from the cavity, and before reaching the surface turn downwards and run just below the epidermis. At the end of each root-like growth is the tube nucleus. When viewed from above the tubes appear as dark lines radiating from a central point. The end of the tube in the cavity is much swollen and contains the developing fertilizing apparatus. It consists of two prothallial cells and a large terminal generative cell (Fig. 15, Pl. LXI). The first prothallial cell is next to the pollen-grain coat. It has a fairly large nucleus and its wall bulges considerably into the second prothallial cell, whose nucleus is thus pushed to one side, and the two thus give the appearance of one cell within another. At the end of the second prothallial cell is the large generative cell, which contains a nucleus 0·03 mm. in diameter (Fig. 16, Pl. LXI). The chromatin of the nucleus is arranged in a definite network, and there is a nucleolus 0·015 mm. in diameter. Situated at opposite ends of the nucleus, generally opposite to an indentation in its margin, are the two blepharoplasts. They are homogeneous bodies with numerous fine radiations arising from them on all sides. When stained with Heidenhain's haematoxylin blepharoplasts and radiations take an intense black. In some cases the pair lie in the plane of the long axis of the pollen-tube, in other cases in a plane at right angles to it.

This is the only stage in the development of the fertilizing apparatus that has been obtained, but the whole structure is so similar to that which has been described for *Zamia*[1] and *Dioon*[2] that it is evident that development has taken place in the same way as in those where the divisions have been followed.

The female prothallus appears as a white glistening mass with a ring of five to seven archegonia opening into an archegonial chamber, which is

[1] Webber: Spermatogenesis and fecundation of *Zamia*. U. S. Dept. Agric. Bull., Bur. Plant Ind., vol. ii, 1901.

[2] Chamberlain: Spermatogenesis in *Dioon edule*. Bot. Gaz., vol. xlvii, 1909.

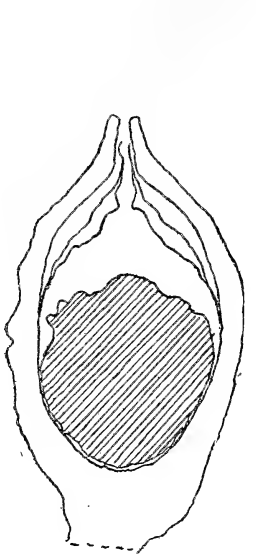
very shallow compared with that in ovules of *Cycas* and *Dioon* (Text-fig. 13). The prothallus is surrounded by the megaspore membrane, which adheres closely to the nucellar tissue rather than to the prothallus. This membrane is still very thin, showing little increase in thickness, if any, from that of the younger ovule. The prothallus tissue consists mainly of large cells with two or three rows of smaller cells at the exterior. In the neighbourhood of the archegonia also the cells are small. They have thin walls and nuclei and no visible stored food material. In the specimens investigated the protoplasm had shrunk considerably from the walls. The archegonium is about 1·7 mm. long and surrounded by a conspicuous jacket layer. It has a pair of arched neck cells above, which are evidently formed by a transverse division of a single cell (Fig. 17, Pl. LXI). The contents of the archegonium consist of foam-like vacuolated protoplasm, with a single nucleus 0·02 mm. in diameter at the apex (Fig. 18, Pl. LXI). The nucleus has an irregular outline and contains numerous masses of chromatin, and various sized granules of a staining substance are scattered around the nucleus in the protoplasm. In Fig. 18, Pl. LXI, two large masses of this substance are seen. It seems very probable that these granules are similar to those described by Ikeno [1] as proteid granules. The central cell of the archegonium is surrounded by a thick 'egg membrane' in which there are pits of various sizes (Fig. 19, Pl. LXI). Through the pits haustoria-like processes of the 'egg' protrude towards the surrounding cells of the jacket layer, the protoplasm of which is very dense and often contains, in addition to the large nucleus, several granules of a substance which stained like chromatin. It has been asserted by several investigators that a thin wall closes the pits of the thick egg membrane. No such closing membrane to the pit can be seen in these ovules with the highest magnification. The preservation of the material may account for this; or it may be, as Chamberlain [1] suggested, that the pit membrane which probably exists in the younger stages is ruptured by the haustoria as they grow out towards the jacket cells.

(*c*) Abnormal Ovules.

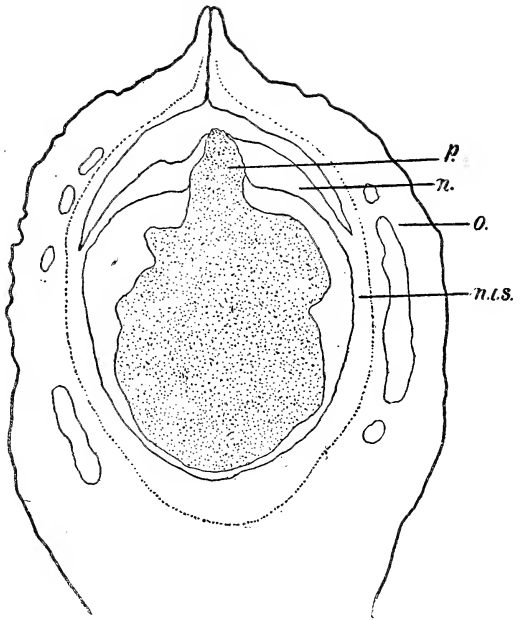
The two stages of abnormal ovules were of use in showing intermediate steps in development, especially as regards the pollen-chamber, between those already dealt with. The main features of these ovules will be described before discussing the development and morphology of the pollen-chamber in Cycads.

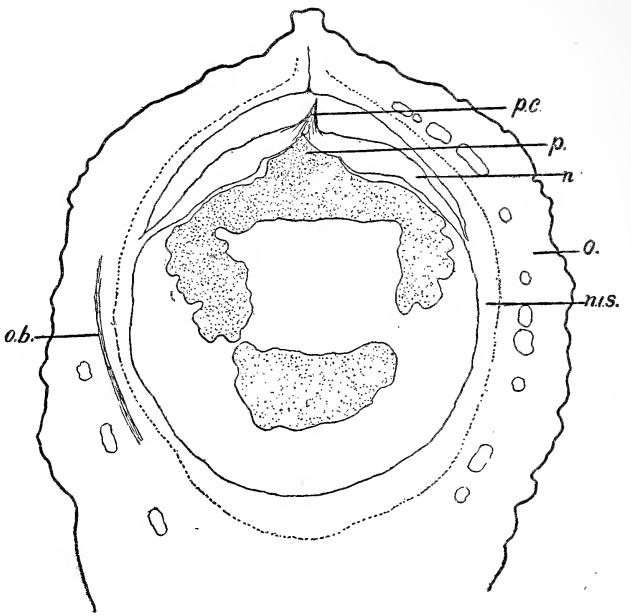
The younger ovules were about 4 mm. long. A vertical section of one of them is represented in outline in Text-fig. 14. In all ovules of this age the pollen-chamber was completely formed, and many of them contained a prothallus with several archegonia; but in all cases it had shrunk, sometimes considerably, from the megaspore membrane. Some of the ovules

[1] Ikeno: *Cycas revoluta*. Jahrb. wissensch. Bot., vol. xxxii, 1898.



TEXT-FIG. 14. Outline drawing of median longitudinal section of young abnormal ovule. × 12.





TEXT-FIGS. 15 and 16. Outline camera lucida drawings of median longitudinal sections of old abnormal ovules. × 10. *p.c.* = pollen-chamber; *p.* = upgrowth of prothallus into nucellus tissue ; *n.* = free part of nucellus; *o.* = outer flesh of integument; *n.i.s.* = fused layers of nucellus, inner flesh, and stone layer ; *o.b.* = outer integumental vascular bundle.

contained no prothallus, for the development of the embryo-sac had evidently been checked. In these cases layers of cells with the appearance of cork have developed in the interior layers of the integument, apparently to cut off the aborting region.

The specimens have a thin integument with, however, a well-developed vascular supply, so that the abnormality does not seem to have been caused by lack of nourishment as in some cases recorded.

The cells of the prothallus are of uniform size, and from their arrangement it seems probable that the tissue has been formed by divisions of a peripheral layer of cells which gradually fill up the hollow cavity centripetally. The apex of the prothallus between the archegonia is elevated, and although the tissue has shrunk away, a complete impression of its shape is seen in the nucellar tissue with which it was in contact. The condition of the nucellar cells in this region indicates that there must have been very close contact between the two. An upward growth of the prothallus at an early stage of development of the archegonia seems constant in Cycads; cf. Coulter and Chamberlain.[1] Later, the region of active growth is removed to a ring of tissue outside the archegonia, and by rapid cell formation the summit of the prothallus becomes sunk in the 'archegonial chamber'. The archegonium is surrounded by a jacket layer and has a pair of flat neck cells (Fig. 20, Pl. LXI). The central cell contains relatively little vacuolate protoplasm with a central nucleus; but with as yet no differentiated egg membrane.

The pollen-chamber in these ovules is a large cavity open to the micropyle above and to the prothallus below (Fig. 21, Pl. LXI). Comparison with pollinated ovules suggests that in a normal ovule of this age the passage would not be completely formed. It seems advisable to call the whole cavity the pollen-chamber and to distinguish it into the *upper pollen-chamber* and *lower pollen-chamber*; such a division seems functionally a natural one. The *upper pollen-chamber* is the narrower part of the cavity in the pointed tip of the nucellus, which extends for some distance up the micropylar tube. Its walls are thin and hardened and taper off to a fine point. The *lower pollen-chamber* is the large cavity in the wider part of the nucellus beneath the tip.

The older abnormal ovules are about 8 mm. long and therefore twice the size of those described above. Two of them are represented in outline in Text-figs. 15 and 16. The integument and nucellus are normally developed and the two parts of the pollen-chamber are seen. The walls of the upper cavity are hard and cutinized and give it the appearance of a dark, pointed cap, similar to that seen in the old pollinated ovule. The lower pollen-chamber is abnormal in its relation to the prothallus, which is of exceptional appearance, often being completely twisted out of shape. The form and

[1] Coulter and Chamberlain: Morphology of Gymnosperms. Univ. of Chicago Press, 1910.

relation of the cells composing this tissue are also extraordinary in some cases. In every ovule examined the prothallus had grown abnormally into the nucellar tissue above, a feature which was remarked upon in connexion with the younger abnormal ovules. In several instances this growth is astonishing. In the ovule in Text-fig. 15, the upper part of the prothallus is a large, cone-shaped mass, the apex of which has pushed into the pollen-chamber. In the ovule in Text-fig. 16, a mass of prothallial tissue has grown out through the pollen-chamber to the cavity between nucellus and integument. In all these cases the tissue of prothallus and integument is very intimately connected.

The cause of the abnormalities in the two sets of ovules just described is probably twofold: first, the unnatural conditions under which the plants grew, and secondly, the lack of pollination. The first reason seems to account for the abnormal appearance of the younger ovules. It is clear from comparing one of these ovules (Text-fig. 14) with a normal ovule just before pollination (Text-fig. 4), that at an early age it must have ceased to follow the usual line of development and yet have produced a prothallus and pollen-chamber. The influence which caused this departure must have been felt before the ovule was normally ready for pollination, for the ovule is actually less in size than the younger ovule with no prothallus and pollen-chamber. The lack of pollination, however, would probably account for the unusual appearance in the older ovules.

Morphology of the Pollen-chamber.

From a comparison of the different stages of development of the nucellus in *Bowenia* and other Cycads it seems probable that the pollen-chamber arises similarly in all cases. Before pollination the nucellus has a central strand of cells differentiated from the cells of the surrounding tissue (Fig. 7, Pl. LXI). About the time of pollination the apical cells of this strand begin to break down. They apparently separate from each other and are then completely dissolved. The first result of this 'resorption' is that a small cavity, such as that figured in *Stangeria*,[1] termed here the upper pollen-chamber, is formed in the apex of the ovule. This small cavity has walls which are cutinized and taper off to a fine point above. It seems probable that pollination would normally take place about the time of the formation of this upper cavity, and that it is by reason of the breaking down of the cells in that region that the pollen ever reaches the nucellus. The passage of a pollen-grain through the micropyle, which has become very narrow at the time of pollination, would be very difficult unless there were some suction from below; cf. Webber on *Zamia*.[2]

In *Bowenia*, before the pollen-chamber has begun to form, the micropylar tube contains mucilage, which probably at a rather later stage assists in

[1] Lang, loc. cit., Fig. 15. [2] Webber, loc. cit.

catching the pollen-grains. The breaking down of the nucellar tissue which would occur about this time, sets up a suction drawing the fluid and the pollen-grains contained in it down towards the nucellus. The grains are then accommodated in the small upper pollen-chamber, but they do not grow any further whilst in that position. By a further deliquescence of the cells of the central strand downwards and also outwards, a larger and wider cavity is formed—the lower pollen-chamber. The pollen-grains drop into this cavity and continue their development in it. There is sufficient room in this lower cavity for the stalk cells and generative cells to grow out from the pollen-grain and also a surrounding tissue of sufficient thickness for the rooting ends to burrow (Figs. 14 and 15, Pl. LXI). The upper pollen-chamber, which is now functionless, appears as a pointed black cap of hard and shrivelled tissue on top of the lower pollen-chamber.

The passage for the sperms to the archegonia is made partly by a further deliquescence of the cells below the lower chamber, and partly by the upward growth of the prothallus and consequent crushing of the nucellar tissue immediately above. In the abnormal ovules this upward growth of the prothallus and absorption of the nucellus is very marked; it takes place to a less extent in the normal ovule, cf. *Dioon*.[1] The part of the nucellus marked out by the central strand in the young ovule, therefore, breaks down and deliquesces, and the remaining part, separating the sperms from the archegonia, is absorbed by the upward growth of the prothallus.

Comparison with the Pollen-chamber and Ovule of Pteridosperms.

Since the living Cycads are practically the only plants in which it is possible to study the actual development and working of the pollen-chamber, it is clearly necessary that the data should be critically examined with what has been described for that interesting region of the seed of Pteridosperms. A comparison of the pollen-chamber mechanism of the Cycads with that of the Lagenostomales[2] emphasizes the relatively simple character of the former. Although two parts have been described above in the pollen-chamber of Cycads, which seem to correspond in position and function with the upper and lower parts of the pollen-chamber of these fossil seeds, they are not such differentiated regions as the 'lagenostome' and 'plinth'. There is no such specialization as one sees in the small cup-shaped lagenostome of *Conostoma* with its sculptured walls, nor the circular cavity with the central cone of tissue in *Lagenostoma*. Again, the development of the pollen-chamber of the Cycads is a much simpler process than that which has been assumed to take place in *Conostoma* and *Lagenostoma*. In the

[1] Chamberlain, loc. cit.

[2] Oliver and Salisbury: Palaeozoic Seeds of the *Conostoma* Group. Annals of Botany, vol. xxv, 1911.

Cycads a lysigenous cavity forms by progressive disintegration of cells and eventually becomes large enough for the storage and growth of the pollen. A very different and more elaborate type of development of the storage cavity or plinth has been assumed to take place in the Lagenostomales. The whole pollination of this group is in striking contrast to the simple development and arrangement of the pollen-chamber of Cycads, in which there is no suggestion at any stage of the previous existence of such a specialized type of nucellus as was present in the Lagenostomales.

A closer comparison can, however, be made with the pollen-chamber of the seeds of the Medulloseae.[1] In *Trigonocarpus, Stephanospermum*, &c., the pollen-chamber consisted of a prominent nucellar beak which engaged with the base of the micropyle, the cavity of which led below to a larger chamber, where the pollen-grains developed. The seeds of the Cordaiteae also had a pollen-chamber of this type. Some of Brongniart's[2] figures of apparently young ovules of Cordaiteae show the method of development of the pollen-chamber. In his Fig. 13, Pl. III, he shows the apex of the nucellus of *Cardiocarpus augustodensis*, in which a median cavity, evidently formed by disintegration of tissue, leads to a larger cavity containing pollen-grains. The upper passage is described as a 'canal conduisant à la chambre pollinique'. From the appearance of the tissues in these figures it seems probable that the development of the pollen-chamber was upon the same lines as has been described in Cycads. Although there are no figures of young stages of *Trigonocarpus* it is probable that the pollen-chamber, which so closely resembled that of *Cardiocarpus*, developed along similar lines, and therefore along similar lines to the Cycads.

As regards structure and development of the pollen-chamber, therefore, the closest parallel to the Cycads among fossil seeds is to be found in seeds of the *Trigonocarpus* affinity, and there seems to be no evidence in either of them that this structure had resulted from simplification of a more complicated type, such as that of the Lagenostomales.

The general morphology of the Cycad ovule has long been a problem of interest, and since the discovery of the relationships of Lagenostoma, comparisons have been made between the two, which scarcely seem justified. The Cycad integument has been described as a morphologically double structure,[3] formed by the fusion of a cupule-like structure with a single integument, the line of fusion being in or near the stone layer. If such were the case it might reasonably be expected that the development of the Cycad integument would show something indicative of its double nature. The integument of *Bowenia* develops as a single homogeneous tissue, which later gradually differentiates into the three characteristic layers, but shows no indication that it is morphologically a double structure. Similar

[1] Scott : Studies in Fossil Botany. Part II, 1909. [2] Brongniart, loc. cit.
[3] Stopes, M.C. : The Double Nature of the Cycadean Integument. Annals of Botany, vol. xix, 1905.

development has been found in all the genera thus far investigated. The facts of ontogeny, therefore, do not support the supposed phylogenetic development of the integument.

The vascular supply of the Cycad ovule was also held to agree closely with that of *Lagenostoma*. In certain genera of Cycads, Stopes[1] found that the bundles of the inner vascular series ran into the free part of the integument, and therefore this supply was held to be the equivalent of the integumental bundles of *Lagenostoma*, while the outer Cycad bundles were the equivalent of the cupule bundles. The vascular supply of *Bowenia* shows evidence of a conflicting nature which makes it impossible to accept the above comparison. In certain cases described the bundles of the inner vascular series ran into the nucellus, certainly only for a short distance; yet, if one accepts such a fact as a criterion, the inner vascular supply of *Bowenia* must be regarded as nucellar. Until more direct evidence on this point appears one can only leave the question of the exact position of the inner vascular series an open one. The general distribution and arrangement of the bundles of the Cycad seed, however, differ considerably from those in *Lagenostoma*. The bundles of the inner series in Cycads are almost concentric in structure, with little phloem, and form together a much branching system around the nucellus; the integument bundles of *Lagenostoma*, which were held to be their equivalent, were collateral with mesarch xylem, and ran without branching almost to the tip of the integument. These facts seem to show that the connexion between the Cycad ovule and *Lagenostoma* is not so close as has sometimes been assumed.

On the contrary, the evidence strengthens the suggested[2] close resemblance of the Cycad ovule to seeds of the *Trigonocarpus* affinity. It was seen above that as regards development and structure of the pollen-chamber Cycads agree closely with *Trigonocarpus*. The close correspondence between the integument of the two was emphasized by Scott and Maslen,[3] for in both it consists of three similarly differentiated layers. The outer fleshy layer of *Trigonocarpus* was lacunar, a condition which may well have led to the formation of mucilage passages, and also in this layer cells similar to the tannin cells of Cycads occur. The sclerotesta was differentiated into two regions of cells such as are found in many Cycads. Considering the vascular system—both have an outer supply of a constant number of unbranching collateral bundles and an inner, much branching system, which in *Trigonocarpus* directly enclosed the nucellus, and in Cycads lies usually in the plane of fusion of nucellus and integument. In both cases these bundles are small and consist only of a group of tracheides with little or no phloem.

[1] Stopes, loc. cit., 1904.

[2] Scott, loc. cit., p. 649.

[3] Scott and Maslen: Structure of the Palaeozoic Seeds *Trigonocarpus Parkinsonii*, &c. Annals of Botany, vol. xxi, 1907.

The great point of difference between *Trigonocarpus* and the Cycad seed is in the degree of union of nucellus and integument, but this does not seem to be a serious obstacle in the general argument. Oliver [1] has pointed out that the free or fused character of the nucellus is probably determined by the method of growth of the ovule, and one assumes that in these two seeds the region of growth in the one case has been apical, in the other basal. Such a difference of growth, then, would account for the greatest feature of dissimilarity between the seeds of *Trigonocarpus* and the Cycads. The general structure of the ovule of *Bowenia*, therefore, in respect to the integument and vascular supply, as well as the pollen-chamber, points to a very close connexion with seeds of the *Trigonocarpus* affinity.

In conclusion I should like to express my thanks to Professor W. H. Lang, F.R.S., for the greater part of the material on which this investigation is based, the facilities he has given me in his laboratory, and his help and criticism during the progress of the work.

Summary.

1. The general development of the ovule of *Bowenia* is found to be similar to that of other genera of Cycads.

2. The embryo-sac arises from the lowest of a row of three cells, which are differentiated from the mass of sporogenous tissue. Free nuclear division proceeds in the embryo-sac, and during the subsequent growth the sporogenous tissue serves as a nutritive layer. This layer is used up by the time the prothallus is formed, i. e. at an earlier period than in other genera of Cycads.

3. The pollen-chamber forms by a solution of the tissue in a region of the nucellus previously recognizable by the arrangement of its cells. It has been distinguished into *upper pollen-chamber*, which developed first, and serves as a storage place for the pollen on entering the ovule, afterwards becoming hard and shrivelled; and the *lower pollen-chamber*, a larger cavity forming later into which the pollen-grains pass, and where they develop the spermatozoids.

4. The male gametophyte consists of two prothallial cells and a large terminal, generative cell with large nucleus and a pair of blepharoblasts. From this one stage observed it is evident that development is similar to that described in *Zamia*.

5. The vascular supply of the ovule is derived from one foliar bundle of the sporophyll, and consists of an outer ring of seven to nine unbranching bundles, collateral with mesarch xylem, which lie in the outer part of the integument; and an inner series of concentric bundles which branch frequently. The most interesting point of the vascular supply is that in

[1] Oliver: The Ovules of the Older Gymnosperms. Annals of Botany, vol. xvii, 1903.

some cases the bundles of the inner series pass beyond the level of the origin of the integument, into the free portion of the nucellus.

6. There is held to be a close agreement of the general structure of the ovule of *Bowenia* with seeds of the Medulloseae.

Cryptogamic Research Laboratory,
Manchester University.
March, 1912.

EXPLANATION OF FIGURES IN PLATE LXI.

Illustrating Miss Kershaw's paper on the Ovule of *Bowenia spectabilis*.

Fig. 1. Mother-cell of the embryo-sac surrounded by cells of sporogenous tissue. × 150.

Fig. 2. Mother-cell of the embryo-sac just before the reduction division. × 150.

Fig. 3. Mother-cell of the embryo-sac divided into three cells, the lowest being slightly larger than the others. × 150.

Fig. 4. Three divisions of the mother-cell of the embryo-sac, the lowest being much larger than the other two. × 150.

Fig. 5. Embryo-sac cell with two nuclei, and the degenerating sister cells above. × 150.

Fig. 6. Embryo-sac with free nuclear division proceeding. × 150.

Fig. 7. Apex of the nucellus of an unpollinated ovule of size represented in Text-fig. 4, showing the median strand of cells which later break down to form the pollen-chamber. × 70.

Fig. 8. Portion of embryo-sac and surrounding sporogenous tissue from a similar ovule. The sporogenous tissue is reduced to a few layers of scattered cells round the embryo-sac, which has a thin lining of protoplasm containing the free nuclei. × 150.

Fig. 9. Sporophyll from a pollinated cone bearing two ovules. Natural size.

Fig. 10. Transverse section of a vascular bundle of the outer series, showing collateral structure and mesarch protoxylem. × 200.

Fig. 11. Micropylar region of old ovule, showing the relation of upper and lower pollen-chamber to the micropyle. The stone layer of the integument is seen, and also the sclerized cells immediately surrounding the micropyle. × 30.

Fig. 12. Section from an old ovule at the level where nucellus and integument become free. The terminal tracheides of a vascular bundle of the inner series are seen to run for a short distance into the free portion of the nucellus. *int.* = integument; *nuc.* = nucellus. × 200.

Fig. 13. Transverse section of a vascular bundle of the inner series, showing the concentric structure and mesarch protoxylem. × 200.

Fig. 14. Section of the apex of the nucellus from an old ovule, showing the beak-like upper pollen-chamber, and the lower pollen-chamber with a developing pollen-grain, and pollen-tube cut obliquely.

Fig. 15. Apex of the nucellus of an old ovule with five pollen-grains developing in the lower pollen-chamber, two of which show the complete apparatus with central generative cell (g) and two prothallial cells (p_1 and p_2). In two cases one of the pair of blepharoplasts is seen. × 100.

Fig. 16. Central cell showing a large nucleus with single nucleolus and a pair of blepharoblasts. × 300.

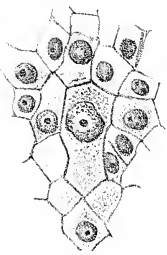
Fig. 17. Neck cells of an archegonium from an old ovule, in transverse section. × 150.

Fig. 18. Median longitudinal section of apex of archegonium, with central cell and nucleus. Close to the nucleus are two small masses of a similarly staining substance. × 150.

Fig. 19. Portion of central cell of archegonium with the surrounding pitted membrane and jacket layer. Haustoria-like processes of the egg pass into the pits which show no closing membrane. × 300.

Fig. 20. Archegonium of young abnormal ovule with central cell and nucleus. × 150.

Fig. 21. Apex of nucellus of young abnormal ovule, showing narrow beak-like upper pollen-chamber (*u.p.c.*) and the larger and wider lower pollen-chamber (*l.p.c.*). × 60.



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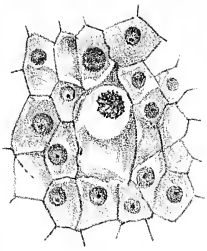

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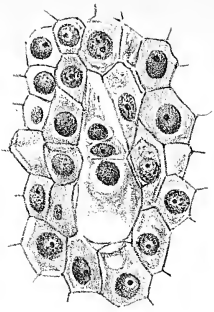

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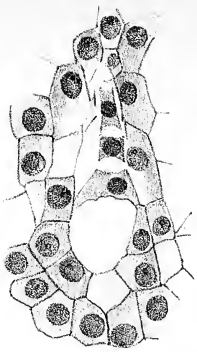

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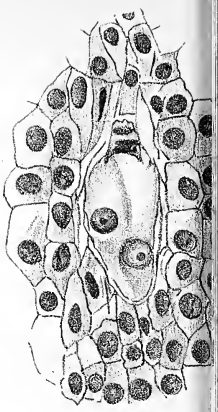

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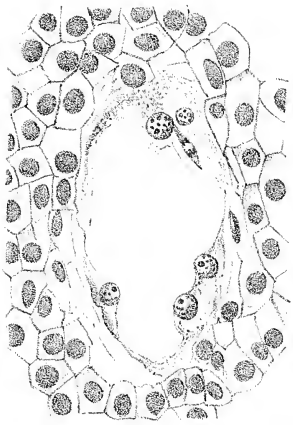

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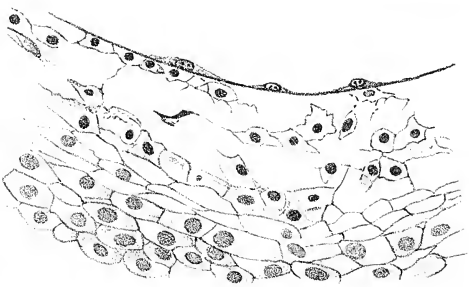

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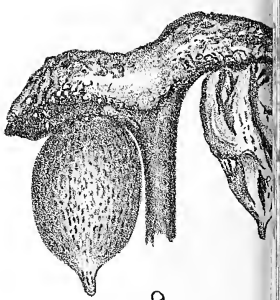

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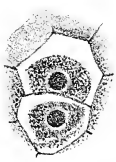

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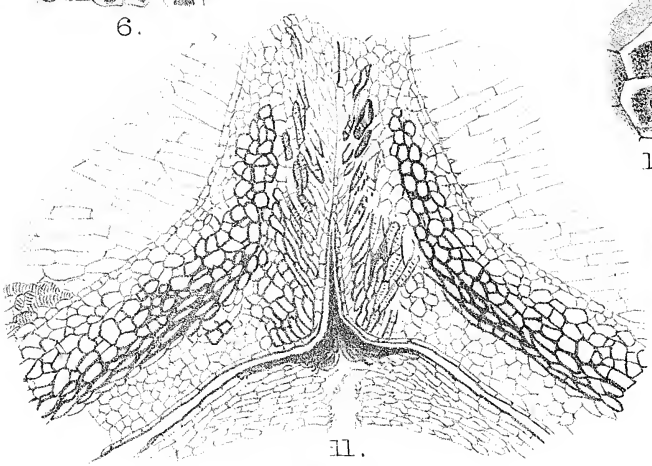

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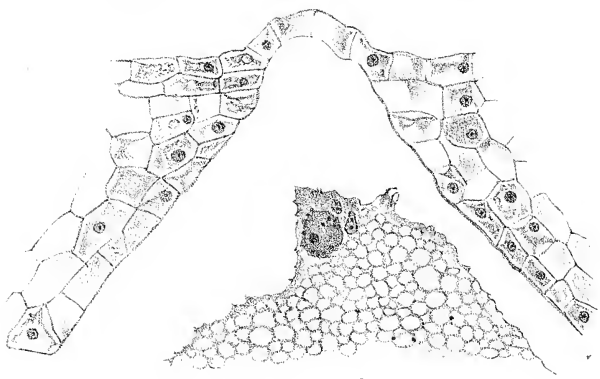

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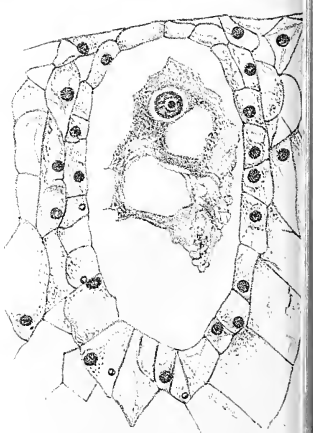

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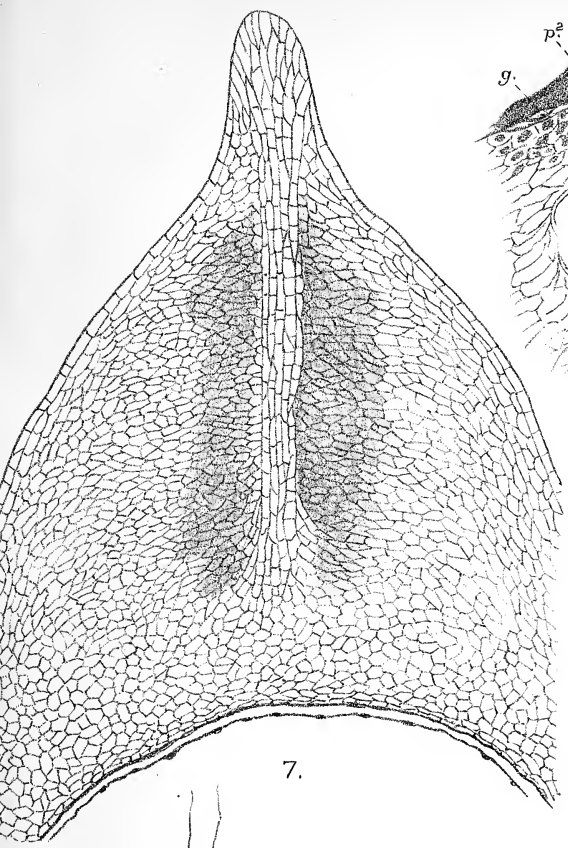

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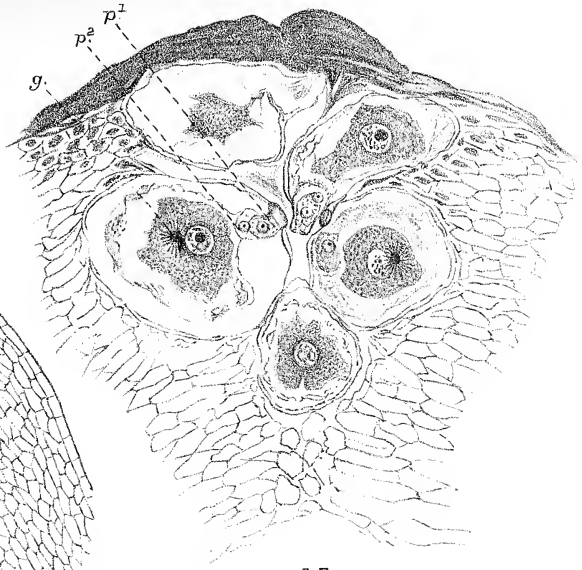

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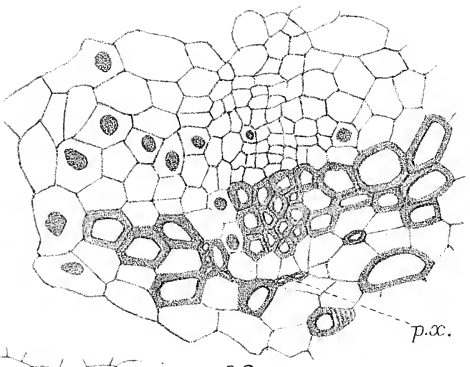

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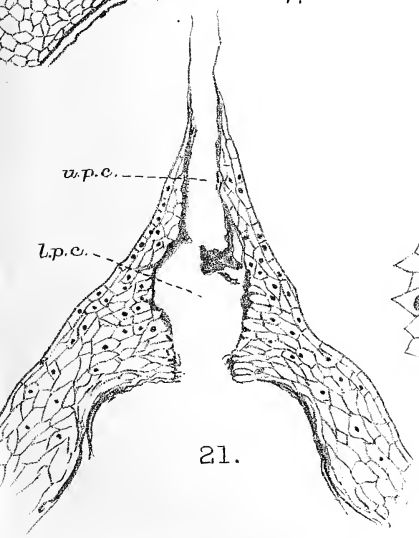

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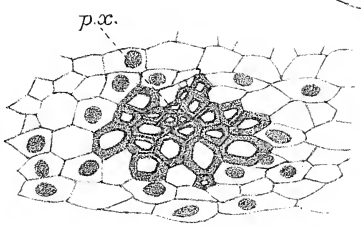

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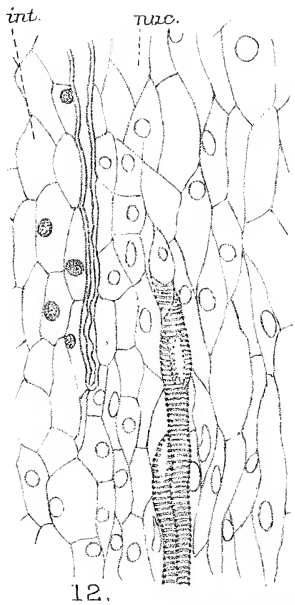

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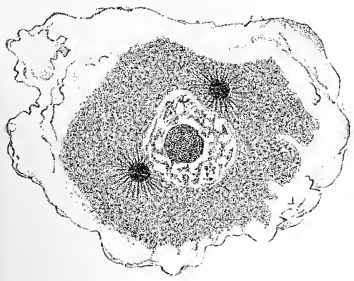

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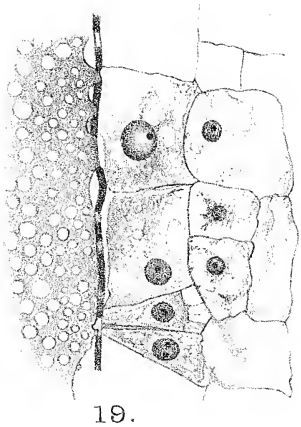

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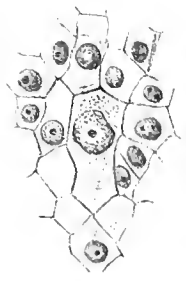

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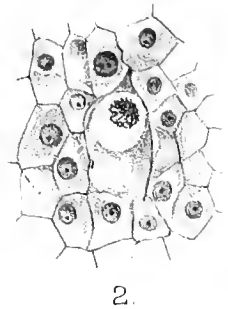

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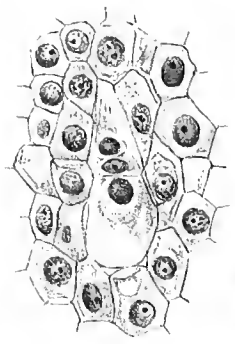

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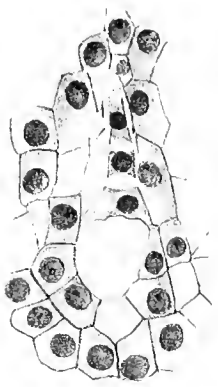

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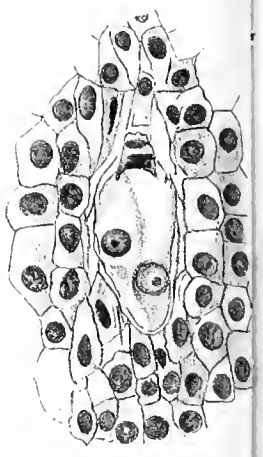

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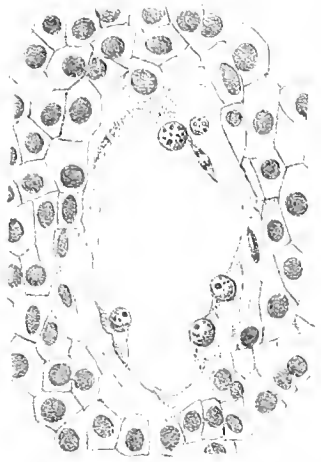

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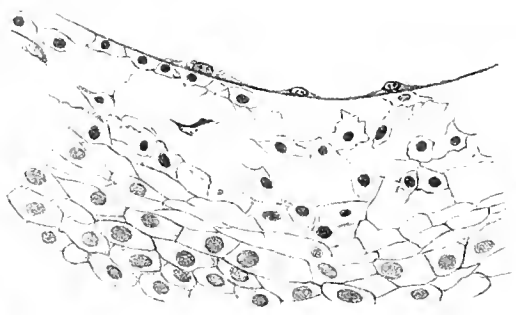

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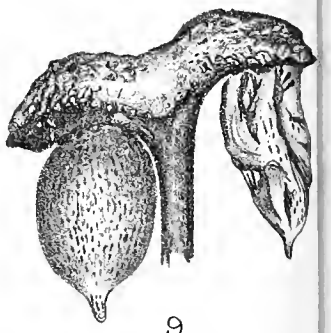

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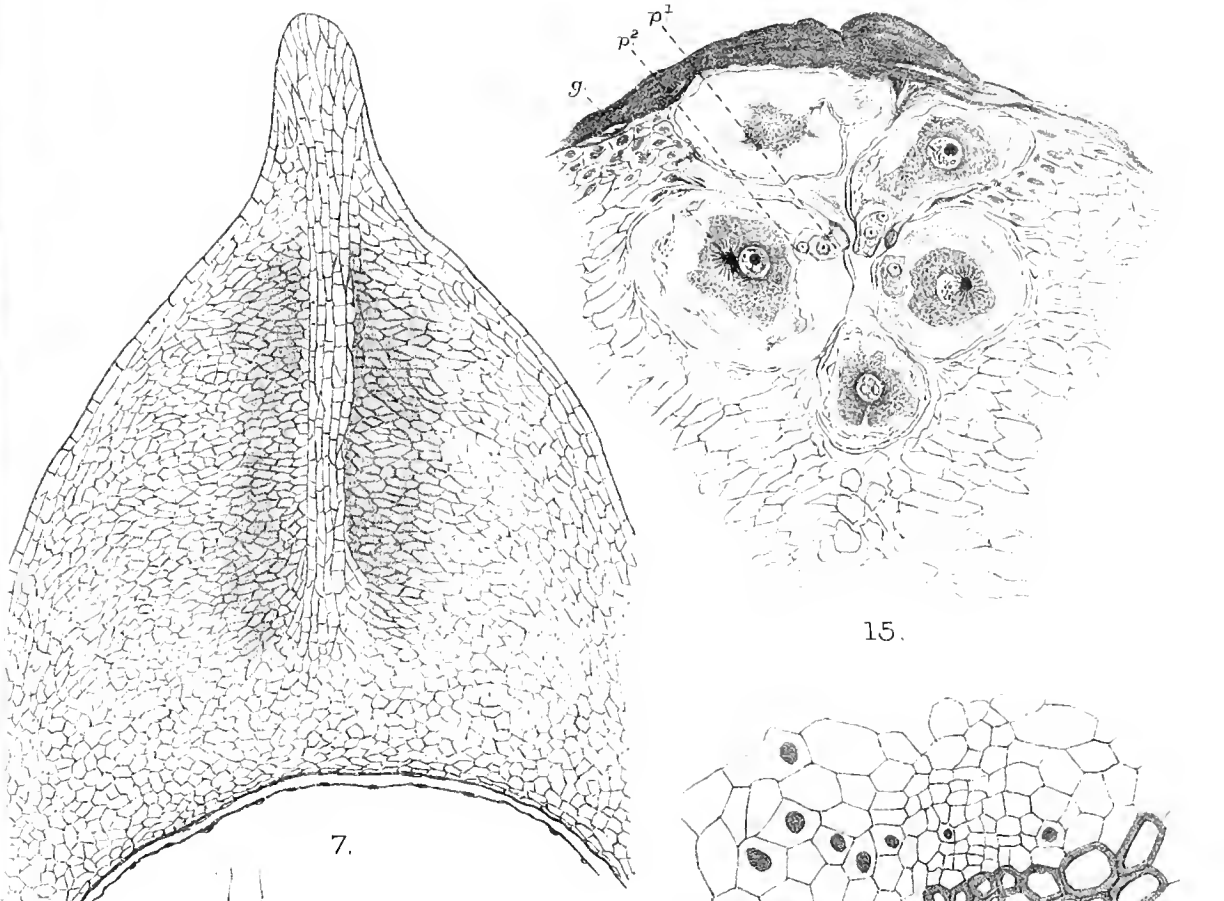

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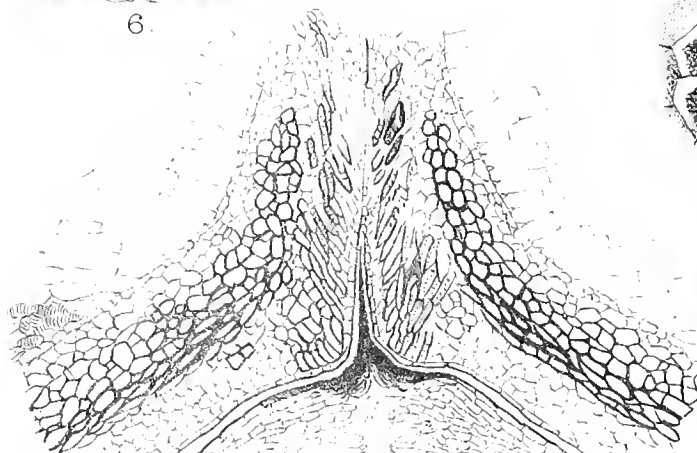

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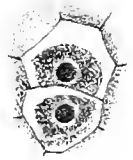

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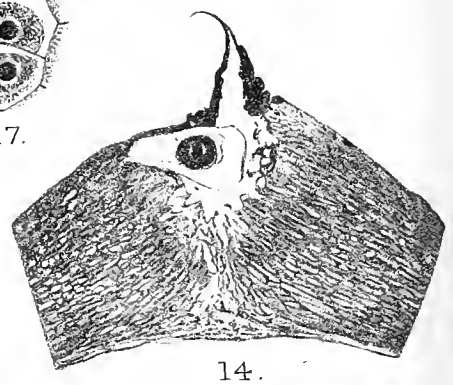

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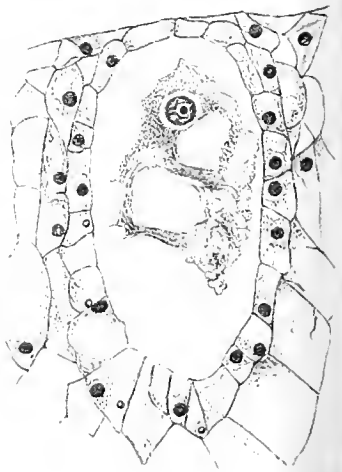

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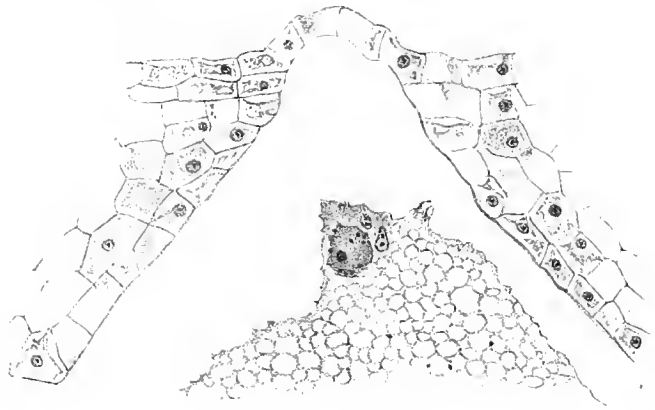

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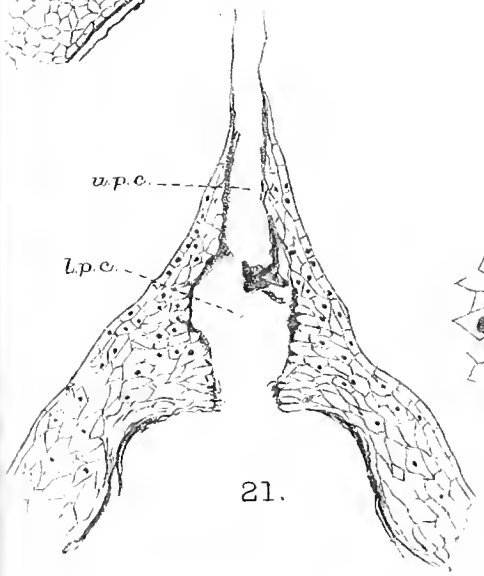

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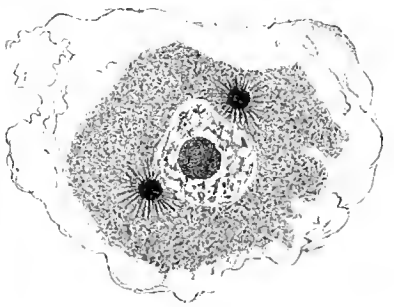

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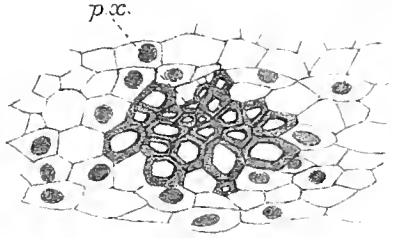

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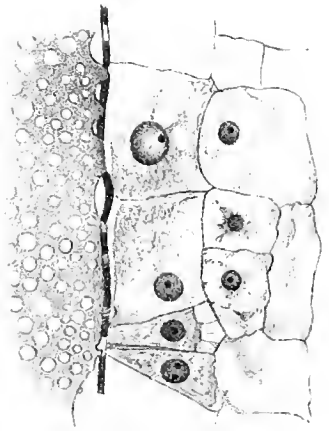

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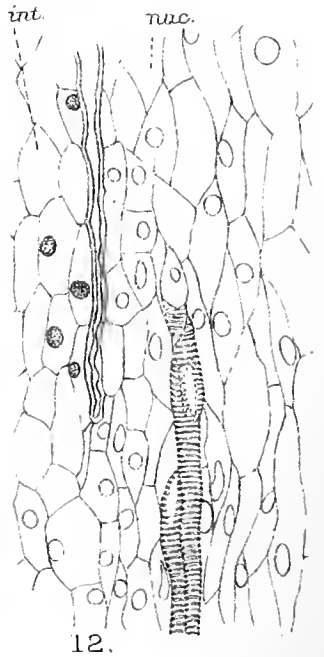

12.

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KERSHAW — BOWENIA.

The Evolutionary History of the Foliar Ray in the Wood of the Dicotyledons: and its Phylogenetic Significance.[1]

BY

IRVING W. BAILEY, A.B., M.F.

Assistant Professor of Forestry at Harvard University.

With Plates LXII and LXIII.

Evolution of the Foliar Ray.

In 1909 Mr. Eames (4) and the writer (1, 2, and 3) conducted a series of investigations upon the broad rays and so-called 'false rays' of the Fagales. The conclusions drawn from these studies have since been published, and may be summarized briefly as follows:

1. Oaks from the Miocene of California are characterized by possessing 'false rays', such as are a distinctive feature of the wood of a large number of living representatives of the Betulaceae.

2. The mature wood of certain Live Oaks of America and Japan possess large rays which are composed of congeries of smaller rays.

3. *Alnus rhombifolia*, Nutt., and *A. maritima*, Muehl, develop in the older wood large homogeneous sheets of ray parenchyma homologous to the large rays of oaks with deciduous foliage.

4. The earliest formed secondary wood of seedling oaks and alders possesses solely uniseriate rays, such as are a characteristic feature of coniferous plants and many other Gymnosperms.

5. In the mature wood of different species of *Alnus* a perfect series of stages occur, which demonstrate the 'building up' of a homogeneous sheet of ray parenchyma from congeries of uniseriate rays and the parenchymatization of fibres included between them.

6. In the development of individual alders and oaks a similar progressive series of compounding stages occur in passing from the younger to the older portion of the stem.

7. Wood which possesses highly developed types of compound ray tissue when injured severely reverts to the primitive uniseriate condition,

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 47.

[Annals of Botany, Vol. XXVI. No. CIII. July, 1912.]

and in subsequent growth a recapitulation of the compounding process occurs.

8. Upon the basis of the comparative and developmental anatomy of living and fossil plants and the phylogenetic significance of seedlings and traumatic regions, the uniseriate ray appears to be the primitive type of ray structure in the Fagales, and the large sheets of ray tissue, either of the homogeneous or aggregate type, have developed from them by a process of aggregation and fusion.

In subsequent investigations by the writer (1, 3) additional evidence was secured in regard to the origin and development of these structures and their effect upon the stem.

1. Small twigs of *Quercus*, *Alnus*, *Carpinus*, and *Betula* revealed upon the removal of the bark a striking and diagrammatic relation between the sheets of aggregate or compound tissue and the traces of the leaves.

2. A study of the development of ray structures in the Fagales made by means of transverse and tangential serial sections cut through the seedling, young and mature twigs, and large stems, showed the leaf to have been the stimulating influence in the formation of these large sheets of storage tissue.

3. The latter originated as aggregations of uniseriate rays in the immediate vicinity of the leaf-traces, and have been 'built up' and extended vertically and horizontally considerable distances from the node.

4. The large sheets of aggregate or compound tissue which may be called *foliar rays*, in view of their origin about the entering trace, have an important effect upon the development of the stem, since their rate of growth is in most cases less rapid than that of the rest of the xylem (Pl. LXII, Fig. 2). This is generally expressed by a strong retarding influence upon the growth of adjacent radii of lignified tissue and produces a marked 'dipping in' of the outline of the annual rings in their vicinity (Fig. 2).

5. The retarding influence of foliar rays is most diagrammatically expressed in small mature twigs of oaks with deciduous foliage, in the stems of certain highly specialized Angiosperms of vine-like and semi-herbaceous habit, and in the fluted trunk of the Blue Beech, *Carpinus caroliniana*, Walt. In the case of the Blue Beech large groups of approximated foliar rays of the 'false type' produce by their retarding effect upon growth the large furrows which are a characteristic feature of the bole of this tree, the ridges corresponding to the segments in which foliar rays are feebly developed or absent. In the mature twigs of oaks with deciduous foliage there are strongly developed foliar rays related to the lateral traces of the leaves. These lateral leaf-trace rays extend downwards through several nodes, and owing to the phyllotaxy of the plant (see Pl. LXIII, Fig. 15), sheets of storage tissue are formed which are relayed from node to node in ten more or less continuous vertical lines along the stem. As is shown in

Fig. 15 the ten sheets of ray tissue are distributed in five pairs of approximated rays. This approximation of the lateral leaf-trace rays enables their concentrated retarding influence upon growth to depress the included segment of tracheal tissue below the general contour of the stem (Pl. LXII, Fig. 3, and Pl. LXIII, Figs. 13, 15). In addition to the lateral leaf-trace rays foliar rays are developed in oak and other plants in relation to the median traces of the leaf. However, in oak these rays are in most cases less strongly developed in the young twig than are the rays related to the lateral traces.

6. The origin and development of foliar rays show clearly that the statements of Sanio, Sachs, and de Bary, pointing to the origin of large rays as inclusions of fundamental tissue or ground parenchyma between putative fibro-vascular bundles and the development of so-called fascicular and interfascicular segments from supposed fascicular and interfascicular cambiums, lead to extremely misleading conclusions.

7. All segments of the stem are essentially 'fascicular', since in the seedling plant of primitive Dicotyledons the stele is an undivided tubular cylinder without indication of the putative fascicular and interfascicular segments and large rays.

8. The development, in relation to the traces of the leaves, of large sheets of storage tissue from congeries of uniseriate rays has a strong dissecting effect upon the stele, producing the supposed fascicular and interfascicular segments of oak, vines, and semi-herbaceous plants.

Eames (5), in an investigation of herbaceous and semi-herbaceous Angiosperms, secured interesting evidence which indicates strongly that herbaceous Angiosperms have been derived from forms which possessed strongly developed secondary growth. In the life-history of many plants there occurs a transition from a tubular stele, in younger portions of the plant, to a ring of separate fibro-vascular bundles in subsequently formed parts of the stem. For example, the prostrate biennial or perennial stems of *Potentilla palustris*, Scop., as well as the seedling plant, possess an unbroken central cylinder, whereas the cylinder of the erect annual stem a short distance above the rhizome breaks down into a typically herbaceous ring of separate bundles. The herbaceous type of central cylinder has resulted, therefore, from the reduction in size of Dicotyledonous plants in later geological periods, coupled with the evolution of foliar rays whose dissecting effect has been progressively increased.

More recently Professor Groom (6) has published the results of an investigation upon the annual ring and medullary rays of *Quercus*. There are certain fundamental objections to the conclusion reached by Professor Groom, that 'it is impossible at the present to decide whether in *Quercus* the broad-rayed or the narrow-rayed type was primitive'.

In studying lines of evolutionary modification in plant structures it is essential not only that a careful study be made of the comparative anatomy

of normal mature plants, but in addition an intensive study is necessary of the development of structures during the life-history of individual plants. Such developmental studies should be made to cover numerous species selected from as many genera and families as possible. Furthermore, since ancestral characters are known to persist in the seedling, reproductive axis, leaf, and first-formed portion of vigorous mature shoots of plants which have suffered vegetative reduction, and to be recalled in traumatic regions of plants which have been similarly reduced, it is essential that these regions be examined with particular care. Finally, palaeobotanical material, when available, must be regarded as an invaluable check upon conclusions of phylogenetic significance reached from living material.

It is unfortunate that Professor Groom's conclusions are based upon rather narrow foundations. The material used by him consisted evidently almost exclusively of mature oak wood, a large part of which was composed of American species cut from a collection in the possession of this Laboratory. An extensive study of the development of ray structures in the life-history of different species of oak (such as was made by Eames in the case of certain vines and species of the Rosaceae, Ranunculaceae, and other families, and by the writer in the case of many species of the following genera of the Fagales, *Quercus*, *Pasania*, *Alnus*, *Betula*, *Corylus*, *Carpinus*, *Ostrya*, *Castanea*, *Castanopsis*, and *Fagus*) was not attempted by Professor Groom. Nor did he examine those regions of the plant which retain ancestral characters.

In view of these facts certain criticisms by Professor Groom of the results secured by Eames and the writer are of interest. Professor Groom states: 'From the point of view of the assumption that the seedling is a seat of phylogenetically early structure features, these facts lose their significance if it be shown that a similar "linking up" of small rays to form large ones takes place in older parts. I find that such a "linking up" does take place, at least in connexion with some of the rays of the inner rings of the twigs of *Quercus Robur*, L.'

It is necessary at this point to emphasize the important fact that the foliar ray has reached varying stages of aggregation and fusion in different species of the Fagales. In *Alnus japonica*, Sieb. et Zucc., *A. rhombifolia*, *A. maritima*, and many evergreen oaks the foliar ray is of the *aggregate type* composed of congeries of smaller rays more or less imperfectly fused. The first-formed wood of the seedling possesses solely uniseriate rays, and the development of foliar rays is a progression in the direction of compounding. In most oaks with a distinctly deciduous foliage and in certain somewhat ring-porous evergreen oaks from the western and south-western part of the United States, the foliar ray is of the *compound type*, composed largely of a nearly homogeneous mass of parenchyma. In the highest types of this group the fused or compound condition of the foliar ray occurs except

in primitive parts, e. g. the seedling. In passing from these regions to subsequently formed portions of the stem the development of the foliar ray tissue is a progression in the direction of compounding. The significant facts to be kept in mind are that in *Quercus* the first-formed wood of the seedling possesses solely uniseriate rays, the development of foliar rays is a progression in the direction of compounding, and more primitive stages of the 'linking-up' process occur in the young plant. The 'linking up of small rays to form large ones in the first rings of the twigs of *Quercus Robur*' does not invalidate seedling evidence, since in this species the solidifying of the foliar ray may not have worked back to the region of the pith in the early wood of the stem and branches.

An unfortunate difficulty has been introduced by Professor Groom: that the broad solid ray may be primitive in oak, since it apparently undergoes subdivision in many cases. In view of the progression in the direction of compounding which occurs in the development of ray structures in the life-history of oak, alder, and other genera of the Fagales it is difficult for the writer to concur in this turning of the *foliar compound ray* inside out. That the foliar ray is dissected into shallower sheets of ray tissue in passing horizontally from the young to the older portions of a thick stem is true for all species of the Fagales. This is as true for species in which foliar rays are of the aggregating type as for those higher types in which nearly homogeneous masses of fused ray tissue are related to the traces of the leaves. In 'exogenous' stems which are increasing rapidly in circumference a multiplication of elements, fibres, vessels, parenchyma, and rays must occur with added layers of growth. Therefore the dissection of foliar rays is essential to maintain the proper proportion of ray tissue in the older zones of the stem. However, the impulse which produces this dissection of foliar rays is fundamentally different from that which results in the evolution of storage tissue in relation to the traces of the leaves, and cannot be considered evidence for believing that the broad, solid type of ray is primitive in *Quercus*. Both phenomena are often active in the same foliar ray. For example, in *Alnus rhombifolia*, *Quercus virginiana*, Mill, or *Q. densiflora*, Hook. and Arn., foliar rays of the aggregate type are gradually 'built up' in passing from the vicinity of the leaf-trace to the mature portion of a wide stem, yet a dissection of this aggregating mass of tissue takes place in the older wood, resulting in the formation of 'lower' and *more numerous* sheets of compounding tissue.

In this connexion Professor Groom's conclusions in regard to '*Quercus* (*Pasania*) *fenestrata* (*Q. spicata*)' are of interest: 'In *Q. fenestrata* these characteristic high rays are often approximated in pairs, and between the two rays forming a pair the boundary of the annual ring is much nearer the centre of the stem than it is elsewhere (Pl. LXXXV, Fig. 12). Moreover, the space between the two rays forming a pair is devoid of vessels. These

three sets of facts denote that in some way the pair of high rays, together with the tissue between them, is equivalent to a single broad ray; and the very considerable inward dip of the annual ring between the two rays finds simplest explanation in the assumption that in the ancestor the complex mass represented solely ray tissue.' This conclusion is somewhat remarkable in view of the facts pointed out by the writer in a former paper, that the depressed segments of the oak stem are produced (see Pl. LXII, Fig. 3, and Pl. LXIII, Figs. 13 and 15) by the concentrated retarding effect upon growth of two closely approximated foliar rays. Since these rays are usually related to the traces of different nodes it is difficult to conceive of their origin from the dissection of a single large ray. The writer recently examined material of *Quercus spicata*, sent from India by courtesy of Mr. R. S. Pearson, Imperial Forest Economist, and is unable to agree with Professor Groom in stating that vessels are absent from the depressed segments included between approximated pairs of foliar rays. Professor Groom for some unexplained reason completely ignores the important relation of the leaf-trace to the formation of large rays, although he refers to the paper by the writer in which this evolutionary process was described.

Since conducting the investigations which have been summarized earlier in this paper a more comprehensive study has been made of Dicotyledonous plants. This investigation has included not only numerous species of the more primitive or supposedly primitive families, but also of the higher or supposedly more recent families. The material used comprised not only species from the temperate regions of North America, Europe, China, and Japan, but in addition representatives of a more tropical flora from India and Australasia. In connexion with instruction upon the anatomy of American woods material of practically all American trees and larger shrubs has been examined. This investigation has confirmed strongly the conclusions reached in regard to the origin, development, and dissecting effect of foliar rays, and has served to emphasize the important part that these structures have played in the evolution of modern Angiosperms. As might naturally be expected in viewing this evolutionary movement from a broader and clearer standpoint, certain obscure and imperfectly understood features have been clarified.

That the evolution of the foliar ray occurred in the early history of Angiosperms is seen from the fact that the most convincing evidence of the compounding process is confined largely to primitive Dicotyledons or to regions of phylogenetic significance, and from the fact that in the majority of woody Angiosperms this primitive type of foliar ray has been changed structurally or completely reduced. This conclusion is further emphasized by the occurrence of well-developed foliar rays in Angiosperms of the upper Cretaceous (11).

In the light of recent investigation the foliar rays appear to have

developed as aggregations of uniseriate rays about the persistent leaf-traces of evergreen plants of the warmer Mesozoic times. These sheets of storage tissue were subsequently extended increasingly greater distances from the leaf-traces, and gradually compacted by the coalescing and enlargement of the uniseriate rays and the parenchymatization of fibres included between them. Rays of this primitive type which occur side by side with uniseriate rays have persisted to the present time in certain of the more primitive Dicotyledonous families, e.g. Fagales, Casuarinaceae.

Diffusion of the Foliar Ray.

With changes of environment during later geological periods the primitive foliar ray was modified in the evolution of the majority of modern trees and shrubs. This change consisted in the diffusion of the constituent smaller rays of the aggregate sheet of foliar ray tissue. Evidence of this transition, as has been shown by Thompson (12), has persisted in certain Dicotyledons and is particularly well shown in the Casuarinaceae and Ericaceae. In certain species of *Casuarina*, e. g. *C. equisetifolia*, L., *C. glauca*, Sieber, &c., the typical primitive foliar ray of the aggregate type exists in the younger portion of the stem, but in passing to the older portion the individual rays of the aggregation are diffused throughout the wood. A similar condition exists in *Rhododendron punctatum*, Andr., *Ledum groenlandicum*, Retz., and *Kalmia angustifolia*, L. In somewhat higher types, *Fagus atropunicea*, Sud., and *Platanus occidentalis*, L., the foliar rays have been diffused throughout the wood and evidence of the former congeries has been lost. However, serial transverse and tangential sections show that the diffused parts of the former aggregate ray are related to the leaf-traces. It is to be emphasized in view of the conclusions drawn by Groom from Jost's (8) study of *Fagus sylvatica*, L., that the so-called broad, high rays of *Fagus* represent diffused and enlarged portions of former *foliar aggregate* rays. In the majority of higher Dicotyledonous trees and shrubs the process of diffusion is so complete that evidence of the former connexion between the multiseriate rays and traces of the leaves has disappeared entirely.

Reduction of the Foliar Ray.

In the wood of a large number of Dicotyledonous plants the foliar ray, either in its aggregate, compound, or diffused form, occurs with the primitive uniseriate ray. There are, however, numerous exceptions to this general rule. Thus the uniseriate ray is seen to disappear entirely from the wood of certain highly specialized Angiosperms (5). Furthermore, individual species of many families of Dicotyledons are characterized by possessing only uniseriate rays. In Pl. LXII, Fig. 1 is illustrated the tangential section of the mature wood of the common American Chestnut, *Castanea dentata*,

(Marsh) Borkh. It will be observed that the wood is characterized by the primitive type of ray structure. A similar condition is found to exist in other members of the Cupuliferae. Thus the mature wood of *Castanea pumila*, (L.) Mill, *Castanopsis hystrix*, A.DC., *C. Indica*, A.DC., *Alnus acuminata*, H.K.B., *A. mollis*, Fernald, *A. yasha*, Matsum, &c., possess characteristically only uniseriate rays. In view of the fact that the first-formed wood of primitive Dicotyledonous plants possesses only uniseriate rays and the foliar ray has been 'built up' from congeries of small rays, it might naturally be supposed that the plants under consideration possessed the primitive type of Dicotyledonous wood from which have been evolved the higher and more complex forms. However, before accepting this supposition as a correct one, a more detailed examination of their structure is necessary. The fallacy of inferring that less complex plants are of necessity more primitive has in recent years been clearly illustrated by the study of the comparative anatomy of living and fossil plants. The pines, which, owing to their complex structure, have been commonly considered the most recent of Coniferous plants, have been shown to be very old geologically, and the supposedly primitive Cupressineae are now known to be of comparatively recent origin, and to have been evolved from more complex ancestors. In other words, in the evolution of living plants may be traced the gradual reduction and disappearance, as well as the origin and development, of specialized tissues. Among Coniferous plants the reduction of resin canals and ray tracheides can be observed in the Abieteae, Cupressineae, and Taxodineae, as well as the evolution of ray tracheides and wood parenchyma in the Pineae. Similarly, among Araucarian plants there exists the reduction of resin canals and Abietineous pitting. It is, therefore, to be expected that among living Dicotyledons reduced forms occur with less complex structures than those of more primitive forms from which they have been derived, and an examination of those regions which are known to retain ancestral characters is essential in determining their real phylogenetic position.

First-formed Wood of Vigorous Mature Twigs.

In Fig. 4 is illustrated the cross-section of a vigorous mature shoot of *Alnus mollis*. It will be observed that in the first annual ring are numerous large rays of the foliar type. These rays gradually die out in the second and third annual layers of growth, and the wood of the older portion of the twig possesses only the uniseriate rays characteristic of the mature wood of the species. In the first annual ring of twigs of normal growth the large rays are absent and the wood resembles that of the mature stem. Since the phylogenetic importance of the first annual ring of vigorous branches of plants which have suffered vegetative reduction has been pointed out by Jeffrey (7) in the recapitulation of resin

canals in *Sequoia washingtoniana*, (Winsl.) Sud., it is to be inferred that the foliar ray has undergone a process of reduction in the alder under consideration. Furthermore, since similar conditions exist in other lower Dicotyledons which possess only uniseriate rays in the mature wood, the conclusion is reached that a considerable number of plants, although not in reality primitive, have reverted by the reduction of the foliar ray to a type of ray structure which characterized primitive Angiosperms.

Vigorous Mature Roots.

Fig. 6 illustrates a cross-section of a vigorous mature root of *Castanea dentata*. Six more or less feebly developed large rays (*x*) radiate from the clusters of protoxylem. A similar condition is illustrated in Fig. 7, a cross-section of a vigorous root of *Alnus yasha*. In this root five much reduced aggregate rays (*x*) are seen to radiate from five clusters of protoxylem. In view of the phylogenetic importance of vigorous mature stems the persistence of large rays in vigorous mature roots is significant.

The Node.

In primitive plants which possess well-developed foliar rays these structures originate in the vicinity of the leaf-trace and gradually 'build up' and enlarge in passing from the earliest formed wood of the node to that of the more mature portions of the plant. Fig. 5 illustrates a cross-section of the node of *Alnus yasha*. The foliar ray, which is strongly developed near the leaf-trace and in the first annual layer of growth, gradually disappears in the older wood. This reduction rather than compounding of ray tissue, which occurs also in *Castanea*, *Castanopsis*, *Alnus mollis*, and *A. acuminata*, indicates, as do the vigorous ramifications, that the foliar ray was once well developed in these plants and has gradually disappeared except from regions which are known to retain primitive characters. The retention of the foliar ray at the node is to be expected, since ancestral characters have been shown by Scott, Jeffrey, and others to persist in the vicinity of the leaf. The nodes of vigorous mature stems are, therefore, particularly favourable regions for the recapitulation of primitive features, and retain indications of the existence of foliar rays after they have disappeared from the rest of the stem.

Traumatic Regions.

Valuable evidence confirming the phylogenetic importance of the recapitulation of primitive characters in the regions just mentioned is afforded by a study of the traumatic reactions of the wood of these plants. It has been pointed out above that severe injuries, in wood which possesses well-developed foliar rays, produce a reversion to primitive stages of aggregation and fusion or to the uniseriate condition. From

this it might naturally be inferred that injuries to the wood of these reduced plants would recall the foliar type of ray. That such is indeed the case is shown by Fig. 8, a cross-section of the wood of *Alnus mollis*, cut in the immediate vicinity of a severe injury. The injury has obviously produced a large aggregate ray which may be clearly recognized by its retarding influence upon the growth of neighbouring elements and the distinct sag in the outline of the annual rings. A similar condition is illustrated in Fig. 9, a cross-section of the mature root of *Alnus mollis*. The wood at the lower side of the photomicrograph, which was formed before the injury occurred, possesses only uniseriate rays, but in the upper portion of the figure may be seen numerous aggregate rays which originate at the injury. Additional illustrations of the traumatic recurrence of large rays have been observed by the writer in *Castanea*, *Castanopsis*, and *Alnus*, as well as in woods which have replaced the compound or aggregate by the diffuse type of foliar ray. Fig. 10, a cross-section of the outer portion of an insect gall in a mature twig of *Ostrya virginiana*, (Mill) Koch., illustrates the recurrence of an aggregate ray in a type of wood that possesses normally only small bi- and triseriate and uniseriate rays.

Contour of Mature Twigs.

There remains for consideration a striking piece of evidence in regard to the reduction of the foliar ray in lower Dicotyledonous plants. A characteristic and important feature of the foliar ray is its retarding influence upon the growth of neighbouring radii of the stem (see Figs. 2 and 3). This influence is so strongly developed in many plants that the depression in the outline of the annual rings which marked the former position of the foliar ray persists for some time after the disappearance of the ray. The writer has shown (3) that the fluted stem of the Blue Beech, *Carpinus caroliniana*, Walt., is produced by congeries of aggregate rays whose concentrated retarding influence upon the growth of certain radii produces the large grooves in the stem. A careful study of this plant shows that the foliar rays of the aggregate type are gradually being replaced by the diffuse condition. (In the closely allied genus *Ostrya* the diffuse condition is dominant.) In specimens growing under unfavourable conditions the writer has found large stems from which the aggregate ray has disappeared almost completely. However, the characteristic flutes remained, although less strongly developed. These plants were later cut down, and during subsequent growth of the stump (by means of stool shoots) congeries of aggregate rays were recalled in the depressed segments of the new layers of growth. The stimulation of the injury and surplus supply of food substances in the root produced evidently a reversion to conditions which existed before the plant suffered vegetative reduction. In Pl. LXIII, Fig. 17, a cross-section of a vigorous mature shoot of *Castanea pumila*,

may be seen five depressed segments. In this twig the foliar ray has disappeared except from the immediate vicinity of the leaf-traces. The normal twig possesses a circular outline, and the foliar ray is not conspicuously developed at the node. This recurrence of depressed segments in vigorous shoots of *Castanea* indicates that five pairs of approximated lateral leaf-trace rays existed formerly in the mature twigs of these plants, and have disappeared except from the nodes of very vigorous stems. In *Castanopsis* indications of depressed segments which were once a well-developed feature of the plant persist in the normal mature twigs. A cross-section of the node of *Castanopsis hystrix* is illustrated in Pl. LXIII, Fig. 16. Two segments (*y*) are more strongly depressed upon the upper side of the photomicrograph, due to the fact that the rays related to the lateral leaf-traces are persistent at the node. Owing to the phyllotaxy of the plant the three remaining segments (*x*) are less strongly depressed. Fig. 14 shows a cross-section of the mature stem of *Castanopsis indica*. In this species the five depressed segments are more persistent between the nodes, and indicate that the reduction of the foliar ray is less complete in this plant than in the preceding species. In Fig. 13 may be seen the cross-section of a mature twig of *Castanopsis tribuloides*, a plant whose structure resembles that of *Quercus*, since the lateral leaf-trace rays are strongly developed in the wood of small twigs. A careful study of the life-history of the plant reveals the fact that the aggregated rays are disappearing in many portions of the root and stem. In Pl. LXII, Figs. 11 and 12 are illustrated the remains of aggregate rays which were once strongly developed by the cambium to maintain the proper proportion of ray tissue in the widening stem and root. These facts, considered in connexion with evidence afforded by the recapitulation of primitive characters which occur in vigorous mature ramifications, nodes, and traumatic regions, point conclusively to the reduction of foliar rays in certain Fagales, and indicate that *Castanopsis* and *Castanea* are reduced members of the oak family, just as *Alnus mollis*, *A. acuminata*, and *A. yasha* must be considered reduced species of the genus *Alnus*.

The uniseriate rayed species of the Salicales (9) and Sapindales (10) have been studied by Miss Holden, and have been found to represent a reduced condition where complex ray structures have been lost in a reversion to the primitive uniseriate type, but among the higher families of Dicotyledonous plants the uniseriate condition has resulted largely from the reduction of foliar rays of the diffused type rather than of the aggregate or compound types.

Summary and Conclusions.

1. The central cylinder of primitive Angiosperms was a tubular cylinder or siphonostele which possessed strongly developed secondary growth.

2. The wood of the most primitive Angiosperms possessed only uniseriate or linear rays, such as are a well-developed feature of the wood of Conifers and other Gymnosperms.

3. During the warmer times of the Mesozoic sheets of storage tissue were 'built up' from congeries of uniseriate rays, about the persistent leaf-traces of evergreen Angiosperms, and were subsequently extended vertically and horizontally considerable distances from the node.

4. This primitive type of foliar ray tissue has persisted, in more or less unaltered form, in certain species of primitive families of the Dicotyledons, e. g. Casuarinaceae, Fagales, &c.

5. With changes of environment in later geological periods storage conditions were fundamentally modified in the wood of Dicotyledons.

6. In the evolution of the majority of living Dicotyledonous trees and shrubs the individual units (varying greatly in size owing to the enlargement and fusion of the original uniseriate rays which formed the incipient aggregation) of the aggregating mass of foliar ray tissue have been diffused more or less uniformly throughout the stem, and evidence of their former relation to the traces of the leaves has disappeared, except from certain primitive forms, e. g. Casuarinaceae, Ericaceae, Fagales, and Platanaceae.

7. In a comparatively limited number of forms the primitive foliar ray of the *aggregate type* has been progressively compounded or solidified, and has resulted in the formation of foliar rays of the *compound type* composed of homogeneous masses of parenchyma, e. g. oaks with deciduous foliage, *Casuarina Fraseriana*, Miq., *Alnus rhombifolia*, &c.

8. In many families of Dicotyledons species exist in which a reversion to the primitive uniseriate condition has occurred as a condition of reduction from foliar rays.

9. Evidence of the reduction of the foliar ray in the Fagales consists of a more or less complete series of progressively reduced species, and of the persistence or recurrence of foliar rays in regions of phylogenetic significance in forms which are very completely reduced.

10. The importance of experimental plant morphology in the study of phylogeny is clearly illustrated by the Fagales. In its later history the family has suffered vegetative reduction. At the same time storage conditions have been fundamentally changed in the family, resulting in modification of the sheets of aggregated ray tissue which originated about the

persistent leaf-traces of more luxuriant ancestors. In modern species the foliar ray of the primitive aggregate type has been or is in the process of being *reduced*, *diffused*, or *compounded*. Rapid accumulation of nutritive substances, such as occurs in mature twigs of unusual vigour or in regions where traumatic stimulation exists, tends to produce a reversion to ancestral structure. Thus in reduced forms the foliar ray is relegated to the first annual rings of vigorous mature roots and shoots. Similarly, in species which have suffered reduction or diffusion, the foliar aggregate ray may be recalled traumatically. Furthermore, species which possess foliar rays of the aggregate or compound type under the stimulus of a slight injury accelerate the 'building-up' process. However, diametrically different results are secured by slow or impaired nutrition. Under conditions of this sort the reduced or diffused condition is hastened by the arrested development of the primitive foliar ray. This is clearly shown in specimens of the Blue Beech which have been grown under unfavourable conditions, and by the fact that the common *Alnus incana* in the northern and colder part of its range has suffered more complete reduction of the foliar aggregate ray than in the warmer southern part. Arrested development due to impaired nutrition occurs also in plants which possess foliar rays that are of the compound or nearly compound type. Thus *severe* injuries in the oak produce a reversion to primitive stages of aggregation and fusion or to the uniseriate condition.

11. *Castanea* and *Castanopsis* are reduced members of the oak family, just as *Alnus mollis*, *A. acuminata*, and *A. yasha* must be considered reduced species of the genus *Alnus*.

12. Owing to the important part that the foliar ray has played in the structural development of Dicotyledons, the study of its evolution and reduction yields evidence significant in a natural classification of Angiosperms.

13. A more detailed consideration of the comparative anatomy and phylogeny of Dicotyledonous plants indicates conclusively that the existing conceptions of the origin and development of the woody cylinder of Angiosperms must be fundamentally modified to agree with actual conditions which exist among the higher seed plants.

In conclusion, the writer wishes to express to Major Gage, I.M.S., Superintendent of the Royal Botanic Gardens at Calcutta, to Mr. G. H. Cave of the Lloyd Botanic Garden at Darjeeling, and to Mr. R. S. Pearson, Imperial Forest Economist at Dehra Dun, his keen appreciation of their courtesy in sending valuable material of Indian plants. To Dr. Jeffrey the writer is much indebted for material and kind assistance in the course of the investigation, and to Mr. James Austin for assistance in photomicrography.

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EXPLANATION OF PLATES LXII AND LXIII.

Illustrating Mr. Bailey's paper on the Foliar Ray.

PLATE LXII.

Fig. 1. *Castanea dentata*. Tangential section of the mature wood, showing the uniseriate rays. × 60.

Fig. 2. *Casuarina*, sp. Transverse section of the mature stem, showing the depression in the outline of the xylem produced by an aggregate ray. × 20.

Fig. 3. *Castanopsis tribuloides*. Transverse section of the mature stem, showing a small segment of wood which is depressed by two closely approximated foliar rays. × 20.

Fig. 4. *Alnus mollis*. Transverse section of a mature vigorous twig, showing numerous rays of the aggregate type in the first and second annual layers of growth. × 20.

Fig. 5. *Alnus yasha*. Transverse section of the node of a mature twig, showing a well-developed aggregate ray in the vicinity of the leaf-trace. The ray is seen to die out in the third annual ring. × 20.

Fig. 6. *Castanea dentata*. Transverse section of a vigorous mature root, showing the retention of aggregate rays. × 20.

Fig. 7. *Alnus yasha*. Transverse section of a vigorous mature root, showing the retention of aggregate rays. × 20.

Fig. 8. *Alnus mollis*. Transverse section of the mature stem in the vicinity of a severe injury, showing a large aggregate ray which has been recalled traumatically. × 40.

Fig. 9. *Alnus mollis*. Transverse section of the mature root, showing aggregate rays recalled by a severe injury. × 20.

Fig. 10. *Ostrya virginiana*. Transverse section of an insect gall in a mature twig, showing the traumatic recurrence of an aggregate ray. The ray produces a sag in the outline of the xylem and is capped in the phloem by a dark-coloured cluster of sclerenchyma. × 100.

Fig. 11. *Castanopsis tribuloides.* Transverse section of the mature wood of the stem, showing the remains of a once strongly developed aggregate ray. × 60.

Fig. 12. *Castanopsis tribuloides.* Transverse section of the mature root, showing evidence of the reduction of aggregate rays in the root. × 60.

PLATE LXIII.

Fig. 13. *Castanopsis tribuloides.* Transverse section of a mature twig, showing the strongly depressed segments produced by well-developed approximated foliar rays. × 10.

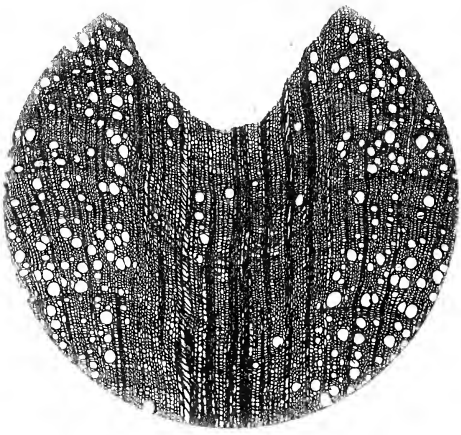
Fig. 14. *Castanopsis indica.* Transverse section of a mature stem, showing the persistence of five well-developed depressed segments. × 10.

Fig. 15. *Quercus velutina.* Transverse section of a mature twig, showing five small depressed segments which are produced by five pairs of closely approximated lateral leaf-trace rays. × 20.

Fig. 16. *Castanopsis hystrix.* Transverse section near the node of the mature twig, showing the persistence of two strongly depressed segments (*y*) which are related to the lateral leaf-traces of this node. Indications of the three other depressed segments (*x*) are more or less feebly developed. × 10.

Fig. 17. *Castanea pumila.* Transverse section of a vigorous mature twig, showing the retention of five depressed segments after the disappearance of the foliar rays. × 10.

1.



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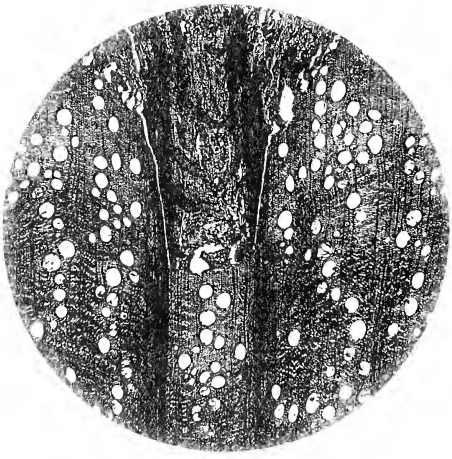

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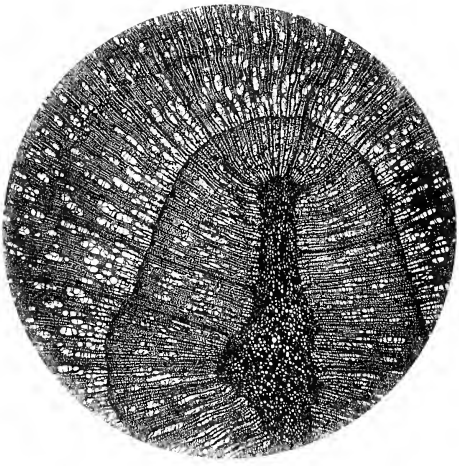

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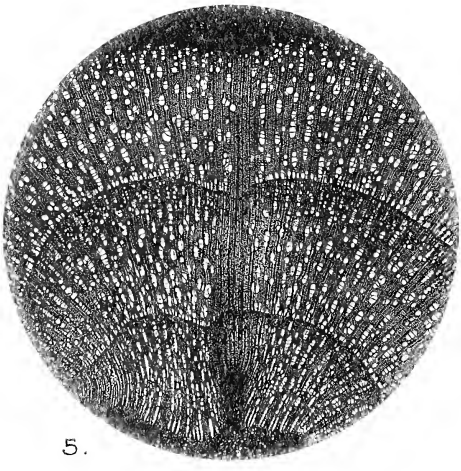

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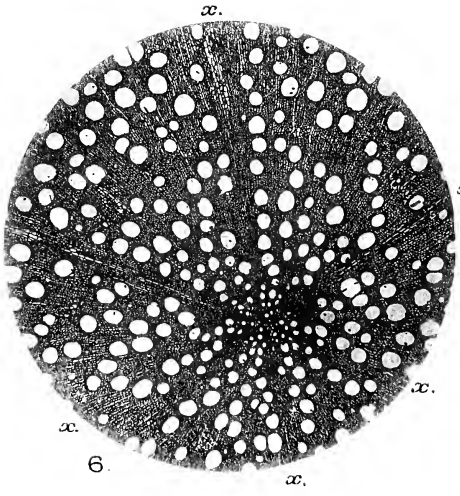

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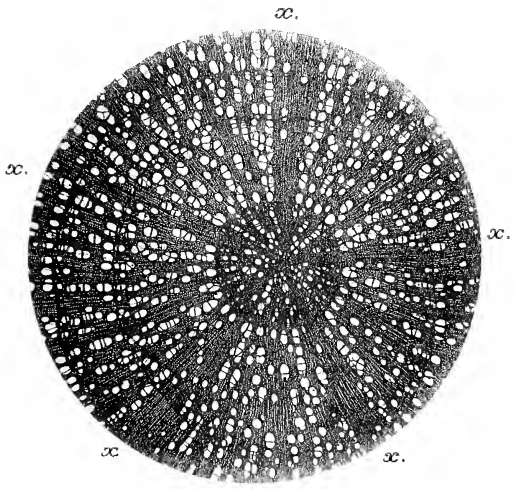

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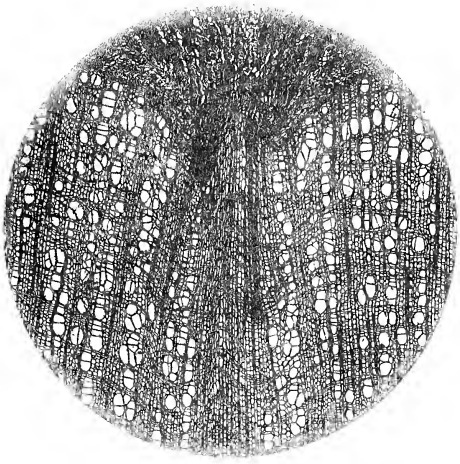

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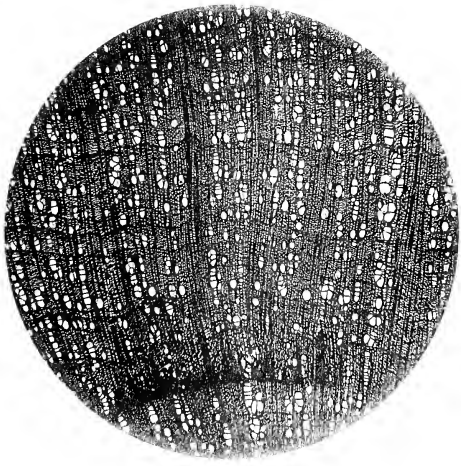

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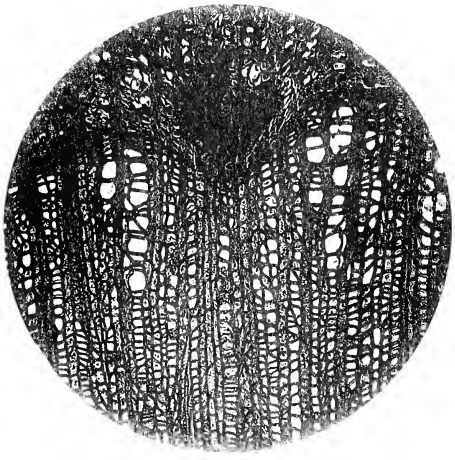

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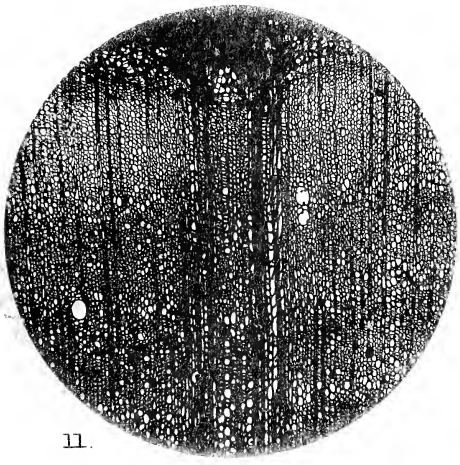

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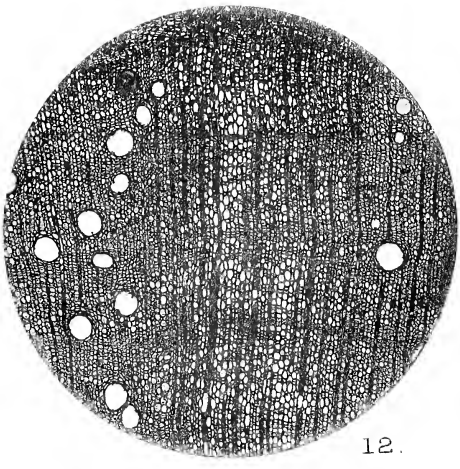

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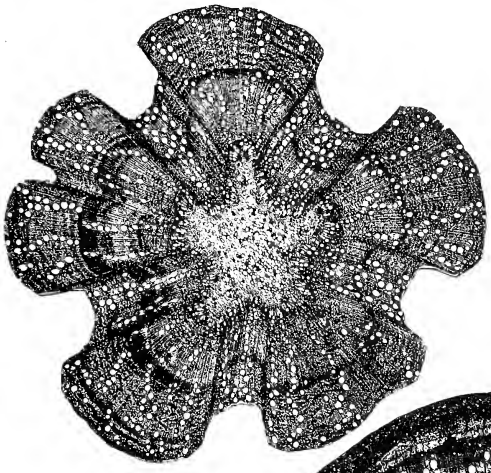

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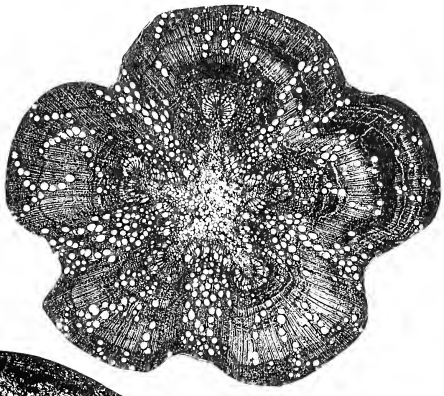

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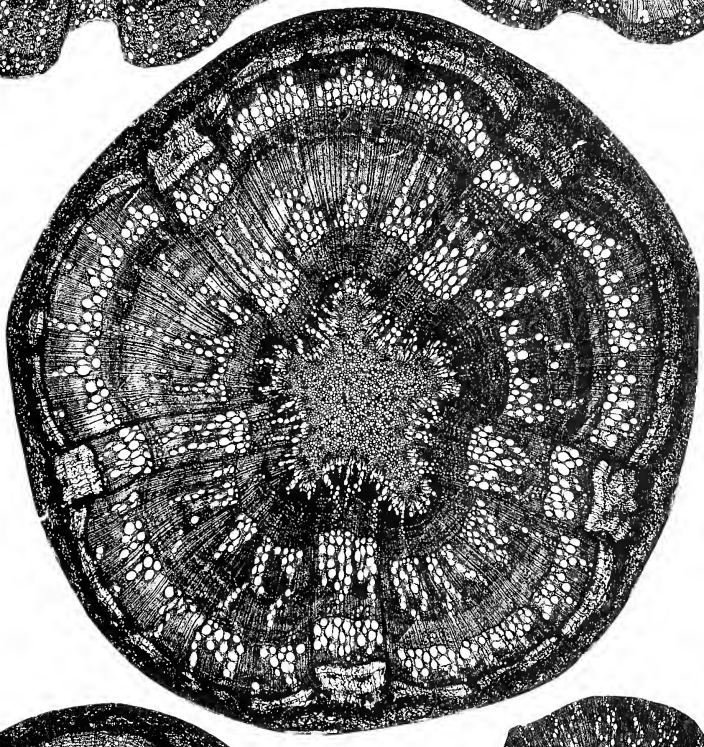

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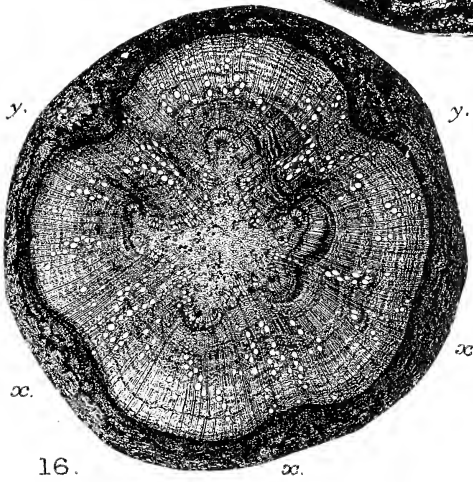

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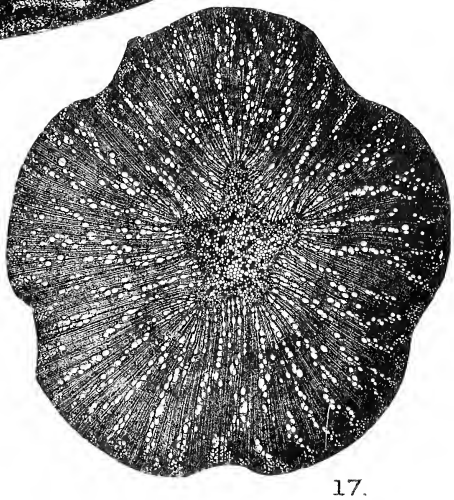

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Contributions to our Knowledge of the Anatomy of the Cone and Fertile Stem of Equisetum.

BY

ISABEL M. P. BROWNE.

With Plates LXIV and LXV and ten Figures in the Text.

Historical Summary.

In 1864 appeared Duval Jouve's 'Histoire Naturelle des Equisetum de France'; this work contained many new and important researches and also gave an excellent summary of the earlier literature concerning the Equisetaceae. In 1865 Milde published his 'Monographia Equisetorum'; in 1867 appeared Pfitzer's account of the structure and distribution of the endodermis in the German species of *Equisetum*; this paper also contains excellent anatomical observations. In 1876 Famitzin and Janczewski, working independently, published more correct accounts of the branching of *Equisetum*; the following year appeared a short paper by Tomaschek, and in 1878 one by Sadebeck on the embryology of the genus. In 1886 Goebel published a short note on the cones of the Horsetails, and in 1888 Buchtien gave a detailed account of the prothallus and young sporophyte. The same year Müller published an account of the leaf-sheaths of the Equisetaceae, written almost entirely from the point of view of resistance to mechanical strains and tensions. In this year, too, Van Tieghem and Douliot treated of *Equisetum* in their paper on the origin of endogenous members, and in 1890 Van Tieghem published additional observations on the endodermis in the genus. Two years later Leclerc du Sablon described the structure of the tubers, and in the following year Cormack investigated the nodal structure of *Equisetum*, describing what he believed to be secondary xylem. In 1893 Bower, in the first of his papers on the Morphology of the Spore-producing Members, dealt with certain features of the Equisetaceae. Campbell's 'Mosses and Ferns', published in 1895, gave a general account of the group, while in 1899 Jeffrey published a paper dealing in detail with the structure and affinities of the genus. In 1901 Gwynne-Vaughan, in a short communication, suggested a fresh interpreta-

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tion of the Equisetaceous bundle; in 1902 a general account of the Equisetaceae was given by Sadebeck in Engler and Prantl's 'Natürliche Pflanzenfamilien'; the same year appeared Chauveaud's account of the anatomy of the root, while the next year the origin and affinities of the Equisetaceae were discussed by Lignier. In 1905 the affinities of the genus were considered by Campbell, and in 1906 Sykes recorded peculiar nodal tracheides in a specimen of *E. maximum*. In 1907 appeared Queva's important paper on the stem and leaf of *Equisetum* and also a short note on the sporangium by Hawkins, while next year was published a second edition of Campbell's 'Mosses and Ferns'. The year 1908 also witnessed the publication of Bower's 'Origin of a Land Flora', in which *Equisetum* is treated at some length, and of Halle's account of the Mesozoic Equisetaceae of Sweden. In the next year Eames published a paper dealing with the anatomy of *Equisetum*, and Jeffrey discussed this subject in a paper entitled 'Are there foliar gaps in the *Lycopsida*?' In this year, 1909, there also appeared a short account of fossil Equisetaceae by Fritel and Viguier, and a note by Stiles on the anatomy of a branched cone of *Equisetum maximum*. In 1911 Ludwigs published an account, largely biological, of the genus, while in 1912 Vidal dealt with the segmentation of the apical cell and the early stages of anatomical differentiation in *E. palustre*.

Material.

The present investigations were confined to the cone and the upper part of the fertile stem in three British species of *Equisetum*: *E. arvense*, *E. palustre*, and *E. limosum*. In the case of the first species two complete cones, henceforward termed A and B respectively, were cut into continuous series of transverse sections. Cone A was very young; it was dug up in October and would, therefore, have remained another five months underground. Nevertheless, the xylem of the cone was almost completely differentiated; this series of sections extended downwards below the cone and included four of the vegetative nodes. Cone B was more or less mature, and the series of sections below it only extended to beneath the annulus. Of a larger cone, C, serial transverse sections were made from below the annulus through the lowest seven whorls of sporangiophores. To confirm the phenomena observed in Cones A and B the upper parts of two cones, D and E, were cut serially; the latter cone, though otherwise normal, had two annuli; satisfactory series through this region of the cone could not, however, be obtained. Another cone, F, also had two annuli, and of this specimen a continuous series of sections extending from below the lower annulus through the three basal whorls of the cone was prepared. The transitional region from below the annulus through the basal whorl of the cone was also studied in two cones: in a very large one, G, the diameter of

the stele of which was about $2\frac{1}{4}$ mm., and in a smaller cone, H. Another cone, I, was cut serially into longitudinal sections. As regards *Equisetum palustre*, a cone which I shall term Cone A was cut serially from above the uppermost vegetative whorl into transverse sections; serial sections extending to below the annulus were made of another cone, B. Of another cone, C, serial sections extending from below the annulus to a point just above the second whorl were made. Cone D, a very small and young cone of the variety *polystachion*, was also cut into serial transverse sections, the series including the uppermost whorl of leaves. Serial longitudinal sections through the base of a young cone were also made.

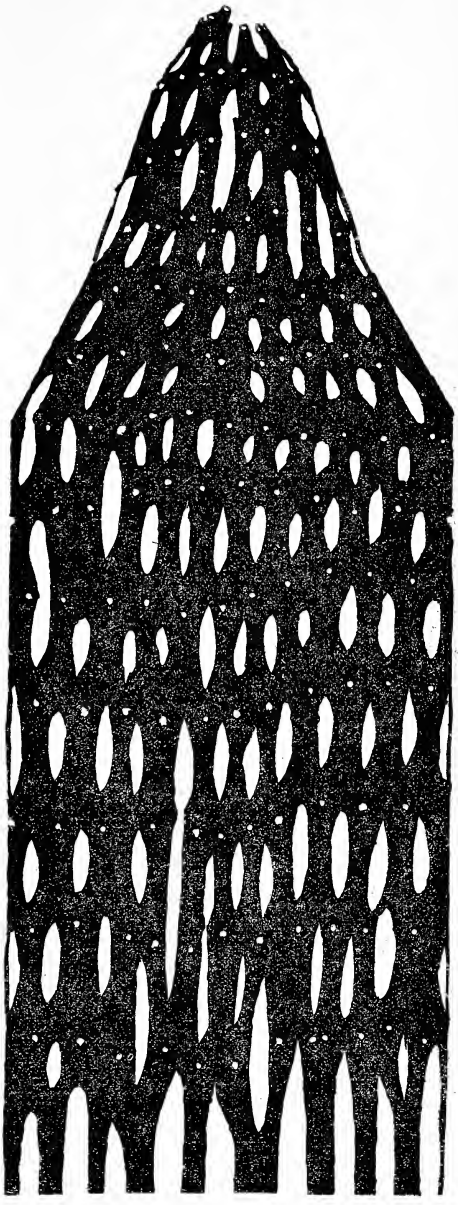
In the case of *E. limosum* a complete young cone, A, was cut into serial transverse sections; these extended downwards to the uppermost vegetative whorl, but did not include quite the whole thickness of the nodal xylem. The whole of a still younger cone, B, with the uppermost vegetative whorl beneath it, was cut up serially into transverse sections. Another series of transverse sections extended from below the uppermost leaf-sheath through the lowest five nodes of an older and much larger cone, C. For all the three species the results obtained were confirmed by numerous longitudinal, transverse, and oblique hand-sections through different cones.

The Cones of E. arvense and E. palustre.

The xylem in the cone of *E. arvense* is relatively much developed compared with that of the cone of *E. limosum*; *E. palustre* is intermediate in this respect between these two species. Taking first *E. arvense* we find that below the basal fertile whorl, but some little distance above the insertion of the annulus, the strands of xylem lose the appearance of typical internodal bundles of the vegetative region. The two lateral groups of metaxylem become united by a curved band of tracheides and the carinal canals begin to diminish in size. This curved band of xylem is several cells thick in a radial direction and its concavity at first faces outwards; its ends, continuous downwards with the lateral bands of metaxylem, project like them slightly beyond and externally to the phloem. The latter also forms a slightly curved band, the concavity in this case facing inwards, so that the phloem lines the interior of parts of the pericycle. As we approach the fertile node the band of xylem straightens out and eventually it comes to form a band slightly curved in the reverse direction, so that the xylem assumes the same contour as the phloem and the stele generally. Meanwhile, the xylem of each bundle begins to narrow in a radial direction, though it may still be locally three to five cells thick. As a rule, too, as we approach a node the xylem of a bundle begins to spread laterally towards the xylem of its neighbours. Generally, many of the bundles of the cone of *E. arvense* become united in the nodal regions by the formation of fresh tracheides; usually these develop in continuity

with the xylem-elements of one or both bundles, until these bundles, spreading towards one another, unite a little higher up; but occasionally an island of tracheides first makes its appearance in the parenchyma between two of these widening strands, and the tracheides of the island increasing in number as we pass upwards, the island becomes united higher up to the neighbouring xylem-strands. In this case the island figures, in a longitudinal reconstruction of the xylem, as a small downward projecting tooth. Such 'interfascicular' xylem has at first no phloem outside it; it not infrequently occupies a slightly more peripheral position than the xylem of the internodal strand. But as we pass upwards phloem very soon makes its appearance; and when this has happened, and when the interfascicular tracheides have joined on to those of the internodal strand (from which they are indistinguishable in form), we get perfectly uniform bands, or in some cases a perfectly uniform ring of xylem. These bands or this ring of xylem is in most parts but one or two cells deep, though locally there may be three or even more cells on the same radius; moreover, in a good many places there are internally to the bands or ring isolated tracheides or little groups of tracheides, usually of small size; these may abut on the bands or ring of xylem, especially where these are relatively thick in a radial direction, or they may be separated from them by as many as three or four parenchymatous cells. Such tracheides or groups of tracheides do not as a rule persist for any considerable distance in a vertical direction; in the internode they occur also internally to the separate strands of xylem (cf. Pl. LXIV, Fig. 3). Some little way above the departure of the xylem of the traces these bands or rings of wood found at the nodes break up, portions of the band or ring ceasing to develop as tracheides. The narrow parenchymatous bands thus formed widen rather rapidly and constitute the parenchymatous meshes seen in a longitudinal reconstruction of the xylem (Text-figs. 1 and 2 and Pl. LXIV, Fig. 4). These meshes are usually situated vertically above traces that have departed (in estimating the superposition of meshes to traces in a longitudinal reconstruction allowance must be made for the narrowing or widening of the diameter of the stele, which in the diagram causes a convergence or divergence of lines passing vertically upwards). A change in the number of members in the next whorl also tends to disturb the superposition of parenchymatous meshes to traces, since the xylem here breaks up into a number of strands different from the number of traces. Other irregularities will be considered later. Usually the parenchymatous meshes arise at a small distance above the traces. A glance at the reconstruction of the xylem in Cone A (Text-fig. 1) will show that the vertical height of the xylem persisting above the trace of a sporangiophore is not very different from the height of nodal xylem persisting above a leaf-trace (allowance must be made for the fact that the apical internodes of this very young cone have elongated even less than the

lower ones). But in some cases a parenchymatous mesh only makes its appearance half-way or more than half-way up the internode (e.g. the meshes appearing above the fourth trace of the first whorl, above the fourth and eighth traces of the fourth whorl, and above the third trace of the fifth whorl of Cone A). In other cases the meshes, though not originating so high up in the internode, yet made their appearance at an unusual distance above the node. In a few cases we pass from a trace of one whorl vertically upwards to the trace of the next whorl without meeting any parenchymatous tissue. Thus we get a relatively wide continuous tract of woody tissue connecting part of one whorl with a portion of the succeeding whorl (e.g. the tract of xylem extending above the fifth and sixth traces of the seventh whorl, that above the third trace of the eighth whorl, and that above the first and second traces of the tenth whorl of Cone A). The extent of such sweeps of xylem, though relatively the same, is of course actually much greater in a mature cone than in so young a one as Cone A. Such absence of one or two meshes throughout an internode is not common in any one cone; but I have found it in all specimens of which I made serial sections of any considerable portion of the cone, even in those that contained relatively little xylem. If, instead of involving one or two xylem-strands of the internode below, these sweeps of xylem extended above all the



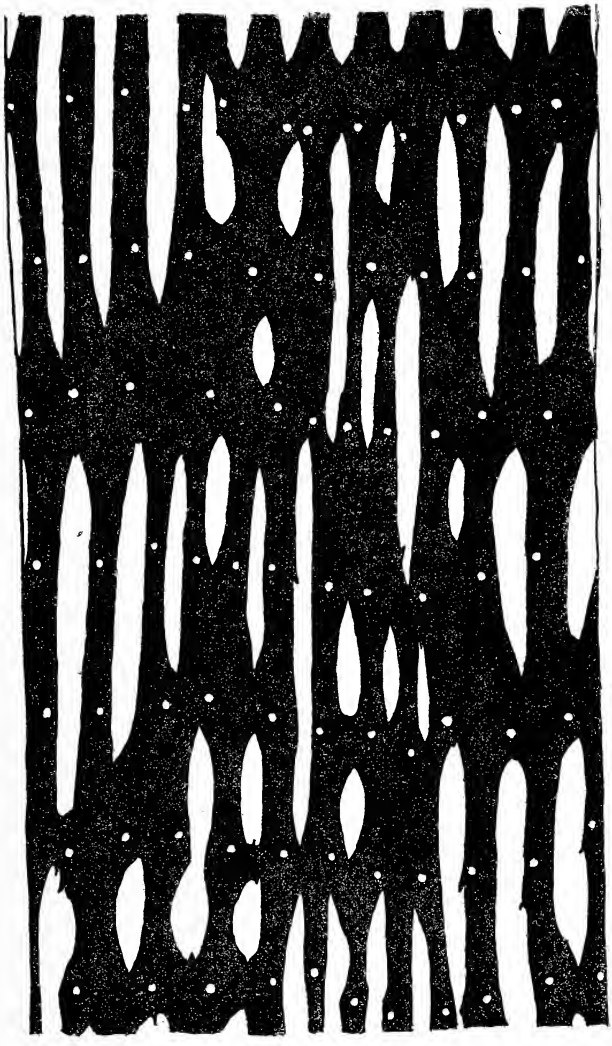
TEXT-FIG. 1. Longitudinal reconstruction of the xylem of Cone A. The xylem of the stele has been cut in two, spread out flat. Axial xylem black; leaf-traces and parenchyma white. Magnification *circa* 16.

traces of a whorl we should have an internodal ring of wood; occasionally, when all the strands become united at the node—and this of course involves the closure of all the parenchymatous meshes of the internode below—we get a nodal ring of xylem and bast (Pl. LXIV, Fig. 1).[1] Such a local ring is present twice in Cone A, twice in Cone B, and once in Cone E; it was not met with in Cones C, D, F, G, and H, but may have occurred in them, since the series of sections did not include the whole of any of these cones. To judge from isolated hand-sections a ring of xylem occurs locally at the nodes of many cones of *E. arvense*. Milde in 1865 stated that at the level of the fertile whorl the xylem-strands become united into a ring. This is too sweeping a generalization, and curiously enough Milde figures this condition diagrammatically in *E. limosum*, a species in which I have never seen even a near approach to a ring (Milde, i, p. 162, Pl. XVI, Fig. 18).

The cases in which a parenchymatous mesh arises some distance up the internode are clearly intermediate between the forms in which the mesh arises at the usual small distance above the node and those rare cases in which no parenchymatous mesh occurs above a trace. The parenchymatous meshes of *E. arvense* do not, as a rule, attain any considerable width; they are frequently narrower, even in their widest part, which is usually in the middle of the internode, than the strands of xylem between which they pursue their course. As regards their vertical extent, by far the greater number are closed at or before the next node, and these meshes I shall, for convenience' sake, term meshes of the first order. Other parenchymatous meshes that extend into two, three, or more internodes will be termed meshes of the second, third, and higher orders. In Cone A there were 90 meshes originating above fertile whorls and closed before we reach the apex of the cone; of these 81 were of the first, 8 of the second, and 1 of the third order. Within the limits of Cone B 37 meshes originated and were closed: 33 of the first, 3 of the second, and 1 of the third order. As only seven whorls of Cone C were cut serially many of the meshes were still unclosed when the series ended; if the figures of all the meshes closed within the series of sections were given, the proportion of meshes of the first order would appear to be larger than it is in nature; for, of the meshes arising above the upper whorls, more of those of the first order would have space, so to speak, to be closed before the end of the series. But all meshes of all orders that arise above the lowest four whorls are closed before the end of the series of sections; by considering these only we may see the relative proportion of meshes of different orders in this Cone C. On this basis we find that out of 22 meshes 10 are of the first, 9 of the second,

[1] Owing to the fact that the sporangiophores are inserted at slightly different levels, all the meshes may be closed at a node and yet no *single* transverse section will show a complete ring of xylem. This tendency is increased by the fact that occasionally meshes are not closed before, but at, or even just after, the departure of the trace.

and 3 of the third order. It will easily be realized that the persistence of a parenchymatous mesh through a node is the result of a poorer development of xylem. Thus Cone C has relatively less xylem than Cones A and B. For instance, the presence of a very little more xylem between the



TEXT-FIG. 2. Longitudinal reconstruction of the xylem of Cone C of *E. arvense*. Axial xylem black; leaf-traces and parenchyma white. Magnification *circa* 12.

fifth and sixth traces of the second whorl of Cone A would lead to the division of a parenchymatous mesh of the second order into two meshes of the first order. When the xylem developed at a node is less markedly deficient, the mesh that persists appears to be sharply constricted in the nodal region; when, on the other hand, the strands show little or no

increase of xylem at the node, the mesh separating them is little or not at all constricted in this region.

Of none of the other cones were the series of sections sufficiently extensive in a vertical direction to afford useful data as to the height of the meshes; Cone E, however, seemed to have about the same relative amount of xylem as Cone C, and Cone F relatively rather less. The proportion of xylem in Cone G seemed to be about the same as that in Cones A and B. The series of sections of the other cones were too short to allow of such generalizations.

The nature of the meshes originating below the lowest whorl of sporangiophores will be considered in discussing the region transitional from cone to fertile stem.

Even a superficial glance at a reconstruction of the xylem of a cone of *E. arvense* in which the wood is relatively well developed, as it is in Cone A, gives the impression of nodal bands or a nodal ring of xylem, broken up in the internodes by meshes of parenchyma and usually broken too at the nodes by the persistence of one or two of these meshes. The cone of *E. palustre* does not give this impression. Here, too, parenchymatous meshes originate above the sporangiophore traces, but many of them persist upwards through more than one internode. Thus Cone A of this species, which had the largest proportion of xylem, had 19 meshes of the first, 9 of the second, 2 of the third, 4 of the fourth, and 1 of the fifth order; Cone B of the same species had 6 of the first, 7 of the second, 1 of the third, 1 of the fourth, 3 of the fifth, and 1 of the seventh order. Cone D of the reduced variety *polystachion* had only four whorls; it had one mesh of the first, one of the second, and one of the third order. Besides these, other meshes originating below the sporangiophores, and therefore below the cone proper, persist through one or more internodes of the cone; they are relatively more developed in Cones B and D, which have less xylem than in Cone A. Their nature will be considered in connexion with the transitional region. The series of sections of Cone C of *E. palustre* was too short to afford data as to the height of the parenchymatous meshes.

In *E. arvense* the sporangiophore traces are never, except at the extremely reduced apical region of the cone, given off from the edge of a strand or band of xylem, though when a relatively wide parenchymatous mesh persists through a node, the traces of the strands abutting on it may depart from a point very near to the edge of the xylem. In *E. palustre*, however, correlated with the less vigorous development of the axial xylem, traces not infrequently arise from the edge of the strand; i. e. at their point of origin they abut on a mesh persisting through the internode, while many other traces depart from a point very near the edge of a xylem-strand (cf. Text-figs. 3 and 4). These kinds of traces may conveniently be referred to as lateral or slightly internal. Traces that depart from what is more or

less the middle of a xylem-strand may be termed median. When the internodal strands remain isolated the median position of the traces is very obvious, but when the bundles are united into bands or into a ring at the nodes, as they often are in *E. arvense*, the median position of the traces is often not so clear (Pl. LXIV, Fig. 1). Such formation of nodal bands giving off median traces also occurs in *E. palustre*, though it is not so marked a feature of this species (e. g. the band from which the third, fourth, fifth, and sixth traces of the first whorl depart in Cone A, and the band giving rise to the fifth, sixth, seventh, and eighth traces of the fifth whorl of the same cone). In reality we find three sorts of structure above median traces. Firstly, there is the form in which the xylem of the axis is most developed ; here there is no mesh, and a relatively wide band of xylem persists through the internode ; this type, never common, occurs in *Equisetum arvense*, and apparently in the upper part of some cones of *E. palustre*. Secondly, a parenchymatous mesh may appear above a median trace ; this is by far the most widely spread form in *E. arvense* ; it is very common too in *E. palustre*, especially in cones with well-developed xylem. The third form of structure is associated with a reduction of axial xylem ; here the trace departs from the middle of a relatively narrow strand ; this often continues its isolated course upwards, and then of course involves the persistence of meshes on either side of it ; above such a trace no mesh is formed. When the meshes on both sides of a strand persist through several internodes the latter pursues an isolated course through several nodes (e. g. the strand arising between the sixth and seventh traces of the fifth whorl of Cone A of *E. palustre*). These conditions are obviously associated with poor development of the xylem. A variation in the behaviour of the strands in the internode above the departure of a median trace of this type occurs when one of its neighbours gives off a median trace of the other type, above which trace there appears a fresh parenchymatous mesh ; or in other

TEXT-FIG. 3. Longitudinal reconstruction of the xylem of Cone A of *E. palustre*. Axial xylem black ; leaf-traces and parenchyma white. Magnification *circa* 9.

words, when two neighbouring strands give off median traces and one forks a little higher up and the other does not. In this case one of the branches of the forking strand may fuse with the strand in which no mesh appeared. This particular variation, fairly common in *E. limosum*, seems to be very rare in *E. palustre*, where I only observed it twice. Another variation which may occur, though rarely, in *E. palustre*, associated with a reduction in number of the strands and traces of the next node and internode, consists in the fusion of two strands, from which median traces not subtending meshes have departed, to form a strand giving rise to but one trace at the next node (e. g. the strands giving rise to the sixth and seventh traces of the ninth whorl of Cone A). In all these variations the absence of a mesh is due to poor development of xylem, and is associated with the persistence of meshes of the internode below. In the cases mentioned first the absence of a mesh is due to the unusually strong development of xylem in the upper internode, and is associated with closure of meshes of the internode below.

TEXT-FIG. 4. Longitudinal reconstruction of the xylem of Cone D of *E. palustre* (var. *polystachion*). Axial xylem black ; leaf-traces and parenchyma white. Magnification *circa* 24.

In *E. palustre* no fresh meshes originate above the departure of a lateral or slightly internal trace ; for though in many cases the xylem-elements superposed to the trace soon die out, the parenchyma which replaces them is laterally in contact with an adjacent parenchymatous mesh originating lower down. The relatively wide band of parenchyma resulting from the congenital fusion of a mesh with one originating lower down may, from a phylogenetic point of view, be considered as biseriate. When the meshes in the cone of *E. palustre* persist through numerous internodes they appear in longitudinal reconstruction to be widened first on one side and then on the other ; this sudden widening usually occurs above a node by the dying out of the xylem superposed to a trace at or near the edge of one of the strands bordering on the mesh. This gives to the parenchymatous meshes of the higher orders a very sinuous outline. As in *E. arvense*, it is sometimes easy to see that the persistence of a mesh into two or more internodes is due to poor development of xylem at the nodes. For instance, the lignification of a small group of cells between the first and second traces of the third whorl and the

fourth and fifth ones of the fourth whorl of Cone A would have closed the meshes between these traces, and we should in both cases have had two meshes alternating, though somewhat irregularly, with one another. In many cases the amount of xylem present at the node, though not so great as in the two cases mentioned, yet produces a marked constriction of the mesh in the nodal region; where there is no perceptible increase of the xylem in the nodal region the mesh remains of the same width at this level. Another phenomenon, only observed once in *E. palustre*, occurs at the apex of Cone A of this species. Here two meshes originating above the tenth whorl fuse in the upper part of their course by the dying out of the strand between them. The conditions are here complicated by the fact that the strand, by the dying out of which the two meshes come into contact, is unusually wide, and gives off two traces; it is clearly one of those stretches of xylem that extend uninterruptedly through a whole internode. Allowing for the convergence of the lines of superposition of the diagram (Text-fig. 3) owing to decrease in diameter of the stele, we can see that the two convergent meshes originate above the third and sixth traces of the tenth whorl.

Another tendency when the xylem is less developed is for a parenchymatous mesh to be slightly decurrent on one side and below the trace above which it may, phylogenetically, be considered to have originated; in such a case a trace-bearing strand appears to branch just below the departure of a trace. Such a trace would have been median and have subtended a mesh had no premature branching of the strand bearing it occurred; it now appears to be lateral and adnate to one of the branches of the strand. This expression of less lignification is common in *E. limosum*, and an example of it may be noted at the eighth node of Cone A of *E. palustre*, where a mesh, apparently associated with the fourth trace, is decurrent some way below the latter and the third trace. (This mesh might at first sight appear to be between the third and fourth traces, but this appearance is due to the fact that the third trace is very nearly lateral; and as the middle line of the mesh is very nearly directly over the fourth trace the mesh presumably belongs to the latter.)

Only one clear case of the decurrence of a parenchymatous mesh was observed in *E. arvense*, and it was of a different type. The second whorl of Cone B of this species had nine traces, of which two were about half the size of the others and entered one sporangiophore. The smallness of these two traces and the fact that the whorl below and the three succeeding whorls each have eight sporangiophores indicate that we are here dealing, not with two fused sporangiophores, but with one of those cases, to be considered later, in which the first division of the vascular supply of the trace is so premature that the trace is double at its point of departure from the axial stele. Such cases of traces originating as two bundles are not rare, but this

one is exceptional in that the two bundles of the trace are not closely approximated at their point of origin, but are separated by a parenchymatous mesh; the two bundles destined to the sporangiophore do not even depart from the edges of the two strands bordering the mesh, but from points slightly internal to the edges of the axial strands. The middle line of the parenchymatous mesh when followed upwards passes midway between the two xylem-strands entering the sporangiophore; below them it only extends downwards through one-third of the internode, and its origin is clearly quite unconnected with the departure of the traces of the whorl below. The conclusion to be drawn would seem to be that here a single mesh, such as commonly appears above the two halves of a bifascicular trace, has, phylogenetically speaking, slipped down between them for a certain distance.

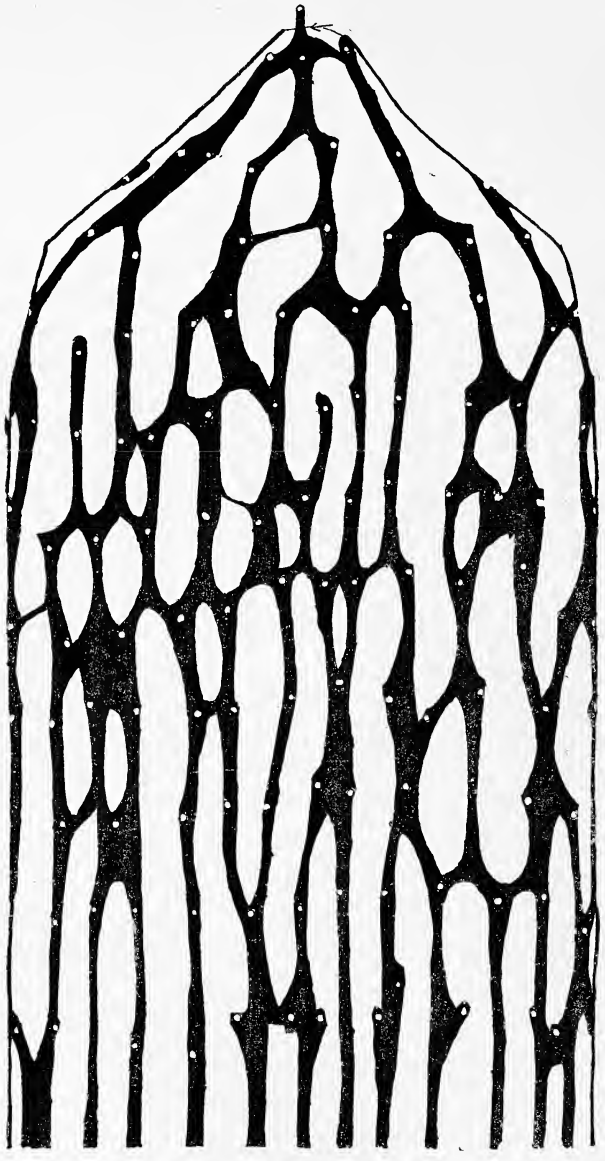
Quite apart from the diminution in number of the sporangiophores of a whorl, due to the narrowing of a cone at its apex, there is a certain irregularity in the number of members in successive whorls of the cone of *Equisetum* (e. g. the seventh and eighth whorls of Cone A of *E. palustre*). Moreover, traces receiving two bundles are by no means rare. Two forms may usually be distinguished. In the first case the sporangiophore receiving the two bundles is very much larger than the other sporangiophores, and obviously represents two approximated and concrescent primordia. Every stage between a complete concrescence and a merely basal fusion of the stalks can be observed with the naked eye if sufficient material is examined, especially of *E. arvense* and *E. maximum*, in which species concrescence is unusually frequent. In this form the two traces, though they usually arise markedly nearer to one another than to the neighbouring traces, are yet not closely approximated at their point of origin. In the second kind of sporangiophore that receives two traces these arise very close to one another, and the sporangiophore itself is hardly, if at all, larger than the other sporangiophores; generally either of the two traces considered by itself is markedly smaller than the other traces of the whorl. In this case we would seem to be dealing with a case in which the division of the trace is so premature that the latter actually originates as two separate strands. This interpretation is supported by the fact that a certain number of traces, though not completely divided into two bundles at their origin, were more or less deeply constricted in the middle; in this case the division of the trace, though still premature, was less markedly so. Another case intermediate between the traces that originate as two bundles and those that only divide in the sporangiophore was found in the upper part of Cone D of *E. arvense*. Here a trace originated as an already markedly constricted bundle and forked in the axial cortex before entering the sporangiophore. The latter received not only these two bundles, but another whole trace; it was thus a 'double' sporangiophore showing in

one of its two fused constituents a case of premature division of the trace. In some cases, however, it is not easy to decide if we are dealing with a rather large sporangiophore whose trace has divided prematurely, or with an exceptionally small 'double' sporangiophore. The position of the meshes in the internode above and the number of members, when constant, in the whorls above and below may afford the means of judging if we are dealing with a single or a double sporangiophore. In normal cones a bifascicular trace is more often due to premature division.

Cone of E. limosum.

If we pass to a consideration of the cone of *E. limosum* we see at once that the xylem is much less developed than in *E. palustre*. Though this poorer development of the xylem is very striking it follows the same lines as the reduction of xylem in *E. palustre*. Meshes arising above traces tend to persist through more numerous internodes; they are widened laterally owing to the dying out of the xylem above lateral or slightly internal traces; they tend to 'decur' below and to one side of the traces above which they lie; meshes originating separately also become confluent by the dying out of the strand separating them. This feature, only observed once in *E. palustre* and unknown in *E. arvense*, is very common in Cone B of *E. limosum*. This cone has relatively to its size less xylem than Cone A; in the latter only two pairs of meshes originally separate become thus confluent; in both cases this confluence took place above the seventh whorl. In Cone A 31 meshes arose, and were closed within the cone; of these 13 were of the first, 7 of the second, 7 of the third, 3 of the fourth, and 1 of the fifth order; two meshes persisted unclosed to the apex of the cone, and eventually fuse above the last whorl round the terminal sporangiophore. In Cone B 12 meshes arose and were closed within the cone; 4 of the first, 5 of the second, 2 of the third, 1 of the fourth order. Ten more meshes arose above sporangiophores, but these remained unclosed; of these, one arose above the lowest whorl, and therefore persisted through all the (nine) internodes of the cone; one unclosed mesh persisted through eight internodes, two through seven, one through six, two through five, and three through four internodes. Yet another mesh was unclosed; this originated above the insertion of the annulus (cf. the account of the transitional region), and therefore persisted through the whole of the cone. These unclosed meshes become confluent with one another in a very irregular manner as we approach the apex of the cone; above the tenth and last whorl the number of independent meshes is reduced to three. But if traced downwards these three meshes have a very different history; one of them, that lying between the second and third traces of the tenth whorl of the diagram, consists only of two fused meshes extending a very long way downwards; they become confluent by the dying out of a strand that pursued an isolated course

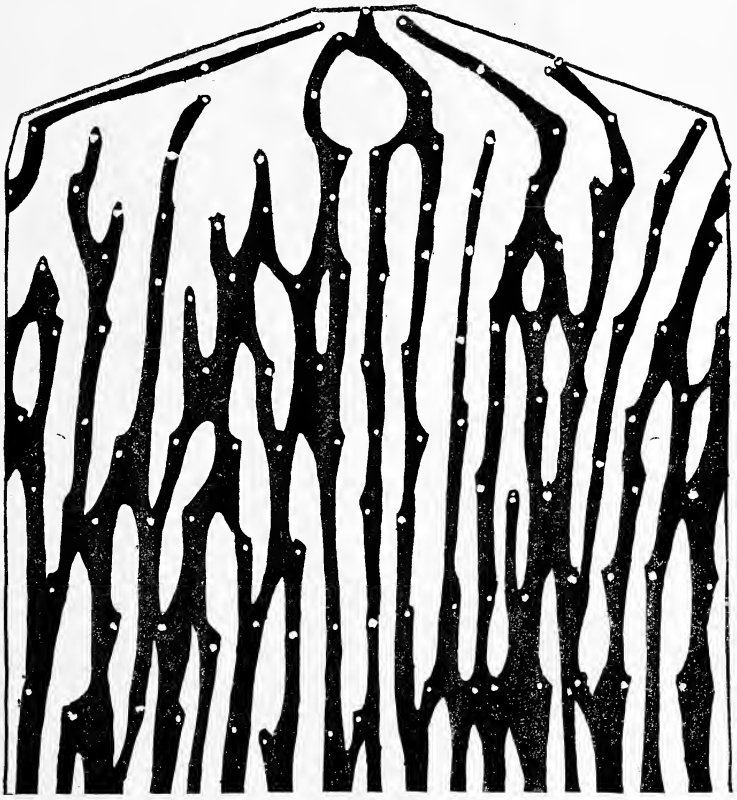
between them through seven internodes. Each of the other two meshes, if traced downwards, is seen to be built up by numerous confluent meshes of very different lengths. Above the three traces of the uppermost whorl the



TEXT-FIG. 5. Longitudinal reconstruction of the xylem of Cone A of *E. limosum*. Axial xylem black; leaf-traces and parenchyma white. Magnification *circa* 16.

xylem dies out, and in this way the three remaining meshes become confluent round the terminal sporangiophore. The confluence of meshes distinct at their origin is not confined to unclosed meshes; it occurs, though

less commonly, between meshes that are closed before the apex of the cone is reached ; the two cases of confluence of meshes of Cone A are of this type, and another example may be seen above the insertion of the third whorl of Cone B. The reason why the confluence of unclosed meshes is commoner than that of closed meshes is that confluence means the dying out of a strand and thus reduces the number of members of the next whorl. Such reduction is, of course, especially characteristic of the upper part of the cone, and meshes arising above the upper whorls of sporangiophores have less



TEXT-FIG. 6. Longitudinal reconstruction of the xylem of Cone B of *E. limosum*. Axial xylem black ; leaf-traces and parenchyma white. Magnification *circa* 16.

chance of being closed, partly because they reach the apex of the cone if they persist through many internodes, and partly because they are continually widened by confluence with other meshes owing to the dying out of strands. Nor are these facts the only ones that indicate reduction of xylem ; the strands of xylem of *E. limosum* are much narrower, compared with the parenchymatous meshes separating them, than those of the other two species studied.

The series of sections of Cone C of *E. limosum* was too short to afford reliable data as to the height of the meshes.

The meshes originating below the cone and persisting into it will be considered in discussing the region transitional from fertile stem to cone.

It has already been shown that where a strand pursues an isolated course through a node, the absence of a mesh above a median trace—or, in other words, the failure of a strand to branch above a median trace—is an effect of poor development of xylem. This is confirmed by the distribution of this type of median trace. In Cone A of *E. limosum*, the reduction in the number of members in a whorl begins above the seventh node; in the seven lowest whorls, 31 median traces out of 40 subtended parenchymatous meshes. Similarly, the seven lowest whorls of Cone B (in which there was no definite reduction, though the number of the traces varied slightly in different whorls) showed 27 median traces, of which 21 subtended meshes. The five lowest whorls of Cone C, which showed no signs of reduction in the number of members of a whorl, had 34 traces, of which 26 subtended parenchymatous meshes. But when we consider the regions of the cone in which reduction of the number of appendages has set in, we find very few parenchymatous meshes above median traces. This region was not available in Cone C, and in Cone B the region of reduction showed no median traces of either sort; but in Cone A there were in the four uppermost whorls only three median traces subtending meshes, while above six such traces the strand pursued its course upwards without branching. As already pointed out, Cone B has relatively to its size much less xylem than Cone A; in its upper part a large number of traces depart from strands so narrow that the traces are attached to the whole width of the strand. Such traces abutting on both edges of the strand cannot be called median; but they clearly represent a further stage of reduction of the axial xylem to which a median trace not subtending a mesh is attached; in the upper part of Cone B they replace the median trace. They are not, however, confined to the upper part of the cone, and an example may be seen in the eighth trace of the second whorl. No examples of such narrow trace-bearing strands were found in the other species or in Cone A of *E. limosum*. Some parts of the latter had rather more internodal xylem than others; this may cause the closure of the meshes a little way below the node; or the greater development of the xylem may take place above the node; for instance, the third and fourth traces of the fourth whorl of this cone depart from the *edges* of a band which persists upwards through the whole of the next internode; if these two traces were median instead of lateral, the absence of a mesh above them would be due, not to the xylem being poorly developed, but to its being well developed. I have not, however, observed such a case in this species.

Occasionally in *E. limosum*, isolated strands pass through a node without giving off a trace (e. g. two of the strands in the lowest whorl of Cone A, two of the strands in the lowest whorl of Cone B, two of the strands of the third whorl, and a strand of the fourth whorl of the same cone). In such

cases, and a few occur also in Cone C, the strand that does not give off a trace is never wide, but it is no narrower than many trace-bearing strands. Such excess of strands over traces must not be confused with an apparent increase in the number of strands due to the forking of a strand above a median trace occurring a little before the fusion of one or both of its branches with a neighbouring strand or branch of a strand, or to the decurrence of a parenchymatous mesh below and to one side of what would otherwise be a median trace. This apparent increase of the strands at a node may be exaggerated by the fact that the sporangiophores of a whorl are not all inserted at exactly the same level. Thus in one transverse section just above a node of Cone C, there were twenty-five strands and parenchymatous meshes, though the number of sporangiophores in the whorls above and below was but twenty. Such apparent increase of strands is, however, strictly localized to the neighbourhood of the nodes, and the supernumerary strands, if traced upwards or downwards for any distance, soon cease to be independent strands, and may thus be distinguished from those much rarer distinct and separate strands that pass through a node without giving off a trace.

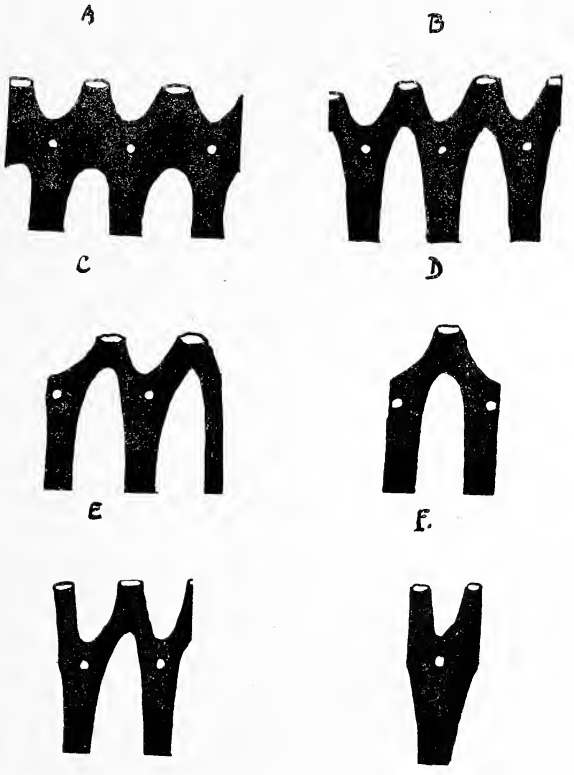
Alternation and Superposition of Whorls of the Cone.

In *Equisetum* generally, the sporangiophores appear externally to alternate with remarkable regularity; in the young state before the internodes have elongated the shields are closely imbricated. But in internal anatomy there is throughout the three species examined no such regular alternation in the insertion of the vascular traces of successive whorls. It is obvious that when a parenchymatous mesh extends through more than two internodes, all the members of a whorl cannot be accurately superposed to those of the second whorl in a downward direction as they are when succeeding whorls alternate regularly. In fact, the greater or less regularity of alternation of traces of successive whorls depends chiefly on the regularity with which successive meshes are closed at the nodes. In cones of *E. arvense*, especially in those that, like Cones A and B, have their xylem well developed, meshes persisting through a node are in a small minority, and the traces are much more often alternate with those of the node below. Even here the alternation is liable to be disturbed by the occasional occurrence of meshes of the second and third orders; by a change in the number of members of a whorl; by the fact that sometimes, though very rarely, a mesh may originate not vertically above a trace, but at a point above and to one side of it; and by the fact that when two sporangiophores become partially concrescent, their traces tend to become slightly approximated at their point of origin. When a trace has divided prematurely into two closely approximated bundles the mesh is superposed to both, and its position is one of the means of distinguishing a case of premature division of a trace (cf. Text-fig. 2, traces 6 and 7 of the first whorl). When by a close approximation of

neighbouring strands a mesh is very nearly closed at the level of a whorl, and when a trace departs from the edge or very near the edge of one of these strands, it may alternate more or less accurately with the traces of the whorl below, despite the persistence of the mesh through the node. When a strand pursues an isolated course through several nodes (i. e. when the parenchymatous meshes on both sides of it extend upwards through several nodes and internodes) the traces which it gives off, whether lateral or median, are necessarily superposed, though not always accurately so. For when the strand is markedly wider than the traces, successive traces may depart from portions of the strand not in the same vertical line ; the traces may, however, be accurately superposed, and when the strand is hardly, if at all, wider than the traces, the superposition is necessarily accurate. The giving off of superposed traces from an isolated strand seems to be an effect of reduction of the xylem, whereby neither of the meshes bordering the strand is closed at the nodes ; it is, therefore, natural that this form of superposition of the traces should be commonest in *E. limosum*, especially in Cone B: but it is common, too, in *E. palustre*, and occurs, though rarely, in *E. arvense* (e. g. the ninth and tenth traces of the second whorl of Cone C are given off from isolated strands, and are superposed respectively to the tenth and eleventh traces of the whorl below ; and the tenth and eleventh traces of the fifth whorl of Cone C are borne by isolated strands, and are superposed to the tenth and eleventh traces of the whorl below).

In *E. limosum*, though the xylem at the nodes is in excess of that in the internodes, it is only rarely sufficient to close a mesh before the departure of the trace at that node, i. e. to unite two trace-bearing strands, as, for instance, the strands giving off the seventh and eighth traces of the third whorl of Cone A are united. More often the strand, though relatively wide, is not wide enough to come into contact with its neighbours ; when a trace departs from the median portion of such a strand a parenchymatous mesh appears a little way above it so that the strand appears to fork just above the node. When two neighbouring strands branch in this way their adjacent branches diverge obliquely and fuse a little higher up to form the trace-bearing strand of the next internode (Text-fig. 7, B) ; we thus get a regular alternation such as obtains in the vegetative stem. But in the latter, generally in the cone of *E. arvense*, and frequently in the cone of *E. palustre*, there is no need for this fusion to constitute fresh strands alternating with those of the internode below, for the strands are, as a rule, laterally in contact with one another at the nodes at or before the departure of the traces, and by the appearance of parenchymatous meshes above the latter, strands arise alternating regularly with those of the internode below (Text-fig. 7, A). In both these cases, represented respectively by Text-fig. 7, A and B, we get a regular alternation of strands at the nodes ; but in the form of alternation illustrated in Text-fig. 7, B, rather less xylem is

in reality developed, and the parenchymatous meshes tend to dovetail into one another at the nodes, since those of the upper internode originate before those of the lower one are closed. In a longitudinal reconstruction of the xylem, the width of the latter at the node is increased by the fact that when the branches of a strand diverge obliquely the vessels are, of course, cut obliquely. A tendency for the parenchymatous meshes of the internode below not to be closed until after the origin of those of the internode above may be noted occasionally in the cone of *E. arvense*, and now and then in



TEXT-FIG. 7. Different forms of course of the strands at the nodes.

the vegetative nodes of the three species that I studied ; but it does not become a marked feature in any of them. According to Pfitzer (Pfitzer, p. 344) this type of alternation, shown in Text-fig. 7, B, is found in the vegetative nodes of *E. variegatum*.

In *E. limosum*, however, it is unusual, except in the fifth and sixth whorls of Cone A, for two or more adjacent strands to give off median traces and fork above their departure. Much more commonly one strand forks and its neighbour does not ; in such cases we get an irregular anatomical alternation between members of successive whorls. Of this irregular

alternation there are three forms: (1) the xylem-strand of the next internode may be formed by the fusion of a branch of a forking strand with a (whole) strand of the internode below (Text-fig. 7, C); or (2) by the fusion of two whole strands (Text-fig. 7, D); or (3) a strand may fork above the departure of a median trace and one (Text-fig. 7, E) or more, rarely both (Text-fig. 7, F), branches may constitute, independent of any fusions, trace-bearing strands of the internode above. The first form of irregular alternation is by far the commonest in *E. limosum*; the second leads to reduction in the number of strands, and is therefore more characteristic of the upper part of the cone; the third naturally leads to an increase in the number of strands in the next whorl unless it is counteracted by the fusion of two whole strands of the internode below. Such irregularities in alternation, when not caused by a change in the number of members of successive whorls, are due to failure to close some of the meshes at the nodes, and therefore to poor development of the xylem in this region; a further reduction of xylem leads, as already pointed out, to the formation of strands pursuing an isolated course through the nodes, and therefore to the superposition of traces.

A case occurred in Cone C of *E. limosum* in which a xylem-strand appeared to branch without any relation to the departure of a trace. A narrow strand, after giving off a trace, comes temporarily into connexion with one of its neighbours, about three-quarters of the way up the internode (and thus closes a parenchymatous mesh); but this connexion only extends for a short distance, and less than half-way up the next internode the strand again detaches itself.

Another exceptional, apparently a unique, irregularity occurs in the lowest whorl of Cone C. Here all the xylem-elements above a xylem-trace do not die out; but some of them pursue an oblique course and fuse with a neighbouring strand, thus closing a mesh of the internode below and initiating one in the internode above.

The lowest whorl in Cones A and C shows further anomalies; in both, while certain strands pursued an isolated course through the node and gave off no traces, pairs of strands united to one another at the node gave off three traces.

Apex of the Cone.

The structure of the apex of the cone differs in the different species. Externally, the terminal sporangiophore of *E. arvense* is larger than the others, and the outer (in this case upper) surface of the shield of the sporangiophore, instead of being flat or slightly depressed in the middle, is prolonged into a drip point; this relatively massive terminal structure never has, as the terminal sporangiophores of *E. palustre* and *E. limosum* have, a single more or less circular trace. Cone A was so very young that the terminal internode had not elongated at all, and the apical phenomena

were therefore indistinct; to ascertain the structure of more mature apices the upper parts of Cones B, D, and E were cut. In all three the last node gave off six traces, but in Cones D and E two of these seem to have entered one sporangiophore. In Cones B and E two unequal bands of xylem, much wider than the ordinary traces, entered the terminal sporangiophore, while in Cone D a similar wide band of xylem and two smaller strands enter the terminal sporangiophore; before entering the sporangiophore these two smaller strands approach very close to one another, but diverge again without fusing. These two small strands certainly seem to correspond to one of the bands that enter the terminal sporangiophore of Cones B and E.

In the cone of *E. palustre*, of the variety *polystachion* as well as of more typical specimens, the proportion of xylem at the apex seems to be relatively great. Here, indeed, it commonly forms a ring extending upwards from a point below the uppermost whorl of sporangiophores. In Cone A such a ring is only formed a little way above the last whorl, and as the stele has already narrowed considerably, the ring is very narrow; but even here the amount of xylem relatively to the diameter of the stele is above the average amount in other parts of the cone. In all three apices of cones of *E. palustre* examined the ring narrows rapidly as we pass upwards, and ere long its condensation gives rise to a small solid circular strand (i. e. it no longer encloses any parenchymatous cells); in the variety *polystachion* this narrowing of the ring and its condensation into a circular strand are effected partly by the fact that certain tracheides pursue an inward, centripetal course. This small circular strand is the trace of the terminal sporangiophore.

In *E. limosum* the trace of the terminal sporangiophore is formed as the continuation of one of the xylem-strands of the internode below. In Cone B the others seem to die out, while in Cone A they give off, after the departure of the traces, contributions to the strand of xylem that persists; the latter then narrows very rapidly, becomes circular and concentric, and passes upwards into the terminal sporangiophore as its trace.

As regards the structure of the sporangiophores, Eames has shown that their traces are concentric and mesarch, and I have nothing to add to his remarks (Eames).

The Structure of the Vegetative Nodes.

Before considering the region transitional from fertile stem to cone a few facts as to the structure of the vegetative nodes must be reviewed. As is well known the bundles in an internode of the stem of *Equisetum* consist of three groups of xylem. Of these the carinal or protoxylem group is situated medianly and at the edge of the pith; its annular or spiral vessels early become disorganized, giving rise to the carinal canal, upon the edge of which abut a few tracheides that have not been torn. The two other groups

are situated at the periphery of the bundle and form bands or groups of elements, the long axis of each group pointing obliquely outwards. They develop some time after the carinal group, and the number of cells in each of these lateral strands varies according to the species from 3 to 15. Quéva states that the lateral xylem is less developed in the rhizome and may even be absent there. According to him, too, the lateral metaxylem is composed entirely of spiral tracheides (Quéva, p. 18); Eames, on the other hand, states that the elements of these lateral strands are in general scalariform, reticulate, or pitted, but that occasionally they contain annular or even spiral tracheides (Eames, p. 589). Bower (Bower (2), p. 389) quotes observations of Gwynne-Vaughan on *E. giganteum*, the details of which have not been published, but which were accessible to him; according to these the lateral strands of this species contain 10–15 tracheides; the smallest of these are at the outside, and they gradually increase in size inwards. The larger elements are coarsely reticulate, the reticulation becoming finer and more regular in the smaller elements until in the smallest it closely resembles spiral thickening.

Gwynne-Vaughan maintained, too, that the lateral metaxylem-strands show indications of centripetal development, especially in the larger species in which the lateral groups are better developed; but he admits he was unable to prove this centripetal development as he had no incompletely developed portions of the stem (Gwynne-Vaughan). Bower endorses Gwynne-Vaughan's opinion with a similar reservation (Bower (2), p. 309). Quéva, however, expressly states that the whole of the lateral metaxylem is centrifugal (Quéva, pp. 25 and 35). Eames also regards the lateral xylem as centrifugal, though he records examples of a certain irregularity in the direction of lignification; I have met with a few cases of similar irregularity in the fertile stem of *E. arvense* (Eames, pp. 591–3). My observations, which were not at all extensive, confirm Eames's view that the prevailing direction of lignification in the lateral strands is from within outwards, but that it is subject to occasional irregularities. In any case the lateral strands seem phylogenetically to represent centrifugal xylem. In the region of the annulus there is no reticulate nodal wood, and as we pass upwards towards the lowest whorl of sporangiophores the lateral groups of xylem become united by elements of a similar nature. Thus in the cone, both in the node and the internode, the xylem of the bundle consists not of three groups, but of a continuous band of tracheides. It seems very probable that in the vegetative internodes the lateral groups of xylem represent the free ends of a more deeply curved band of xylem. In that case the position of the tracheides of the lateral groups of metaxylem in a more or less radial series is due to the more or less marked curvature of a band of which only the carinal tracheides and the free ends are lignified. The primitive form of internodal bundle would then be that of the cone, an interpretation

in accordance with modern ideas as to the conservatism of reproductive axes. Quéva has figured and described a curved band of xylem in the vegetative bundles of the upper end of the internode of *E. littorale* (Quéva, p. 23, Fig. 21). The xylem of the internode of the cone, where the latter contains a circle of more or less equidistant strands, often forms curved bands with the concavity facing outwards, though the curvature is less than we should have to assume for the vegetative node if the lateral strands are supposed to mark the ends of such a band. When wide tracts of xylem involving more than one strand of the internode below persist through an internode of the cone, the curvature of the band of xylem is slightly in the inverse direction, so that the xylem forms segments of a circle concentric with the outline of the stele. Traced downwards the terminal tracheides of the curved xylem-strands of the cone are continuous with the tracheides of the lateral metaxylem of the internode below the cone. We may add here that according to Leclerc du Sablon the xylem in the bundles of the tubers of *Equisetum* is not broken up into a carinal and two lateral groups, but vessels and parenchyma are mixed irregularly (Leclerc du Sablon).[1] Milde has figured a similar bundle of a tuber (Milde, Pl. I, Fig. 22). In the tubers of the fossil Equisetaceae recently described by Fritel and Viguier, the xylem of each bundle appears to consist of an oval mass of parenchyma mixed with tracheides. Quéva has shown that the elements of the nodal wood are lignified after those of the carinal group, but before the lateral metaxylem of the internode. In 1901 Gwynne-Vaughan, from a study of *E. gianteum* and *E. hiemale*, concluded that the lateral strands may be traced through the node and across the upper surface of the ring of nodal xylem ; that after passing above and across the latter they fuse with the similar adjacent strand of a neighbouring bundle to form the alternating strands of the internode above (Gwynne-Vaughan). Quéva, however, writing six years later, seems to be unaware of Gwynne-Vaughan's remarks ; he states that the nodal reticulate tracheides are ' in direct relation to the metaxylem, the elements of which show above the node transitional markings ' (Quéva, pp. 18–19). According to this botanist, therefore, the tracheides of the lateral strands are gradually replaced by reticulate nodal elements as we pass upwards ; higher up this xylem dies out, except at the points where the wood persists as the lateral metaxylem-strands of the internode above, and at these points the reticulate thickening of the elements is gradually replaced by spiral. Ludwigs in a recent paper seems to be of this opinion, though he expresses himself somewhat differently, saying that metaxylem-elements are initiated on both sides of the bundle, and are especially numerous at the level of the nodal diaphragms ; that after the departure of the protoxylem into the leaf the two groups of metaxylem

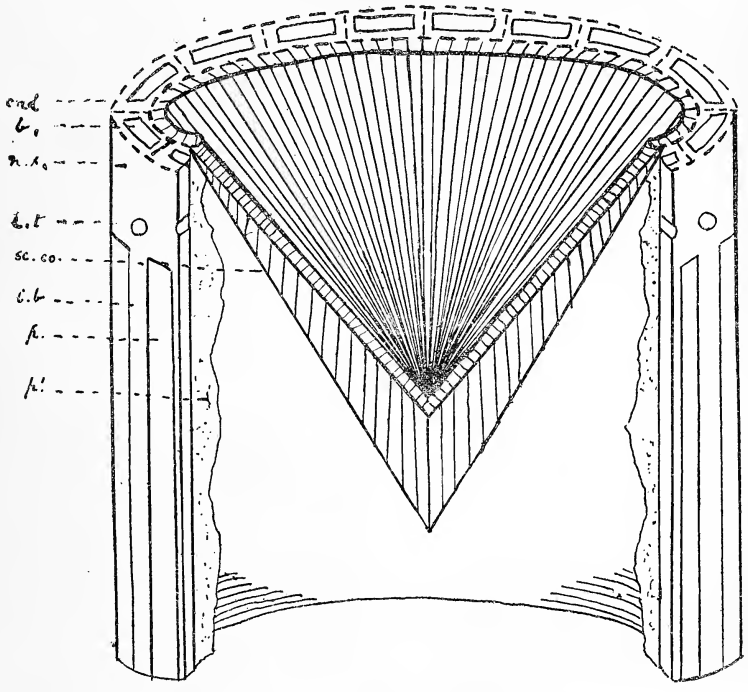
[1] This author also states that throughout the tuber the bundles form an irregular network, a point which would repay further investigation.

fuse and fill up the carinal canal (Ludwigs, pp. 401–2). Quite recently, Vidal has recorded all forms of thickening intermediate between reticulate and annular in the stem of *E. palustre* (Vidal, p. 24). My observations, so far as they go, are in complete agreement with those of Quéva on this point and in contradiction to those of Gwynne-Vaughan ; but they were not extensive, and were moreover limited to the fertile stem of three relatively small species in which the lateral metaxylem groups are not particularly well developed.

In 1893 Cormack suggested that there were traces of secondary xylem in the nodal region of *E. maximum* (Cormack). Quéva, after a careful study of the ontogeny, pointed out that though the ' zone génératrice ' of the bundle persists later in the nodal region its activity is strictly limited, the divisions of its cells being merely the continuation of the earlier procambial division of the bundle ; thus its secondary nature is not proved (Quéva, pp. 33 and 36). Ludwigs is of the same opinion (Ludwigs, pp. 400–1). Nevertheless, Eames states that a few of the living species of *Equisetum* have been shown by Cormack to possess the remains of secondary xylem, and that Quéva confirms the presence of such wood in *E. maximum* and records its occurrence in *E. arvense*. Eames further remarks that he has seen evidence for this ancestral character in both these species, in *E. hyemale* and in *E. hyemale*, var. *robustum*, A. A. Eaton. Finally, Ludwigs, speaking of the reticulate nodal tracheides, says : ' These vessels are supposed to be formed (" sollen sich . . . bilden ") from without inwards by the activity of a cambium and represent that which Eames would have us regard as centripetal wood ' (Ludwigs, p. 400). It is hard to see how xylem-elements formed internally by a cambium could be anything but centrifugal ; nor does Eames regard the nodal xylem as centripetal. On the contrary, he expressly states that so far as he knows no question has arisen as to the centrifugal development of the nodal or supranodal wood, as he, in agreement with Jeffrey, calls it. He adds that it is clear that the innermost elements are protoxylem (Eames, p. 590). It seems clear that Ludwigs' statement that Eames regards the nodal wood as centripetal is incorrect ; nor does Ludwigs himself express any agreement with this view. Whether the nodal wood is secondary or not is less clear ; on the whole its secondary character seems not to be proved ; though from the size of the Mesozoic Equisetales we should *a priori* regard secondary growth as an ancestral character, traces of which might well remain in certain species.

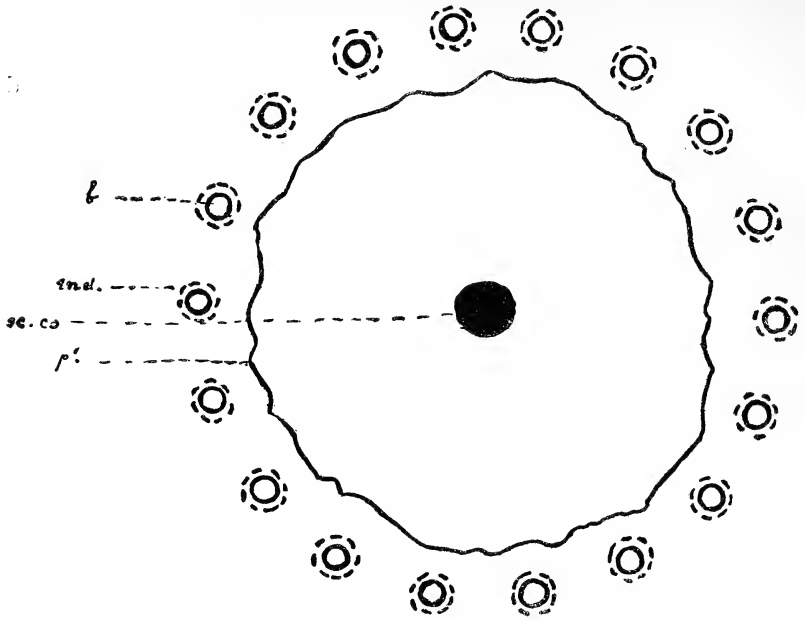
At the level of the uppermost vegetative whorl of the fertile stem of *E. limosum* a diaphragm of the usual type was not found. At the node, owing to the junction of the xylem of the separate bundles into a continuous ring, the separate endodermes of the internodal bundles are replaced by common inner and outer endodermes, this change being brought about

in the manner already described by Pfitzer (Pfitzer, pp. 336–7). The same author has noted that in the nodal region the cells of the outer endodermis have for a little distance thicker walls (l. c., p. 332). I have observed the same phenomenon in *E. limosum*. A little above the departure of the leaf-traces, where the ring of nodal xylem breaks up, the endodermis consists of relatively thick-walled cells that stain very deeply with Bismarck brown; as the breaking up of the ring proceeds these dark brown cells become involuted round the bundles through the parenchymatous

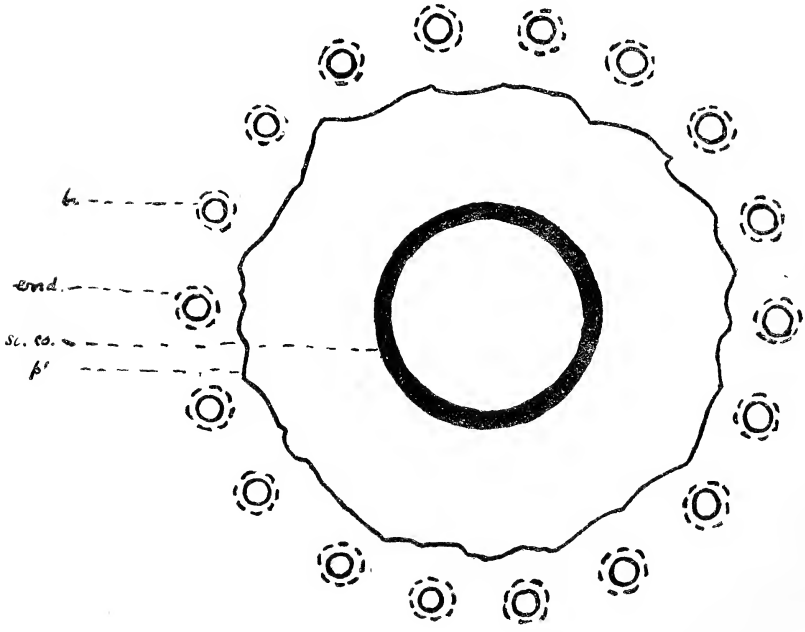


TEXT-FIG. 8. Reconstruction of uppermost part of fertile stem of *E. limosum*. *n.x.* = nodal wood, breaking up into the bundles (*b*) of the internode above; *l.t.* = leaf-trace; *i.b.* = bundle in the internode below; *p.* = parenchyma between the bundles; $p.^1$ = torn parenchyma on the inner side of the bundles; *end.* = endodermis; *sc.co.* = inverted hollow cone of sclerenchyma.

meshes of the upper internode; thus we get a ring, three to four cells deep, of these dark brown cells lying inside the circle of strands; traced upwards this ring communicates through the parenchymatous meshes with the dark brown cells of the outer endodermis. Traced downwards into the lower internode the ring of thickened cells narrows rather rapidly; at first it is connected with the narrow band of parenchyma that persist on the inner side of the vascular bundles; but as we pass downwards it narrows so rapidly that in the mature stem is soon ceases to be in contact with this parenchyma, and in a transverse section lies free in the middle of the central cavity (Text-figs. 8 and 10). The thin-walled elements internal to this ring



TEXT-FIG. 9. Lettering as in Fig. 8.



TEXT-FIG. 10. Lettering as in Fig. 8.

also become torn as the stem increases in width, though not infrequently the torn remains of such thin-walled cells may be seen adhering to the inner side of the thickened ring. As the latter narrows, passing down the internode the cavity which it encloses becomes smaller, until we get a solid core of thickened cells lying free in the central cavity (Text-fig. 9). These phenomena were observed in Cones A and C, in which they were closely similar; the series of sections below Cone B was not sufficiently extensive to show the form of the mass of dark brown cells, but the presence of such cells was ascertained.

Pfitzer has described dark brown cells in the interior of the branch at its insertion on the stem, but he does not seem to have noticed similar phenomena in the main shoots. His description, which, *mutatis mutandis*, agrees with the description given above, runs thus: 'In a radial longitudinal section through the origin of the branch the brown internal mass of the cylinder is seen to widen out laterally . . . in such a way that it comes into connexion with the outer endodermis of the cylinder.' The cylinder ('Röhre') mentioned here is, of course, the ring of xylem found in the lower part of the branch; upwards this cylinder of xylem only breaks up into separate strands above the insertion of the first leaf-sheath of the branch, while below it is attached to two of the cauline bundles; its insertion here is also oblique with regard to the main axis.

E. palustre has in the internode only an external endodermis, but Pfitzer has shown that throughout the node there is also a common internal endodermis, which traced upwards and downwards passes over gradually into the typical pith without coming into contact with the external endodermis. He remarks that in the old stems of *E. palustre* all the cells within the inner endodermis are thickened and dark brown, and says that they constitute the brown diaphragm. In my young specimens the diaphragm was not at all brown. Pfitzer also gives a curious description of the brown cells within the base of the cylinder of the branch of this species. The branch has an inner endodermis, the inner longitudinal walls of which are thick and dark brown; the cylinder also contains thickened dark brown cells; these appear first in the middle of the cylinder, and their development is continued upwards and downwards; when it reaches its highest grade 'the whole interior of the cylinder is filled with a dark brown mass of cells which either shows a dilation ("Anschwellung") above or below or below only, and is surrounded by the unthickened external longitudinal walls of the (inner) endodermis'. If this description is accurate the form of the brown mass of cells would be that of a solid dumb-bell or of a solid normally oriented cone. Pfitzer also describes dark brown cells in the branch of *E. littorale* and in the stem of *E. variegatum*. In the latter species, when the nodal wood breaks up above the departure of the traces, the outer and inner endodermes are replaced by special endodermes round the bundles,

and the endodermal cells lying on the inside of the bundles are thickened, and the thickenings are dark brown. This thickening and colouring of the layers of thickening spreads a little higher up to the pith cells, so that we get a polygon of which the blunt ends project between the special endodermes. Thin-walled cells are often found inside this polygon (Pfitzer).

Jeffrey, working on *E. hiemale*, also records sclerized brown cells. He writes of his Fig. 2, Pl. XXIX, representing a tangential section of the nodal region of *E. hiemale*: 'The magnification is sufficient to show that the medulla of the branch is composed of brown sclerenchymatous cells' (Jeffrey, 1). As the photograph is of an obliquely oriented organ it is not easy to make out the exact form of the sclerenchymatous mass; from the above quotation it might be thought that the whole medulla is sclerized, but it is hardly safe to conclude this from a passing reference like that quoted above. Jeffrey states, too, that the diaphragm of the first node of the branch is very deep, and somewhat sclerified at the ends; this diaphragm does not appear to be connected at the origin of the branch with the above-mentioned mass of sclerenchyma. He also found that in the main stem of *E. hiemale* and *E. limosum* the diaphragm was partially sclerified; this he regarded as sclerified periderm and compared it to the non-sclerified periderm in the diaphragm of certain *Calamites*.[1]

The Transition from Fertile Stem to Cone.

The annulus seems to mark the position of a reduced node, and the reasons for this must be examined. We have seen from the anatomy of the cone and stem that fresh parenchymatous meshes arise above the nodes; usually they arise at a relatively small distance above a trace that has departed; we have also seen that the closure of meshes of the internode below is effected in the region of the node. This closure of parenchymatous meshes and the formation of fresh ones in the region of the node causes a branching of the vascular strands at this level. We have seen also that when the xylem is much reduced at a node certain of the strands pursue an isolated course upwards into the next internode because sufficient xylem to close the meshes of the internode below is not present, and because the trace-bearing strand remains too narrow to give rise to an independent parenchymatous mesh above the node. We may generalize as to the fluctuating conditions obtaining in the fertile nodes by saying the greater the reduction of *nodal* xylem the larger the proportion of strands that pass through a node without branching. In the vegetative axis in which the nodal xylem is well developed and forms a complete ring (though the internodal xylem is less developed than in the cone), all the meshes are

[1] Since writing the above I have come across H. H. Thomas's record of a nodal diaphragm, hollow in the middle, in a Jurassic species of *Equisetites*. Cf. Mémoires du Comité Géologique de Saint-Pétersbourg, N. S. 71, 1911.

closed at and fresh ones originate above the node. A branching of strands independent of the formation of a parenchymatous mesh above a trace that has departed is very rare. It is true that in cones with relatively little xylem, or in parts of cones with little xylem, a mesh may arise below and to one side of a trace ; but in such cases its form and position seem to show that it has, phylogenetically speaking, 'decurred' downwards below the trace to which it corresponds (cf. p. 673). True branching of strands, independent of the formation of meshes above traces, does occur, though rarely, both in cones and stems where the number of members in the next whorl is increased, and still more rarely owing to inexplicable irregularities. But even in cases in which an increase in the number of members causes branching of the strands the area of disturbance of superposition of meshes to traces is narrowly restricted. Branching of some of the strands above the annulus is common, and strongly suggests that we are dealing with a reduced node ; in *E. arvense*, however, the number of sporangiophores in a whorl often considerably exceeds the number of leaves in the fertile stem, and the formation of fresh meshes above the insertion of the annulus might be regarded as the expression of the increase in the number of appendicular members. In this species, too, the number of strands that pass uninterruptedly through the annular node is relatively large. The transitional region was studied in Cones A, B, C, F, G, and H of this species. In Cone A four out of nine strands forked, giving rise to fresh meshes above the annulus. Below the annulus of Cone B there were seven strands, and all passed through the annular region without branching ; in Cone C one only of the eleven strands branched above the insertion of the annulus. Cone F was exceptional and will be considered later ; in Cone G three out of nine strands forked above the insertion of the annulus, while in Cone H one of eleven strands forked. The forking observed in this last cone was the more significant as there were only ten sporangiophores in the lowest whorl of the cone ; thus the branching of the strand was not correlated with an increase of appendicular members, and seems clearly to be a vestigial nodal character. In Cones A, B, and D of *E. palustre*, too, the number of members in the uppermost vegetative whorl was equal to the number in the lowest whorl of the cone ; yet in all these cases a certain number of meshes were closed, and others originated in the neighbourhood of the annulus. In the four cones of *E. palustre* examined, after the closure of certain of the parenchymatous meshes in the region of insertion of the annulus, a band of xylem which effected this closure persisted upwards through the 'internode' lying between the annulus and the sporangiophores ; in this region the absence of meshes was due, as it appears occasionally to be due in the cone (cf. p. 671), not to poor development of nodal wood, but to unusual development of internodal wood.

Cases of closure of meshes of the internode below in the neighbourhood

of the annulus are difficult to observe in *E. arvense*. In Cone A of this species the meshes below the first and second traces of the lowest whorl of sporangiophores are closed, and a fresh mesh of the first order originates between them (cf. Text-fig. 1). In this restricted region the branching gives rise to the formation of a mesh alternating with that of the internode below, and the nodal appearance is most clearly marked in a longitudinal reconstruction, though, of course, the fresh meshes are not subtended by traces. In other cases a mesh is closed in the neighbourhood of the insertion of the annulus, but no fresh mesh originates higher up alternating with it; this may, perhaps, be due, as in *E. palustre*, to the persistence of a band of xylem through the internode above the annulus. This seems to be the case with certain of the strands of Cone C of *E. arvense*. Here the first, the second, the third, the fourth traces, the two bundles composing the prematurely divided sixth trace, and the eleventh trace, are all inserted not vertically above the middle of the strands of the uppermost vegetative internode, but lie more or less above the point of junction of two strands. Thus the meshes above these traces do not alternate with those of the uppermost vegetative internode, but lie more or less above them; on this assumption the meshes corresponding to the third and fourth traces have 'decurred' a little below the traces to which they phylogenetically belong. If parenchymatous meshes had appeared in this band of xylem above the annulus and had been closed again at the insertion of the sporangiophores they would be about half the height of the meshes of the first order of the cone. In Cone A, where two meshes actually arise above the annulus and are closed before the insertion of the basal whorl of the cone, their height is about half that of the meshes of the first order found in the lower and less immature region of the cone. The absence of branching in the region of the annulus in Cone B may perhaps be due, at any rate in part, to this same relatively great development of xylem in the internode above the annulus. The xylem is much more developed in this cone relatively to its size than in Cone C, and at the level of the basal whorl all the strands are united into a ring of xylem. But the fusion of the xylem of the bundles forming a ring takes place at very different levels, and some of the strands fuse as far down as 0·9 mm. below the sporangiophores; if meshes originated just above this lowest fusion and were closed at the height of the sporangiophores they would be, like the meshes of the first order arising above the annulus of Cone A, about half the average height of the meshes of the first order of the cone of the specimen in which they occur. In Cone B, however, it was difficult to be sure if the meshes above the sporangiophores were superposed to those of the internode below, for the diameter of the stele narrowed abruptly at and above the insertion of the basal whorl of the cone, and this narrowing confused the relations of alternation and superposition of the meshes. It is quite possible, therefore,

that in Cone B we are dealing merely with a case of early closure of certain meshes, and parallel cases of early closure are by no means rare in the cone of this species. But in Cone C such an explanation would not account for the fact that the traces of the first whorl seem in some cases to lie above and between the strands of the internode rather than above and in the middle of them; in this cone, too, one fresh mesh originates above the annulus and very much at the level at which some of the other meshes are closed.

To return to *E. palustre*, in which the percentage of closure and of origin of meshes in the annular region is highest and in which the nodal appearance of this region in longitudinal reconstruction is therefore most striking, we find that in Cone A six out of seven, in Cone B four out of eight, and in Cone D four out of six meshes are closed in the neighbourhood of the insertion of the annulus. Notwithstanding the persistence of bands of xylem into the internode above the annulus, four fresh meshes originated above the annulus in Cone A, three in Cone B, and three in Cone D. Cone C was exceptional; though there were eight strands below the annulus and nine sporangiophores in the second fertile whorl, the basal whorl of the cone consisted of but three sporangiophores. The increase in number of trace-bearing strands to nine in the second fertile whorl is due to the fact that above the lowest whorl of the cone a band consisting of three strands (that with two other single strands gave off no traces at this node) breaks up into four strands; thus the increase in the number of strands takes place between the first and second whorls of the cone and not between the annulus and the lowest whorl. Nevertheless, six out of eight meshes were closed and four originated in the neighbourhood of the annulus. It is curious that none of the fourteen meshes originating above the annuli of the cones of *E. palustre* examined were of the first order. It must, however, be remembered that meshes of the first order are much rarer in the cones of *E. palustre* than in those of *E. arvense*.

In *E. limosum* the indications of the nodal structure of the axis at the insertion of the annulus are less marked; in Cone A we notice one case of the origin of a mesh above the annulus. In Cone B there are four meshes which I interpret as arising above the annulus; like the mesh in Cone A they are of the second and higher orders. The mesh below the second trace of the lowest whorl of this cone appears to be closed in the region of insertion of the annulus and markedly below the level of the basal whorl; this is true, too, of the meshes above the twelfth and thirteenth leaf-traces; but this cone is still so young and has elongated so little that it is exceedingly doubtful if we are dealing with bands of xylem persisting through an unelongated supra-annular internode or with early closure of a mesh before the first fertile node. Cone C of this species showed two clear cases of meshes originating above the annulus. This tendency of meshes to be closed slightly above a node was noticed also in various cones (cf. pp. 680–1),

and seems to be one result of a lesser development of xylem. The latter being insufficient to bring the strands laterally into contact at the node, their connexion is effected higher up by the oblique course of the tracheides present.

Strong confirmation of the view that the annulus marks the position of a reduced node was afforded by the region transitional from the fertile stem to the cone in Cone F of *E. arvense*. This specimen had two annuli; the upper one was normal in appearance, the lower one was rather more developed than is usual in this species, especially on one side where the edge was slightly lobed. The insertion of this lower annulus on the axis was remarkably oblique, a phenomenon which may occasionally be observed in normal annuli. According to Duval Jouve the possession of more than one annulus and irregularities of the annulus are very common in *E. arvense*, *E. maximum*, *E. littorale*, and common in *E. sylvaticum*, *E. limosum*, and *E. ramosissimum* (Duval Jouve, pp. 150 and 154). Milde goes so far as to describe the cone of *Equisetum* as having one to two annuli (Milde, p. 161). But I know of no account of the anatomical relations of such abnormal forms. A little distance below the lower annulus of Cone F the anatomy of the stem was that of a typical internode of the vegetative region. There were seven more or less equivalent bundles, each with a large carinal canal and two small lateral groups of metaxylem. A little higher up reticulate tracheides, resembling those of a vegetative node, make their appearance in the adjacent halves of two bundles; these two groups of tracheides and the phloem external to them pursue a course so oblique that about 30 to 40 μ higher up they come into contact. The reticulate wood thus formed is of the same radial extent as that of an ordinary vegetative node; but in the direction of the circumference of the stele it at first occupies only the portion of the stele lying between the carinal canals of two adjacent bundles. The lateral groups of metaxylem at the free ends of these bundles persist for a little distance in the condition of typical internodal lateral metaxylem; but a little higher up they and the cells beyond them become connected with and assume the character of nodal wood. There results a band of reticulate tracheides extending over rather more than a quarter of the circumference of the stele. This band of xylem, however, very soon breaks up into three bundles by the dying out of portions of the woody band. About 320 μ above the first appearance of the reticulate tracheides the band of xylem has broken up into three normal sized bundles; the middle one is already in a typical internodal condition, each of the others consists of a curved band of typical 'nodal xylem'. Traced upwards these reticulate tracheides, the free ends of the band of reticulate wood, do not die out, but pursue an obliquely transverse course, passing in opposite directions round the circumference of the stele (Fig. 5, Pl. LXIV). Thus a mass of tracheides appears at different levels in all the bundles. When the two strands of reticulate tracheides and the phloem outside them have

girdled nearly the whole circumference of the stele they find themselves in adjacent bundles, and after becoming very closely approximated they die out without coming into absolute contact at a height of about 1·6 mm. above the first appearance of reticulate tracheides. When they die out the two strands are separated only by one or two parenchymatous cells. Each strand consists of the equivalent of a bundle, but while each may sometimes occupy the position of a single bundle it has of course to pass from one bundle to another, and frequently occupies a position between two bundles. While the masses of nodal wood have been journeying round the stele the number of bundles has increased from seven to ten, an increase very similar in amount to that found in many normal cones of *E. arvense*. It is interesting to note that this lower annulus, which only faintly recalled a leaf-sheath by its lobing on one side, possessed reticulate tracheides apparently identical in structure with those of an ordinary node; the distribution of these is of course different from their distribution in an ordinary node; but this is presumably due, in part at least, to the oblique insertion of the annulus. In one of the two bundles that first develop reticulate tracheides, a bundle situated on the side on which the annulus was most developed, there seemed to be indications of a reduced and abortive trace. A few phloem cells bend out into the cortex, and endodermal cells with exceptionally well-marked bands of cutinization may be seen cut longitudinally a little way out in the cortex. Moreover, one or two tracheides bulge out through the opening in the phloem and seem to be passing out into the cortex; unfortunately, two sections, each 10 μ thick, above the section showing these phenomena, were lost in mounting. If one or two tracheides did, as seems likely, pass out into the cortex they must have died out almost at once, for no further trace of them can be seen in other sections. The parenchymatous stelar cells that bend outwards, and those with endodermal markings, also die out ere long in the cortex; but for several sections they are distinctly discernible owing to the fact that they are cut longitudinally or very obliquely. No further indications of traces were observed in any of the other bundles.

Below the upper normal annulus there were ten strands, and the lowest whorl of the cone consisted of ten sporangiophores, so that there was no increase in appendicular members; yet six new meshes were formed above this annulus, two of which were closed at or just above the level of the lowest fertile whorl. No less than seven out of the ten meshes existing below the upper and normal annulus were closed in the region of its insertion. Thus the number of meshes closed and initiated in the region of the annulus was in the case of Cone F exceptionally high for *E. arvense*.

We may add that though annuli do not seem to have been described in most of the cones of the fossil Equisetales, Thomas records a possible example in *Calamostachys* (Thomas), and a structure which appears to

be of the same nature may be seen in one of Kidston's figures of *Pothocites* (Kidston, Pl. XII, Fig. 14).

General Considerations.

Equisetum is so isolated among existing plants that comparisons of the structure of its cone with the cones of recent plants seem at present to be futile from a morphological point of view. But the phylum of the Equisetales was well developed in the past, and a comparison with the varied cones of this great phylum offers the most promising field for conclusions as to the comparative morphology of the cone. The cones of the Equisetales may be divided into two great groups, those consisting only of fertile appendages, provisionally called sporangiophores, and those in which the cone also contains sterile appendages. Leaving abnormalities out of consideration the former group includes the cones of the recent *Equisetum*, those of the species of *Equisetites* in which the reproductive organs are known, namely, the carboniferous *E. Hemingwayi*, Kidston, the Liassic *E. Nathorsti*, Halle, and the Rhaetic *E. suecicum*, Nathorst (Halle, pp. 27–31); it also includes the cones of *Autophyllites furcatus*, Grand'Eury, *Bornia pachystachya*, Bureau, and perhaps of *B. transitionis*, Grand'Eury; these three species come from the Carboniferous (Jongmanns, pp. 265–7). In the cones forming the so-called genera *Calamostachys* (including Renault's *Calamodendrostachys Zeilleri*), *Palaeostachya*, *Huttonia*, *Cingularia*, and in *Volkmannia pseudosessilis*, Grand'Eury, sporangiophores and sterile bracts succeed one another on the axis of the cone (Jongmanns, p. 279). These types of cones are of Palaeozoic age; most of them certainly, probably all of them, represent cones of the Calamariae in the widest sense of the word. In *Calamostachys* bracts and sporangiophores form equidistant whorls; in *Palaeostachya* the sporangiophores are inserted in the axils above the bracts, while in *Huttonia*, in *Cingularia*, and in *Volkmannia pseudosessilis* they are situated below the bracts. *Archaeocalamites* (including *Pothocites* and *Bornia*, excepting perhaps *B. pachystachya* and *B. transitionis*) appears to be intermediate between the two groups, for its cone consists of whorls of sporangiophores, sometimes at least interrupted at considerable intervals by a whorl of sterile bracts. The best way of elucidating the anatomy of a cone is certainly by the study of its internal structure. But nearly all the cones mentioned above are known as impressions only; we are, however, acquainted with the anatomy of some species of *Calamostachys* and *Palaeostachya*. It has been customary of late years to regard the sporangiophores and bracts of these two genera as phylogenetically lobes of a compound leaf of sporophyll. Such a view is admittedly based partly on an analogy with the Sphenophyllales, in which group the sporangiophore, when present, is usually inserted on a sterile bract. This view has been somewhat widely held, and different modifications of it were

favourably considered by Scott (Scott, pp. 158–62)[1], Lignier (Lignier), Jeffrey (Jeffrey (1), p. 185), and others. Bower, however, was strongly opposed to it, regarding the sporangiophore as an organ *sui generis*, not only in the Equisetales, but also in the Sphenophyllales (Bower (2), pp. 381–4), while Seward supported him as far as regarding the sporangiophore, at least provisionally, as an organ *sui generis* (Seward, p. 15). There are certainly much stronger reasons for doubting that the sporangiophore represents a lobe of a leaf in the Equisetales than in the Sphenophyllales. In the former group we are at once met with the difficulty of explaining those cones in which the bracts are either absent or present only at considerable intervals. Those that regard the sporangiophore as a lobe believe that it has been displaced in *Calamostachys*; some years ago Dr. Scott pointed out that if such displacement occurred in a form in which, as in *Sphenophyllum fertile*, both lobes of the sporophyll bore sporangia, we should get a near approach to the *Equisetum* arrangement (Scott, p. 162). Jeffrey also remarks that there are many reasons for regarding the sporophyll in the Equisetaceous series as dorsiventrally lobed. He suggests that in this case the sporangiophores of *Equisetum* represent the result of fusion of ventral and dorsal segments, or that they are ventral segments, the dorsal having become obsolete. No known cone seems to show such an obsolescence of bracts, and the sporangiophores of *Equisetum* do not seem to be dorsiventrally double.

I was at first inclined to accept Dr. Scott's tentative suggestion that in *Equisetum* each sporangiophore might represent a separated lobe of a dorsiventrally lobed sporophyll; in this I was influenced by the analogy with the Sphenophyllales, but principally by the fact that I felt unable to escape the conviction that the sporangiophores of *Archaeocalamites* are the homologues of those of *Calamites*, and these sporangiophores seem very closely to resemble those of *Equisetum* (Browne, p. 16). But a comparative study of the cone of *Equisetum* seems to show that the sporangiophores are whole appendages and that their insertion marks the position of a node. It is true that though the cone has more internodal xylem than the stem, the ring of wood at a fertile node is only exceptionally complete; such a ring is, however, by no means rare in *E. arvense*. Moreover, at the insertion of the whorled appendages there is (except in cases of great reduction of the xylem) a marked increase in the amount of xylem, leading to the formation of bands or of a ring of xylem. Above the departure of the traces of the sporangiophores, and often very much at the same height as the breaking up of the xylem above the leaf-traces, these bands or this ring of xylem breaks up owing to the appearance of parenchymatous meshes.[2] In this

[1] Dr. Scott subsequently pointed out that the spore-bearing organ might be a whole leaf or a lobe of a leaf (Studies in Fossil Botany, 1909, p. 623). I have been led to adopt this view.

[2] The nodal xylem of the stem of course differs markedly from the xylem at a fertile node, for, except for the protoxylem, it consists of large reticulate elements and has a considerable depth radially, while the xylem at a fertile node consists entirely of relatively small annular and spiral tracheides and is very narrow radially.

respect, however, the cone varies much more than the stem, for a portion of the band of xylem may be continued upwards through the greater part or the whole of the internode, or it may break up relatively near the departure of the traces.

Thus the anatomy of the cone supports the view that the sporangiophores of *Equisetum* are whole appendages. On the other hand, the anatomy of the cone of *Palaeostachya* and *Calamostachys* seems to show that in these genera the sporangiophores and bracts are lobes of a leaf (Hickling, 1 and 2). This is a very fundamental difference, and it would seem that these genera do not lie at all near the Equisetal line of descent. The anatomical evidence is at present insufficient to decide whether the sporangiophores of *Archaeocalamites* were lobes, as were those of *Calamostachys*, or whole appendages, as those of *Equisetum* seem to be. The latter view seems to me the more natural. The bracts found at irregular intervals in the cone of *Archaeocalamites*, which I was formerly inclined to regard as indications of sterilization of fertile appendages (Browne, p. 17), would seem more probably to be reduced vegetative leaves, and their occurrence in the relatively ancient *Archaeocalamites* might be a primitive character, indicating want of definition of the strobilus.

The parenchymatous meshes found in the cone, like those in the vegetative internodes of *Equisetum*, do not, except at the reduced or immature apex of the cone, originate immediately above the traces. Thus they are not foliar gaps as defined by Jeffrey. Speaking of the tracts of parenchyma of the internode of the stem of *Equisetum* this author says, 'They lack, however, one important feature of foliar gaps, for they do not occur *immediately above the traces*, as should be the case with true foliar gaps. All other foliar gaps with which we are acquainted show this feature'[1] (Jeffrey (2), p. 252). If this definition of a foliar gap is to be applied in all cases, the tracts of parenchyma above the traces of certain recent Osmundaceae are not foliar gaps. Sinnott, seeking to defend Jeffrey's view that the traces of siphonostelic and dictyostelic Pteropsida always leave gaps, claims that when in recent Osmundaceae a trace appears to leave no gap in the wood at its departure the trace-bearing xylem-strands always break a little further up while the trace is in the cortex. This is exactly what occurs in the axis of the cone when a strand branches a little way above the departure of a trace; very much the same thing occurs in the stem when the nodal wood breaks up a little way above the insertion of the leaf-sheath. In cones which, like that of *E. limosum*, have very little xylem, the 'gap' or break usually occurs very close to, though not immediately above the trace, and thus the mesh of parenchyma approximates more closely to Jeffrey's conception of a gap. In the cones of *E. arvense* and *E. palustre*, when the xylem is broken above a trace, pith and cortex remain separated

[1] The italics are Jeffrey's.

by the endodermis, which in these species surrounds the stele. In the stem of *E. limosum* (and presumably in all cases in which the vascular strands are surrounded by separate endodermes) even this barrier is broken down ; for at the breaking up of the nodal ring of wood the external and internal endodermes found in this region become involuted round each bundle, so that pith and cortex are in direct communication (Pfitzer, pp. 336–7). Definite endodermal markings are, however, very difficult to distinguish in the cone of this species. It would seem that, phylogenetically speaking, parenchymatous tissue first arose in the internodal part of the siphonostelic xylem of the cone of *Equisetum*. It is not surprising that this parenchyma should have arisen vertically above the traces, since the current of water, being deflected into the sporangiophores, would be diminished above them. From the dimensions of the fossil *Equisetites* we have good reason to believe that existing species of *Equisetum* are reduced, and reduction of the xylem would tend to make the mesh arise closer to the trace. This is exemplified in the cone of *E. limosum*, in which there is very little xylem ; here the traces are often only surmounted by a very few lignified elements, though even in this species the parenchymatous mesh sometimes originates at a considerable distance above the trace.

In the preceding pages we have traced the phylogeny of the irregular network of strands of the cone of *E. limosum* from the more regular stele of *E. arvense*, which is imperfectly siphonostelic at the nodes and dictyostelic in the internodes. Considered separately, the irregular network appears to baffle interpretation ; considered in the light of a comparative study of the cones of *E. palustre* and *E. arvense*, we see that it has arisen in the phylogeny by the reduction of xylem, this reduction being manifested by extension of the parenchymatous meshes upwards, downwards, and laterally.

In 1899 Jeffrey published a photograph of a cone of *E. arvense* cut longitudinally in two ; this photograph was intended to show that the majority of the bundles of the cone do not alternate at the nodes (Jeffrey (1), Pl. XXX, Fig. 3). This feature was, he said, more or less marked in the cones of all the species of *Equisetum* that he had examined. Some years later he returned to this point, and after commenting on the greater conservatism of reproductive axes as compared with vegetative ones he proceeded : ' It appears to have been shown above and beyond any doubt that the equisetaceous strobilus perpetuates both the alternating strands and the complete absence of foliar gaps of the oldest Calamitean forms ' (Jeffrey (2), p. 254). While, as Jeffrey remarks, it seems obvious from the data of Stur, which have never been called in question, that the oldest Calamariae were without alternation of the strands at the nodes, and while, according to Hickling, the bundles of the cone of *Calamostachys* did not alternate at the nodes (Hickling, 2, p. 10), and those of *Palaeostachya vera* seem to show no regular pectination, but probably occasional and irregular communication

(Hickling (1), p. 375), the non-alternation of the bundles in the cone of *Equisetum* seems clearly, from the comparative anatomy of the cone, not to be a primitive feature. Moreover, Renault says of his *Volkmannia gracilis*, which is clearly a *Palaeostachya* (cf. Jongmanns (1), p. 332), that the vascular bundles alternate from one internode to the next. It seems shown by the full discussion above that this non-alternation of the bundles at the nodes of the cone of *Equisetum* is due to the persistence of parenchymatous meshes on either side of a strand of xylem, and that this persistence is the direct result of poor development of xylem. More generally, when there is no marked reduction of xylem, and when the position of traces is not disturbed by changes in the number or size of members of successive whorls, the traces of successive whorls alternate with one another. When owing to reduction of xylem in the axis of the cone the traces are superposed, the sporangiophores which they supply still alternate regularly. This is, no doubt, due partly to exigencies of space in the young unelongated cone, but it may possibly be in part due to the retention of an ancestral feature. In Jeffrey's photograph of a cone of *E. arvense* cut in two, some of the strands may not have alternated at the nodes owing to persistence of parenchymatous meshes, while other strands may appear to be continued upwards above the node because the meshes of parenchyma originating above traces are generally narrow in this species, so that a longitudinal cut would not pass through many of them.

Summary of Results.

1. The xylem at the node of a cone of *Equisetum* consists of a woody ring or of woody bands of varying width; in the internode the xylem usually breaks up into definite strands separated by parenchymatous meshes; these generally arise vertically above the points of departure of the traces, often close above them, but never, except perhaps in the reduced apical region, immediately above them.

2. When the xylem is relatively well developed these meshes are closed again in the neighbourhood of the next node; when less xylem is present some of the meshes tend to persist upwards through two or more internodes. When still less xylem is developed meshes of parenchyma tend to arise a little below and to one side of a trace; they also frequently become widened laterally by fusion with a mesh that has originated independently or by congenital fusion with a mesh above a trace departing from the edge of a strand of xylem.

3. The cones of *E. arvense*, *E. palustre*, and *E. limosum* form a series showing increased reduction of the xylem; *pari passu* with this decrease of the xylem the stele comes to form a more and more irregular network of strands.

4. The non-alternation of some of the strands of the cone at the node is due to poor development of xylem in this region causing the persistence of the meshes on either side of a strand.

5. The anatomy of the axis supports the view that the sporangiophores are whole appendages and not lobes of a sporophyll or leaf.

6. The branching of some of the vascular bundles in the neighbourhood of the insertion of the annulus affords an indication of nodal character at this level. This is confirmed by the anatomy of an abnormal specimen of *E. arvense*.

7. In the upper part of the fertile stem of *E. limosum* a diaphragm of the usual type was not found; instead there was an inverted hollow cone of dark brown cells, hanging freely down for a little distance in the central cavity and continuous upwards, above the breaking up of the nodal xylem with the locally somewhat thickened and dark brown endodermis.

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EXPLANATION OF PLATES LXIV AND LXV.

Illustrating Lady Isabel Browne's paper on the Anatomy of the Cone of *Equisetum.*

FIG. 1. Transverse section of the stele of a node of *E. arvense*, showing the bundles united into a ring of xylem and phloem. At the upper side of the photograph the ring is not quite complete; the complete ring of xylem persists a little way below and above the departure of the traces; on one side the section passes through a region where the ring of xylem is complete and no traces are departing. × 80.

FIG. 2. Transverse section of part of the stele at the node of a cone of *E. arvense.* This photograph illustrates a case in which the traces depart from strands unusually far apart, and there is no formation of a ring or bands of xylem. A trace has just departed from a bundle on the reader's left hand. × 80.

FIG. 3. Transverse section of part of the stele of the internode of the cone of *E. arvense.* The separate bundles are wider, compared to the parenchyma between them, than in *E. palustre* or in *E. limosum.* × 80.

FIG. 4. Tangential longitudinal section through part of the cone of *E. arvense.* This photograph illustrates the fact that sometimes the strands alternate at the nodes and sometimes they pass uninterruptedly through the nodes. × 22.

FIG. 5. Transverse section of part of the stele of the axis at the insertion of an abnormal annulus in *E. arvense.* Some of the bundles are in the condition typical of the vegetative internode, while two masses of reticulate tracheides and the phloem outside them are passing in opposite directions and obliquely round the stele. × 65.

FIG. 6. Transverse section of the stele at the node of the cone of *E. palustre.* Two of the trace-bearing bundles are united, the others are separate though more approximated and larger than in the internode. In this species the strands often become united just above the node by their forking, and by the fusion of adjacent forks of neighbouring bundles; all the bundles do not give off traces at exactly the same level, so that the photograph of any single section does not show as much xylem as is present in the nodal region. × 65.

FIG. 7. Transverse section of part of the stele of the internode of *E. palustre*, showing small bundles. × 80.

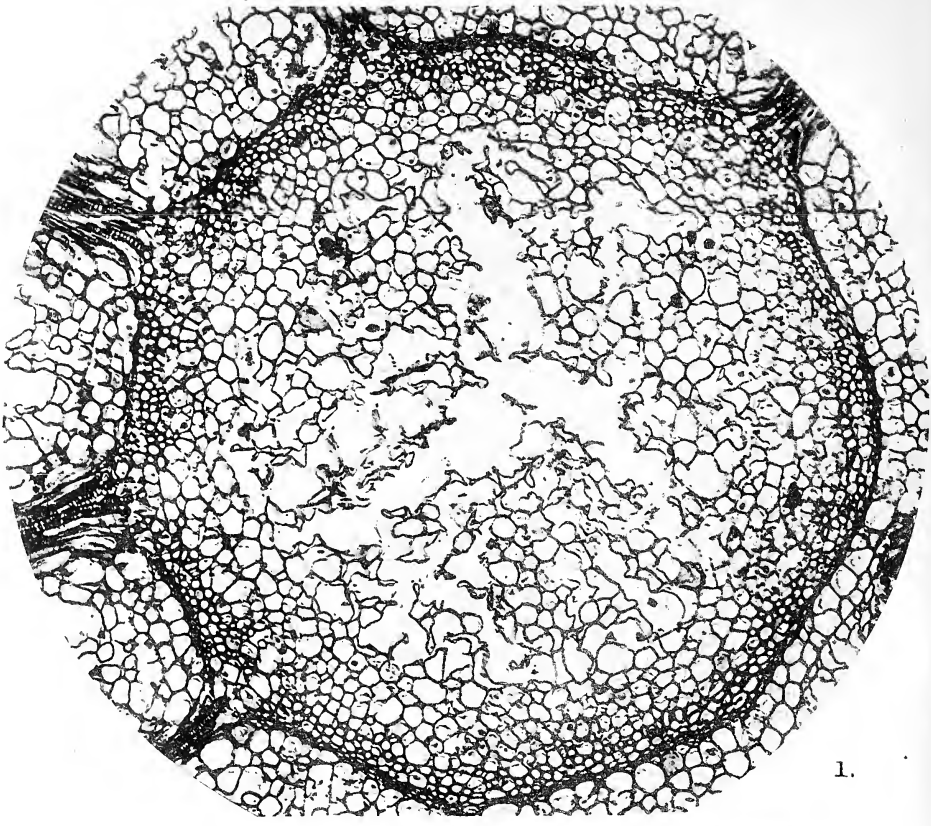
FIG. 8. Transverse section of part of the stele of the internode of a large cone of *E. limosum.* × 80.

FIG. 9. Transverse section of the stele in a small young cone of *E. palustre*, var. *polystachion*, showing ring of separate bundles. × 80.

FIG. 10. Transverse section of stele just above the annulus of small young cone of *E. palustre*, var. *polystachion*, showing the formation of bands of xylem owing to fusion of bundles, unconnected with the departure of any traces. × 80.

FIG. 11. Transverse section of the stele at the node of a small young cone of *E. palustre*, var. *polystachion*; as in the more typical specimens, some bundles are isolated and others fused. × 80.

FIG. 12. Transverse section of the stele of large, mature cone of *E. limosum.* Some of the bundles are giving off traces, but owing to the irregularity of insertion of the sporangiophores, others have done so lower down or will do so higher up. Some of the bundles have already forked above the departure of traces and the branches have begun diverging, but have not yet fused with their neighbours (e. g. the two small bundles in the middle of the photograph on the reader's left); this accounts for the slight increase in number of the bundles; in the internode below there were eighteen, and this is also the number of the traces. × 55.



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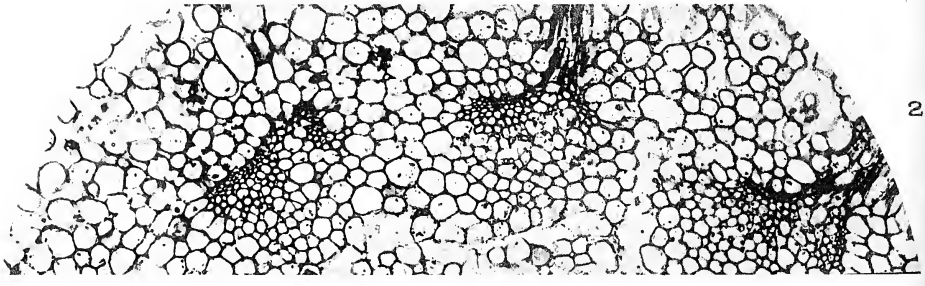

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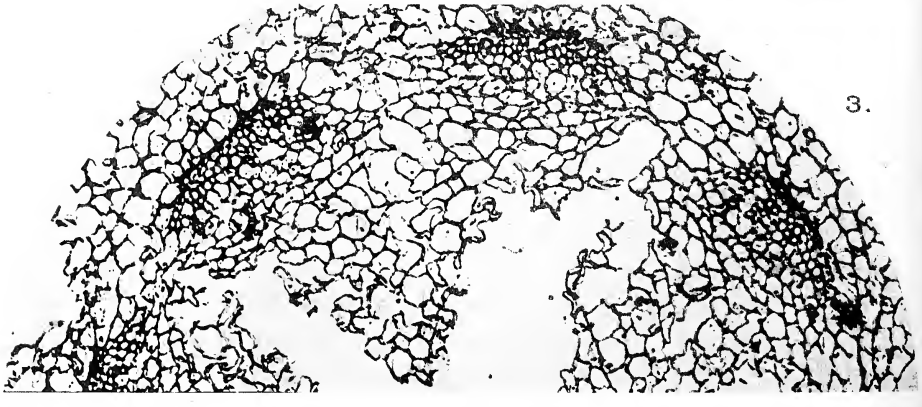

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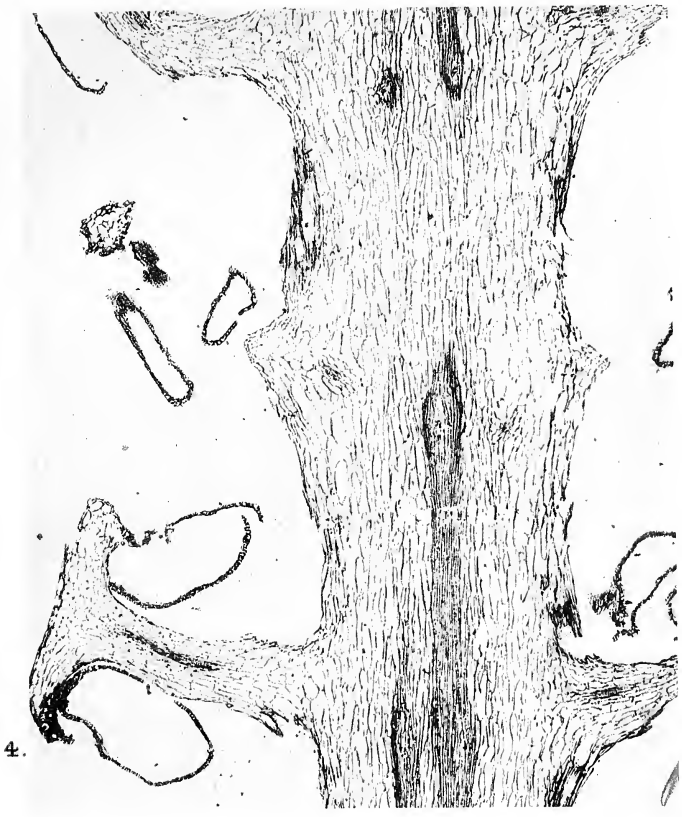
BROWNE — EQUISETUM.



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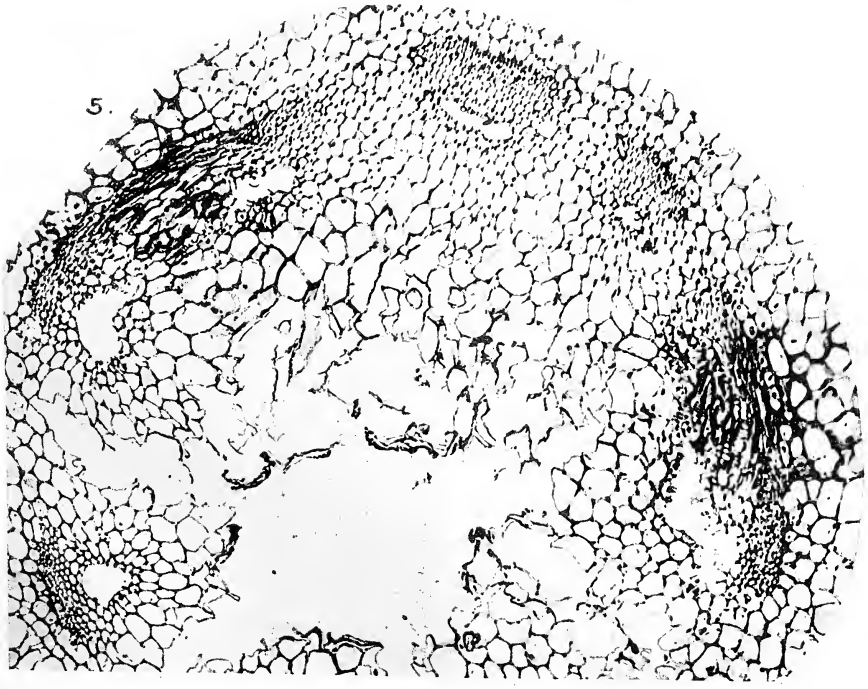

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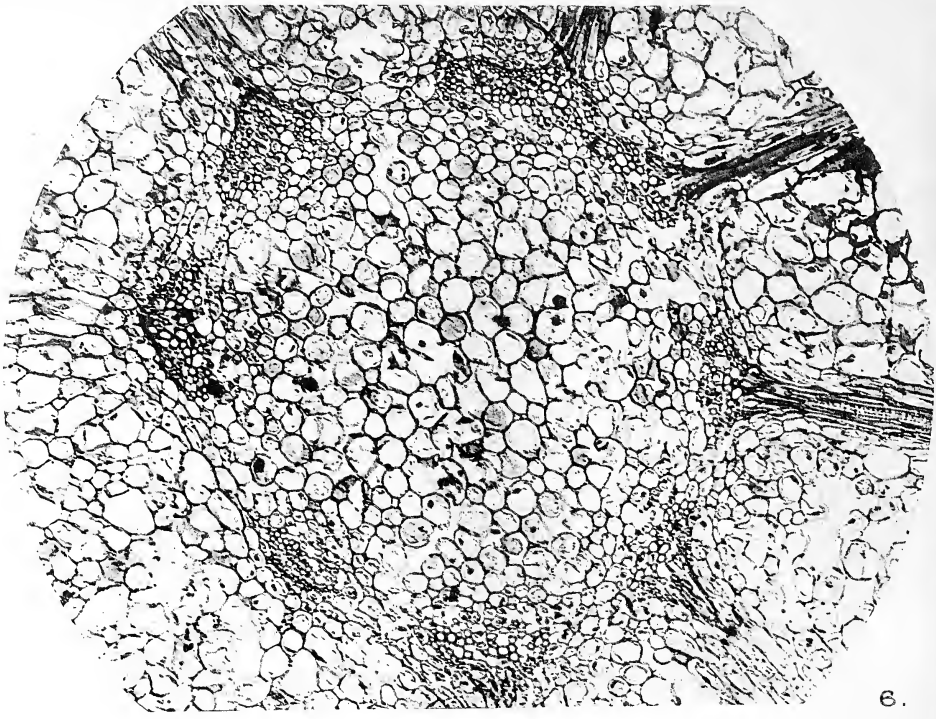

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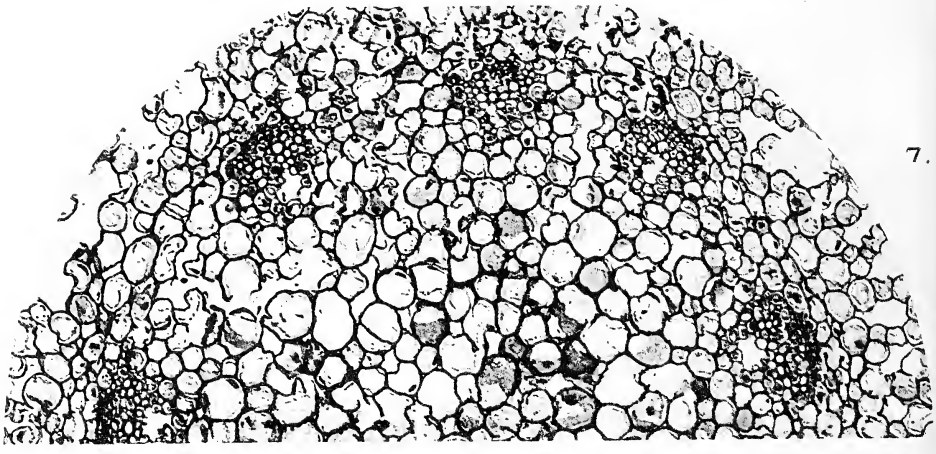

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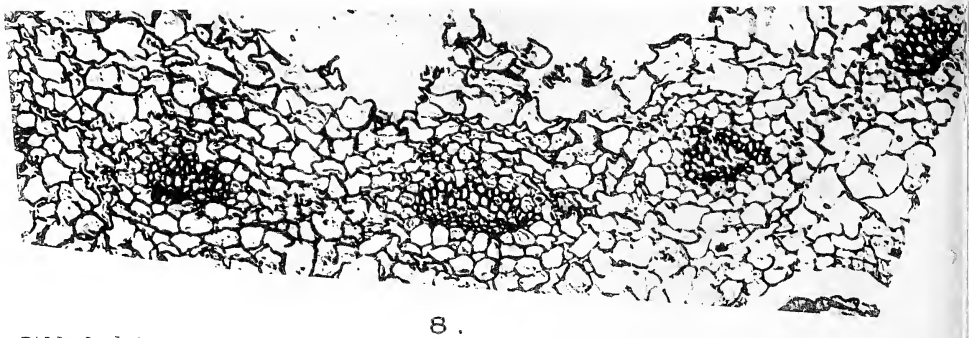

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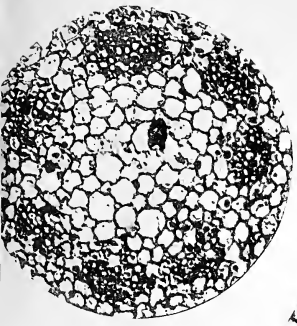
BROWNE — EQUISETUM.



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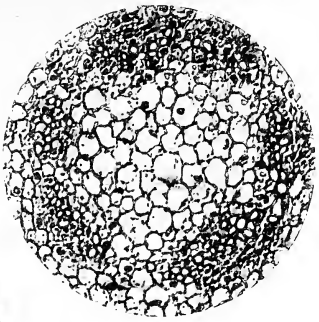

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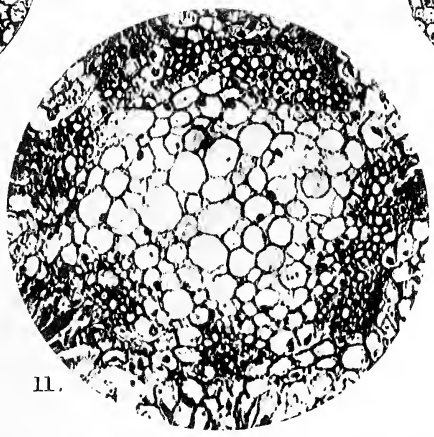

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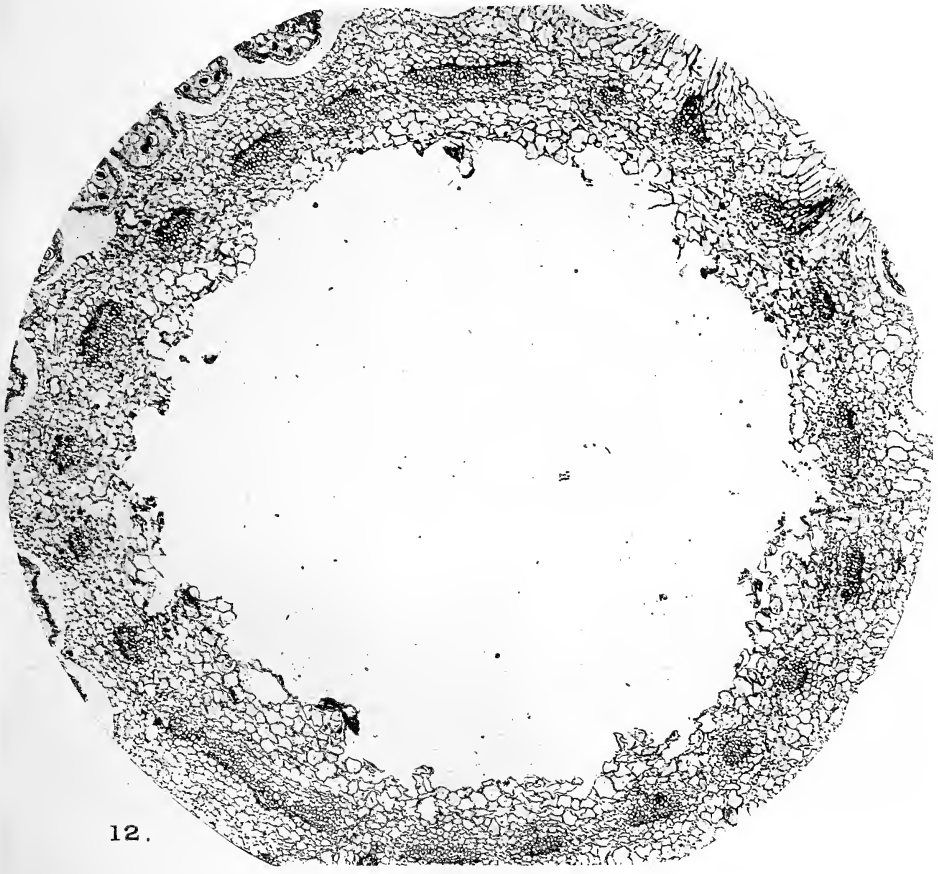

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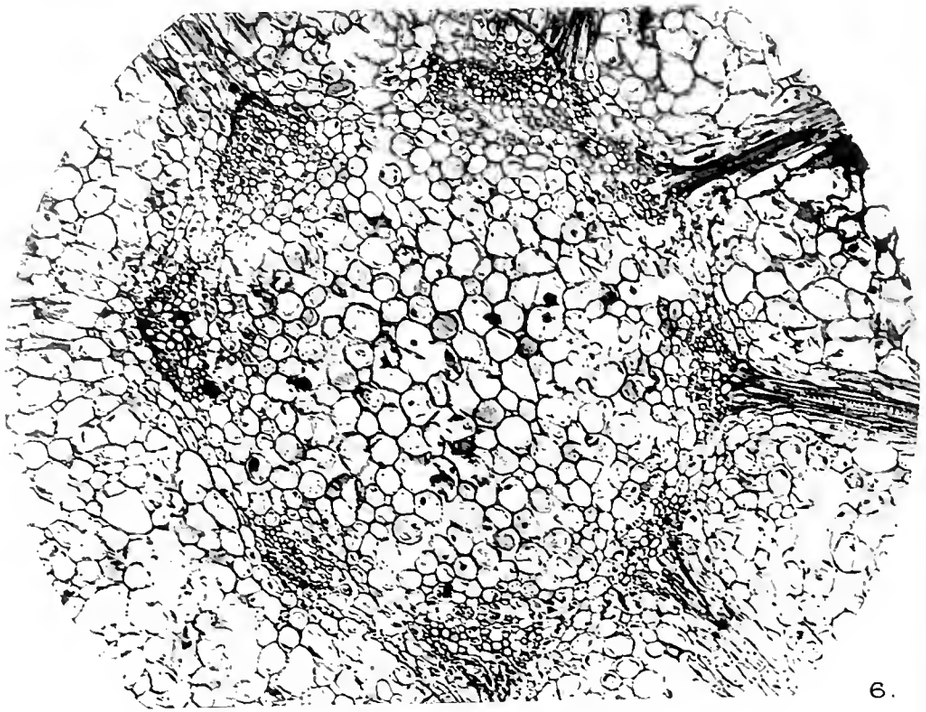

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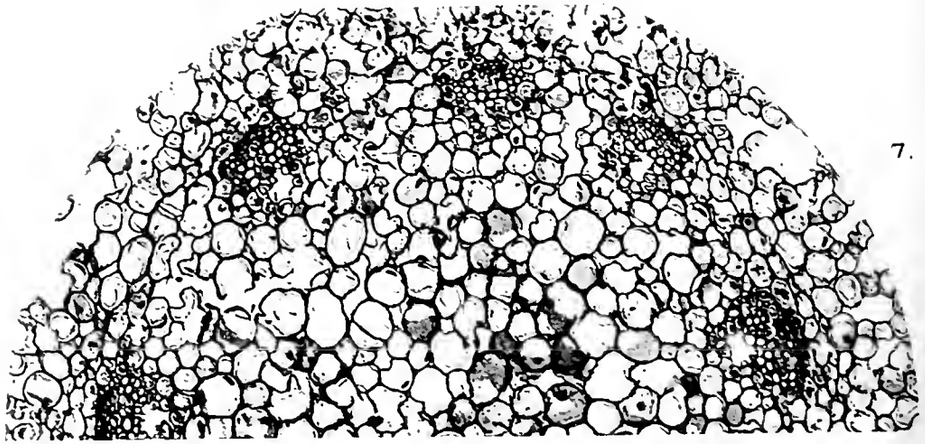

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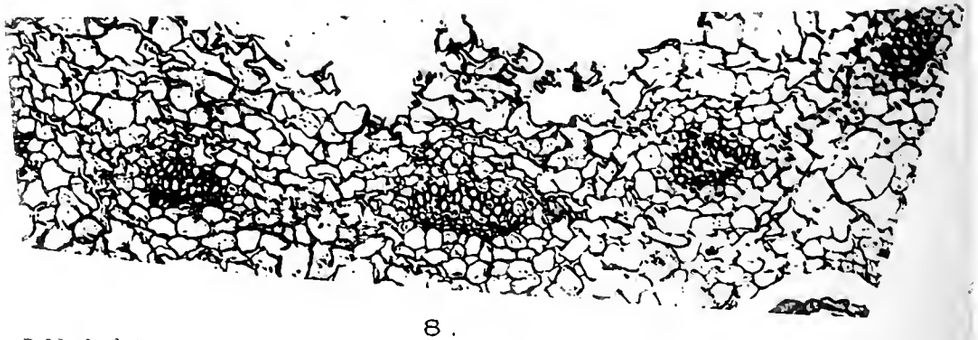

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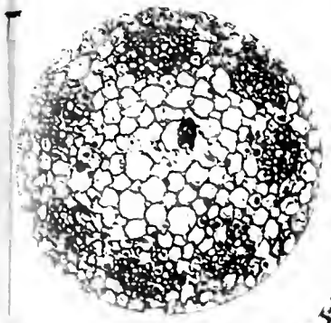

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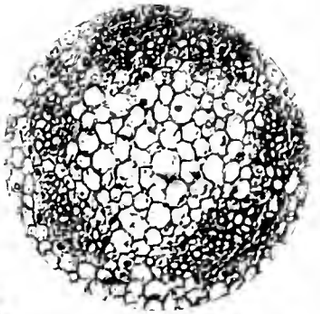

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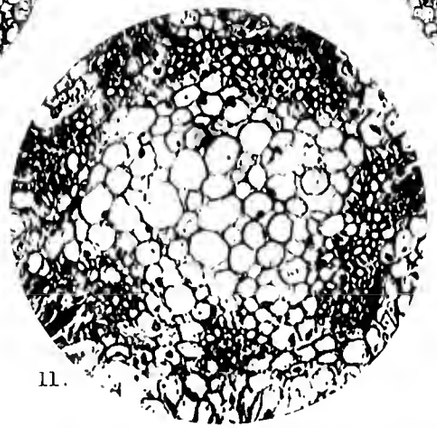

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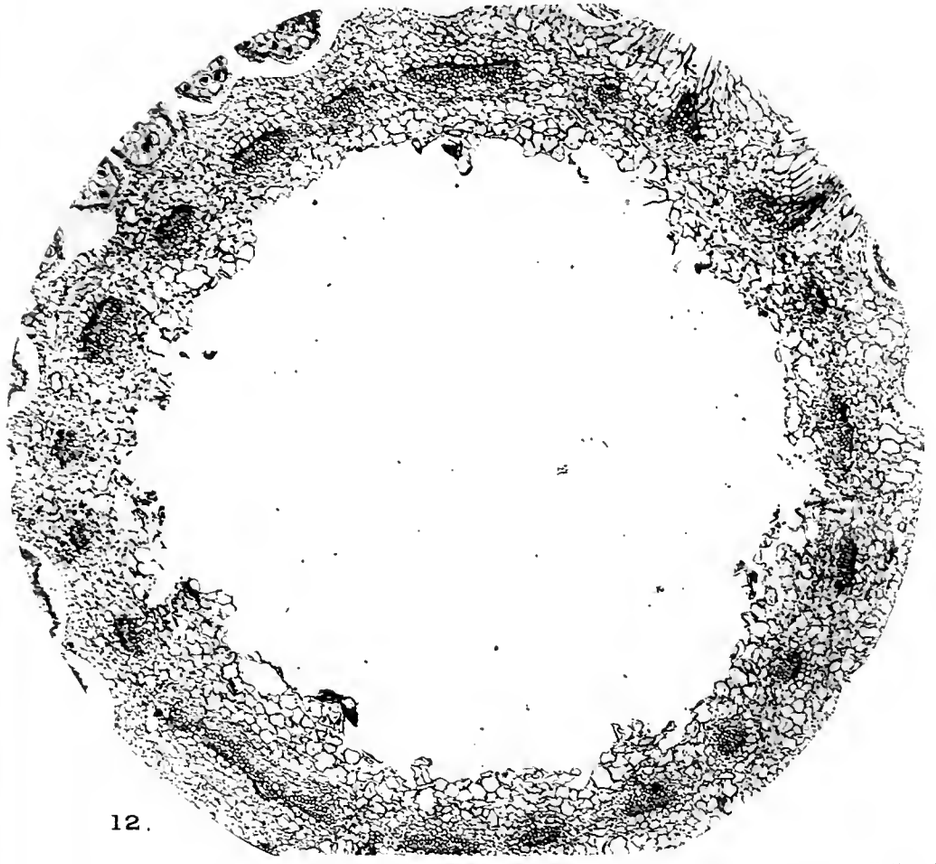

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Studies in Spore Development.

II. On the Structure and Division of the Nuclei in the Compositae.[1]

BY

RUDOLF BEER, B.Sc., F.L.S.

With Plates LXVI and LXVII.

IN 1900 Merrell (16) published an account of the life-history of *Silphium*, in the course of which some reference is made to the nuclear phenomena, both in connexion with the development of the embryo-sac and with that of the pollen-grains. He found that eight is the reduced number of the chromosomes in this plant.

In 1905 Juel (13) added materially to our knowledge of the nuclear divisions taking place in the Compositae by his studies of *Taraxacum*, *Hieracium umbellatum*, and *Crepis tectorum*. In the last-named species he found the reduced number of chromosomes to be only four.

Previous to this work, Juel had published (12) an interesting account of his investigation of *Antennaria dioica* and *A. alpina*, in which the mitotic phenomena were also noticed. The most comprehensive work, however, which has recently been published upon the nuclear phenomena of the Compositae is that which has been carried out by Rosenberg (19 and 20) and Lundegardh (15).

A number of species were examined in these investigations,[2] with the result that they were believed to afford strong evidence in favour of the existence of prochromosomes in these plants and to furnish striking examples of the parasynaptic origin of the chromosomes. A condition resembling that of the second contraction described by Farmer and Moore (5) was recorded by both Rosenberg and Lundegardh in several species of Compositae, but they regard it merely as a chance arrangement of the nuclear contents without any essential significance in the development of the heterotype chromosomes.

[1] I am indebted to the Government Grant Committee of the Royal Society for the loan of a Zeiss 2 mm. apochromatic objective (1·40 aperture) used during the research.

[2] *Hieracium venosum*, *H. Auricula*, *Tanacetum vulgare*, *Crepis virens*, *Calendula officinalis*, *Achillea millefolium*, *Anthemis tinctoria*, and *Matricaria Chamomilla*.

My own observations upon the pollen development of the Compositae were undertaken with the primary object of studying the structure and development of the membrane of these bodies.

I saw no reason to doubt the correctness of the results reached by the Swedish investigators in their study of the reduction divisions, and I, therefore, turned to my preparations of the dividing pollen mother-cells with the full expectation of finding that these would confirm the previous observations. I soon found, however, that my sections presented so many and such notable points of difference from what I had anticipated from Rosenberg's descriptions, that it was no longer possible to dismiss the nuclear phenomena of these plants in a few words, but that they would require a very careful re-examination.

I have, therefore, decided to divide my account of the pollen development of the Compositae into two parts, and to deal in the present communication with the nuclear divisions (both meiotic and somatic) alone, whilst I reserve the detailed description of the pollen-wall for a separate part of these 'Studies'.

In dealing with the meiotic phase I have examined *Tragopogon pratensis*, *Matricaria Chamomilla*, and *Crepis taraxacifolia*, throughout the different stages. In addition to these, I have also carefully examined the earlier prophases of the heterotype division in *Doronicum plantagineum*, *Calendula officinalis*, and *Anthemis Cotula*. The later stages in the telophase of both the heterotype and the homotype division were also studied in the case of *Crepis virens*.

For comparison with the meiotic phase I have also made a careful study of the somatic divisions in *Crepis virens*.[1]

Presynapsis.

In *Doronicum plantagineum*, as the nucleus passes over from the telophase of the last premeiotic division to the 'resting' condition, we find that the gradual vacuolation of the chromosomes and the dispersal of their substance lead to the formation of a coarse reticulum. At one spot this reticulum consists of only delicate fibres, at another the strands of the network are coarse and thick, and the chromatic material may even be aggregated into irregular clumps. There can be little doubt that these thickenings upon the reticulum and the irregular clumps of material are derived from the incomplete dispersal of the chromosome bodies of the last somatic division. There is, however, no constancy in their number, size, form, or arrangement, and they cannot be regarded as prochromosomes in the sense in which this word is used by cytologists (Pl. LXVI, Fig. 1). The nucleus at this time contains either one large nucleolus or two to three some-

[1] The material was fixed, with all precautions, in the stronger Flemming's solution, and in an alcohol and acetic acid mixture.

what smaller ones. The nuclear reticulum has a peripheral arrangement and the nucleolus (or nucleoli) appears to lie in a cavity. When such a nucleus is viewed in section one can occasionally find two chromatic aggregates or two filaments of the reticulum which lie parallel with one another. This, however, is merely due to the sides of the meshes of the network appearing to be closely approximated when seen in section. In a surface view of such a nucleus, this appearance of a parallelism of its parts usually vanishes altogether. Nuclei of *Doronicum* at this stage are shown in Fig. 1, *a* and *b*.

In slightly older nuclei, that is to say, in nuclei which are nearer to synapsis, the reticulum has become somewhat finer and smoother. The chromatic aggregates have become much less evident and the thickened bars or meshes of the network tend to become more even. The nucleolus still lies in a clear cavity. It will be seen from Fig. 2, which represents such a nucleus, that the filaments of the reticulum often have a dotted appearance. This is due to the points of crossing of the filaments or the nodes of the network being seen in optical section, and does not represent a granular arrangement of chromatin particles within the linin threadwork. At this stage the delicate meshwork shows no distinction between chromatin and linin.

In *Tragopogon pratensis* the behaviour of the nucleus during pre-synapsis is very similar. Here also a rather coarse network tends to become smoother and more even before synapsis is entered upon.

In *Tragopogon* the reticulum often appears to reach up to and to be attached to the nucleolus, so that this body is not in such cases surrounded by a clear space. Fig. 3 shows this relation between the nucleolus and the reticulum. Mottier (18) has described a somewhat similar appearance in some of the nuclei of *Lilium Martagon* at a corresponding stage. He mentions, however, that the chromatin lumps are collected round the nucleolus in *Lilium*, but I do not find this in *Tragopogon*.

Calendula officinalis quite agrees with the Compositae which I have already described. A rather coarse network with chromatin aggregates which are irregular in size and form, and of no constant number, becomes more even and with less obvious chromatin lumps upon it before synapsis is reached. Fig. 4 shows these nuclei. *Crepis virens*, *Crepis taraxacifolia*, and *Anthemis cotula* are in no way different.

In *Calendula* and in both species of *Crepis* the nuclear reticulum is peripherally arranged and a clear space lies round the nucleolus.

The description which I have given above and the drawings which illustrate it will show the striking differences which exist between the appearance of my preparations of these stages and the figures and account given by Rosenberg and Lundegardh.

For example, I have searched my preparations of *Calendula* repeatedly for a resting nucleus of a pollen mother-cell containing a number of regularly

paired prochromosomes such as Lundegardh (15) has illustrated in his Fig. 1, Tafel 2. My search has, however, always been in vain, and I have found no single instance in which the chromatic aggregates are regularly arranged in pairs as we see them in Lundegardh's figure, or in which they show any fixed relation to the chromosome number of this plant.[1] I have found, on the other hand, that the structure of these nuclei conforms much more nearly to Davis's account of the presynaptic nuclei of certain Evening Primroses (1, 2, 3). Here he finds a number of chromatic bodies derived from the chromosomes of the last premeiotic division gradually merging their substance with that of the reticulum, from which they cannot finally be differentiated. This reticulum, without any apparent chromatic bodies upon it, passes into synapsis.[2]

In the Compositae the vacuolization and breaking up of the chromosomes at the conclusion of the last premeiotic division are usually fairly complete, and by the time the prophases of the heterotype division are entered upon, any correspondence between the number of chromatic aggregates and that of the chromosomes of the last somatic division is quite lost. The small, irregular chromatic lumps and thickenings upon the reticulum during the early stages of the prophase of the division are, nevertheless, the last remains of the somatic chromosomes which have very nearly, but not quite, distributed their substance over the general reticulum of the nucleus. Before synapsis is entered upon there is usually a still further smoothing out of the reticulum, although there is some variation in the degree to which this progresses. Although, so far as the presynaptic pollen mother-cells of the Compositae[3] are concerned, I find definite prochromosomes or gamosomes to be non-existent, there is no reason to doubt that in the cells of other organisms the chromosomes of the preceding division may possess a more marked persistence, and remain as clearly recognizable bodies which occur in the same number as the chromosomes. We have an example of this in the meiotic cells of the testis of *Triton* described by J. E. S. Moore and Miss Embleton[4] (17).

Synapsis.

The nucleus, containing this usually fine and smooth reticulum, next passes into synapsis. During this process there is an enlargement of the nuclear cavity, and the reticulum becomes gradually drawn together on one

[1] Lundegardh found the somatic chromosomes to number twenty-eight in this plant.

[2] Small, deeply staining bodies often occur in the nuclei of the vegetative cells and archesporial cells (previous to meiosis) of *Calendula*. These are sometimes single and sometimes paired. They are derived from the fragmentation and median vacuolization of the chromosomes of the preceding division. These bodies are, however, always absent from the nuclei of the pollen mother-cells shortly before synapsis (see Pl. LXVII, Fig. 77).

[3] i. e. those species which I have examined.

[4] Compare also the behaviour of the chromosomes of *Matricaria* during the interkinesis of the meiotic phase which is described below.

side of the nucleus. In the majority of cases there is now only a single large nucleolus, which sometimes lies pressed against the nuclear wall, but more often lies on the opposite side of the contracting reticulum. The most important alteration which can be seen to take place in the nuclear contents during the earlier stages of synapsis is the gradual transformation of the reticulum into a long, exceedingly delicate, and closely wound spireme (Figs. 5, 6, and 7). In no case have I found any indication of the occurrence of chromatic bodies which could be interpreted as gamosomes or zygosomes. The nuclear cavity contains no other visible constituent besides the nucleolus and the system of delicate, closely coiled threads. The threadwork is so closely wound together that it is quite impossible for any one to find in it the slightest evidence in support of the occurrence of two systems of threads lying parallel with one another. It would be just as reasonable to claim that a dozen threads could be counted lying parallel with one another in this coil as to assert that two such can be seen thus arranged. Moreover, the few loops which, in some cases, stretch from the margin of the coil into the nuclear cavity, are most certainly single structures. It is only necessary to glance at Fig. 6, which gives a true representation of a nucleus of *Doronicum* at this stage, to fully realize that there is neither any evidence for the existence of two parallel spiremes nor for that of paired gamosomes within the synaptic coil.

During synapsis the spireme becomes gradually thicker and at the same time shorter (compare Fig. 5 with Fig. 8, both of *Calendula*). In many cases this thicker thread appears quite uniform in structure, but where the differentiation of the stain has been carefully carried out, it can be seen that as the synaptic coil loosens and begins to open out, the filament exhibits an alternation of darker coloured areas (chromomeres) with lighter ones. The synaptic knot continues to unfold and passes into the stage of the hollow spireme (Fig. 11).

Hollow Spireme.

As in the later stages of synapsis, so here also the thread often appears homogeneous, but in both cases, when the differentiation of the stain has been properly regulated, the filament can be seen to have a definite structure. In these cases there is an alternation of darker and lighter areas which no doubt signifies that, as is the case with many other plants which have been studied by cytologists, the spireme of the Compositae also consists of a linin thread in which chromatic bodies (chromomeres) are embedded. I have observed this structure of the spireme in all the species of Compositae which I have closely examined. Fig. 9 shows a portion of the spireme of *Calendula* which exhibits this structure very clearly.

The coils of the hollow spireme extend over the entire nuclear space as a series of broad, open loops.

I have found it very difficult to determine whether this spireme forms a continuous thread or whether it is segmented into several parts. In the majority of cases I can find no evidence of the existence of free ends (except where these were obviously due to the microtome knife), and the spireme appears as one long, continuous, much-wound thread. A few nuclei were, however, met with in which the existence of a continuous unsegmented spireme appeared somewhat more doubtful (Fig. 10). It is possible, however, that another explanation may be found for these rather exceptional cases in which free ends of the threads were apparently present. In any case, the segmentation of the meiotic spireme of these plants, if it really occurs at this stage, is very difficult to see, and contrasts strikingly with the obviously segmented spireme of the somatic divisions of the same plants. In the lily, as well as in a large number of plants and animals, a longitudinal split has been described in the spireme. This longitudinal division of the spireme has received very contrary interpretations at the hands of various investigators. Some regard it as a precocious division of the spireme which only reaches its consummation during the homotype separation of the chromosomes. Other cytologists look upon the split in the spireme, not as a true division of that filament, but as the approximation and conjugation of two separate and independent threads. In the case of the Compositae a longitudinal split of the spireme has been described, and very conspicuously figured by both Rosenberg (19, 20) and Lundegardh (15). These writers adopt the view that the division in the spireme is not a true division, but that it really represents the approximation of two independent spiremes.

I have devoted much time and care to the study of this matter, but although many hundreds of sections of pollen mother-cells containing the hollow spireme, either completely developed or still unfolding from the synaptic knot, have been passed in review, I have not succeeded in finding a single unquestionable example of a split spireme! The filament is comparatively thin in these plants, and it is not easy to determine whether the row of chromomeres is single or double; but, however this may be, I have never found an instance in which the spireme was divided into two halves which divaricated in the slightest degree from one another. Other plants belonging to quite different orders to the Compositae have been very thoroughly studied by other cytologists, and the same difficulty in demonstrating a longitudinal division of the spireme has been met with. In the species of *Oenothera* examined by Davis (3), that writer was unable to find a longitudinal split in the spireme. After pointing out that such a split may still quite possibly be found in the future, he adds, 'but, as previously noted, the writer has found no evidence that such a division takes place or that there is a fusion of parallel spiremes.'

It would seem then that there is a good deal of difference in the

behaviour of the spiremes of different plants. In some, such as the lily, the longitudinal separation of the spireme into two halves is a conspicuous and unmistakable phenomenon, whilst in others, such as several species of *Oenothera* and in many (or possibly all) Compositae, this division is either entirely wanting at this stage or the two halves remain so closely pressed together that it is most difficult to demonstrate them.

In any case, whether the spireme of the Compositae obscurely divides longitudinally or not, the facts offer very little support to those who believe in the existence of two independent spiremes which are supposed to approach one another and to become closely approximated. No chromatin bodies (gamosomes) and no threads can be seen approaching one another in order to become intimately associated.

A fine reticulum, without prochromosomes or any other such definite chromatic aggregates upon it, passes into synapsis; here the reticulum becomes transformed into a spireme which is, at first, very delicate, but which gradually thickens and becomes shorter. No definite parallelism of the filaments can be seen other than is inevitably connected with any dense coil of thread. The close synaptic coil gradually unfolds and passes into the hollow spireme, but never once do we find parallel threads coming together to meet laterally in order to become intimately approximated.

This spireme has every appearance of being undivided, but should it prove to be longitudinally split there can be no doubt that the two halves are so closely merged together from the first that no other explanation is possible, but that they have arisen from the real longitudinal division of a previously single thread.

As will be seen below, the later stages of the second contraction often simulate the appearance of a longitudinally divided spireme when the long loops, with closely approximated sides, are viewed in thin sections. It is conceivable that some confusion between the two stages has taken place in the past and that some of the illustrations of a split spireme may more correctly be referred to the later stage.

Second Contraction.

During the next stage in the history of the nucleus the hollow spireme again draws itself together to form the so-called 'second contraction', which has been met with by many observers of the reduction phenomena. The interpretation which has been placed upon this condition of the nucleus is, however, very contradictory.

Some writers, such as Farmer and Moore (5), Mottier (18), Lewis (14), Miss Digby (4), and Dr. Fraser (7, 8), regard this stage as an important one in the development of the bivalent chromosomes.

Other cytologists, however, amongst whom are Rosenberg (19, 20) and

Lundegardh (15), place no importance upon this contraction and consider it to be merely a chance arrangement of the nuclear thread.

Lundegardh (15), writing of *Trollius europaeus*, says, 'Ich will unten zu zeigen versuchen, dass die "second contraction" eine ganz sekundäre Erscheinung ist, die sich in Kernen kurz nach der Spaltung des Kernfadens zeigt, und dass ich ihr nicht wie Mottier u. a. eine tiefere Bedeutung für die Entwicklungsgeschichte der heterotypischen Chromosomen zuerteilen kann.' Again, in dealing with the Compositae he further writes of the 'second contraction', 'Ebensowenig wie bei *Trollius* habe ich bei den Compositen gefunden, dass dieses Stadium etwas spezifisch Eigentümliches mit sich bringe.'

Rosenberg, although he has not infrequently noted a second contraction in the Compositae, is equally sceptical of the importance of this phenomenon in the development of the heterotype chromosomes of these plants.

In my preparations a 'second contraction' has made such a regular and constant appearance at one particular stage in the history of the heterotype division that it seems impossible to regard it as a chance occurrence which has no importance. Moreover, it shows such a striking relation to the development of the bivalent chromosomes that prejudice alone can induce one to deny its significance in this process. I have confined my study of this stage to the three species *Crepis taraxacifolia* (Figs. 12–14), *Tragopogon pratensis* (Figs. 16, *a* and *b*, and 17), and *Matricaria Chamomilla* (Fig. 18), in all of which the contraction is very clearly and regularly shown.

During this stage the coils of the spireme have become arranged in a number of loops, the ends of which are drawn together at one point at which the nucleolus is very frequently situated. In some cases these loops are very long and bent round to accommodate themselves to the nuclear space. In these cases the ends of the loops can often only be seen by altering the focus, or, if the sections are very thin, they must be sought for in the succeeding section of the microtome series (Figs. 13 and 17). In other cases the loops are shorter and their entire extent falls within the field of vision (Figs. 12 and 18). A certain amount of twisting of the arms of the loops about one another can be seen, but this becomes more obvious at a later stage. One or occasionally two nucleoli occur in these nuclei.

It frequently happens, especially where the loops are long, that they tend to close together, and their sides become closely approximated. In such cases the long loops bending through the narrow limits of the nuclear cavity bear a striking resemblance to a hollow spireme consisting of two parallel parts which here and there separate from one another, whilst at other places they merge together. Where the sections are thin and the closed ends of the loops have been severed by the microtome knife the resemblance between the two stages is still further heightened. As was

pointed out before, it is not unlikely that some confusion may have arisen in the past through this behaviour of the loops and their resemblance to a double spireme.

In Fig. 15 I have drawn a long loop of *Crepis taraxacifolia* in which the sides have come closely together. In Fig. 14 is shown a nucleus in which several such loops and their simulation of a double spireme will be evident at once. It was quite easy, nevertheless, to entirely satisfy oneself that the structures in this nucleus were actually closed loops.

In some sections one can without difficulty observe the separation of the loops from their meeting point at the common centre (Fig. 19). At first they remain grouped together with their proximal ends free from one another but still directed towards a common centre. The distal ends of the loops at this time usually have their limbs still united. As the loops become separated from the common centre from which they all radiated just before, the two limbs tend more frequently to become twisted about one another than was the case at an earlier stage.

The isolated loops continue to separate from one another and to travel towards the periphery of the nucleus. Many remain as loops closed at one end, others break across the loop and the two limbs are then independent of one another, although they remain more or less closely associated together (Fig. 20).

Diakinesis.

The two limbs of the loops are for a time long and comparatively slender, but as they reach the nuclear membrane, and there take up the position characteristic of the nucleus during diakinesis, they shorten and become very much thicker. The two arms of the loops are variously arranged with regard to one another. They sometimes appear as crosses, or as rings, or loops still unbroken at one end, or as two rods which lie side by side or which have become closely twisted together. In fact, all the different forms which have been described by cytologists in dealing with the bivalent chromosomes during the diakinesis of various plants, can be found in the appearances presented by the two arms of the loops of the Compositae. It appears to me impossible to doubt that the arms of the loops of these plants correspond to single chromosomes, and that each association of the two limbs of a loop forms a bivalent chromosome (Figs. 20–3).

In *Matricaria* (Fig. 23) by far the most usual form assumed by the chromosomes during diakinesis is that of closed rings. In *Tragopogon* and in the two species of *Crepis*, however, the paired chromosomes occur as often in the shape of a cross, or a loop open at one end, or twisted rods free at both ends, as in that of the closed ring.

The diakinetic chromosomes can often be seen to be joined together by a number of delicate filaments (Fig. 23).

Metaphase, Anaphase, and Telophase.

Following on diakinesis we find the development of the spindle taking place in the usual manner. In Fig. 24 I have shown the six bivalent chromosomes of *Tragopogon* irregularly placed upon the multipolar, polyarch spindle. A layer of denser cytoplasm lies just outside the spindle rudiment. The multipolar spindle later becomes bipolar in the way so often described in other plants. The chromosomes have meanwhile contracted very considerably, and some of their forms in this stage are shown in Fig. 25 for *Tragopogon*. In *Matricaria* the contraction of the bivalent chromosomes, which are nine in number, is even more noticeable, and they present considerable uniformity of shape in this plant. During the metaphase the univalent members of the bivalent chromosomes become drawn apart, and are distributed to the two poles of the spindle during the anaphase.

In Fig. 26 I have represented the end of the metaphase (or beginning of the anaphase) in *Matricaria*. The almost diagrammatic regularity in the form of the chromosomes is very noticeable in this drawing, and has not been at all exaggerated.

In Fig. 27 is seen a later stage in the anaphase of *Matricaria*, whilst in Fig. 28 the chromosomes have reached their destination at the two poles, and are very closely aggregated together. The early and late anaphase in *Tragopogon* are illustrated in Figs. 29 and 30, respectively.

In *Tragopogon*, as well as in *Crepis*, the chromosomes are at first very closely arranged together at the poles (Figs. 31 and 28). There is no nuclear wall about the groups of daughter chromosomes at this time, but a little later such a limiting membrane can be seen to have developed (Figs. 32 and 33*a*).

Just before the new nuclear wall is formed the chromosomes begin to separate slightly from one another. The appearance suggests that an unstained fluid is collecting between and around the chromosomes, and that this presses these bodies apart. Almost coincident with the separation of the chromosomes is the appearance of the new nuclear wall enclosing both the chromosomes and the fluid which has collected round these.

In the later history of the nucleus there can be no doubt that an interchange of materials takes place between the cytoplasm and the karyolymph, and that at least some of the constituents of this fluid are derived from the cytoplasm. The manner in which the *first* nuclear sap collects between the massed chromosomes suggests, however, that this fluid is secreted within the chromosomes themselves, and probably within the vacuoles which can so often be seen in their substance at this stage. The fluid passes out from

the interior of the chromosome bodies and surrounds these with the first karyolymph. Where this karyolymph meets the cytoplasm a precipitation membrane is deposited, and this constitutes the first nuclear wall. Subsequent to this, materials not only pass through the membrane from the nucleus to reach the cytoplasm, but the karyolymph is without doubt reinforced by materials which reach it from without.

As the chromosomes of *Tragopogon* and *Crepis*, which were at first crowded so closely together, begin to separate, their viscous substance remains attached at certain spots, and at these points of union the material of the chromosomes becomes drawn out into connecting bars and arms which become longer and finer as the chromosomes move further apart. At the same time that this is taking place vacuoles appear within the bodies of the chromosomes. These vacuoles not infrequently occupy the central region of the chromosome, and their gradual growth and union tends, in these cases, to divide the chromosome into two longitudinal halves (Fig. 34). This corresponds to the behaviour of the chromosomes observed by Miss Digby (4) during the telophase of the heterotype division in *Galtonia*.

In other cases, however, such a regular arrangement of the vacuoles along the middle of the chromosomes is not apparent, and these bodies resolve themselves into an irregular reticulum without any distinct separation into two longitudinal halves (Fig. 35). As the telophase proceeds the material of the chromosomes gradually becomes more and more distributed along the anastomosing bars and filaments, and at the same time opens out by the increase in alveolization. Complete 'rest' does not appear to be reached in any case, however, for distinct traces of the chromosome bodies of the preceding division can be seen right up to the time that the prophases of the homotype division are entered upon. Figs. 36 and 37 represent two daughter nuclei of *Tragopogon*, as near the 'resting' stage as they ever come.

In *Tragopogon* and *Crepis*, however, the resolution of the chromosomes goes comparatively far, but in *Matricaria* the chromosomes maintain almost their entire independence during interkinesis. In this case we might justifiably speak of the existence of prochromosomes in the nucleus.

On first reaching the poles of the spindle the chromosomes of *Matricaria* are closely crowded together, but a little later they again separate somewhat from one another. During this separation portions of their viscous substance remain adherent and become drawn out as the chromosomes move apart, so that these bodies are connected together during interkinesis by filaments of chromatic material. The formation of these connecting branches often gives the chromosomes an irregular, almost amoeboid, appearance during interkinesis, but they show no indication of alveolization or of becoming otherwise broken up (Fig. 33 *b*). The

karyolymph, if secreted by the chromosomes, is in this case excreted immediately without ever collecting in visible vacuoles within the chromosome bodies. The fact that these chromosomes enlarge somewhat as they move apart is probably due to the formation of karyolymph diffused within their substance, but which does not collect in definite, visible vacuoles.

Homotype Division.

Some difference in the behaviour of the nuclei during this division is shown between *Matricaria* on the one hand, and *Tragopogon* and *Crepis* on the other. The prophases of this division, to a great extent, retraverse the ground gone over in the telophase of the heterotype division, and since *Matricaria* maintained the individuality of its chromosomes almost intact throughout the period of interkinesis, fewer steps are required in their reconstruction and their arrangement upon the equator of the spindle. The fine, connecting branches joining together the chromosomes during interkinesis are withdrawn, and the substance of the chromosomes becomes concentrated into short, rounded bodies. These take up their position upon the equator of the spindle, and it can then be seen that each chromosome is divided into two halves (Fig. 38). The two halves of each chromosome are separated from one another during the anaphase of the division and travel to the poles. During this passage to the poles they remain short, stumpy bodies. Reaching the poles the daughter chromosomes become closely arranged together, as was the case at the conclusion of the heterotype division. A little later a colourless nuclear sap collects between and round the chromosomes, and separates these from one another, but, as in the preceding division, the chromosomes remain adherent at certain spots (Fig. 39). A nuclear wall is deposited, and the chromosomes move more widely apart. Fig. 40 *a*, Pl. LXVII, represents this stage. In the later history of these nuclei no vacuolization of the chromosomes is to be seen, but their substance appears to become gradually distributed along the anastomosing branches which connect them together. A successive series of stages is represented in Fig. 40, *a–d*.

There is some variation, however, in the degree to which the substance of the chromosomes becomes distributed in these nuclei. Sometimes, as in Fig. 40 *d*, the distribution is fairly complete, whilst in other cases the chromatin remains[1] aggregated in masses of varying number and size. Fig. 41 *a*, Pl. LXVI, is drawn from a young pollen-grain at the same stage of development as Fig. 40 *d*, whilst Fig. 41 *b* is from quite a late stage in pollen development (but some time previous to the division of the nucleus of the pollen-grain).

These differences in the state of aggregation of the chromatin no doubt

[1] Or it may possibly have again become aggregated into such masses after first having been distributed.

depend upon the physiological condition of the cell at the time when it was fixed. One or two nucleoli have, meanwhile, made their appearance in these nuclei.

In *Tragopogon* and *Crepis* (both species) the prophases of this division are not quite so simple. The material of the chromosomes of these plants, which was more or less distributed during the telophase of the heterotype division, now again becomes concentrated to reconstruct the chromosomes. In a number of cases this concentration is seen to take place from two sides, so that the young chromosomes are built up of two longitudinal halves with a clear space between them (Fig. 42). In these cases, therefore, the developing chromosomes (and probably also the fully developed chromosomes as well) are longitudinally divided. This is strikingly different from what was seen to occur during the heterotype prophases and, as will be seen below, resembles the behaviour of the somatic chromosomes of these plants. It must be pointed out, however, that this concentration of the homotype chromosomes in two longitudinal halves is not always apparent. In several cases the chromosomes appear to concentrate as single, more or less homogeneous bands (Fig. 43). This may, perhaps, in some instances be due to the aspect from which the chromosomes are viewed. Seen from a lateral or oblique direction the split would often be hidden by one of the sides, especially in those cases in which the chromosome material was still very diffuse. The chromosomes continue to become more concentrated, and they gradually assume a regular, smooth, and compact appearance. They become arranged in the form of a spireme (Figs. 44 and 46), which is probably discontinuous, although it is difficult to be quite certain of this point. In nuclei which are at a slightly more advanced stage the chromosomes are, however, very distinctly separated from one another (Fig. 45).

At first they are long and slender, but they gradually contract into shorter and thicker bodies. The rod-shaped chromosomes which, after the disappearance of the nuclear membrane, are drawn upon the spindle in *Tragopogon* are seen in Fig. 47. The metaphase of the division is illustrated in Fig. 48, which shows that the chromosomes of *Crepis taraxacifolia* are rather longer than those of *Tragopogon*. The early anaphase in *Crepis virens* is represented in Fig. 49. A later stage of the anaphase in *Tragopogon* is shown in Fig. 50, from which it will be seen that the chromosomes of the plant become much drawn out and elongated during their passage to the poles of the spindle. In Fig. 51 the chromosomes of *Tragopogon* have reached the poles, and they then shorten somewhat again. A late anaphase in *Crepis virens* is shown in Fig. 52. There are some points of difference between the telophase of these plants (viz. *Tragopogon* and *Crepis*) and that of *Matricaria*. There is the same coherence of the chromosomes with one another, and the same separation of their bodies by the accumulation of a fluid between them, as is the case in *Matricaria*, but in *Tragopogon* and

the two species of *Crepis* very obvious vacuolization of the chromosome bodies occurs at the same time.

Moreover, the vacuoles tend much more regularly than in the previous division to occupy a central position, and thus to divide the chromosomes into two lateral halves. Figs. 53, 54, and 55 show these facts in the cases of *Tragopogon*, *Crepis taraxacifolia*, and *Crepis virens*.

It will be observed from these figures that by the time that the division is completed and the daughter nuclei entirely reconstructed, the substance of the chromosomes has become more or less distributed through the nuclear cavity. As in *Matricaria*, there is some variation in the degree to which this dispersal of the chromatin attains, and in both cases the state of chromatin aggregation no doubt depends upon the metabolic activity of the cell.

No definite prochromosomes are, however, to be seen in the young pollen-grains. I have illustrated these nuclei in the young tetrads of *Crepis taraxacifolia* (Figs. 57–9).

From the time of the telophase of the first meiotic division until the second division is completed and the pollen-tetrads formed, the cytoplasm contains a number of deeply staining granules or droplets in *Crepis taraxacifolia* (Figs. 56–8), and also in *Crepis virens* (Fig. 55). In *Matricaria Chamomilla* deeply staining particles are also very obvious in the cytoplasm during, and for a short time after, the heterotype telophase, but in the later stages these seem to disappear in this plant (Figs. 28 and 33 *a*). The presence of chromatic granules in the cytoplasm during these stages of mitosis has often been described in other plants, and in one case at any rate the origin of these particles in the cytoplasm has been ascertained. In *Polypodium vulgare* Professor Farmer and Miss Digby (8) succeeded in tracing at least some of these droplets through the nuclear membrane into the cytoplasm.

In the two species of *Crepis* I have spent much time in the attempt to trace the origin of these granules lying in the cytoplasm during the later stages of the meiotic phase. Granules or droplets of chromatin often appear to be passing through the nuclear membrane, but in the great majority of these cases a careful change of focus shows that the granules are really lying against one side of the membrane and are not actually passing through. A few rare instances were, however, met with in which even with the most careful focusing the chromatin droplet still gave the impression of being in the act of passing through the nuclear membrane. I have drawn such a case in Fig. 60. In *Matricaria* several cases more or less similar to the one represented in Fig. 33 *a* were met with. Here the chromatin droplet is on the outside of the nuclear membrane, but it strongly suggests that it is derived from the nuclear chromatin, perhaps before the nuclear wall has been deposited, and that it is passing further into the cytoplasm of the cell. From these facts I believe that the chromatin granules lying in the cyto-

plasm of both the species of *Crepis* and of *Matricaria* may have a nuclear origin. The nucleoli which are cast into the cytoplasm during the divisions are no doubt also responsible for some of these granules.

Somatic Divisions.

In order to make my account of the nuclear phenomena of the Compositae more complete, and for comparison with the meiotic phase described in the foregoing pages, I have also studied the somatic divisions in *Crepis virens* and *Tragopogon*. As the results were essentially similar in the two plants I will restrict my description to *Crepis virens* alone. This plant is particularly well adapted for our purpose on account of the low number of its chromosomes, viz. it possesses six somatic and three heterotype chromosomes respectively, as Rosenberg (20) has already pointed out.

The somatic divisions were studied partly in the vegetative cells of the anther and partly in the other vegetative tissues of the capitulum. The resting nucleus contains, besides the nucleolus, a very delicate reticulum. In some cases this reticulum is so fine that it appears more as a cloudy precipitate of chromatin than as a network (Fig. 61). When such a nucleus is about to divide we find that the chromatin tends to aggregate along certain lines. The manner of this aggregation is, however, not always the same. In what is probably the majority of cases the chromatin gathers together at certain spots from two directions, so that a double line of stainable material is formed (Fig. 62). In this way a spireme gradually condenses out from the indefinite reticulum of the resting nucleus, and this thread is composed of two parallel halves throughout its development (Fig. 65). This corresponds very closely to the descriptions which have been given of this process in other plants by Grégoire (10, 11), Miss Digby (4), and Dr. Fraser and Mr. Snell (9). In other nuclei of this plant (and also in *Tragopogon* and *Crepis taraxacifolia*) the chromatin appears to concentrate along the lines of the future spireme as an undivided whole in which no parallel halves are to be seen (Fig. 63). As was pointed out in the case of the homotype division, this may in some instances be due to the angle from which the spireme rudiment is viewed, but I believe that in both divisions there are cases in which the chromosomes develop as single undivided structures during the early stages. It is more particularly in those nuclei in which the chromatin has the appearance of a cloudy precipitate that this type of development is met with. Both Grégoire (11) and Miss Digby (4) have described an uneven concentration of the chromatin to form a spireme which has a corkscrew-like appearance. I have observed a similar zigzag arrangement of the developing chromosome bands in *Crepis virens* (Fig. 64).

Whichever plan of concentration is followed it results in the formation of a spireme which is most certainly discontinuous, and which is more or less clearly divided longitudinally (Fig. 66, *a* and *b*). Dr. Fraser and Mr. Snell (9)

have found in *Vicia Faba* that the somatic chromosomes form a continuous spireme which subsequently segments into the constituent chromosomes. Both *Allium* and *Galtonia*, upon the other hand, resemble *Crepis virens* in possessing no continuous spireme. This is clearly shown in the writings of Grégoire (11) and Miss Digby (4).

Although not always to be seen, a certain amount of polarization of the developing spireme is sometimes noticeable in *Crepis*. An exceptionally marked case of such polarization was observed in the premeiotic division of one of the archesporial cells of the anther of this plant (Fig. 67).

The chromosomes next proceed to shorten and thicken; a spindle develops in the usual way, and the chromosomes are drawn upon the equator of this structure. At this stage the form and number of the chromosomes are very clearly to be seen. In a large number of cases there appears to be, as Rosenberg has shown, a constancy in the relative sizes of these bodies. In Fig. 68 it is seen that there are present two large chromosomes, two small ones, and two of an intermediate size. Each chromosome is distinctly divided longitudinally. My figure corresponds almost exactly with Rosenberg's Fig. 4, Plate I (20). It must be pointed out, however, that this definite distinction in the sizes of the chromosomes is not always so easy to see. In Fig. 69, which shows the metaphase of another nucleus of *Crepis virens*, these relative sizes of the chromosomes are not nearly so clearly expressed. In such cases the action of the fixative may most likely have had some effect upon the form of the chromosomes and the direction from which they are viewed (whether slightly obliquely or not) may also influence the result. On the whole I am inclined to agree with Rosenberg that the six chromosomes of this plant occur constantly in the relative sizes which he has described. The two halves of each chromosome are drawn apart and distributed to the two poles. At this time (late anaphase) some of the daughter chromosomes have the form of bent rods with one long limb bearing a short hook at the end (Fig. 70 *a*). At the poles the chromosomes shorten considerably and become very closely pressed together (Fig. 70 *b*). They next again separate slightly from one another and become alveolized. No doubt the same causes which are at work in separating the meiotic chromosomes during the telophase are also active here.

A nuclear wall is then formed round the chromosomes and the karyolymph which has begun to accumulate between them (Fig. 71). In by far the greater number of cases which have been observed the alveolization of the chromosomes takes place in such a way as to divide these bodies into two longitudinal halves (Fig. 71). Moreover, during the loosening of the ball of daughter chromosomes their viscid substance has remained adherent at certain spots, and it becomes drawn out into connecting filaments as the separation of the chromosomes proceeds. In a much smaller proportion of cases the central vacuolization of the chromosomes is not so apparent, and

there seems to be instead a general opening out of the substance of the chromosomes so that they assume a spongy or porous appearance (Fig. 72). In either case the result is the same, and the material of the chromosomes becomes gradually dispersed through the nucleus until it again reaches the state of a more or less even reticulum, or assumes the appearance of a cloudy, flocculent precipitate which we have already seen characterizes the 'resting' nuclei of this plant. No definite aggregates of chromatin (prochromosomes) can be seen in such a resting nucleus (Fig. 73). It should be mentioned that throughout this division the chromosomes, or the bands of concentrating chromatic material which precede them, show no distinction of chromatin and linin. During the heterotype division the spireme could be very clearly seen to be composed of chromatin particles (chromomeres) embedded in a clear linin thread, but in the somatic divisions nothing of this sort is apparent.

From what has been said above it will be seen that in *Crepis*, as in *Galtonia* (examined by Miss Digby), there is no stereotyped plan which is invariably followed by the somatic chromosomes in their development or in their passage back to the resting state. Apart from minor variations, however, we may say that it is the general rule in this plant for an extremely early longitudinal division of the chromosomes or chromosome rudiments to provide for the separation of the daughter chromosomes during the anaphase of mitosis, and that in consequence of this precocious division a general parallelism of parts is often to be seen. In a large number of cases observed the daughter chromosomes themselves became centrally vacuolated and divided at the telophase of a division.

During the prophase of the following division it is very usual to find the chromosomes reconstructed in two halves, and this dual nature of the chromosomes is maintained until the separation of these halves is effected at the anaphase. In such cases as this, it does not appear to be straining the facts to consider the median division of the daughter chromosomes at the telophase of one division as an extremely early preparation for the separation of their longitudinal halves at the conclusion of the following division. This is quite in accordance with what has already been observed by both Miss Digby (4) and Dr. Fraser and Mr. Snell (9) in other plants. But I must again repeat that there are other instances in which no such distinct central vacuolization of the chromosomes can be detected during the telophase, and in which we must assume that their longitudinal division is deferred until a later stage.

The frequent occurrence of parallel filaments and bands during the somatic divisions of these plants forms a contrast to what is seen in the heterotype division in which such parallel parts are much more rarely met with.

In the preceding pages I have exclusively devoted my attention to the

nuclear phenomena of the Compositae, in so far as these are to be seen in the pollen mother-cells and (for comparison) in certain somatic cells of these plants. I have reserved all details regarding the structure of the pollen-membrane for a separate account, but I should like, before I conclude this article, to add one word concerning a general feature of the pollen-wall of the Compositae.

Among all the species which I have examined for this purpose I have found constantly two different types of pollen-wall.[1] In the one type the exine is flat and unfolded, whilst in the other type it is thrown into a number of distinct folds. These two types appear to be constant for the two great divisions of the Compositae. All the members of the Tubuliflorae which I have examined possess pollen-grains with an unfolded exine (Figs. 74, 76), whilst all the Liguliflorae which I have observed exhibited pollen-grains with a folded exospore (Fig. 75).

Summary.

1. During the period just preceding synapsis the nuclei of those Compositae which have been examined were found to contain a more or less fine reticulum. No definite aggregations of chromatin which could be regarded as prochromosomes were in any case found upon this reticulum.

2. The reticulum passes into the synaptic contraction, during which it is gradually converted into a very long, delicate spireme. No chromatic aggregates were observed during this stage, and no definite parallelism of the threads occurs other than is inevitable in any closely coiled system of filaments.

3. The spireme which emerges from the closely wound synaptic knot has become thicker, and, in favourable examples, shows a series of chromatic particles (chromomeres) embedded in a less deeply stained linen thread. The spireme, during the hollow spireme stage, either remains unsplit or may be very obscurely divided longitudinally. No evidence was found of the existence of two parallel spiremes.

4. The spireme again draws itself together in the 'second contraction'. In doing so it forms a series of loops, radiating from a common centre. The loops become detached, thicken considerably, and pass towards the periphery of the nucleus. Here they assume the shape of rings, crosses, parallel rods, twisted rods, or remain as loops.

There can be no doubt that the loops which are formed in this way are actually the bivalent chromosomes of the heterotype division, and that they are constituted of two univalent chromosomes joined end to end.

[1] I have examined up to the present the following species: *Tragopogon pratensis, Matricaria Chamomilla, Doronicum plantagineum, Crepis taraxacifolia, C. virens, Arctium Lappa, Olearia Haastii, Sonchus asper, S. oleraceus, Cnicus arvensis, Hieracium Pilosella, Tagetes plumilla, Gaillardia* sp., *Coreopsis* sp., *Hypochaeris radicata, Bellis perennis, Senecio vulgaris, Anthemis Cotula, Artemisia vulgaris, Tussilago Farfara, Petasites fragrans, Taraxacum vulgare.*

5. A spindle develops, the bivalent chromosomes are drawn upon it, and the single chromosomes separate from one another and travel to the two poles of the spindle.

6. Here they remain either almost unchanged in the daughter nuclei during interkinesis (*Matricaria*) or they may undergo partial dispersal of their substance (*Tragopogon*, *Crepis*). A condition of complete 'rest' is, however, not reached in any of these forms. In *Matricaria* there is no vacuolation of the chromosomes at this time, but in *Tragopogon* and *Crepis* vacuoles do make their appearance in these bodies. In these cases the vacuoles may occupy the central line of the chromosomes, and thus divide these structures into two longitudinal halves (as in *Galtonia*), or quite often no such regular disposition of the vacuoles is to be observed and the breaking up of the chromosome bodies takes place less regularly.

7. The homotype division which follows resembles, in most respects, an ordinary somatic division. The halves of the longitudinally divided chromosomes are separated during the anaphase of this division.

8. The somatic divisions were studied in *Crepis virens*, which was a convenient object on account of the low number of its chromosomes (6 somatic). Considerable variety was found to exist in the manner in which the chromosomes develop during the prophase of division. In a large number of cases this takes place by the condensation of the nuclear reticulum along the two sides of certain lines or bands. This gives rise to condensation-areas (rudimentary chromosomes), which consist of two parallel halves. Condensation may, however, take place differently in other cases, but sooner or later it leads to the formation of longitudinally divided chromosomes.

9. No continuous spireme is formed during the somatic divisions.

10. The daughter chromosomes of the somatic division are distributed to the poles, where they become joined together by delicate bars and filaments of chromatin and their substance undergoes vacuolization. By the continuation of this process the substance of the chromosomes gradually becomes dispersed over a delicate reticulum. No prochromosomes are to be seen in the resting nuclei which are in this way produced.

In the majority of cases the vacuolation of the chromosomes takes place along a median line so as to divide these bodies into two longitudinal parts.

There is reason to believe that this division of the chromosomes during the telophase may be identical with the one which so often reappears at the prophase of the following mitosis, and which marks the line of separation between the daughter chromosomes which are drawn apart during the anaphase.

It should be noted, however, that just as there was found to be considerable variation in the details of the condensation phenomena of the

prophase, so also is there no hard and fast line which is invariably followed by the chromosomes in the dispersal of their substance at the telophase.

11. There are two types of pollen-wall in the Compositae. All the Tubuliflorae which have been examined possess pollen-grains with an unfolded exine, whilst the exospore of all the Liguliflorae observed is thrown into a number of distinct folds. The details of the structure of the pollen-membrane is reserved for a separate part of these 'Studies'.

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EXPLANATION OF PLATES LXVI AND LXVII.

Illustrating Mr. Beer's paper on the Structure and Division of the Nuclei in the Compositae.

All figures were drawn with the aid of the camera lucida. Either Zeiss's apochrom. objective 2 mm. (aper. 1·40) or Leitz's $\frac{1}{10}$ inch objective was used with various compensating oculars.

Figs. 1–37 refer to the heterotype division.

Figs. 38–60 refer to the homotype division.

Figs. 61–73 refer to the somatic divisions of *Crepis virens*, with the exception of Fig. 63, which represents the somatic division of *C. taraxacifolia*.

Fig. 1, *a* and *b*. *Doronicum plantagineum*. Nuclei during presynapsis. *a*. Nucleus seen in section. *b*. Nucleus in surface view. × 2,600.

Fig. 2. *Doronicum plantagineum*. Nucleus shortly before synaptic contraction. × 1,900.

Fig. 3. *Tragopogon pratensis*. Nucleus previous to synapsis. × 2,600.

Fig. 4, *a* and *b*. *Calendula officinalis*. Nuclei previous to synapsis. × 2,000.

Fig. 5. *Calendula officinalis*. Early synapsis. × 2,000.

Fig. 6. *Doronicum plantagineum*. Synapsis. × 2,600.

Fig. 7. *Doronicum plantagineum*. Synapsis, with a rather looser coil than in Fig. 6. × 2,600.

Fig. 8. *Calendula officinalis*. Synapsis; later stage. × 1,900.

Fig. 9. *Calendula officinalis*. Portion of spireme showing chromomeres. × 2,600.

Fig. 10, *a* and *b*. *Crepis taraxacifolia*. Hollow spireme. × 2,600.

Fig. 11. *Matricaria Chamomilla*. Unfolding of synaptic coil. × 1900.

Fig. 12. *Crepis taraxacifolia*. Second contraction. × 2,600.

Fig. 13. *Crepis taraxacifolia*. Second contraction. Sides of loops rather near together and ends of loops out of the field of vision. × 2,600.

Fig. 14. *Crepis taraxacifolia*. Second contraction. Sides of loops have become closely approximated, and the long, bent loops give the impression of a longitudinally divided spireme. × 1,900.

Fig. 15. *Crepis taraxacifolia*. Single loop of second contraction with closely approximated sides. × 2,600.

Fig. 16, *a* and *b*. *Tragopogon pratensis*. Second contraction. × 1,500.

Fig. 17. *Tragopogon pratensis*. Second contraction. Sides of loops approximated and their ends not in field of vision. × 1,500.

Fig. 18. *Matricaria Chamomilla*. Second contraction. × 1,500.

Fig. 19. *Matricaria Chamomilla*. Loops (= bivalent chromosomes) separating from their common meeting point. × 2,600.

Fig. 20. *Tragopogon pratensis*. Loops (= bivalent chromosomes) soon after they reach the periphery of the nucleus. × 1,500.

Fig. 21. *Crepis taraxacifolia*. Single loop (= bivalent chromosome) in which the closed end of the loop is just breaking across. The arms of the loop (= univalent chromosomes) joined by delicate chromatic processes. Nucleus at about the same stage as that shown in Fig. 20. × 2,600.

Fig. 22. *Crepis taraxacifolia*. Two bivalent chromosomes during diakinesis. × 2,600.

Fig. 23. *Matricaria Chamomilla*. Diakinesis. The chromosomes have all assumed the form of rings which are joined together by delicate filaments. × 1,900.

Fig. 24. *Tragopogon pratensis*. Multipolar spindle with six chromosomes upon it. × 2,600.

Fig. 25. *Tragopogon pratensis*. The six chromosomes during metaphase. × 2,600.

Fig. 26. *Matricaria Chamomilla*. Commencement of anaphase. The regular form of these chromosomes is to be noted. × 1,900.

Figs. 27 and 28. *Matricaria Chamomilla*. Two stages of the anaphase. × 1,900.

Figs. 29 and 30, *a* and *b*. *Tragopogon pratensis*. Commencement of anaphase and a late stage of the same respectively. Fig. 30 *b* shows a polar view of daughter chromosomes. × 1,500.

Fig. 31. *Tragopogon pratensis*. Late stage of anaphase. × 1,500.

Fig. 32. *Tragopogon pratensis*. Telophase. A nuclear membrane has developed round the daughter chromosomes. × 1,500.

Fig. 33, *a* and *b*. *Matricaria Chamomilla*. *a*. Telophase of heterotype division. *b*. Interkinesis. × 1,900.

Fig. 34. *Crepis taraxacifolia*. Telophase with median vacuolization of chromosomes. × 1,500.

Fig. 35. *Tragopogon pratensis*. Telophase with diffuse vacuolization of chromosomes. × 2,600.

Figs. 36 and 37. *Tragopogon pratensis*. Interkinesis. Fig. 36 × 2,600; Fig. 37 × 1,500.

Fig. 38. *Matricaria Chamomilla*. Metaphase of homotype division. × 1,900.

Fig. 39. *Matricaria Chamomilla*. Telophase of homotype division. × 1,900.

Fig. 40, *a–d*. *Matricaria Chamomilla*. Four successive stages of homotype telophase. *d* is from a young pollen-grain with its own wall round it. × 1,900.

Fig. 41, *a* and *b*. *Matricaria Chamomilla*. Nuclei from young pollen-grains. 41 *a* is from a pollen-grain of approximately the same stage as 40 *d*. × 1,900.

Fig. 42. *Crepis taraxacifolia*. Homotype division. Concentration of chromosomes in two longitudinal halves. × 1,900.

Fig. 43. *Tragopogon pratensis*. Homotype division. Concentration of chromosomes as undivided bands of chromatin. × 2,600.

Fig. 44. *Crepis taraxacifolia*. Homotype division. Spireme. × 1,900.

Fig. 45. *Crepis taraxacifolia*. Homotype division. Spireme. Chromosome segments distinct. × 2,600.

Fig. 46. *Crepis taraxacifolia*. Homotype division. Spireme. × 1,500.

Fig. 47. *Tragopogon pratensis*. Homotype division. Chromosomes on equator of spindle. × 2,600.

Fig. 48. *Crepis taraxacifolia*. Homotype division. Metaphase. × 2,600.

Fig. 49. *Crepis virens*. Homotype division. Early anaphase. × 2,600.

Figs. 50 and 51. *Tragopogon pratensis*. Homotype division. Two successive stages of the anaphase. × 2,600.

Figs. 52. *Crepis virens*. Homotype division. Late anaphase. × 2,600.

Figs. 53, 54, and 55. Telophase of homotype division in *Tragopogon pratensis, Crepis taraxacifolia*, and *C. virens* respectively. Central vacuolization of chromosomes. Fig. 53 × 1,900; Figs. 54 and 55 × 2,600.

Figs. 56, 57, and 58. *Crepis taraxacifolia*. Homotype division. Telophase. Chromatin droplets in cytoplasm. × about 2,000.

Fig. 59. *Crepis taraxacifolia*. Two nuclei from very young pollen-grains (still united in the tetrad). × 1,900.

Fig. 60. *Crepis virens*. Homotype division. Telophase. Chromatin droplet passing into the cytoplasm. × 1,900.

Fig. 61. Resting nucleus from anther wall. × 2,600.

Fig. 62. Prophase. Concentration of chromosomes in two halves. × 2,600.

Fig. 63. Prophase in *Crepis taraxacifolia*. Concentration of chromosomes as single, undivided bands. × 2,600.

Fig. 64. Prophase. 'Corkscrew' spireme. × 2,600.

Fig. 65. Spireme concentrating in two longitudinal halves. Anther wall. × 2,600.

Fig. 66, *a* and *b*. Spireme longitudinally divided. × 2,600.

Fig. 67. Prophase of premeiotic division in an archesporial cell. Polarization of parts is to be noted. × 2,600.

Fig. 68. Metaphase. Two long chromosomes, two short ones, and two of intermediate size occur in this nucleus. × 2,600.

Fig. 69. Metaphase. Six chromosomes from another nucleus. × 2,600.

Fig. 70, *a* and *b*. Two stages of the anaphase. × 2,600.

Fig. 71. The two daughter nuclei in telophase. × 2,600.

Fig. 72. Daughter chromosomes in telophase. × 2,600.

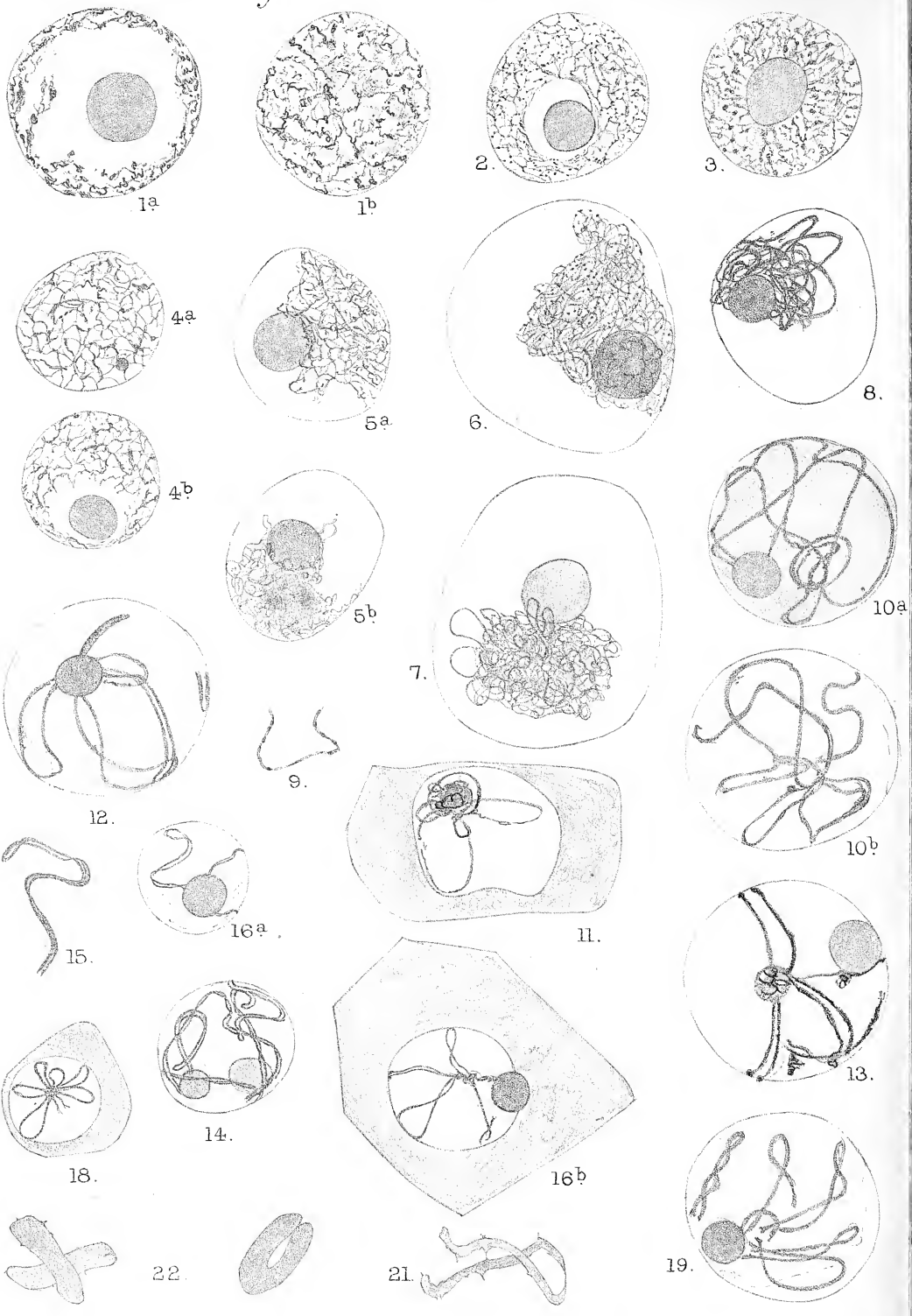
Fig. 73. Conclusion of telophase. Daughter nuclei passing into 'resting' state. × 2,600.

Fig. 74. Portion of pollen-grain of *Matricaria Chamomilla* (Tubuliflorae). × 1,900.

Fig. 75. Pollen-grain of *Tragopogon pratensis* (Liguliflorae). × 1,500.

Fig. 76. Pollen-grain of *Artemisia vulgaris* (Tubuliflorae), with smooth, unfolded exospore. × 1,100.

Fig. 77. 'Resting' nucleus from the anther wall of *Calendula*. × 1,900.



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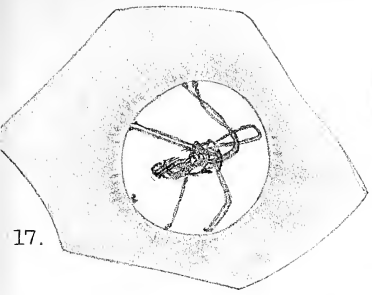
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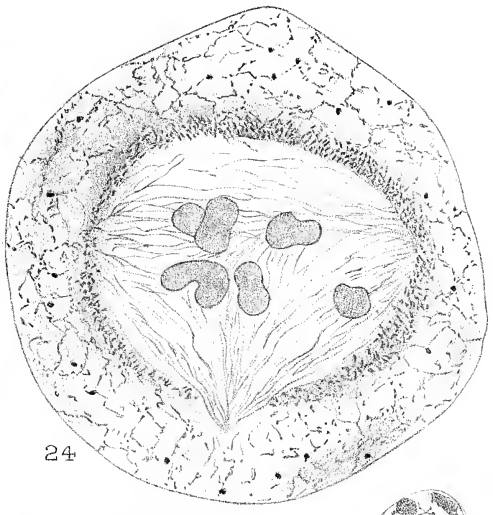
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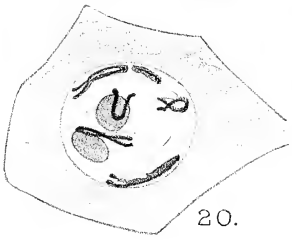
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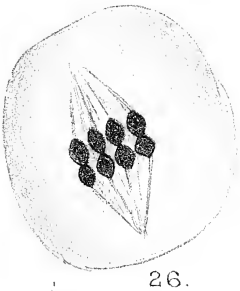
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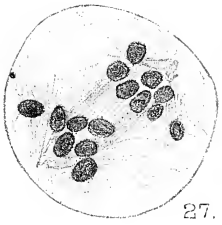
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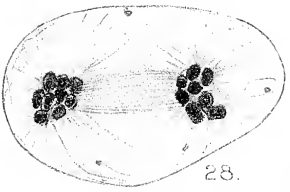
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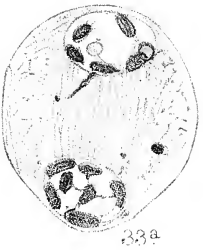
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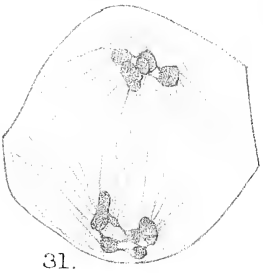
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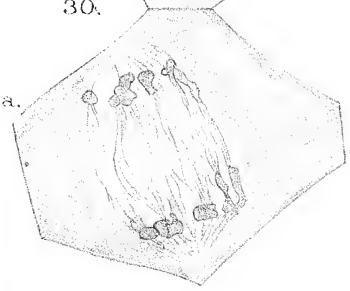
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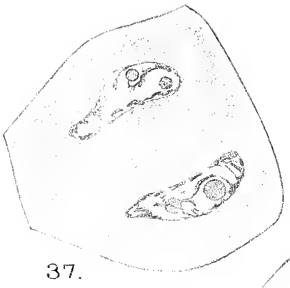
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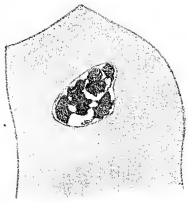
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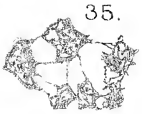
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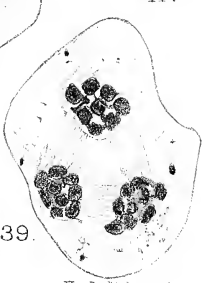
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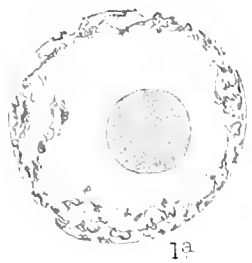
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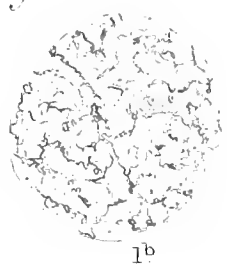
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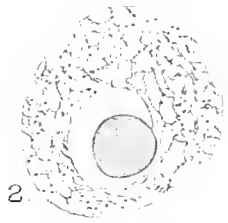
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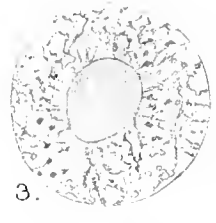
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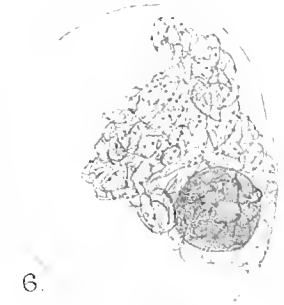
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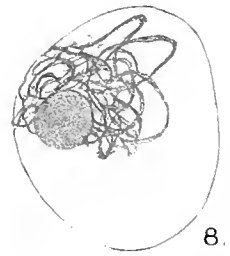
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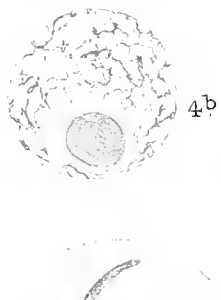
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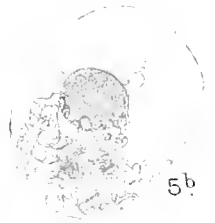
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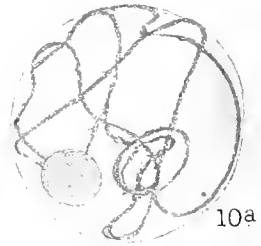
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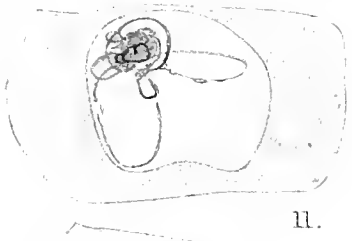
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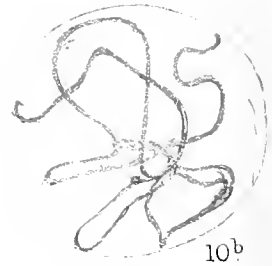
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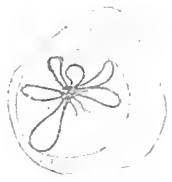
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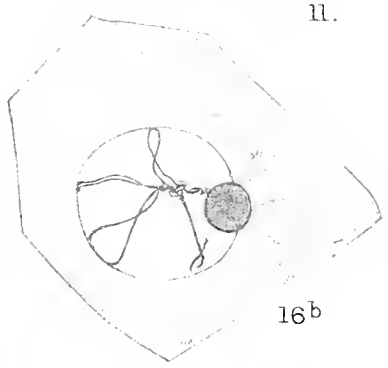
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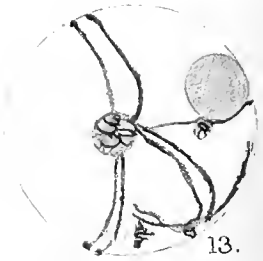
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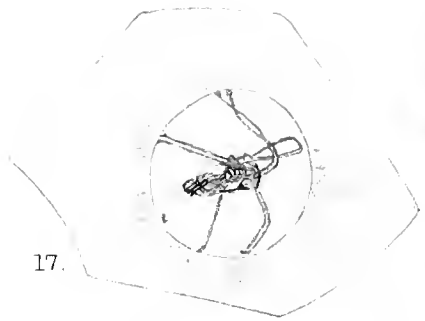
BEER —— SPORE-DEVELOPMENT.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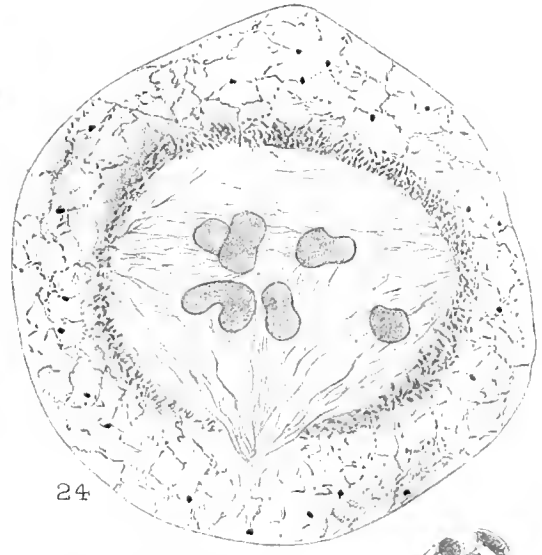
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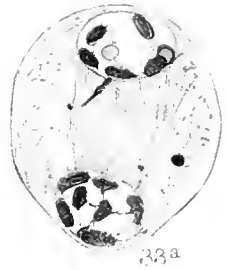
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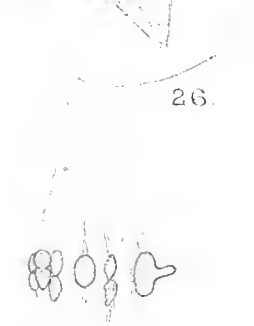
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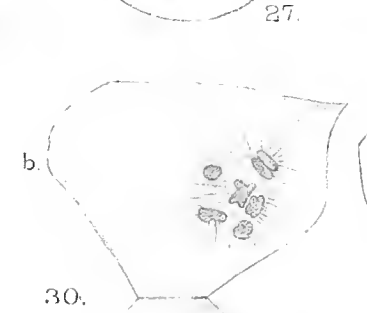
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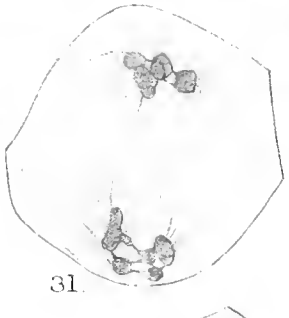
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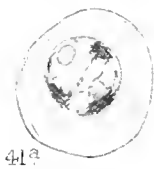
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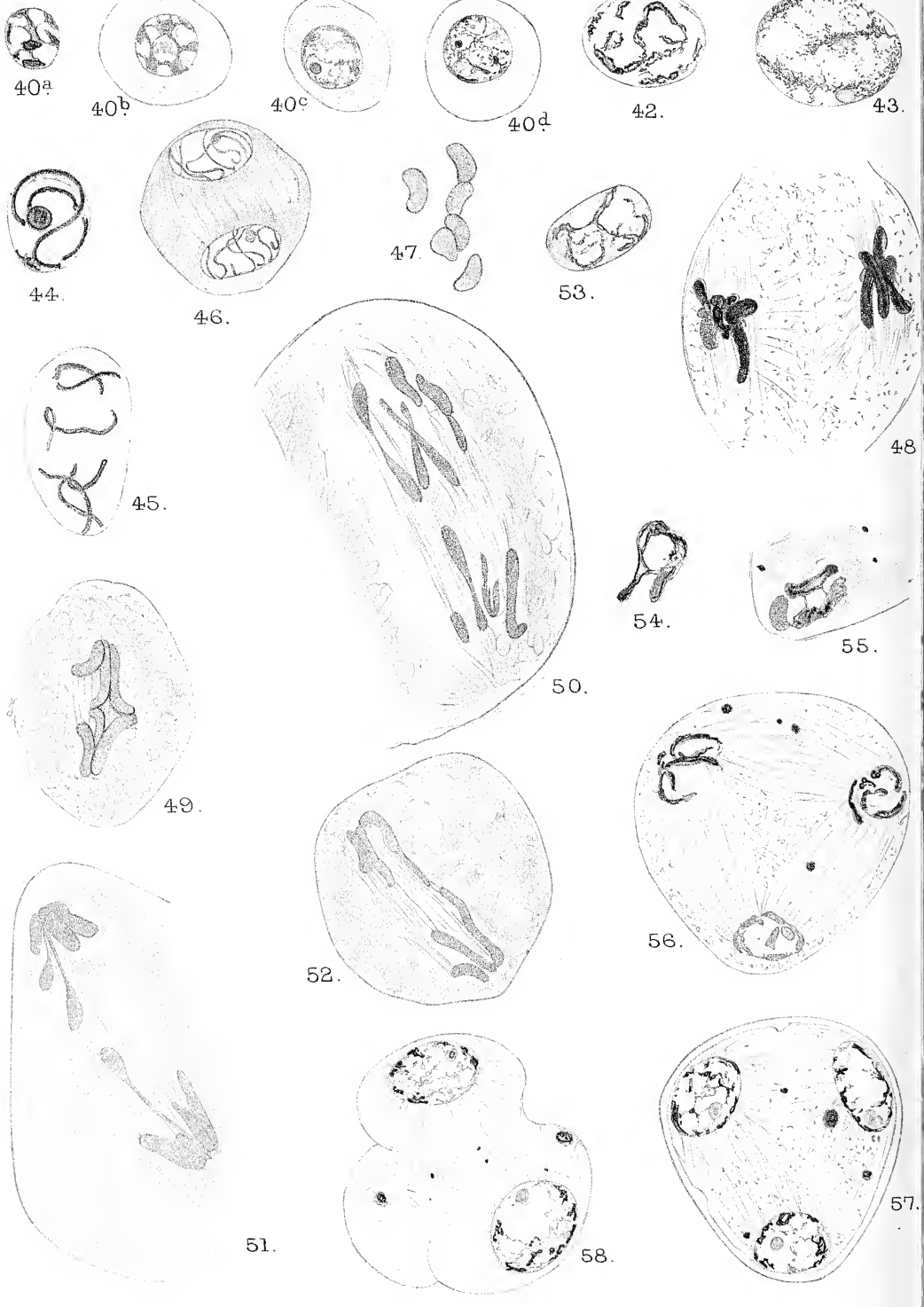

BEER.—SPORE-DEVELOPMENT.

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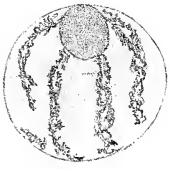
63.



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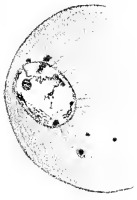

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66a.

66b.

70a.

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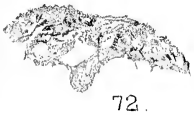

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70b.

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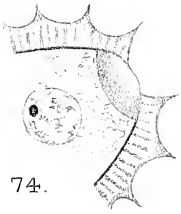

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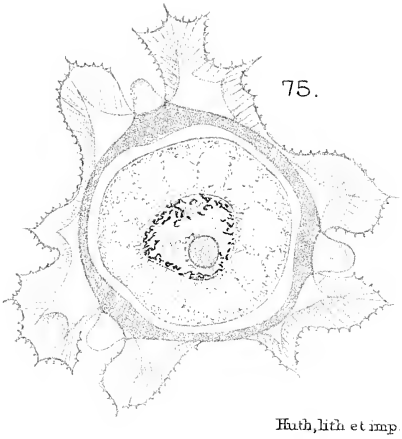

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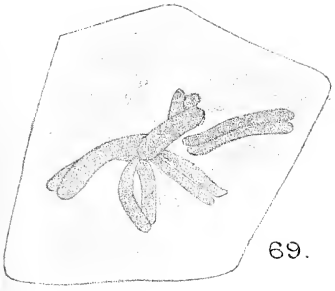

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Observations on the Seedling Anatomy of Certain Sympetalae.

I. Tubiflorae.

BY

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With Plate LXVIII and eight Diagrams and Figures in the Text.

Historical.

DURING recent years our knowledge of seedling anatomy has been extended in nearly every direction. The researches of Van Tieghem (25, 26) about 1870 were followed by the published observations of many continental workers (chiefly 5, 7, and 28); but, as was almost inevitable in an unexplored branch of the subject, the researches carried out during this initial stage, with one notable exception (Vuillemin, 28), concerned only scattered species from the largest classes of plants. As published papers on this subject became more numerous, the method of attack changed. The publications of Sargant (13, 14, 15, 16) resulted in the systematizing of the observations on seedling anatomy. This has been especially the case with British workers, who have taken definite groups and have conducted their observations on large numbers of species within each group. The result is that, in place of the isolated observations often so favourable to theorists, there is now a solid body of evidence rather disturbing to certain phylogenetic hypotheses, but undoubtedly valuable in other directions. The recent work of Chauveaud (2) has shown that seedling anatomy may possess an evolutionary value quite different from that hitherto assigned to it; while the physiological aspect of this subject still remains almost untouched.

Hitherto systematic research on seedling structure of Dicotyledons has concerned chiefly certain groups among the Archichlamydeae of Engler (4, 6, 8, 9, 11, and 20). Apart from the work of Vuillemin (28) on the Compositae, observations on the Sympetalae have, for the most part, been scattered. In 1849 Clos, who, perhaps, may be described as the first

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seedling anatomist, showed that the *collet* could be defined anatomically, and referred very briefly to the vascular anatomy of a few species among the Sympetalae (3); and about twenty years later Van Tieghem (25, 26) described the transition phenomena in a few examples from the same group. A decade later was published an account of the careful work of Gerard (7), which included a description of the seedling structure of nearly a score of examples belonging to the Sympetalae. Several of the same species have been included in the present research, which, except in details, confirms and extends this branch of Gerard's work. Of Vuillemin's memoir on the Compositae (28) more will be said in a future paper on that group. P. A. Dangeard (5), who extended Gerard's observations on the Sympetalae, classified his results on the basis of the number of strands present in the root; while Van Tieghem, in defining his three types of transition (27), also included a few examples from the same group. In 1890 Scott (18) described the seedling anatomy of *Ipomoea versicolor*; and a little later Scott and Brebner, in their paper 'On Internal Phloem in . . . Dicotyledons' (19), and Lamounette (12), working independently on the same subject, incidentally included a description of the transition phenomena in various Sympetalae. It remains only to mention the more recent publications of Tansley and Thomas (21, 22), and Thomas (23), in which the seedling anatomy of certain members of this group is described in a more general manner.

Scope and Methods.

In the present paper only a few of the better-known orders of the Tubiflorae will be considered; in these the seedling anatomy of selected species will be described, and the results of other workers will be included. The methods adopted are similar to those described by Hill and de Fraine (10). When possible, series of transverse sections of three seedlings of each species were cut with the microtome, and longitudinal sections were also made for comparison. For staining, combinations of gentian violet and vesuvin, and safranin and lichtgrün, were found to give the best results.

For the majority of the seedlings and for much kindly advice as to methods I am indebted to Mr. T. G. Hill. The greater portion of the material was grown at the Chelsea Physic Garden, and to Mr. W. Hales, the Curator, I wish to express my sincere thanks.

Description of Species.

Convolvulaceae.

Convolvulus tricolor, L., var. *major*. Seedlings large, each with massive hypocotyl (often more than 6 cm. long) and two slightly unequal cotyledons (Pl. LXVIII, Fig. 1, A). Each cotyledon consists of a long slender petiole

bearing a large thin lamina with retuse apex. A definite midrib traverses the blade, and gives off a pair of branches before bifurcating near the apex. Thus each lobe of the cotyledon is supplied with two principal branch veins. Near the apex are ramifications of smaller vascular strands consisting solely of spiral vessels or tracheides, which end in curious cavities forming a kind of hydathode (24). The point of attachment of the seed-leaves is marked by a large swelling, but there is no cotyledonary tube.

Transverse sections of the lamina show the midrib with the lateral veins and their branches (Diagram 1, Figs. 1 and 2). The mesophyll is differentiated into palisade tissue and spongy parenchyma, and at various points are large secretory cells with vascular connexions. Coming down the lamina the lateral strands join on to the midrib. Anastomoses occur at the junction of blade and petiole and occasionally lower down, and in the

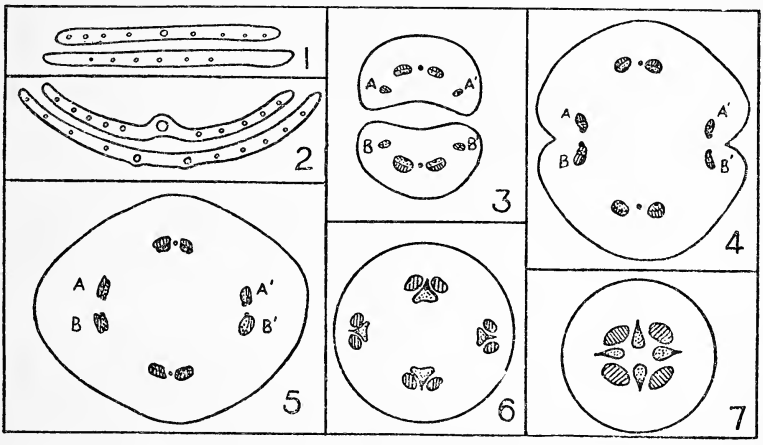

DIAGRAM 1. *Convolvulus tricolor*, var. *major*. Transition from cotyledons to root. In Figs. 3–7 and in the following diagrams, protoxylem is shown in black, metaxylem dotted, and phloem hatched.

upper part of the latter there is a large median bundle and two small laterals, all simple and collateral in structure. While still in the petiole the median strand divides, two groups of xylem and phloem moving away right and left, leaving a small protoxylem strand between them. In this condition (Diagram 1, Fig. 3) the bundles enter the hypocotyl, where the small lateral strands (A and A′), increased in size by the appearance of metaxylem elements, move towards their fellows (B and B′) from the other cotyledon, the protoxylems turning outwards during the process (Diagram 1, Fig. 4). The protoxylem groups of these bundles become detached from the metaxylem masses, and taking up a position between the latter, they fuse together, and traverse nearly the whole of the hypocotyl in this condition (Diagram 1, Fig. 5). The group corresponding to the midrib, on the other hand, decreases in size, and while travelling down the hypocotyl its constituent parts assume different positions, and are not always well defined.

During the greater part of the transition, the four vascular groups in the hypocotyl are equal in size and similar in configuration, and it is impossible structurally to distinguish between the intercotyledonary strands and those in the plane of the cotyledon. The phloem is extremely small in amount, and at no point can internal phloem be recognized with certainty—an

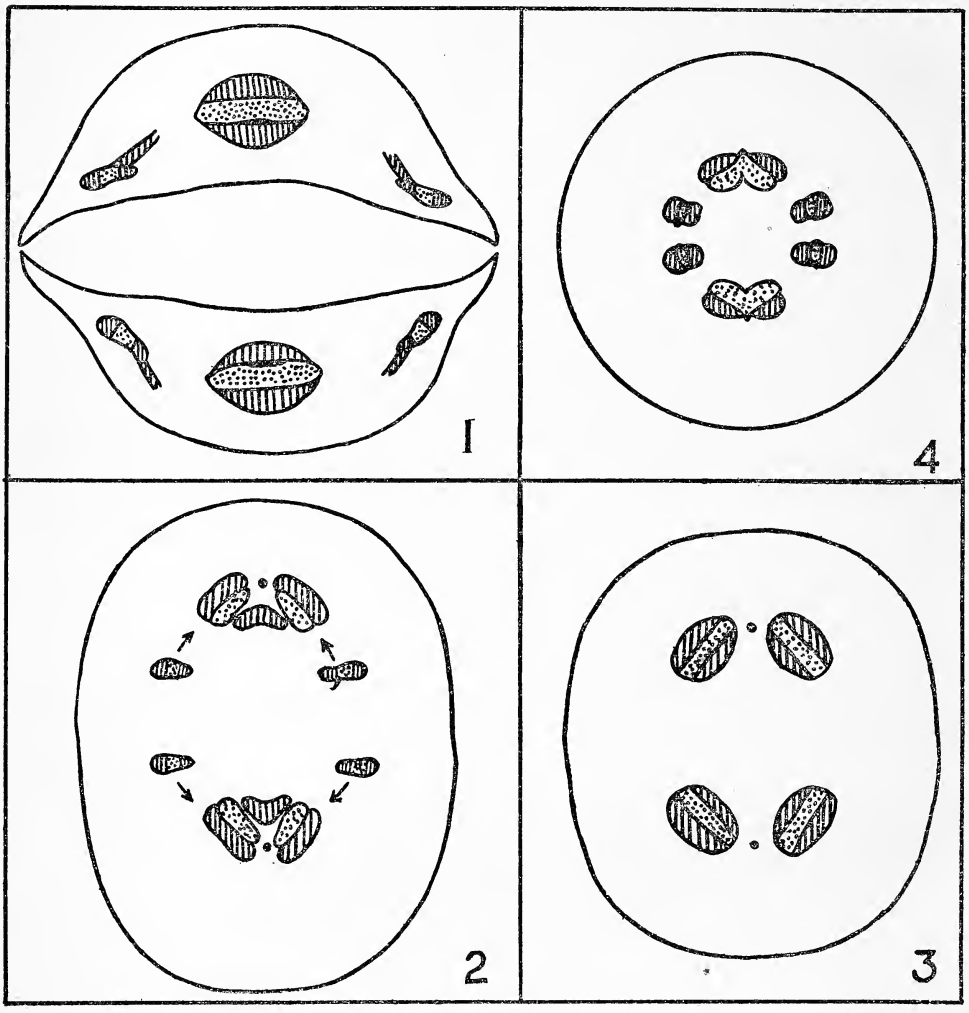

DIAGRAM 2. *Convolvulus tricolor.*

observation which emphasizes the late appearance of this tissue noticed by other observers.

Finally, the protoxylem in each case becomes definitely external, and the metaxylem groups fuse; the xylem masses move towards the centre, the corresponding phloem groups meet and fuse, and a tetrarch root is

produced, in which the pith and endodermis are characteristic features (Diagram 1, Fig. 6).

Convolvulus tricolor, L. In this example, seedlings of which were obtained after the above description had been written, one or two differences must be noted. Externally the young plants are very similar, though in *C. tricolor* there is a tendency to form a slight cotyledonary tube. Internally, also, the arrangements in this species and the var. *major* show a general similarity in the broad features. In the petiole anastomoses occur between the median bundle and the lateral ones, and often strands of phloem can be detected connecting the bundles together (Diagram 2, Fig. 1). In the upper part of the hypocotyl the two lateral bundles approach the median one, and finally more or less complete fusion occurs (Diagram 2, Figs. 2 and 3). At a lower level the two lateral strands again separate, and, as in var. *major*, approach their fellows from the opposite cotyledons, giving rise to the intercotyledonary bundles of the tetrarch root (Diagram 2, Fig. 4, and Diagram 1, Figs. 6 and 7).

Another difference consists in the presence, in the petiole and hypocotyl, of abundant internal phloem. This fact was noted by Lamounette (12, p. 219), who, however, appears to have doubted the connexion between internal and external phloem traced by other observers (cf. Scott (18) and Scott and Brebner (19)). In the upper part of the hypocotyl, during the rearrangements in the cotyledonary strand so that the protoxylem becomes external, the internal phloem masses of that bundle fuse, and the fused mass gradually passes out between the metaxylem groups (before the latter meet to form the root bundles) and joins on to the external phloem. The same events take place at a lower level in connexion with the intercotyledonary bundles, so that finally an ordinary tetrarch root is produced.

Dangeard (5) investigated several examples belonging to the Convolvulaceae, but in most cases only a general description of the genus is given. Among others, he described the following:

Convolvulus,
Calystegia,
Quamoclit coccinea,
Ipomoea,

all of which follow the method already given. One difference, however, must be noted. Speaking generally of the Convolvulaceae, Dangeard described the cotyledonary traces as being bicollateral in the hypocotyl. In the present research, however, nothing of the nature of internal phloem was found in any part of the seedling of *C. tricolor*, var. *major*.

Ipomoea versicolor, Meissn. From the description by Scott (18,

pp. 174, 175) the transition phenomena appear to be almost exactly as described above for *Convolvulus tricolor*. In this case also internal phloem is present both in cotyledons and in hypocotyl. As the xylem groups of each bundle come together at the base of the hypocotyl, the corresponding phloem mass passes out between them and joins the external phloem.

Ipomoea leucantha was described by Lamounette (12, p. 220), and appears to differ from *I. versicolor* only in that 'internal' phloem is absent from the vascular bundles in the cotyledons.

Ipomoea purpurea, investigated by Gerard (7, p. 369), is not essentially different from *I. versicolor*.

Cuscuta Epilinum, Weihe. Much is generally known concerning the anatomy of *Cuscuta*. Seedlings of various species were re-examined, however, in the hope that a knowledge of the seedling structure of other genera might throw some light on that of *Cuscuta*. Unfortunately, no new observations can be recorded. Special attention was devoted to *C. Epilinum*. The seedlings investigated were 2–5 cm. in length, were quite undifferentiated, and had the appearance of a piece of fine string. In one case (Text-fig. 1) the upper part twice encircled the stem of the host, and haustoria were beginning to form. Internally, most of the tissue was cortical, the vascular elements being aggregated into an insignificant axile strand. The tissue composing the latter was much reduced, and in some cases (lignification being at a minimum) it was impossible, in transverse sections, to distinguish between wood and phloem. The sieve-tube tissue occupied the periphery of the stele, and enclosed the undifferentiated vessels, in which, however, no order of development could be recognized. In the upper portion of the larger seedlings the vessels became arranged into more or less definite groups, each with a little phloem. Up to the present it has been impossible to find anything even remotely resembling transition phenomena.

TEXT-FIG. 1. *Cuscuta Epilinum* on *Linum usitatissimum* (nat. size).

Polemoniaceae.

No examples from this order were examined during the present research, but in *Polemonium caeruleum*, described by Gerard (7, p. 373), the transition follows Type 3. The same remark applies to

Hydrophyllaceae,

of which Gerard investigated *Hydrophyllum canadense* (7, p. 373);

Boraginaceae,

the same worker taking *Lithospermum gremil* as his example (7, p. 374);

Labiatae,

of which two species were examined:

Phlomis fruticosa
Galeopsis ladanum (7, pp. 379–80).

In all these cases the seedlings were small and slender, and the transition, with certain variations, followed Type 3. The two genera, *Phlomis* and *Ocimum* (with special reference to *O. basilicum*), were investigated by Dangeard (5), whose description agrees in all essential particulars with that of Gerard referred to above.

Solanaceae.

Nicandra physaloides, Gaert. Seedlings very small; cotyledons shortly stalked, equal in size, and comparatively fleshy. There is no cotyledonary

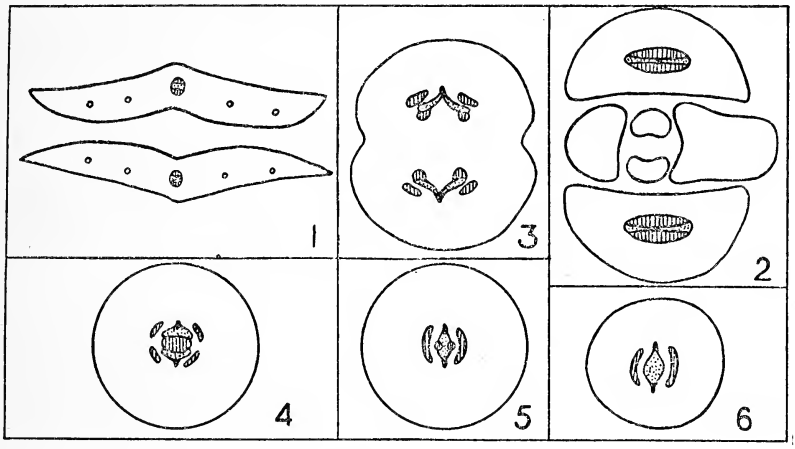

DIAGRAM 3. *Nicandra physaloides.*

tube. The various aerial parts are covered with the pedicellate glandular hairs characteristic of the Solanaceae.

The lamina is traversed by a rather large vascular strand which gives off numerous small branches (Diagram 3, Fig. 1). Near the base of the cotyledon this midrib consists mostly of phloem, arranged on the upper and lower sides of a flattened strand of tracheae, of which the middle ones represent the protoxylem (Diagram 3, Fig. 2). As the strand enters the hypocotyl the internal and external phloem groups bifurcate, and at the same time the protoxylem becomes definitely exarch (Diagram 3, Fig. 3) by moving outwards in the cotyledonary plane. At this stage there are two strands of xylem with eight phloem groups, four of which are internal, the remaining and much larger groups being peripheral and situated on diagonal lines. Rearrangements continue to go on gradually; the vascular groups

close in towards the centre, the pith disappears, and the xylem strands are only separated by the internal phloem masses, which have now fused together (Diagram 3, Fig. 4). Finally, a large trachea appears in the centre connecting the two xylem masses, and dividing the internal phloem into two parts (Diagram 3, Fig. 5) which pass out right and left and join on to the external phloem. The simple diarch condition seen in Diagram 3, Fig. 6, is thus obtained; and the transition is according to Type 3 of Van Tieghem.

Atropa Belladonna, L. This species was also investigated by Gerard (7, p. 377) and by Lamounette (12, p. 211). The latter, however, described the internal phloem as being quite distinct from the external phloem even at the base of the hypocotyl. The present observations agree with those of Gerard. The seedlings are larger than in *Nicandra physaloides*, but the transition is almost identical in the two species. In view of the statements by Lamounette it should be emphasized that, before disappearing, the internal phloem is in direct connexion with the peripheral phloem groups. In many cases, at one stage there is no line of demarcation between the internal and external phloem systems (Diagram 4).

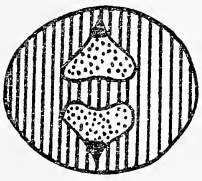

DIAGRAM 4. *Atropa Belladonna.* In the hatched portion the phloem cannot be distinguished from the ground tissue.

Solanum guineense (Garden form of *S. nigrum*). Transition exactly as in *Atropa Belladonna*, i. e. Type 3.

Solanum nigrum. From the description by Lamounette (12, p. 211), the seedling anatomy of this species is identical with that of his *Atropa Belladonna*, i. e. Type 3, with internal and external phloem systems quite distinct.

The genera *Solanum*, *Capsicum*, and *Lycopersicum* were investigated by Dangeard (5), and in the main the transition resembles that given above for *Atropa Belladonna.*

Datura Stramonium, L. This species was first investigated by Gerard (7, p. 375), with whose description the following agrees, except in one or two details. The genus was also generally referred to by Lamounette (12, p. 211).

The fairly large seedling has two fleshy, slightly unequal cotyledons, with swelling at point of attachment, but without any signs of a cotyledonary tube. The lamina, which passes gradually into the short petiole, has a definite midrib with slender branches which end near the periphery of the leaf. The mesophyll shows the usual differentiation.

In the lamina the midrib consists of a triangular mass of tracheae with the protoxylem at the upper apex, surrounded by phloem, the upper ('internal') part of which disappears near the apex of the cotyledon. All except the smallest branches are bicollateral in structure (Diagram 5, Fig. 1).

At the base of the lamina, often only a single bundle is present. In this strand, rearrangements begin in the upper part of the petiole, by the disappearance of the phloem elements from the sides simultaneously with the lateral elongation of the xylem. Almost immediately the lower ('external') phloem bifurcates, and traverses the remaining part of the petiole and the hypocotyl as two distinct groups (Diagram 5, Fig. 2).

In some cases the rearrangement of the xylem results in the production of three masses—a small median strand of protoxylem, and two lateral strands consisting chiefly of metaxylem, in which protoxylem elements can be distinguished for some distance. Even in the clearest cases, these

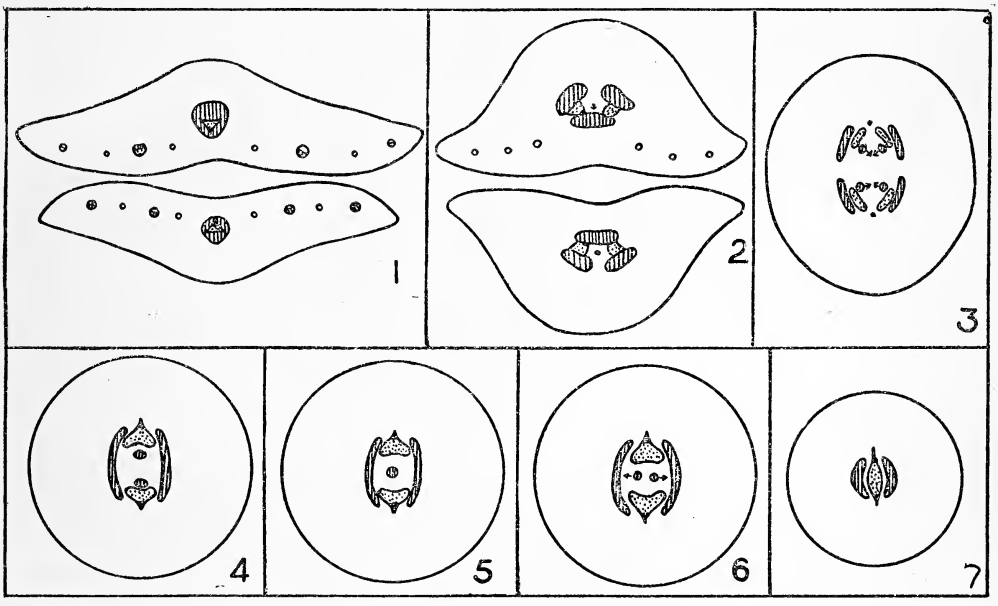

DIAGRAM 5. *Datura Stramonium.*

lateral protoxylem groups disappear after a longer or a shorter course, while in other examples the only definite protoxylem is the median one. The lateral xylem groups diverge, and take with them the corresponding external phloem mass and also part of the internal phloem, and further rearrangement in the upper region of the hypocotyl results in the production of the structure shown in Diagram 5, Fig. 3.

Further changes are due to the closing in of the various tissues. Each external phloem mass joins its fellow from the opposite cotyledon; the whole of the xylem derived from each cotyledonary bundle moves towards the centre of the hypocotyl, and at the same time the metaxylem strands of each bundle fuse and take up a position internal to their respective protoxylems (Diagram 4, Fig. 4). Finally, the two xylem bundles meet at the centre, and a typical diarch plate results (Diagram 4, Fig. 5).

The behaviour of the internal phloem groups is interesting. While still in the petiole, the internal phloem undergoes equal division, one part going to each of the lateral xylem masses. In these positions, and with occasional anastomoses, the whole of the hypocotyl is traversed. Previous to entering the root, however, the two groups belonging to one midrib come together and fuse (Diagram 5, Fig. 4); then, passing to the centre, they join on to the group derived from the other cotyledon (Diagram 5, Fig. 5). Occasionally, anastomoses with the external phloem are seen. Finally, the fused phloem mass elongates in the intercotyledonary plane, bifurcates, and the two branches pass out right and left to join the external phloem (Diagram 5, Fig. 6). A little pith is present at this time, but this soon disappears, and a solid xylem core results (Diagram 5, Fig. 7).

Many variations on this arrangement were found. The definite fusion and division of the internal phloem are not always so clear as has just been described. Fusion may take place irregularly, and one part may pass out and join the external phloem long before the other. In addition, the internal phloem masses are often more numerous than in the simple case described (cf. Gerard, 7, p. 375). Blind endings are seldom found, and even when they apparently occur, it is only after anastomosis with another phloem mass.

Datura Metel, L. Seedlings rather larger than in *D. Stramonium*, and the details of transition are better shown than in the last species. The division of the midrib xylem into three is more definite, as is also the possession of protoxylem by the two lateral masses thus produced. One difference is noteworthy, though there are indications of the same phenomenon in *D. Stramonium*, viz. the division of the internal phloem of the cotyledonary trace into three parts, one going to each of the three xylem masses. Anastomoses take place not only between the different internal groups, but also between the latter and the external phloem. In other cases the internal phloem groups were more numerous. The final result is the same; all the internal phloem masses come together in the centre, the fused bundle divides, and the two parts diverge and join the peripheral phloem. Another peculiarity was seen in one specimen in which the three protoxylem groups derived from one cotyledonary bundle remained distinct for a long time after the root was reached (Pl. LXVIII, Fig. 2). The lateral protoxylems were at first stronger, but after a time they decreased in size and finally disappeared. The metaxylem became more compact, and the median protoxylem assumed the exarch position in the plane of the cotyledons.

Nicotiana alata, Link and Otto. In this species the seedlings are very small, and the transition, which takes place in the upper part of the hypocotyl, is according to Type 3. This species differs from the rest of the Solanaceae examined in the scanty supply of phloem, this tissue being entirely absent from the inner side of the vascular bundle, which, therefore, throughout the

cotyledons and hypocotyl, are of the collateral type. Lamounette (12, p. 211), on the other hand, describes the genus *Nicotiana* as being like *Solanum nigrum*, in which internal phloem is present.

Salpiglossis sinuatus, Hook. The seedlings of this species are very minute, and even in the smallest the transition phenomena are obscured by the presence of secondary thickening. The chief features, however, appear to be similar to those described for *Nicandra physaloides*.

Petunia violacea, Lind. Seedlings very small, cotyledons slightly unequal, with trace of cotyledonary tube. The poorly developed vascular tissue suggests that the seedlings are immature, a condition which probably accounts for the absence (as in *Nicotiana alata*) of internal phloem. The transition, which begins in the petiole, is quickly completed, and conforms to Type 3.

Browallia viscosa, H.B. and Kth. This species was not examined during the present research. From the description by Scott and Brebner (18, p. 267) the transition is according to Type 3, but differs from the plants already described in the behaviour of the internal phloem. In the upper part of the hypocotyl there are 10–12 internal phloem masses. Passing down towards the root the pith gradually disappears, and the internal phloem strands successively pass out between the converging xylem bundles and one by one reach the external phloem strands. The two xylem bundles unite to form the diarch plate, and at this point the last of the internal phloem strands passes out and joins the normal phloem.

Schizanthus pinnatus, Ruiz. et Pav. Seedlings not more than a centimetre in length, and on the whole, both externally and internally, very similar to *Nicandra physaloides*. A few minor differences may be noted. The small epicotyledonary strands, two in number and arranged in the intercotyledonary plane, are peculiar in that they persist down to the base of the hypocotyl, the xylem groups especially remaining quite distinct until they fuse with the root bundles just as the latter approach to form the diarch plate. The xylem of the cotyledonary traces is rather irregularly disposed, a condition which is emphasized by the presence of intercellular spaces near the two protoxylems. The preliminary rearrangements which the internal phloem groups undergo are similar to those in *Nicandra physaloides*; but after fusion in the centre and subsequent division, the two phloem masses appear to die out instead of merging into the external phloem. With the disappearance of the last remnants of the pith, the two xylem bundles become continuous, and a diarch root is produced.

Nierembergia gracilis, Hook. Apart from the slightly larger size of the seedlings this species resembles *Schizanthus pinnatus* in all particulars. In one seedling, in which the primary tissues in the hypocotyl were not fully developed, there was scarcely a trace of 'internal' phloem in the cotyledons; while in another, whose hypocotyl showed much secondary

wood, the midrib of the seed-leaf was conspicuously bicollateral in structure. In all cases the transition was according to Type 3, and the internal phloem died out in the root.

Scrophulariaceae.

Verbascum pulverulentum, Vill. Seedlings about two centimetres long, with definite hypocotyl and two unequal cotyledons. Each cotyledon consists of a large lamina which passes gradually into the definite petiole. There is no cotyledonary tube.

In the lamina the midrib sends out small branches, but enters the petiole as a single rather small vascular bundle. The rearrangement of the vascular tissue begins before the strand enters the hypocotyl, and is completed very quickly. The transition, which is according to Type 3, is masked by the presence of two epicotyledonary traces situated in the intercotyledonary plane, which persist throughout the hypocotyl and enter the root. Thus for a long distance in the upper part of the root the arrangement appears to be tetrarch when it is really diarch, a condition which is indicated by the origin of the lateral rootlets. These are given off in four rows from the two ends of the diarch plate.

Alonsoa. As described by Dangeard (5) the transition in this genus appears to conform to Type 3.

Diascia Barberae, Hook. In most Scrophulariaceae examined, the rearrangement of the vascular tissue begins in the upper part of the petiole, and one of the first indications is the separation of the xylem strand into three more or less definite portions, of which the median one is the protoxylem. In *D. Barberae* the xylem remains throughout quite continuous. In the upper part of the petiole, the vascular bundle divides into two parts (Diagram 6, Figs. 1 and 2) connected towards the upper surface by means of protoxylem elements. Each semi-strand possesses its own phloem mass. The transition takes place by the passage of the phloem groups to a position in the intercotyledonary plane, where they fuse with their fellows from the other seed-leaf, and by a simple twisting on the part of the xylem, so that the protoxylem takes up an external position (Diagram 6, Fig. 3).

Linaria origanifolia, DC.
Linaria heterophylla, Desf. } In both species the minute seedlings are provided with two equal petiolate cotyledons. There is no cotyledonary tube.

The transition, which is according to Type 3, commences in the petiole, and takes place very quickly.

Nemesia floribunda, Lehur.
Nemesia versicolor, E. Mey. } With the exception of their larger size, the seedlings of these species are exactly like those of *Linaria*. The method of transition is the same, and the rearrangements take place in the upper part of the hypocotyl.

Antirrhinum Orontium, L., differs from *Linaria* externally only in the larger size of the seedlings and the slight inequality of the cotyledons. The region of transition is, on the whole, rather shorter ; in the root the xylem plate is quite inconspicuous.

Scrophularia nodosa, L., in all essentials resembles *Linaria origanifolia*. In the petiole the xylem elements are arranged in a single row parallel with the upper surface of the leaf, while the diarch plate also consists of a single row of elements, but arranged in the cotyledonary plane.

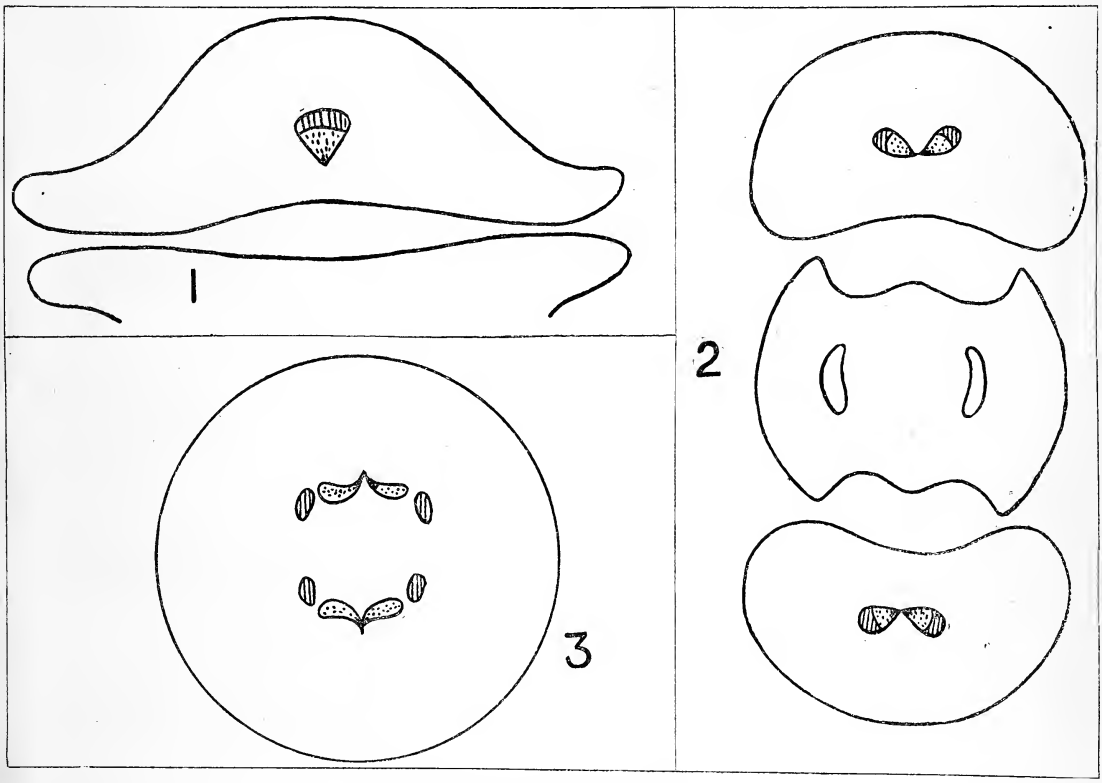

DIAGRAM 6. *Diascia Barberae.*

Torenia asiatica, L.
Torenia Fournieri, Linden. } In both species the transition is according to Type 3, and resembles that of *Antirrhinum Orontium* in all respects.

Mimulus luteus, L. Apart from the presence of a cotyledonary tube, the seedlings of this species, both externally and internally, are very similar to those of *Antirrhinum Orontium*.

Dischisma arenarium, E. Mey. Seedlings small, with narrow, rather fleshy cotyledons which form a slight tube at point of attachment. The rearrangements, which are according to Type 3, take place as in

Antirrhinum Orontium, but the region of transition is shorter, and is confined to the upper part of the hypocotyl.

Tetranema mexicanum, Benth. As in *Mimulus luteus*.

Zaluzianskya capensis, Walp. Seedlings differ in no respect from those of *Mimulus luteus*.

Veronica longifolia, L.
Veronica hederaefolia, L. } (also described by Gerard (7, p. 378)). Exactly similar to *Mimulus luteus*. .

Rehmannia angulata, Hemsl. Both externally and internally similar to *Linaria origanifolia*.

Digitalis ferruginea, L.
Digitalis lanata, Ehrh. } Seedlings are much larger than in *Antirrhinum Orontium*, and consequently the vascular tissues throughout are more definite. There is, however, no essential difference in the transition phenomena of these three species.

Bignoniaceae.

Incarvillea Delayvei, Bur. et Franch. Seedlings large, with two slightly unequal cotyledons. Hypocotyl long and fairly stout, with a prominent swelling at the point of attachment of the cotyledons, where also a distinct cotyledonary tube is formed. Apart from this and the presence of peculiar glandular hairs, the seedlings are very similar to those of *Convolvulus*.

In the seed-leaves the mesophyll tissues are well differentiated. Each lamina has a large midrib, which is half-moon-shaped in section, and which gives off numerous branches in regular order (Diagram 7, Fig. 1). All the branches fuse with the median strand before the latter enters the long petiole, and as no further branching occurs a single laterally elongated bundle passes into the hypocotyl from each cotyledon (Diagram 7, Fig. 2). Previous to entering the hypocotyl, and often before the cotyledonary tube is reached (Diagram 7, Fig. 3), the phloem of each bundle bifurcates. In some cases also, the xylem shows distinct division into a middle portion consisting of protoxylem, and two lateral parts in close connexion with the phloem groups, and consisting of metaxylem with a few protoxylem elements (Diagram 7, Fig. 2 *a*). This condition, interesting for comparison with other species, is not constant ; it was found in one specimen, and even there did not persist for very long. In all cases, in the upper part of the hypocotyl, there are two bundles of xylem each with a single protoxylem group (Diagram 7, Fig. 4), and the rearrangements that now occur take place very gradually. As each bundle passes down the hypocotyl, its protoxylem divides into three, each branch at first consisting of 1–3 elements (Diagram 7, Fig. 5, and Pl. LXVIII, Fig. 3). The middle one passes almost directly outwards and takes up an exarch position in the plane of the cotyledons. The lateral groups traverse an oblique path, and finally come

to rest near the periphery of the stele in the intercotyledonary plane, where each one fuses with its fellow from the other seed-leaf bundle (Pl. LXVIII, Fig. 5). There are thus formed four protoxylem groups, of which the two in the cotyledonary plane are much the stronger, and in these the metaxylem closes in behind so that typical root bundles are produced. At a lower level metaxylem also appears on the inner side of the other protoxylem groups, and a tetrarch root results, in which the metaxylem always preponderates in the intracotyledonary strands (Diagram 7, Fig. 6). No other condition was found in any root.

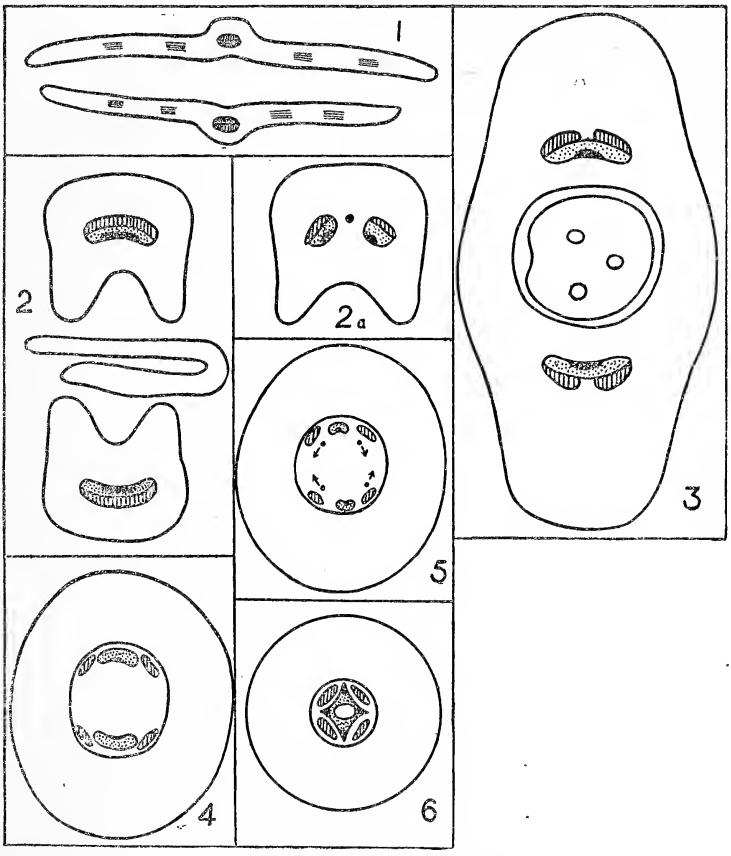

DIAGRAM 7. *Incarvillea Delayvei.*

It is to be noted that the transition in this species is remarkably like that of *Anemarrhena* (13), in both of which a tetrarch root structure is obtained from two cotyledonary traces. This type of transition has hitherto been found, among dicotyledons, only in certain Cactaceae (6), and possibly in *Eranthis* and *Podophyllum*.

Eccremocarpus scaber, Ruiz. et Pav. Only a single seedling of this species was available. This specimen was much smaller than those of *Incarvillea Delayvei*, and resembled the latter in no particular. The

cotyledons were petiolate, retuse, and markedly unequal. A number of small veins supplied the lamina, and these were collected at the base into a single bundle which entered the petiole. The transition, slightly obscured by the presence of secondary thickening in the hypocotyl, was of Type 3, and began in the petiole. In the shorter petiole, the protoxylem became external by simple rearrangement of the xylem elements. In the other the xylem divided into three portions, the median one (protoxylem) taking up an external position. The metaxylem in both cases moved towards the centre, became fragmentary, and finally disappeared, leaving only the protoxylem groups of the original cotyledonary xylem strands. These gradually closed in, and with the reappearance of the metaxylem a small diarch plate was produced.

Acanthaceae.

The only member of this order of which the seedling anatomy is known is *Acanthus*, which was investigated by Dangeard (5). The transition is essentially the same as in the Scrophulariaceae.

Theoretical Considerations.

On the present occasion it is not proposed to give a full discussion of the bearing of the above observations on the various phylogenetic theories. Quite recently, in discussing the theoretical importance of the seedling structure of the Cactaceae, de Fraine (6, p. 164) gave a summary of the principal theories based on, or influenced by, the seedling anatomy, which it is hardly necessary to repeat here. Briefly, the observations on the Tubiflorae entirely support the general conclusions of that author.

In the Tubiflorae examined there are two fairly well marked methods of transition:

(1) Characterized by possession of a diarch root throughout. This method, known as Van Tieghem's Type 3, has been found in the majority of the plants investigated, e. g. Polemoniaceae, Hydrophyllaceae, Boraginaceae, Labiatae, Solanaceae, Scrophulariaceae, some Bignoniaceae, and Acanthaceae.

(2) In which the root is tetrarch in structure. These can be further classified according to the number of bundles in the upper part of the hypocotyl.

(*a*) In the Convolvulaceae, a 'double' midrib and two small lateral bundles enter the hypocotyl from each cotyledon and become orientated to produce the tetrarch root. This is obviously merely a modification of Van Tieghem's Type 2.

(*b*) In some Bignoniaceae (e. g. *Incarvillea Delayvei*) a single bundle enters the hypocotyl from each cotyledon, where it undergoes division and orientation which result in the production of a tetrarch root structure.

The great majority of the species comprised in the Tubiflorae are herbaceous, and in many respects this extensive group is generally regarded as a remarkably natural one. Engler and (especially) Bentham and Hooker recognized certain sub-groups; and in the latest attempt to deal with the Tubiflorae, Wernham (29), discussing the question from the point of view of floral evolution, appears to hold that certain of these sub-groups are not only well defined, but possibly had a quite different course of evolution. In this connexion, it is perhaps worthy of note that in the principal sub-groups, i. e. in Polemoniales, Personales, and Lamiales of Bentham and Hooker, the prevailing method of transition is Type 3, while in each of the groups Polemoniales and Personales a single Natural Order shows (in part) a different arrangement. Although it is not possible to advance any external cause as being responsible for this difference in the seedling anatomy, the cases quoted serve to emphasize the fact that orders, and even genera (e. g. of Bignoniaceae) which are generally regarded as being closely allied, show different methods of transition. Conversely, just as de Fraine demonstrated in the delimiting of Cactaceous *genera*, so here with regard to the *sub-groups* and (rarely) *orders* of the Tubiflorae, no assistance is derived from seedling anatomy.

Perhaps the chief interest which emanates from the present study of the seedling structure or the Tubiflorae attaches to the occurrence of the *Anemarrhena* type of transition. Previous to the discovery of this type in the Cactaceae (6), it had been found only in certain Ranunculaceae among dicotyledons (13, 17), and on its occurrence here, as well as on other features common to this order and to certain monocotyledons, had been founded a theory of the origin of the latter group from a dicotyledonous ancestor. In opposing this view to the light of her discoveries in the Cactaceous genera, *Opuntia* and *Nopalia*, de Fraine concludes (6, p. 167): 'It cannot be considered that the resemblance of *Opuntia*, for example, to *Anemarrhena* is the result of a close genetic relation between the two; nor can it be conceded that it is due to the response of two unrelated forms to similar conditions; hence we cannot but conclude that the resemblance is accidental. This being so, then it is quite possible that the similarity of *Eranthis* and *Podophyllum* to *Anemarrhena* is also accidental.' The conclusion arrived at is that 'the evidence on which the "fusion" hypothesis [of Sargant (14)] was based has been considerably weakened by the discovery of the *Anemarrhena* type of seedling in such a specialized order as the Cactaceae'.

This conclusion, however, is not generally admitted. It has been called into question by (Mrs.) A. Arber (1) on the ground that the Cactaceae, while showing a high degree of specialization in their vegetative characters, may really be closely allied to the Ranales. A comparison was made between the floral structures in the two orders, Nymphaeaceae and

Cactaceae, the writer inclining to the view that the two groups 'are derived from the same ancestral stock', and that 'it is no longer necessary to look upon [the occurrence of the *Anemarrhena* type in the Cactaceae] as accidental. Among the Cactaceae this type of transition occurs in those genera whose seedlings are least modified, and it is in such cases that we should naturally expect to find that ancestral characters had been retained.' The conclusion of A. Arber is 'that Miss Sargant's view that the various monocotyledonous and dicotyledonous types of seedling anatomy were all originally derived from the *Anemarrhena* type is strengthened, rather than weakened, by the discovery of the ancestral type among the Cactaceae.'

In making this criticism the writer admits that the question depends very largely upon the systematic position of the group in question. The force of the criticism is, in fact, proportionate to the real distance between the Cactaceae and the group from which it was derived, which is assumed—an assumption that is itself not unquestioned—to have given origin also to the Nymphaeaceae and the other members of the Ranalian plexus. If, therefore, there exists a group which shows specialization in the 'parts of prime importance as regards affinities', i. e. 'in the reproductive organs, and not the vegetative features of the mature plant', and which also possesses the *Anemarrhena* type of seedling structure, it will perhaps be conceded that some ground exists for the view that the resemblance between the seedlings of this group and those of *Eranthis*, &c., is accidental. Such a state of affairs is found in the Bignoniaceae. Even assuming that this order was originally derived from the same group as the Ranales, there can be no doubt as to the want of affinity between the two, and that any character which is common to *Incarvillea Delayvei* (Bignoniaceae) and the Ranales serves to indicate nothing more than an analogy, and is, to use de Fraine's term, quite 'accidental'. Neither can there be any reasonable doubt that the discovery of the *Anemarrhena* type in this highly evolved group has not only 'considerably weakened the evidence on which the "fusion" hypothesis was based', but also has placed 'Sargant's view that the various monocotyledonous and dicotyledonous types of seedling anatomy were all originally derived from the *Anemarrhena* type' beyond the region of probability.

It is highly probable that many other species, more or less remote from the Ranunculaceae or Liliaceae, will be found to possess the *Anemarrhena* type of seedling anatomy. The observations on *Convolvulus* recorded above indicate the absence of any sharp line of demarcation between the *Anemarrhena* method and Van Tieghem's Type 2. What is practically the latter is seen in *Convolvulus tricolor*, var. *major*. If, in this example, the lateral cotyledonary strands fused with the median one, and, at a lower level, separated and became orientated to form the tetrarch root, the *Anemarrhena* type, with, however, two cotyledons instead of one, would

be obtained. As a matter of fact, this sequence is found to occur in *Convolvulus tricolor*, which, therefore, serves to connect Type 2 with the *Anemarrhena* type.

Summary.

1. The present paper deals with the seedling anatomy of the following Natural Orders of the Tubiflorae: Convolvulaceae, Polemoniaceae, Hydrophyllaceae, Boraginaceae, Labiatae, Solanaceae, Scrophulariaceae, Bignoniaceae, and Acanthaceae.

2. Much variation occurs in the *size* of the seedlings belonging to different species. Broadly speaking, in the smaller seedlings the transition region is short, and the rearrangements are concluded in the upper part of the hypocotyl; while in the larger seedlings the region of transition is very extended (cf. Compton (4)).

3. Cotyledons invariably two in number, and while the members of each pair are often unequal in size, this apparently has no effect on the essential transition phenomena.

4. Cotyledonary tubes are present in members of all the orders examined, but their presence appears to have no bearing on the type of transition. Nearly related species having the same type of seedling structure show marked differences with regard to the cotyledonary tube.

5. The prevailing type of transition, which is present in all the smaller seedlings, is Van Tieghem's Type 3, and occurs in Polemoniaceae, Hydrophyllaceae, Boraginaceae, Labiatae, Solanaceae, Scrophulariaceae, some Bignoniaceae, and Acanthaceae. The larger seedlings possess a tetrarch root. In *Convolvulus tricolor*, var. *major*, the transition is a modification of Van Tieghem's Type 2, while the *Anemarrhena* type exhibited by *Incarvillea Delayvei* is approached through *Convolvulus tricolor*.

6. Internal phloem is present in all Solanaceae and Convolvulaceae examined with the exception of *Convolvulus tricolor*, var. *major*, *Nicotiana alata*, *Petunia violacea*, and *Nierembergia gracilis* (one seedling). Indications are not wanting that the absence of internal phloem in these species is owing to the incomplete development of the tissues in the specimens examined.

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EXPLANATION OF FIGURES IN PLATE LXVIII.

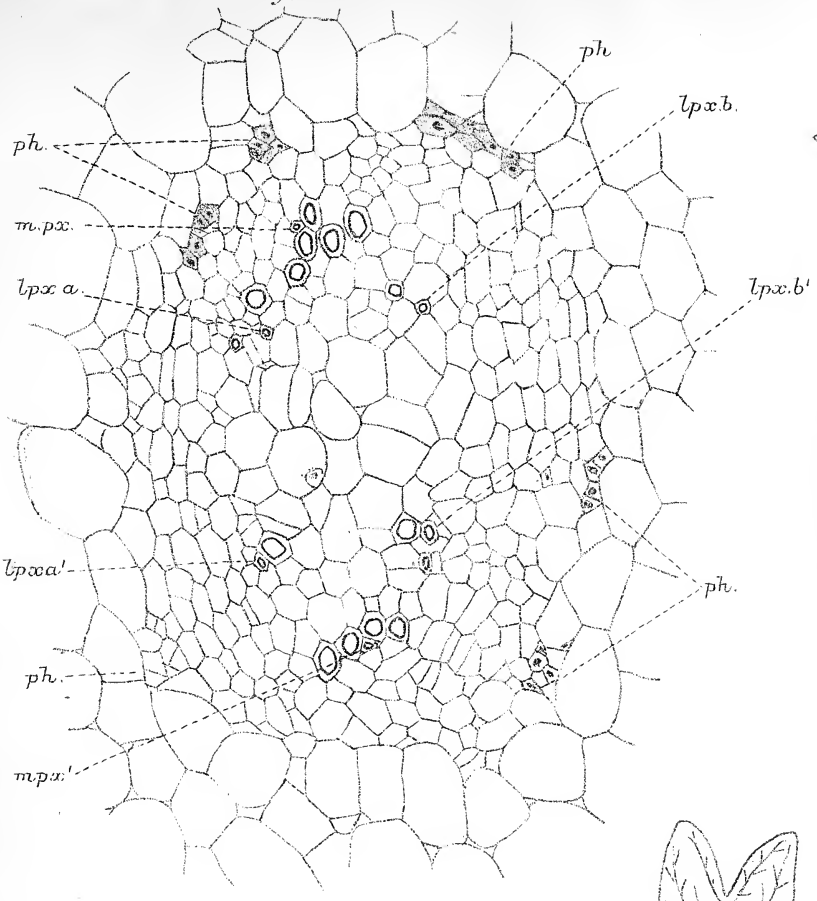
Illustrating Mr. Lee's paper on the Seedling Anatomy of the Tubiflorae.

Fig. 1. *Convolvulus tricolor*, var. *major*. A, whole seedling (½ nat. size). B, single cotyledon (nat. size).

Fig. 2. *Datura Metel*. Portion of transverse section of upper part of root, showing three protoxylem groups. (x, y, z and x', y', z') in each xylem strand.

Fig. 3. *Incarvillea Delayvei*. Transverse section of hypocotyl, showing division of the protoxylem of each cotyledonary bundle into three (*m.px*, *l.pxa*, *l.pxb*, and *m.px'*, *l.pxa'*, *l.pxb'*, respectively). For description see text (p. 740). *ph.* = phloem.

Fig. 4. *Incarvillea Delayvei*. Transverse section of root, showing tetrarch structure derived from condition seen in Fig. 3.



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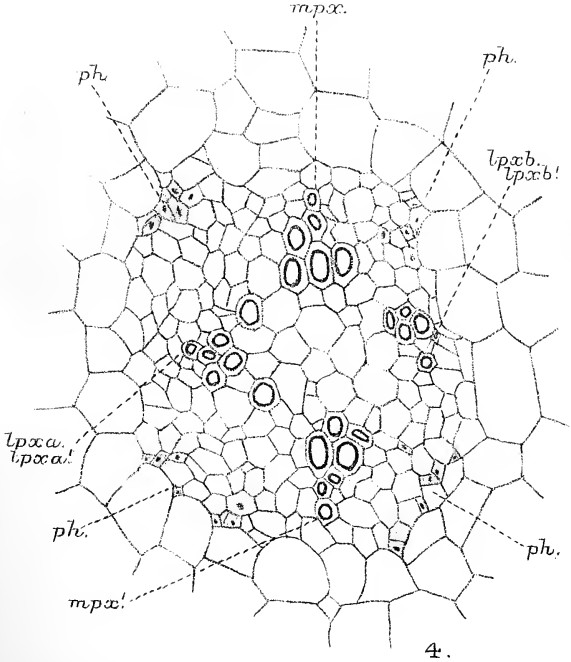

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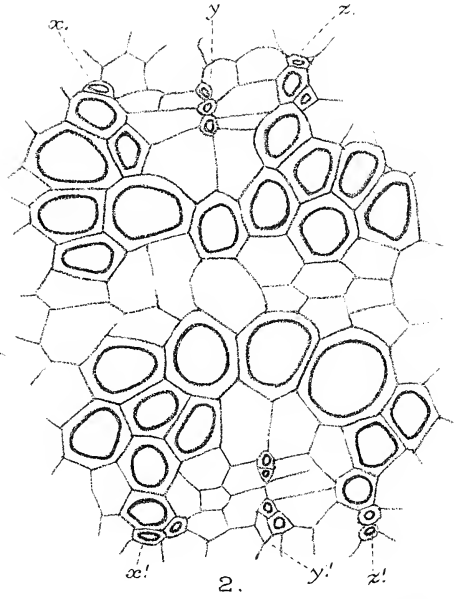

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E.Lee, del.

LEE — SEEDLING ANATOMY.

Huth, lith et imp.

A New Type of Spermogonium and Fertilization in Collema.

BY

FREDA M. BACHMANN.

With Plate LXIX.

THE question as to the true nature of the so-called spermatia in Lichens, many Pyrenomycetes, Rusts, &c., connected as it is with the general question of functional sexuality in the Ascomycetes, still remains an open one in the minds of some students of the Fungi.[1] I have undertaken further studies on the Collemaceae with a view to throwing light on both these problems.

What Tulasne has called the spermogonia of Lichens were thought by Fries (15) to be aborted apothecia. Flotow (14), a few decades later, describes them as perithecia without asci or paraphyses and containing only atom-like sporidia. Itzigsohn (21) believed the spermatia were motile and similar to the male cells of *Polytrichum* and *Marchantia*, hence he called them spermatozoids and called the spermogonia antheridia. A few months later, in a second paper (22), he confirms his earlier observations, but also adds that Rabenhorst and Kützing had written him of their failure to see any movement of these small cells, and that Flotow had observed a movement of the cells but, finding the same in material which had been in the herbarium for twenty years, had concluded that it was only a molecular movement. A year later, in a letter to Itzigsohn (23), Rabenhorst writes that he saw such a movement as had been described by Itzigsohn.

Tulasne (35), failing to see any resemblance between these flask-shaped filamentous structures in Lichens and the antheridia of Mosses and Hepatics, and not finding their contents motile, proposed to call them spermogonia and the small cells produced in them and set free through the ostiole spermatia. He figured the spermogonia and spermatia for about fifty

[1] Klebahn, H.: Die wirtswechselnden Rostpilze. Berlin, 1904. Bitter, Georg: Zur Morphol. u. Systematik v. *Parmelia*, Untergattung *Hypogymnia*. Hedwigia, xl, 1901, pp. 171–274.

[Annals of Botany, Vol. XXVI. No. CIII. July, 1912.]

different species of Lichens. He found that the size and shape of the spermatia vary in the different species, but in every case he found them borne on specialized hyphae, the spermatiophores, in pocket-like depressions in the thallus, the spermogonia, and finally extruded through the ostiole of the spermogonium on the surface of the thallus. In a later paper (36) he described and figured spermatia in Fungi other than Lichens. Tulasne thought spermatia differed from conidia in that they would not germinate. He believed the spermatia were male cells, and the asci female cells.

Cornu (8) succeeded in germinating the spermatia of a number of Ascomycetes other than Lichens, and concluded from his observations that spermatia are a kind of conidia. He continued to use the word spermatia, but would have it apply only to very small conidia which because of their very small size could easily be carried long distances.

Stahl (30) was the first to recognize the true female reproductive organ in the Lichens and to determine the real function of the spermatia. In the gelatinous Lichens which he investigated, *Collema pulposum*, *C. microphyllum*, *Physma compactum*, and *Synechoblastus conglomeratus*, the female organ or carpogone, as Stahl calls it, is a septate, spirally wound hypha at its base with a long terminal portion directed towards and protruding somewhat above the surface of the thallus. Stahl called this terminal part the trichogyne, a name which had originally been given by Bornet and Thuret (7) to a similar structure in the red Algae. In these Lichens Stahl found the spermatia to be small oval cells borne in spermogonia. Stahl says it is easy to believe that the spermatia which are extruded in large numbers from the neck of the spermogonium are spread over the surface of the thallus by means of rain-drops. At least he finds the end cell of the trichogynes which protrudes above the surface of the thallus with several spermatia clinging to it. He also finds that the spermatia become attached very firmly to the trichogyne, so that neither water nor a jarring of the cover-glass will detach them. He has figured three cases in which the wall has been dissolved between the spermatium and the trichogyne, resulting in a continuity of the protoplasm of the two cells. Stahl found that after a spermatium had fused with a trichogyne, the cross-walls of the trichogyne became gelatinized, and the cells of the spirally wound portion, or ascogone, increased in size. From this basal portion, as Jancewski (24) had described for *Ascobolus* and Kihlman (25) for *Pyronema*, came the ascogenous hyphae. That the spermatia are the male cells of Lichens and that we have in spermatia, trichogyne, and carpogone a genuine functional sexual apparatus seems conclusive, in spite of the attempts of Brefeld, Möller, and others many times reviewed,[1] to

[1] Blackman, V. H.: On the Fertilization, Alternation of Generations, and General Cytology of the Uredineae. Annals of Bot., xviii, 1904. Harper, R. A.: Sexual Reproduction in *Pyronema confluens* and the Morphology of the Ascocarp. Annals of Bot., xiv, 1900. Baur, Erwin: Die Anlage und Entwickelung einiger Flechtenapothecien. Flora, lxxxviii, 1888, pp. 319–32.

weaken the evidence. Stahl notes that *Collema pulposum* is frequently entirely sterile, though well provided with trichogynes. In these cases spermogonia were lacking or were represented only by a few small rudiments embedded in the thallus. Whether such cases indicate an approach in the European forms of *C. pulposum* to the conditions which I shall describe below for the Wisconsin material is an interesting question.

Stahl's work on the Collemaceae was later confirmed by Sturgis and Baur. Sturgis (31) noted the presence of spermogonia in some species which he investigated, and very briefly and incompletely described the spermatia and the origin of the spermogonia. In *Collema chalazanum*, Ach., *C. pulposum*, Ach., *C. nigrescens*, (Huds.) Ach., and *Leptogium myochorum*, Tuck., he found the ascogones, trichogynes, and spermogonia exactly as described by Stahl. Baur's (4) results on *Collema crispum* are likewise in agreement with Stahl's. Stahl's observations have also been confirmed wholly or in part for a number of Lichens other than the gelatinous types in the work of Lindau (26), Baur (5 and 6), and Darbishire (10). Spermatia have not been found in all Lichens. Fünfstück (16) concludes that the genera *Peltigera* and *Peltidea* have no spermatia, and he described a vegetative origin of the apothecia in *Peltigera malacea*, *P. canina*, *Peltidea apthosa*, and *P. venosa*.

In all Lichens in which spermatia have been found up to the present time these cells are borne near the surface of the thallus in large numbers in cup-like depressions, the spermogonia, as described by Tulasne in 1850. The trichogynes, wherever they have been found, grow up to the surface of the thallus, protrude a short distance, and there fuse with the spermatia which are brought to them probably by rain as suggested by Stahl. In the *Collema* which I have studied I find no superficial spermogonia.

My material was obtained from a sandstone cliff overlooking Lake Mendota and from the bluffs along the Wisconsin River near Lone Rock, Wisc. The lichen has also been collected at Blue Mounds, Wisc. There has always been plenty of material for fixing and sectioning, but never an abundance for herbarium specimens. Only small amounts collected at different times have been preserved dry. *Preissia*, *Conocephalus*, *Marchantia*, Mosses, and a few small flowering plants, mostly in the sunnier places, are found in the same habitats. The *Collema* material grows in the more shaded parts or where it is partly shaded by flowering plants. Growth of the thalli is interrupted only in the winter and during very dry weather in summer. In the spring, as soon as the snow melts and the ground is thawed, the thalli resume growth.

Prof. Farlow has very kindly examined my material and finds that it agrees very well with Tuckerman specimens of *Collema pulposum*, (Bernh.) Ach., noting, however, that it approaches *C. tenax*, (Sw.) Ach. In view of the new results which I have obtained and which are unlike anything

found by Stahl, Sturgis, Tulasne, or others who have described either the carpogones or spermogonia of *C. pulposum*, the identification of the species has become a question of prime importance. It appears that what is known as *C. pulposum*, (Bernh.) Ach., varies considerably. Acharius (2) gives five varieties: *crispum*, *cristatum*, *aphaneum*, *granulatum*, and *prasinum*. Schaerer (29), in 1842, gives two varieties, *vulgaris* and *prasina*. Nylander (28) gives five: *formosum*, *compactum*, *hydrocharum*, *prasinum*, and *tenax*. Tuckerman (34) writes of *C. pulposum*, 'It is without doubt largely represented in North America, but abounds peculiarly in difficulties which do not appear to be as yet resolvable in Europe; as certainly not here. With present knowledge, beside what may vaguely be taken for true *C. pulposum*, the group may be considered as represented with us by the five following, at least sub-species, the claims of which to higher rank are left open.' These five are: *C. tenaxum*, (Tuck.), *C. tenax*, (Sw.) Ach., *C. crispum*, Borr., *C. limosum*, Ach., *C. coccophorum*, Tuck., and *C. plicatile*, Schaer.

The form of *C. pulposum* which I have studied may be described as follows: Thallus, when young orbicular, later becoming quite irregular, 15 to 60 mm. long, upper surface smooth or sometimes granuloso-lobulate, especially about the immersed apothecia; when wet gelatinous but quite firm, dark olive green above and below, or lead-coloured or yellowish green; when dry brittle, much shrivelled and almost black, or, especially in case of yellowish green thalli, the shape and colour little changed in drying; attached to the soil by numerous light to dark brown simple or branched rhizoids 4 to 7 microns in diameter; marginal lobes more or less expanded, sometimes repand-crenate, sometimes imbricated, 1 to 1·5 mm. thick, sometimes showing a rosulate arrangement, or both marginal and central lobes somewhat erect; Nostoc filaments more numerous near the surfaces; apothecia developing quite close together and almost covering the central part of the thallus, rounded, 1·5 to 4·5 mm. in diameter; when young immersed in slightly raised areas of the thallus, sessile, at first concave, later concave or convex; disc at first pale green, later reddish-brown, margin from entire becoming rugose-crenate; hypothecium almost colourless to light brown; hymenium light brown above; paraphyses brown in potassium iodide, septate, 2 to 3 microns wide and 110 to 160 microns long, simple or branched near the base, tips somewhat enlarged; asci clavate, blue in potassium iodide, 18 to 25 microns wide and 95 to 140 microns long; spores 8, hyaline, brown in potassium iodide, oblong-ellipsoid, muriform, 10 or 18 celled, 17 to 30 microns long and 7 to 13·5 microns wide.

Stahl and those who have described the spermogonia in *Collema pulposum* may have had what Tuckerman (34) designates as the 'true *C. pulposum*'. Sturgis worked with some American form. He states that his observations agree in all respects with those of Stahl. He gives but one

figure, and this is of a young carpogone which has not yet developed a trichogyne.

The technique necessary for a cytological study of the Lichens has been found very difficult by most investigators. Baur found *Endocarpon miniatum* the only lichen which he could cut in paraffin. For others he found it necessary to cut in celloidin after first saturating the block with glycerine. In this way he succeeded in getting sections 10 to 25 microns thick.

For killing and fixing my material I have used Flemming's medium solution of chrom-osmic acetic acids and a 1 per cent. solution of platinic chloride with very satisfactory results. After washing in water, the material was dehydrated in the different grades of alcohol. Better results in sectioning were obtained if the dehydration was somewhat rapid up to 95 per cent. alcohol. The use of cedar oil makes the material less brittle than xylol or chloroform. The difficulties of other students in this respect were probably due to failure to dehydrate perfectly and to secure perfect infiltration. I have left the material from eight to twelve hours in cedar oil and for the same length of time in equal parts cedar oil and 45° paraffin. The temperature of the paraffin bath should not greatly exceed the melting point of the paraffin. Perfect infiltration has been secured in four to five days in melted paraffin before embedding. Serial sections 5 to 25 microns thick were cut without any difficulty. Some of the slides were stained with Heidenhain's iron-hematoxylin, but the triple stain of Flemming gave the most satisfactory results.

In microscopic section the thallus is seen, as is well figured by Stahl, to be composed of a network of anastomosing fungal hyphae embedded in the jelly of the algal symbiont, Nostoc. The Nostoc filaments are much more abundant near the surface of the thallus, but are also scattered throughout its entire thickness (Pl. LXIX, Fig. 1).

Ascogones are formed as soon as growth begins in the spring, and in May or June are very abundant in both early and later stages of development. In lobes of small fruiting thalli about half of the sections (25 microns thick) show parts of ascogones. In order to facilitate tracing the ascogones and trichogynes I have cut some sections quite thick. With some practice in staining, these thick sections stain very well for cytological as well as morphological study. The ascogones are found singly or more often in groups of three or four and are usually about a fourth of the thickness of the thallus below the upper surface. Carpogones have been found in groups in a number of Lichens. In *Lecanora subfusca*, L., Baur (6) and Lindau (26) found the carpogones in groups of five to ten; in *Endocarpon miniatum*, L., Baur (6) found three to eight. Except in one instance in the *Collemas*, Stahl found that the apothecia arise from a single carpogone, and he has figured the carpogones of *Collema* as isolated. In *Physma compactum* he found several trichogones in a single apothecium. In this

species the apothecium is compound, since it develops from several carpogones. Very often at least, the filament which is to bear one or more carpogones is differentiated from the other hyphae by having shorter, broader cells. From this filament branches arise which coil at once. The cells of a coil are short and broad, isodiametric or very slightly elongated. As the carpogone grows, the cells composing it elongate somewhat and become two to three times as long as they are wide (Figs. 1 and 2). There are from fifteen to twenty-five cells in a coil. Stahl found the number varied from twelve to twenty in *Collema microphyllum*, and for *Collema crispum* Baur (4) gives the number as fifteen to twenty. The number of spirals varies from two to about three and a half. Sometimes the spiral is loosely and unevenly coiled (Fig. 4), more often it is coiled quite evenly (Fig. 3). This is in agreement with what has been found in other species of the genus.

Very shortly after the spirals are formed, the terminal cell begins to elongate. It is 3 to 5 microns wide and reaches a length of 175 to 280 microns (Figs. 2, 3, and 4). The terminal cell of a carpogone is always easily distinguished from the vegetative hyphae in a stained section by its colour. It is coloured more deeply blue than the other hyphae. It is sometimes reddish if the exposure to the safranin has been prolonged, but very seldom appears stained by the orange. Also the width of the cell is slightly greater than that of most vegetative hyphae, and these two facts, with the absence of cross-walls, make it very conspicuous. Tracing this cell from section to section one finds that it does not grow vertically upwards towards the surface of the thallus, but more or less horizontally or parallel to the plane of the thallus. It is not straight, but often somewhat winding. Often several of the cells next to the terminal trichogyne cell are elongated more than those further back in the coils of the carpogone.

Stahl has distinguished three parts in the carpogone—the terminal cell which is the receptive portion, the ten to twenty cells (number varying with the species) next the terminal cell which is the conductive portion, and the coils at the base or ascogone from which later the ascogenous hyphae develop. The carpogone, then, according to Stahl, is composed of ascogone and trichogyne, and the latter again is divided according to function into a receptive and conductive part. In my material these three parts may be readily distinguished. The long terminal cell is the receptive cell, but of course it is largely concerned in conducting the male nucleus to the ascogone. Even before fertilization, then, the carpogones are easily recognized and distinguished from the vegetative parts in the section by the coiled basal portion and the long terminal cell.

As noted above, no superficial spermogonia have been observed in my material. The spermatia are, however, found abundantly in the same thalli, and in the same part of the thallus in which the carpogones are found.

There are no flask-shaped spermogonia such as have been described for other Lichens. The spermatia are simply borne in scattered groups embedded in the thallus at a depth of 100 to 300 microns (Fig. 1). They are oval or pear-shaped cells, often more or less constricted in the centre (Figs. 2, 4, and 5). They are 2 to 3 microns wide and 9 to 12 microns long. They stain lightly. There may be few in a group—only two (Fig. 3), or as many as fourteen or fifteen may be found in a single cluster. They are borne terminally if only one or two are present, or they may be both terminal and lateral if more are present. They arise by what appears to be a process of budding from certain slender lightly staining hyphae. The cells of the hyphae bearing them are often much shorter than the spermatia; sometimes the spermatia are long and narrow. Lindau (26) has described the spermatia of *Anaptychia ciliaris* as small cells, borne on many-celled sterigmata from which they are constricted off. He does not figure them. In *Collema pulposum*, Ach., according to Tulasne, the spermogonia are sunken in the thallus and contain many branched septate filaments, the spermatiophores, which bear the spermatia laterally and terminally. In *Collema cheileum*, Ach., the filaments are more branched and the spermogonia not sunken in the thallus. In Tulasne's figure the spermatia are borne at the sides and ends of the intercalary cells very much as I find them in my material. Tulasne's figure of *C. pulposum* shows in some cases two spermatia arising from the same cell. The spermatia of *C. jacobaeifolium* and *C. pulposum* are small oval cells, sometimes constricted in the centre. Tulasne (35) gives the length of the spermatia of *C. nigrescens* as 4 to 5 microns. The spermatia of *C. microphyllum*, according to Stahl, are often constricted in the centre; those of *Physma compactum* are ovoid. In *C. crispum*, Ach., Baur (4) found the spermatia to be small ovoidal cells. He does not give the size nor tell how they are borne.

Sometimes the spermatia in my material form such dense groups that the exact manner of their origin is difficult to determine. Very commonly they are found free, suspended in the jelly of the Nostoc. Apparently when mature they are not at all firmly attached to the filament on which they are borne. There is no evidence in such sections that they have been torn away by the process of fixing and cutting.

As noted above, the trichogynes in my material do not grow vertically upwards to the surface of the thallus, but remain embedded in it, extending horizontally or in the plane of the thallus. If we follow them in their course the striking fact is at once noted that they grow towards the groups of spermatia. In fact, the groups of spermatia are most easily found by tracing the long terminal cells of trichogynes which are growing towards them. These trichogynes may, of course, run through several sections 25 microns thick. As one is nearing that section in which the spermatia are present, frequently more and more parts of trichogynes are

seen. Where there are only one or two spermatia in a group, a single trichogyne grows in that direction (Fig. 3); if a larger number of spermatia are present there is a correspondingly larger number of trichogynes converging towards them (Figs. 2 and 5). Frequently the trichogynes from ascogones originating rather close together grow towards different groups of spermatia.

We have thus a complete submergence of the sexual apparatus in the tissues of the thallus and the consequent complete disappearance of the protective spermogonium about the groups of spermatia (Fig. 1). The trichogynes seek out the spermatia which have lost even their passive motility by means of water or insects.

The end of the trichogyne is oval, and becomes closely appressed to and flattened against the wall of the spermatium. In some cases the tip of the trichogyne coils closely about half-way around the spermatium, and the opening later between the trichogyne and the spermatium is at the tip of the trichogyne and about in the centre, and on the side of the spermatium. This is similar to the behaviour of the conjugating tube in *Pyronema* as described by Harper (17). Here, too, the egg-cell has produced a structure which grows out to the antheridium and coils partly around it before the conjugation pore is formed. Harper says the conjugating tube applies itself closely to the surface of the antheridium and becomes curved, and sometimes even hook-shaped, to conform to the surface of the antheridium. This behaviour is, in many cases, exactly like that of the trichogyne in the form of *Collema pulposum* here described. In other cases it does not coil around the spermatium, but becomes attached at the side or at the end of the spermatium (Fig. 5). In one case a trichogyne was found with two free spermatia attached to it. The figures of Stahl and Baur show the spermatium attached to the trichogyne a short distance from the tip of the latter and the opening at the side and near one end of the spermatium. In this species there is no coiling of the trichogyne around the spermatium.

The several cells next the terminal cell of the trichogyne now begin to exhibit characteristic changes. Up to this time the cross-walls have appeared thin and sharp in outline. As Stahl observed, however, the cross-walls now apparently gelatinize and become very much thickened. This thickening is greatest in the centre of the septum, so that biconvex lens-shaped walls result; only occasionally are they biconcave (Figs. 3 and 4). This process begins in the most distal of these cells and progresses towards the ascogone. The walls nearest the ascogone appear as a thick gelatinous plate with a broad opening in the centre. My results on these points are in agreement with those of Stahl and Baur. In my preparations made with the triple stain these swollen gelatinous cross-walls take a deep orange colour with even a very short exposure to orange G. This makes these cells extremely conspicuous and easy to find in the sections. The changes

in the thickness of the walls and their affinity for the orange is very similar to that described by Harper (18) for the antheridium of *Phyllactinia*, where, after the conjugation pore is closed, the wall of the antheridium increases in thickness by what seems to be mucilaginous degeneration. The swelling, too, is towards the interior of the cell. There is, however, a difference in that it is only the cross-walls of the trichogyne which gelatinize; the side walls remain as before. In *Phyllactinia* the thickening is less in the region of the closed conjugation pore and greatest on the wall of the antheridium opposite this pore. The blue-staining terminal cells and the brown cross-walls of the trichogynes are very conspicuous even after the apothecium has been formed. The cells of the trichogyne maintain their cylindrical shape for some time, but by the time the thickening of the cross-walls is greatest the protoplasm of the cells has become denser, the plasma membrane is pulled away from the side walls, and these walls begin to bend in towards the centre of the cell, giving the cell somewhat the shape of an hour-glass. The cells are even more conspicuous now because their apparently disintegrating protoplasmic masses stain deep red. The cross-walls are gelatinized but remain as wide as ever.

This change in the shape of the trichogyne cells was observed by Stahl, who thought it was connected with a loss of water and hence of turgidity. The disintegrating masses of protoplasm have the same appearance as that described for the antheridial cell of the Mildews and of the conjugation tube of *Pyronema*. Harper found that the cell sap disappears, allowing the denser portions to form a homogeneous mass which has then an affinity for the safranin. Harper also observed that as disintegration continued the mass stained less deeply, but that the brown walls were still conspicuous. This is true for my material. The brown cross-walls are still evident when the part of the cell between is scarcely noticeable.

As to the sexual nature of the structures described, my investigations confirm those of Stahl, Baur, and Darbishire, and leave no doubt as to the functions of the trichogyne and spermatia. The behaviour of the trichogyne in this *Collema* should put an end to the idea of the trichogyne being either a respiratory apparatus or a boring organ. It is very evident here that the trichogyne is exactly what it is in the red Algae, a structure developed from the egg-cell to conduct the male nucleus to the egg-cell. The very apparent attraction of the trichogynes to the spermatia, and the later changes in the ascogone and trichogyne, show plainly also that the reproductive organs are functional.

We may summarize the differences in the sexual apparatus of this form of *Collema pulposum* and that of all other Lichens so far described as follows: (1) The spermatia are reduced in numbers and are not enclosed in spermogonia. (2) The spermatia are embedded deep within the thallus and are never set free on its surface. (3) The trichogyne has fewer cells

and an unusually long terminal cell. (4) The trichogyne grows towards and seeks out the male cells. In all other Lichens the spermatia are borne in large numbers in specialized organs, the spermogonia, and are extruded through the ostiole on the surface of the thallus, then to be carried by rain, dew, or possibly wind or insects, to the trichogyne, the tip of which is also above the surface of the thallus. If fertilization by means of water is more primitive, these then have more nearly retained or reverted to the aquatic habits of their ancestors in the production of large numbers of male cells and in the method by which the egg-cell is fertilized.

The conditions found in *Collema pulposum* have probably been developed with the land habit and approach those found in *Pyronema* and the Erysipheae. The greater activity of the trichogyne results in a certainty of fertilization which makes the production of a large number of male cells entirely unnecessary. There is here the same certainty of fertilization which exists in *Pyronema*, where there is usually but one and never more than two male cells for each female cell. The disappearance of large spermogonia is correlated with the certainty of fertilization and the diminished number of spermatia. It is plain, however, that this is not a reduced or vegetative fertilization in Blackman's sense. The male cells are just as highly specialized as in other Lichens or the Red Algae. Whenever it has been possible to be certain of the manner in which the spermatia are borne, this has been found to be similar to that of other Collemas as well as of many other Lichens. The short, broad, faintly staining cells of the spermatiophores with delicate cross-walls are quite different in appearance from the elongated, easily stained cells with heavy septa of the vegetative hyphae. These spermatia are also similar to those borne in spermogonia in the apparent readiness with which they are separated from their place of origin. The limited size, the shape, the often constricted centre, the attraction for the trichogynes, and the fact that they are formed only at a particular stage in the life-history of the lichen, all show that they are specialized cells with a definite sex function. The trichogyne does not fuse with the vegetative hyphae, but seeks out these specialized cells exactly as in the case of all other sexual fusions.

In view of what has been described of the relation of spermatia and trichogynes by Stahl, Sturgis, Lindau, Baur, and Darbishire, it would seem as if the sexual nature of spermatia could not be doubted. Still Fink (13), after noting Möller's results in germinating spermatia, writes: 'This would seem to indicate that the spermatia, if they are sexual cells, have become so degenerate in certain Lichens as to lose their sexual function, becoming capable at the same time of reproducing vegetatively.' It seems very clear that the spermatia in my material are entirely homologous with those borne in the spermogonia of other Collemas, and it is impossible to conceive, after what has been described above of their behaviour in this

species, that they are asexual conidia. Möller's results as to germination may or may not be correct, but the conditions in *Collema pulposum* certainly give a final and complete demonstration that the spermatia of the Lichens are male gametes and not asexual conidia. Even the author of such a tenuous hypothesis as that of the boring function of the trichogyne will hardly argue that these few cells buried deep in the thallus and never set free have their natural function as spores for the rapid asexual spread of the fungus.

The conditions in the lichen here described throw new light on the old and much discussed question as to the relationship of the red Algae and the Ascomycetes. Harper (17), as have others before, has emphasized the fact that *Pyronema*, like the Laboulbeniaceae, forms an interesting link between the Lichens and the red Algae because of its conjugating tube. In those red Algae which have been investigated, the trichogyne is a tubular outgrowth from the egg-cell. In *Pyronema* this outgrowth is separated from the egg-cell by a wall. In the Lichens there are perhaps several cells between the receptive portion and the egg-cell. *Collema pulposum* is intermediate between those Lichens in which the trichogyne is composed of many small cells and *Pyronema*, where it is but one cell. But this species resembles *Pyronema* more than other Lichens, especially in the greater activity of the trichogyne and the manner in which it grows towards and coils about a spermatium, and in the reduced number and greater fixity of the spermatia, thus making them more like antheridia. In attempting to homologize the spermatia of the Collemaceae and the antheridia of *Pyronema*, de Bary (11), with his usual morphological acumen, pointed out that 'If the spermatia of *Physma* remained fixed to their spermatiophores in order to conjugate with the archicarp, the only difference between the two forms would be one of conformation'. In the *Collema* I have described we have just such a form, and one still more useful for the homology because the spermatia are not borne in spermogonia and are so few in number. In the number and nature of its spermatia and the manner in which they are borne, this species of *Collema* forms about the most perfect conceivable connecting link between the aquatic red Algae with many non-motile male cells, which are, however, set free, and such terrestrial Ascomycetes as *Pyronema* and the Mildews, where the male cells are reduced in number to one or two which remain permanently attached.

The conditions as to sexual reproduction in *Collema pulposum* are also quite the same in principle as those in some of the Laboulbeniaceae, as fully described by Thaxter (32 and 33). In *Zodiomyces* one to three antherozoids bud from the tips of the antheridial appendages. From here the antherozoids fall and are then found lying loose about the bases of the perithecial stalks. The behaviour of the trichogyne is like that of *Collema pulposum* in that it grows towards where the spermatia are formed and there

becomes attached to one of them. In *Rhynchophoromyces rostratus* the small rod-like antherozoids bud distally and laterally from intercalary and undifferentiated cells of the appendages. In some species of *Ceratomyces* the single rods are replaced by slender long filaments which eventually break up into rods that probably function as antherozoids. Through the motion of the plant the trichogyne is brought into contact with the antherozoids, and Thaxter says it is probable that such antherozoids, in order to be functional, must become detached at the moment when they come in contact with the trichogyne. In the discussion above it was suggested that the greater activity of the trichogyne and the permanent attachment of the spermatia are a result of the land habit. Thaxter suggests that the adherence of the antherozoids to the mother-cell or to one another is an adaptation to ensure fertilization where the antherozoids might otherwise be lost because of the rapidly moving host. This seems entirely reasonable for the special case in question; still, in general, it seems that the production of a large number of free male cells which are carried to the trichogyne in water is characteristic of the aquatic Algae, while the production of few and permanently attached male cells is equally a feature of the terrestrial Fungi. It may be that we have in the genera above mentioned land forms which have later become parasitic on aquatic insects, or it may be, as Thaxter (32) suggests, that we have here an adaptation to an isolated and rapidly moving host.

In a following paper I will describe the development of the apothecium, together with certain nuclear phenomena.

I am greatly indebted to Prof. R. A. Harper, at whose suggestion this work was begun, for helpful advice and criticism.

Summary.

1. The spermatia of *Collema pulposum* are not borne in spermogonia, but are few in number and are borne terminally and laterally on a hypha below the surface of the thallus. They are completely embedded in the thallus and are never set free. They are entirely homologous with the spermatia borne in spermogonia in other species.

2. The carpogones, as in other Lichens, are embedded in the thallus. These consist of a coiled basal portion, the ascogone, and a long terminal structure, the trichogyne. The trichogyne, the end cell of which is exceedingly long, does not grow towards the surface of the thallus and protrude from it, but instead grows more or less horizontally within the thallus towards the region where the spermatia are borne. The sexual apparatus is thus completely submerged in the tissues of the thallus.

3. There is a very evident attraction of the spermatia for the tricho-

gynes, which is seen in the manner in which the latter converge about a group of spermatia. In growing towards a spermatium and often coiling about it the trichogyne shows a greater activity than that which has been described for other Lichens.

4. The spermatium fuses with the trichogyne to which it has become attached. After this fusion the cross-walls of the cells next to the long terminal cell exhibit the characteristic changes which have been described by other investigators.

5. It is very evident that the spermatia and trichogyne are functional, and that there is not merely a reduced form of fertilization in *Collema pulposum*.

6. In the number of male cells and the manner in which they are borne *Collema pulposum* forms an interesting link between the red Algae and such Ascomycetes as *Pyronema* and the Mildews. In its reproductive structures there are many points of resemblance to such representatives of the Laboulbeniaceae as *Zodiomyces* and *Rhynchophoromyces*.

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February, 1912.

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EXPLANATION OF FIGURES IN PLATE LXIX.

Illustrating Miss Bachmann's paper on *Collema*.

Figs. 2–5 were drawn with the aid of a camera lucida, and have a magnification of 520 diameters.

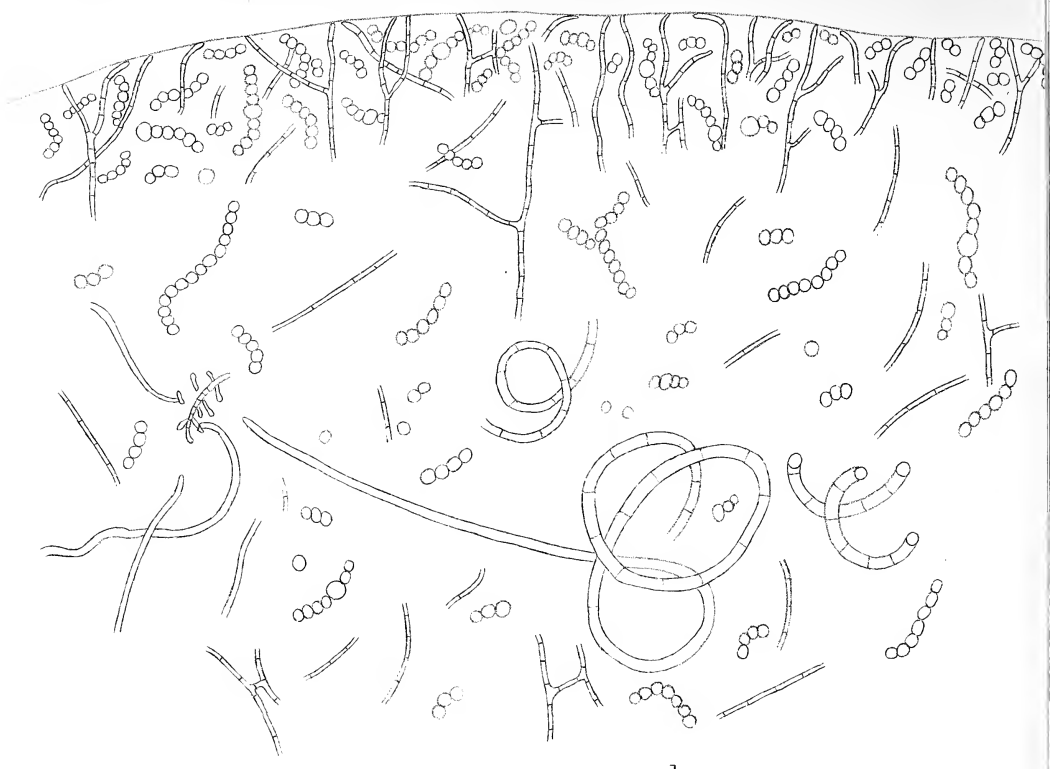
Fig. 1. Diagrammatic representation of cross-section of thallus, showing location of sexual organs and growth of trichogynes towards spermatia.

Fig. 2. Trichogynes converging about a group of spermatia. End of trichogyne fused with spermatium at *a*.

Fig. 3. Few spermatia in group. Cross-walls of cells below terminal cell of trichogyne much thickened. Cells of ascogone somewhat enlarged.

Fig. 4. Several spermatia in group. Ascogone and trichogyne as in Fig. 3.

Fig. 5. Growth of trichogynes towards spermatia. End of trichogyne fused with spermatium at *a*.



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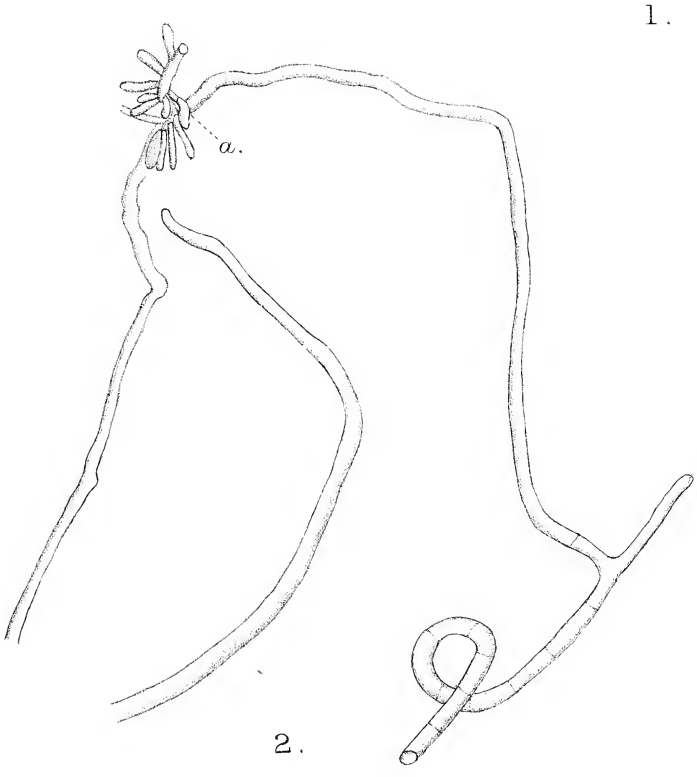

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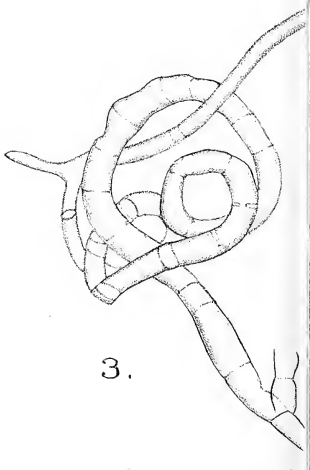

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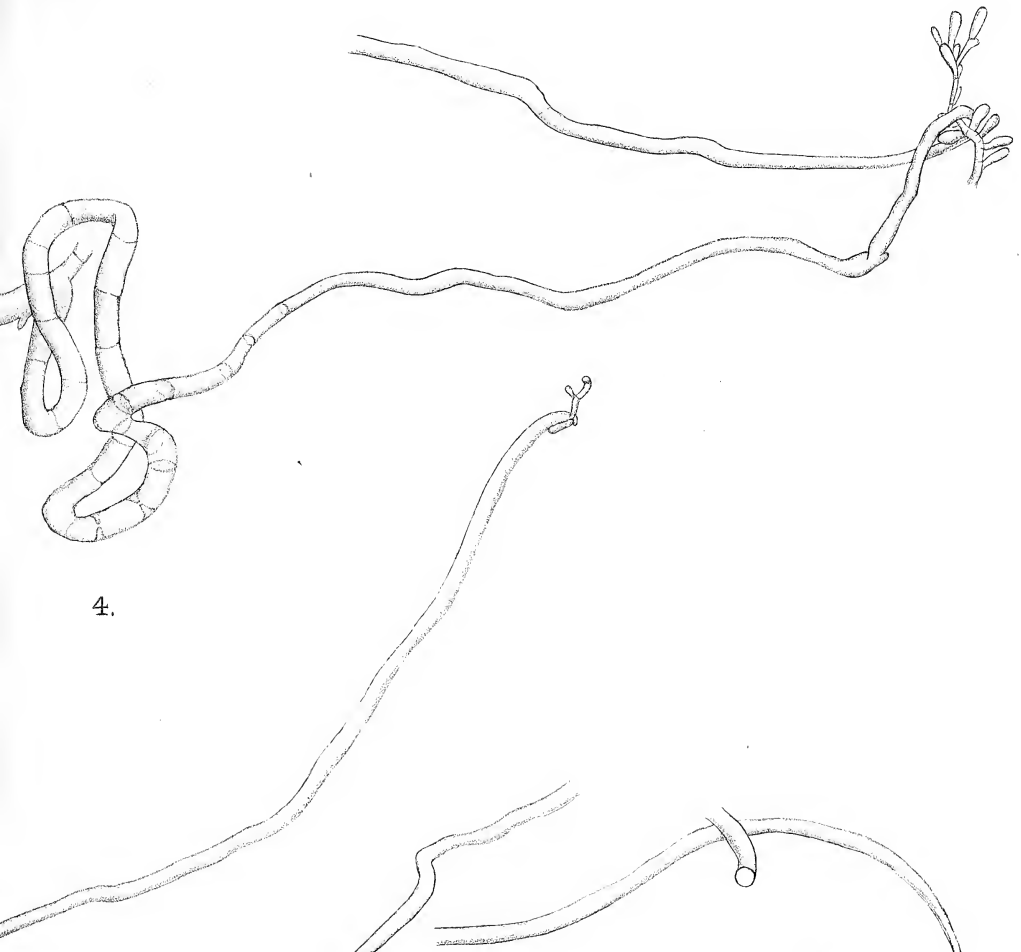
BACHMANN—COLLEMA.



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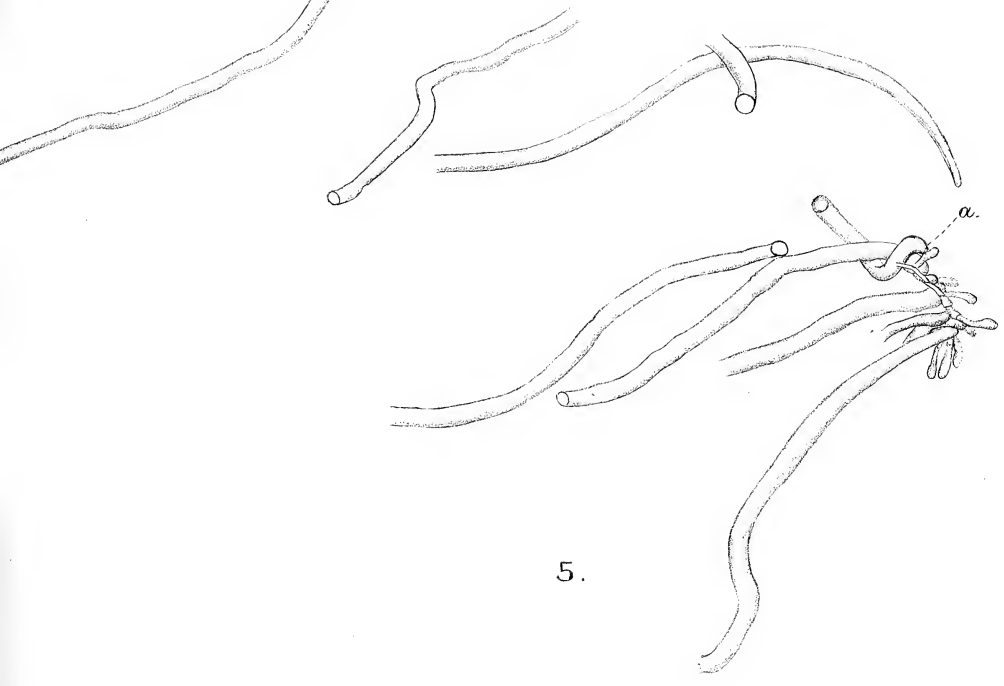

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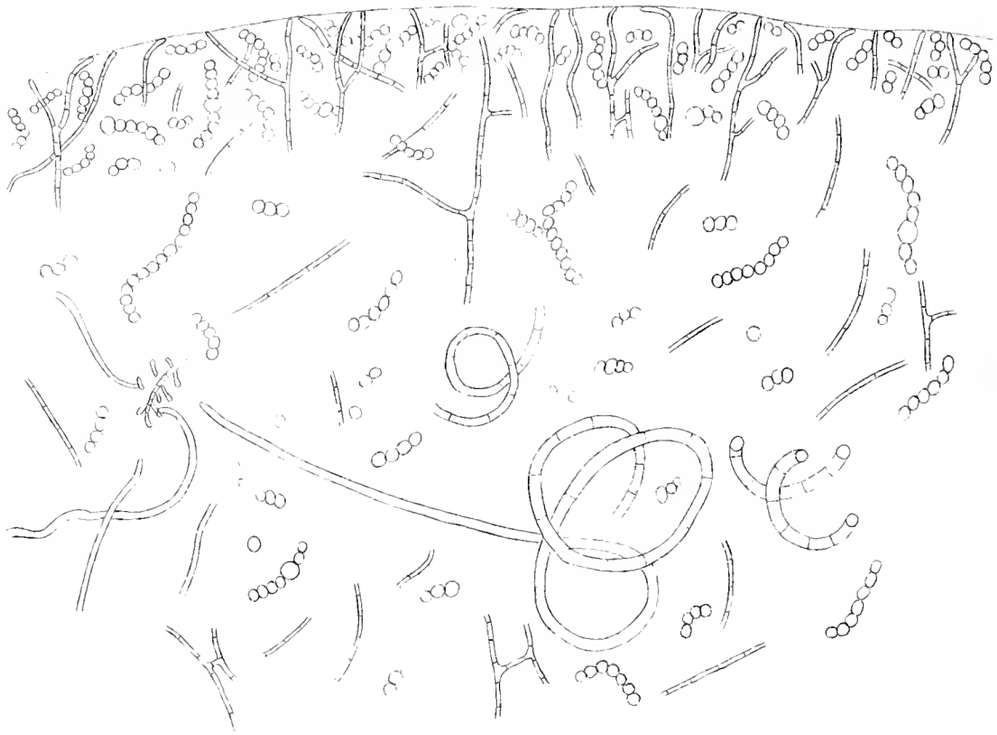

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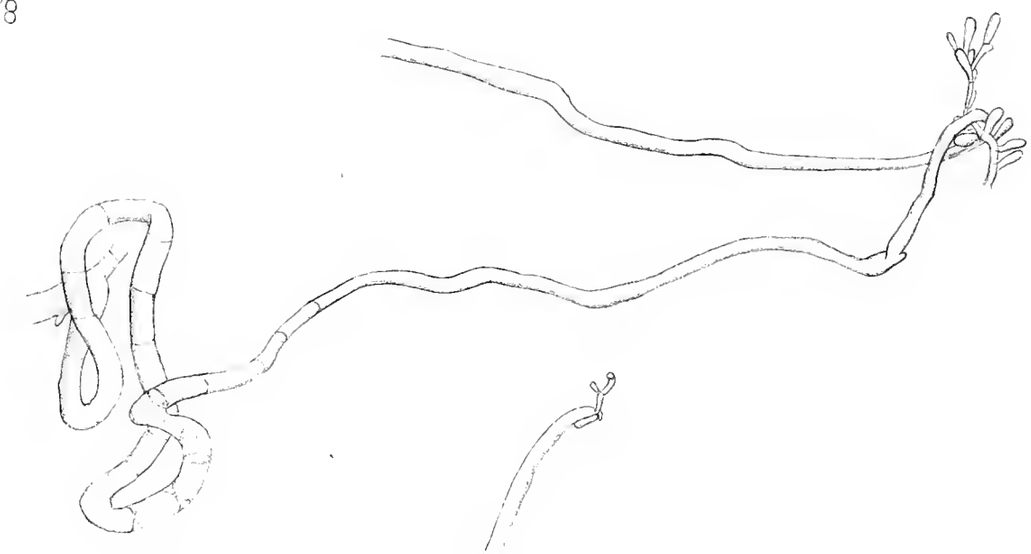

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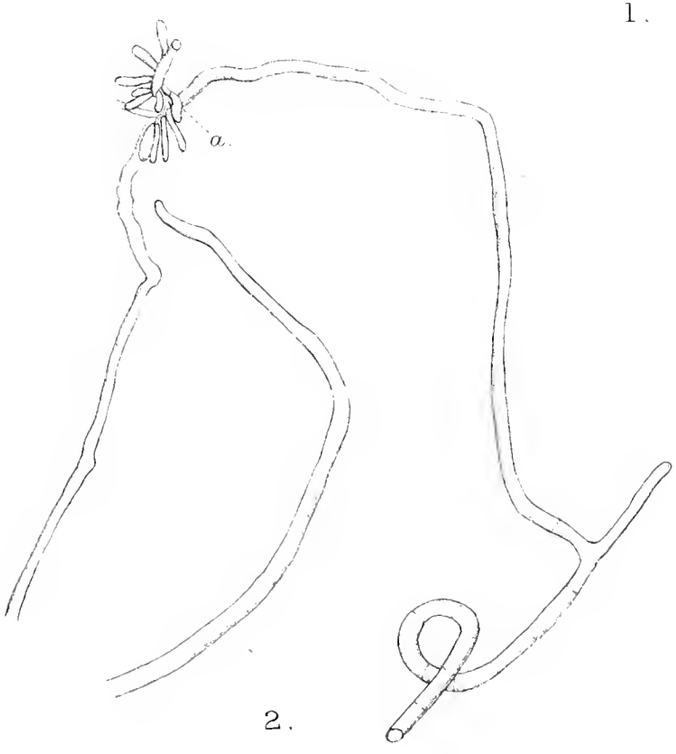

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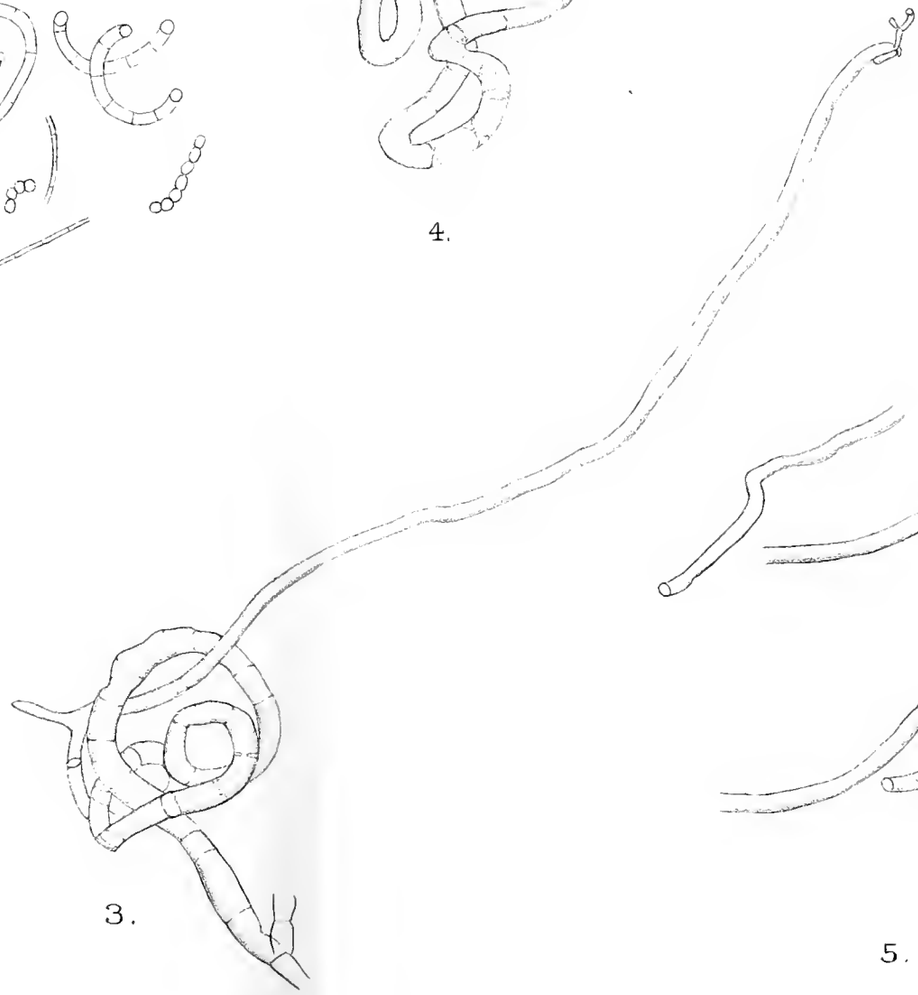

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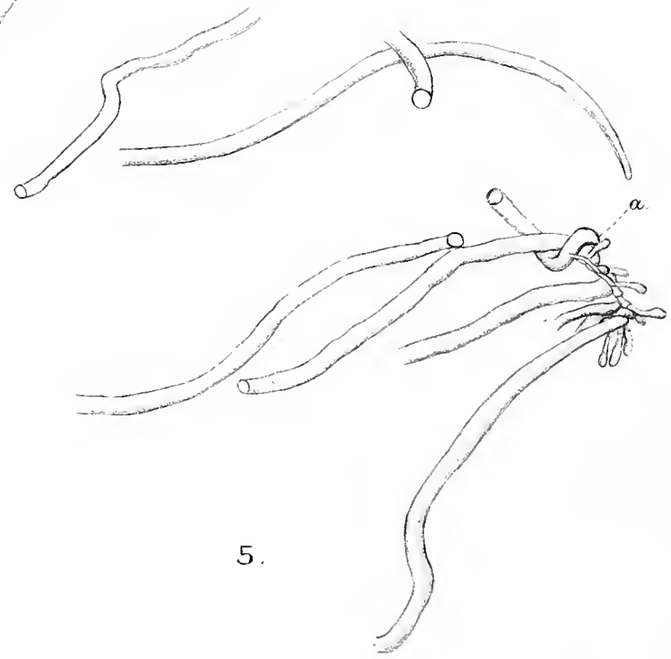

5.

BACHMANN — COLLEMA.

Huth lith. et imp.

The Development of the Perithecium of Polystigma rubrum, DC.

BY

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With Plates LXX and LXXI.

OUR knowledge of the details of ascocarp development in the Pyrenomycetes, when compared with that of other groups of the Ascomycetes, is extremely scanty. Only a few forms have been investigated in full cytological detail, such as *Aspergillus* (7), *Gnomonia* (6), and the forms examined by Dangeard (3). An examination of *Polystigma* seemed then particularly desirable not only on this ground, but also from the fact that the earlier investigations of Fisch (5) in 1882 and Frank (6) in 1883 suggested that this form was of particular interest. Their observations indicated the existence of well-marked, coiled, multicellular ascogonia with trichogynes, and also of spermatia of peculiar form. Furthermore, Frank believed that he had obtained evidence of a fusion between spermatium and trichogyne indicating the occurrence of a normal sexual process. It seemed then possible that a knowledge of the cytology of this form might throw considerable light on the general question of the sexuality of the Ascomycetes and of the Lichens in particular, for the problem which apparently faces us in the Lichens, that of the fertilization by a spermatium of a *multicellular* ascogonium, has in no case been completely solved.

With these hopes, which were, however, doomed to disappointment, some tentative observations were made as far back as 1905, but material in any quantity was not available till 1908. Difficulties in obtaining complete stages and in elucidating the behaviour of the complicated ascogonia, together with a change of posts, have delayed the completion of the work.

[Annals of Botany, Vol. XXVI. No. CIII. July, 1912.]

Polystigma rubrum is a fungus parasitic on *Prunus spinosa* and *P. insititia*, and on the cultivated plum. Its attack is confined to the leaves, on which it produces orange-yellow sclerotial patches. These patches are each the result of a separate infection and they only spread to a limited extent; with the fall of the leaf in autumn the host plant is freed from the disease. The life-history is almost identical with that of the leaf-scorch fungus (*Gnomonia erythrostoma*) of the cherry, but while the affected leaves of the cherry remain on the tree during the winter, the leaves attacked by *P. rubrum* fall at the usual time. This difference is no doubt related to the fact that in *Polystigma* there is a sclerotium, in which the perithecia develop as the leaves lie upon the ground, while in *Gnomonia* this structure is absent, so that if the leaves fell and rotted on the ground there would remain no basis for perithecial development.

In this country *P. rubrum* does not seem at all abundant. Though it is found in small quantities in various places the only locality known to us in which it occurs in any abundance is at Palling-on-Sea, Norfolk; our attention was first drawn to the fungus in this locality by Mr. George Massee.

Mycelium. The stages of actual infection of the leaf by a germ-tube from the secondary spore, which develops from the germinated ascospore, have been sufficiently investigated by Fisch (5) and Frank (6), and were not studied. The mycelium in its early stages shows thin-walled hyphae with cells containing a small number of nuclei, usually one to three. The hyphae push their way between the host cells (Pl. LXX, Fig. 1) gradually, forcing them apart and bringing about their disorganization. In a mature sclerotial patch the host tissue is reduced to a few isolated cells with tannin-like contents which stain deeply; the rest of the cells having been completely absorbed (Fig. 2).

The walls of the hyphae are at first thin (Fig. 1), but early in development the thin walls are modified into thick, gelatinous membranes which encroach on the cell cavity of the hyphae; these membranes usually show fine pits (Fig. 3). The gelatinous wall is no doubt of the nature of reserve material, for it appears to be in part absorbed during the later development of the perithecium after the fall of the leaf. No storage of reserve material in the form of special cell contents was to be observed.

In many well-developed sclerotial patches the hyphae form a continuous mass between the upper and the lower epidermis, the only remains of the mesophyll being the few scattered cells already mentioned. The hyphae generally contain in the fresh state an orange pigment which gives the bright colour to the sclerotial patches; it is easily soluble in alcohol. It is to be noted that in the early stages of mycelial development the hyphae congregate, especially in the intercellular spaces beneath the stomata, *and often push their way through the stomatal pore* (Figs. 4 and 5).

Spermogonia. These arise from a group of interwoven unthickened hyphae found usually beneath a stoma. From this mass there develops the flask-shaped spermogonium (Fig. 6), which is often large enough to extend across the leaf from one epidermis to the other. The periphery of the spermogonium is formed of densely interwoven hyphae and is lined internally by the spermatia-bearing hyphae. The spermatia are borne terminally on these hyphae, which, like the spermatia, are uninucleate; but while the nuclei of the special hyphae are only slightly elongated, those of the spermatia become very long and narrow (Fig. 8 *c*). The spermatial nucleus appears at maturity as a narrow band, staining nearly homogeneously and occupying the lower half or two-thirds of the cell (Figs. 8 *a* and 8 *b*). The spermatia narrow at their free ends and show the peculiar curvature which has been described by earlier workers (Figs. 7 and 8). The nucleus appears in many cases to undergo early disorganization, for it may show a nodulose appearance (Fig. 8 *a*) while the spermatia are still enclosed within the spermogonium. The spermatia are carried out of the spermogonium by a mucilaginous material which oozes from the mouth of that organ, and thus the spermatia become distributed over the surface of the leaf.

No relation of any kind was observed between the spermatia and the female reproductive organs, and attempts to bring about the germination of the spermatia ended, like those of Fisch, in failure. The spermatia must, then, be considered *functionless* structures, like the similarly named structures in the Uredineae.

Ascogonia.[1] These structures develop from the rapidly growing ends of ordinary hyphae; they were multinucleate in the earliest stages observed (Fig. 9). This hypha soon becomes curved and septate (Fig. 10), and gradually assumes the more or less closely coiled appearance of the mature ascogonium (Figs. 11, 12, and 13). Surrounding each ascogonium is a mass of small-celled hyphae, which are uninucleate, and with walls slightly thickened. There is a great variety in the length of the ascogonia and their degree of 'coiling' (Figs. 12 and 13). One end of the ascogonium, the base, usually can be traced into a vegetative hypha, while the other ends freely in the mycelial mass (Figs. 12 and 13). A simple slightly coiled ascogonium is shown highly magnified in Fig. 12, and a more complicated one is drawn on a smaller scale in Fig. 13.

The ascogonia are usually found in the neighbourhood of a stomata (Pl. LXX, Fig. 11, and Pl. LXXI, Fig. 14), and the stoma shows the

[1] Owing to the abortive nature of these coiled structures designated ascogonia by Fisch, it is impossible to say whether, primitively, they were wholly ascogonial in nature, or whether they represent archicarps of which only a portion was fertile, i. e. gave origin to the ascogenous hyphae. Under these circumstances the older name, ascogonium, may be retained, although by analogy with other cases (e. g. Lichens) we should expect the terminal portions of the coil to be sterile in nature, when the term archicarp would more fittingly describe them as a whole.

projecting hyphae already described, but in no case could the ascogonial hypha be followed through the stoma in the form of a specially differentiated trichogyne, as described by Fisch and Frank. As already stated, the apex of the ascogonium usually ends blindly in the tissues (Pl. LXX, Figs. 12 and 13) some distance from the stoma. In fact, of all the hundreds of ascogonia examined, in only three or four cases could the ascogonium be traced upwards towards the stoma, and even then it did not project as a differentiated trichogyne. In one of these cases the ascogonium lost itself in an ordinary hypha a little below the stoma (Fig. 11). In another case (Pl. LXXI, Fig. 14) the ascogonium was clearly abnormal, for it was much branched towards the epidermis, and gave origin to several slightly differentiated hyphae which could be traced upwards towards the hyphae projecting from the stoma. The projecting hyphae were unusually long, but were of ordinary vegetative nature and not comparable with the trichogynes described by Fisch. Only in one case was a broad hypha of a trichogyne-like nature seen projecting from the stoma, and then there was no evidence of its connexion with an ascogonium. In all other cases the projecting hyphae (Pl. LXX, Fig. 5) were clearly of vegetative nature, and in no case was a spermatium found attached to any of them.

The number and size of the cells which make up the ascogonium is very variable, but the majority of the cells are multinucleate, a few uninucleate cells being occasionally found. The basal cell, which connects the ascogonium with an ordinary vegetative hypha, is commonly larger than the others, and contains a large number of small nuclei (Pl. LXXI, Fig. 15); the majority of the other cells contain about four nuclei. The nuclei usually show a chromatin network and a well-marked nucleolus, but the nuclear membrane is not generally to be distinguished. In a few of the cells of each ascogonium there is generally to be found—either alone or with other ordinary nuclei—a nucleus without a distinct chromatin network, but with a huge nucleolus (Pl. LXX, Fig. 12, and Pl. LXXI, Fig. 16 *b*). The origin of these special nuclei could not be traced. No convincing evidence of fusion of ascogonial nuclei could be obtained, though the common close association of the nuclei in pairs and the difference in the size of the nuclei of a single cell (Fig. 16) suggest that such fusions may still take place in spite of the abortive nature of the ascogonia.

In some cases two ascogonia may develop together, but this occurrence was not nearly so common as Fisch's description would lead one to expect.

The outgrowths of the large cells of the ascogonium figured by Fisch and described as ascogenous cells were not observed. Our observations show that the cells of the ascogonia do not become emptied of their contents, as in the functional ascogonia of *Ascobolus*, *Humaria*, &c., but retain their contents during the process of disorganization, and so appear as darkly staining masses; this is well seen in Figs. 17 and 18 and particularly clearly in Fig. 19.

Perithecium. This structure, though not in structural connexion with the ascogonium, arises in its neighbourhood, one perithecium being usually found in association with each ascogonium. The perithecium is first to be distinguished as a group of special hyphae which arise from the small-celled hyphae surrounding the ascogonium. These special hyphae are characterized by their long finger-like shape; together they soon take the form of a conical mass with its apex pointed towards the lower epidermis (Figs. 17 and 18). Their cells may have the nuclei arranged in pairs (Fig. 18). This conical mass increases in size and develops into the flask-shaped perithecium (Fig. 24). During the early stages of development the disorganizing cells of the ascogonium can be clearly seen at its periphery (Figs. 17, 18, and 19).

At a stage a little later than that shown in Fig. 18, ascogenous hyphae become differentiated towards the base of the young perithecium. They have no connexion with the ascogonia, but arise by differentiation from the perithecial hyphae, which are of vegetative origin as described above. The ascogenous hyphae are at first not very sharply marked, but later they are easily distinguished by their larger size, denser contents, and larger nuclei (Fig. 19). If a section be taken through the base of the perithecium at a time when the ascogenous hyphae are differentiating there may be seen (Figs. 20 and 21), besides the nuclei in pairs mentioned earlier, larger nuclei. Nuclei in close contact are also seen, and in one case what appeared to be a stage of nuclear fusion (marked thus * in Fig. 20). There are thus indications that at this stage a nuclear fusion occurs which replaces a normal sexual fusion now lost.

The details of the formation of the asci and the ascospores were not followed, since for such work this form is not a favourable object, but a few stages were observed showing that the ascus is formed in the normal way with fusion of nuclei in the penultimate cell of an ascogenous hypha (Figs. 22 and 23).

Conclusion.

Polystigma rubrum possesses well-marked, coiled, multicellular ascogonia. The ascogonia disorganize without producing ascogenous hyphae. The spermatia also are functionless, and appear in some cases to show signs of nuclear disorganization while still within the spermogonia. The ascogenous hyphae arise near the ascogonia by differentiation from vegetative hyphae. There is a nuclear fusion in the ascus, and some evidence for an earlier nuclear fusion in the ascogenous hyphae at the time of their differentiation. Vegetative hyphae push through the stomata, but there are no trichogynes.

The divergence from the results obtained by Frank and Fisch are no doubt to be explained by the deficiencies of the primitive technique employed by earlier investigators.

P. rubrum is to be added to the already long list of Ascomycetes in which the normal sexual process is absent. It is, however, to be distinguished from the majority of such forms by the fact that well-marked male and female reproductive organs are produced, but *both* are abortive. It resembles *Gnomonia erythrostoma* in this respect, but the ascogonia are much more clearly marked than in that form. It is probable that *Xylaria* is similar to *Polystigma*, for in that form according to Fisch the ascogonia degenerate later. In *Poronia*, Miss Dawson (4) finds at the base of the young perithecium ' a group of stouter deeply-stained hyphae, presumably the remains of the coil, and now representing the young ascogenous hyphae '. It is clear from this quotation that a reinvestigation of *Poronia* might show that the ascogenous hyphae really arise independently of the ascogonia, as in *Polystigma*. In *Claviceps*, which requires, however, further investigation, there are no ascogonia, according to Fisch (5).

The evidence to be drawn from a study of *Polystigma*, though not very strong, gives no support to the contention of Claussen (2) that there is only one nuclear fusion in the Ascomycetes, that in the ascus.

The expenses of this investigation were mainly defrayed by grants from the Government Grant Committee of the Royal Society.

List of Papers.

1. Brooks, F. T.: The Development of *Gnomonia erythrostoma*, Pers. Annals of Botany, vol. xxiv, 1910, p. 585.
2. Claussen, P.: Zur Entwicklungsgeschichte der Ascomyceten, *Pyronema confluens*. Zeitsch. f. Botanik, 4. Jahrg., 1912, p. 1.
3. Dangeard, P.: Sur le développement du périthèce chez les Ascomycètes. Le Botaniste, vol. x, 1907, p. 1.
4. Dawson, M.: On the Biology of *Poronia punctata*. Annals of Botany, vol. xiv, 1900, p. 245.
5. Fisch, C.: Beiträge zur Entwickelungsgeschichte einiger Ascomyceten. Bot. Zeit., Jahrg. 40, 1882, p. 850.
6. Frank, B.: Ueber einige neue oder weniger bekannte Pflanzenkrankheiten. Ber. d. deutsch. bot. Ges., Bd. i, 1883, p. 58.
7. Fraser, H. C. J., and Chambers, H. S.: The Morphology of *Aspergillus herbariorum*. Ann. Mycologici, vol. v, 1907, p. 419.

EXPLANATION OF PLATES LXX AND LXXI.

Illustrating the paper by Professor V. H. Blackman and Miss Welsford on *Polystigma rubrum*.

PLATE LXX.

The preparations are mostly from material fixed in Flemming's strong fluid (half strength) or acetic alcohol and stained with iron-alum-haematoxylin.

Fig. 1. Leaf-cells of host with hyphae of fungus pushing between them; the cell on the right is dead and has darkly staining contents. × 1,200.

Fig. 2. Later stage in the formation of a sclerotial patch. The host cells, with deeply staining tannin-like contents, are dead, and have been pushed apart by the action of the fungus. × 1,200.

Fig. 3. Older hyphae of sclerotial patch with thick walls, showing fine pits. × 1,800.

Fig. 4. Aggregation of hyphae beneath the stomata on the under surface of the leaf. × 900.

Fig. 5. Section through leaf in the plane of the guard cells, showing one guard cell and hyphae projecting through the stomatal pore. × 800.

Fig. 6. Section through leaf, showing a spermogonium. × 300.

Figs. 7 *a* and 7 *b*. Two spermatia unstained and mounted in a watery medium. × 1,800.

Figs. 8 *a* and 8 *b*. Stained spermatia, showing the elongated nucleus. × 1,800.

Fig. 8 *c*. Spermatial hypha with base of spermatium above, with elongated nucleus. × 1,800.

Fig. 9. Very early stage in the development of an ascogonium; the terminal portion of a vegetative hypha shows four nuclei and is the future ascogonium. × 1,800.

Fig. 10. Later stage in the development of the ascogonium, which has now become coiled, and shows at least one septum. × 1,800.

Fig. 11. Section showing coiled ascogonium in the neighbourhood of a stoma through which ordinary vegetative hyphae are seen projecting. The four irregular masses are disorganized mesophyll cells. × 900.

Fig. 12. Mature ascogonium of simple form, showing details of cells and nuclei. The basal end can be traced into a vegetative hypha, while the other ends freely. Some of the nuclei show a large nucleolus and little chromatin; others show a small nucleolus and ample chromatin. × 1,300.

Fig. 13. A more complicated ascogonium than that of Fig. 12. × 800.

PLATE LXXI.

Fig. 14. Abnormally branched ascogonium. One or more of the branches apparently projects from the stoma as an ordinary vegetative hypha. × 800.

Fig. 15. Basal cell of ascogonium, showing a large number of small nuclei. × 1,200.

Figs. 16 *a* and 16 *b*. Several cells of an ascogonium, showing the distinction of size and appearance of the nuclei. × 1,200.

Fig. 17. Early stage of perithecium as a cone-shaped mass of vegetative hyphae. The disorganizing ascogonial cells are seen at the periphery and at the centre. × 800.

Fig. 18. Young perithecium a little older than that of Fig. 17. The ascogenous hyphae are not yet clearly to be distinguished, but many of the nuclei of the perithecial cells are clearly arranged in pairs. The darkly stained masses at the periphery are the disorganizing cells of the ascogonium. × 800.

Fig. 19. Later stage of young perithecium with ascogenous hyphae well differentiated by their larger size, denser contents, and larger nuclei. Remains of ascogonium still visible. × 1,200.

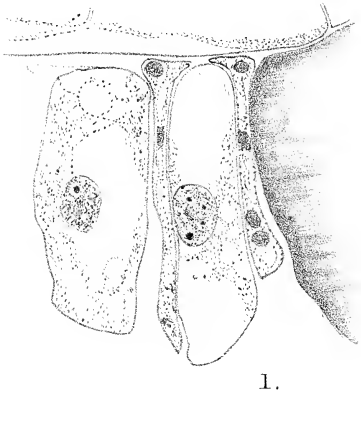
Fig. 20. Section through base of young perithecium at a time when the ascogenous hyphae are becoming differentiated. There are indications of nuclear fusions, especially at the point marked with a star, and also in the paired condition of many nuclei and the larger size of others. × 1,800.

Fig. 21. Cells at base of young perithecium with two nuclei in contact, indicating a stage before fusion. × 1,800.

Fig. 22. Young ascus still binucleate, occupying the typical position at end of an ascogenous hypha. × 2,700.

Fig. 23. An ascus in a stage somewhat later than that of Fig. 22. The two nuclei have fused, but the nucleoli remain distinct. × 1,800.

Fig. 24. Section through mature perithecium. Many of the younger asci are disorganized. × 300.



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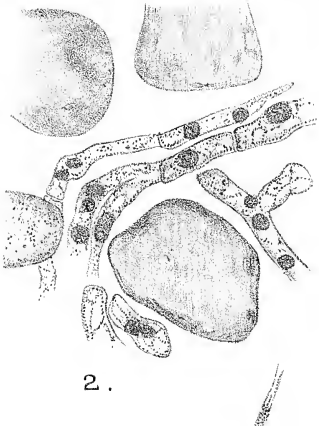

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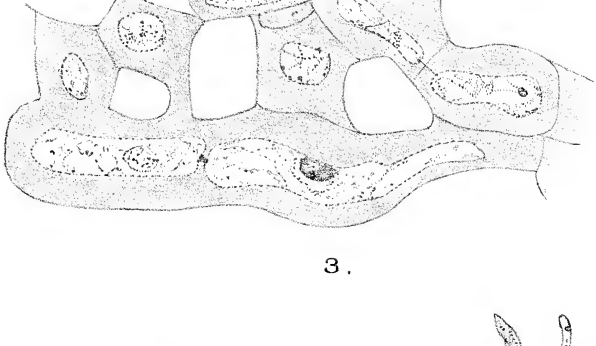

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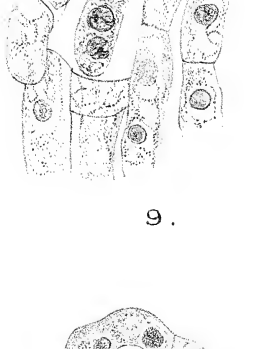

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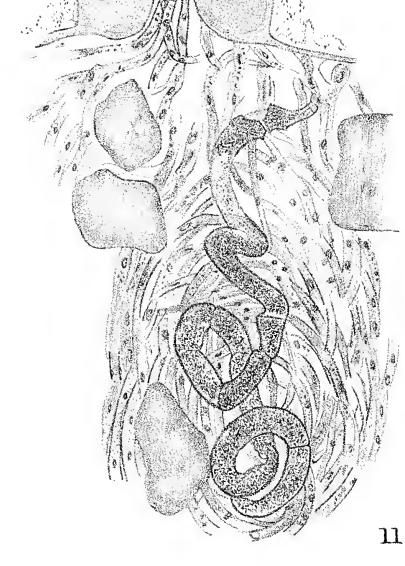

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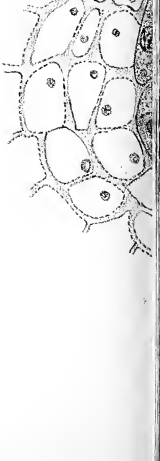

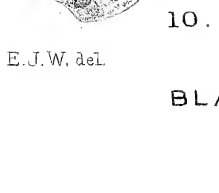

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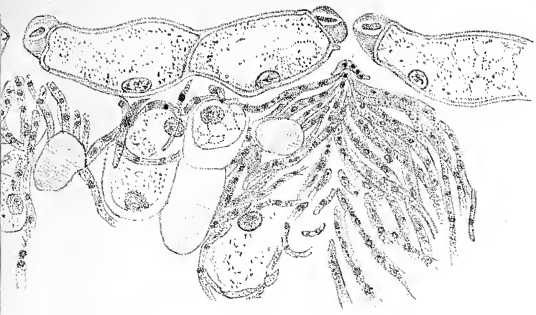
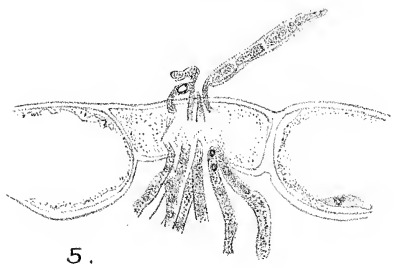
BLACKMAN & WELSFORD—POLYSTIGMA.





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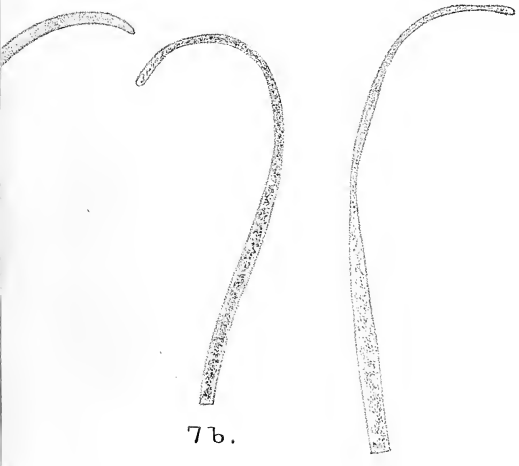

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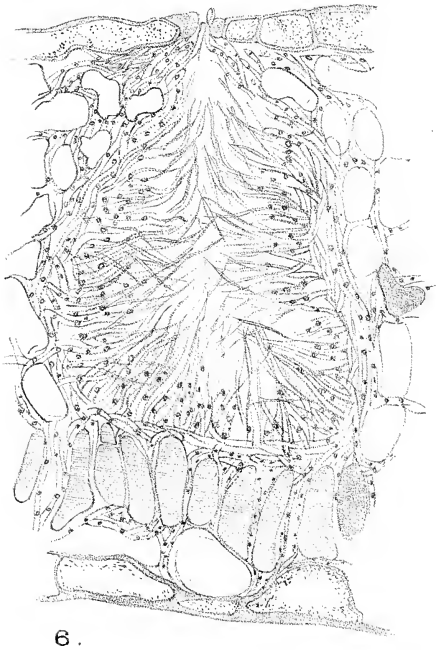

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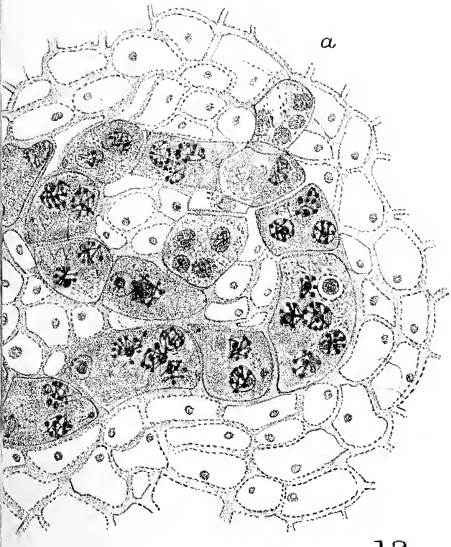

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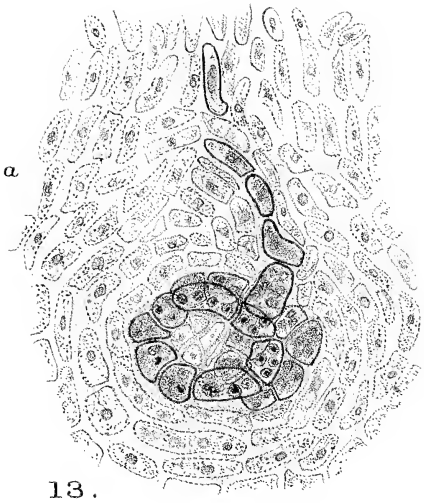

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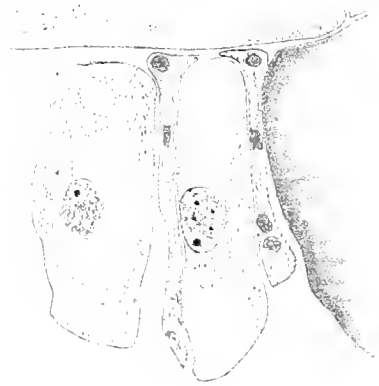

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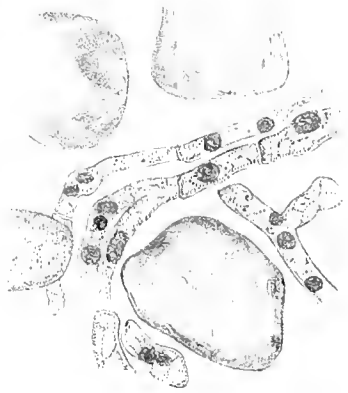

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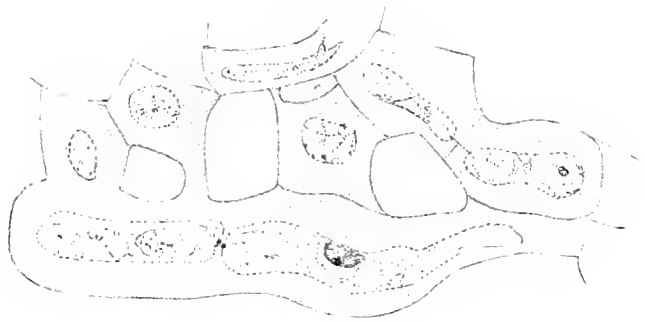

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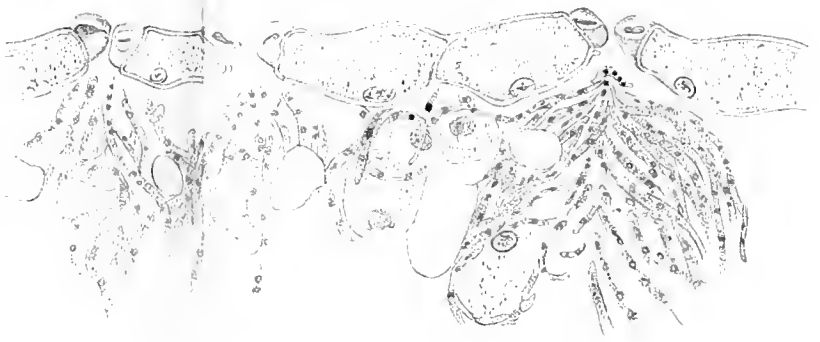

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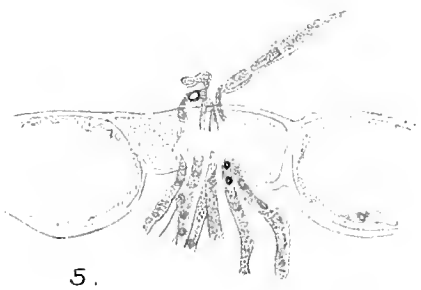

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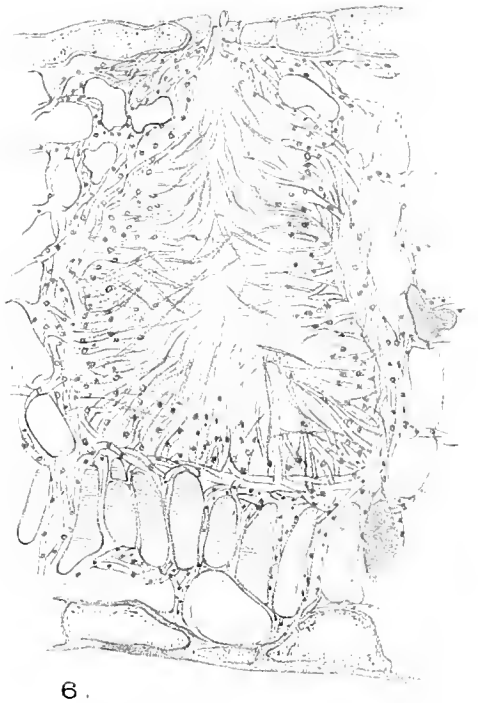

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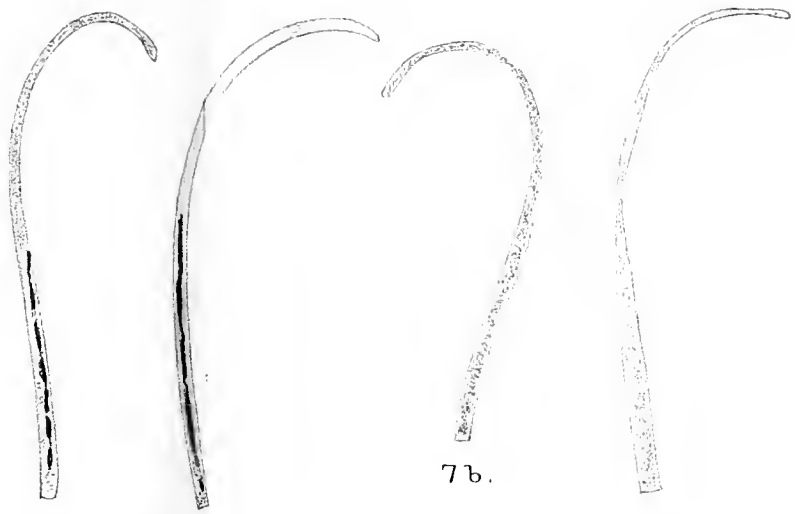

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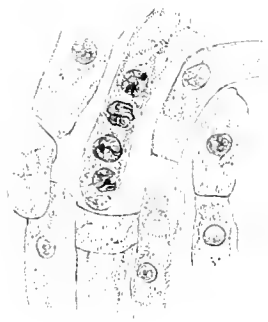

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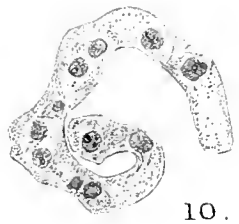

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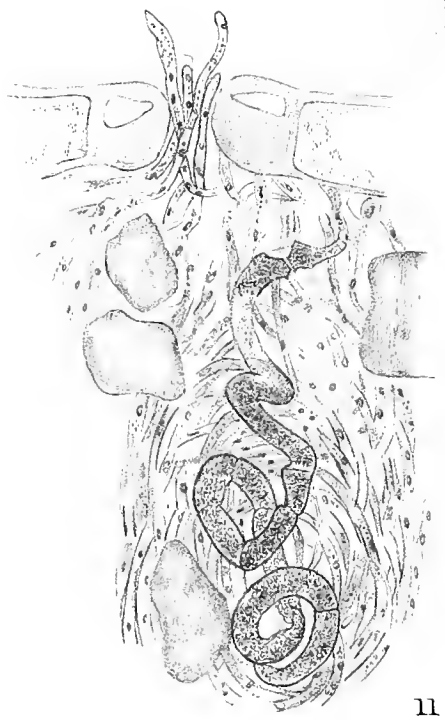

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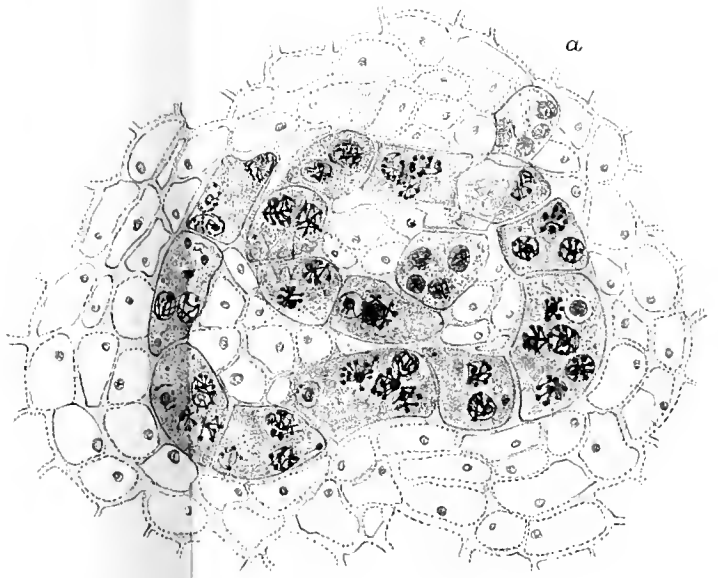

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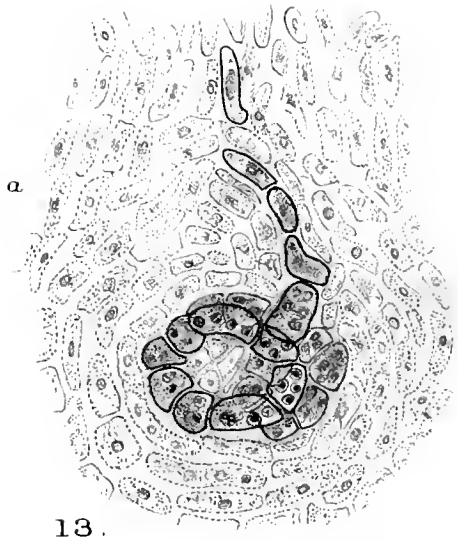

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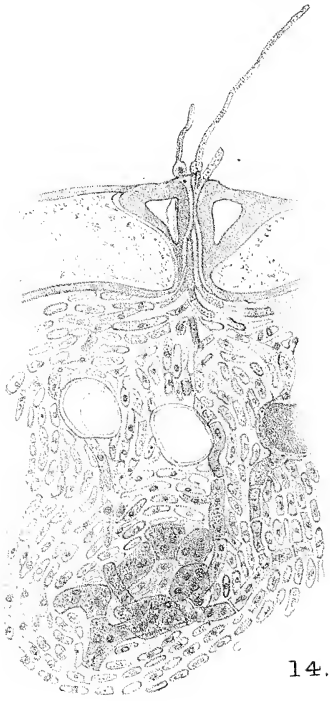
BLACKMAN & WELSFORD—POLYSTIGMA.



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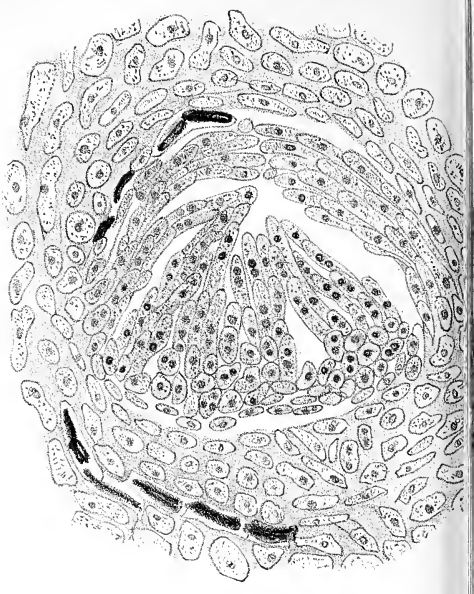

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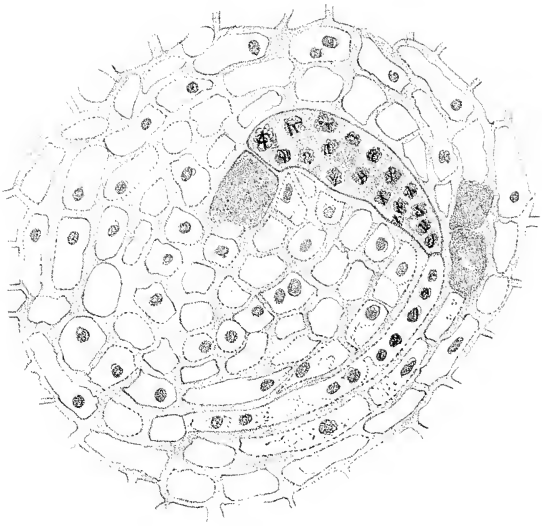

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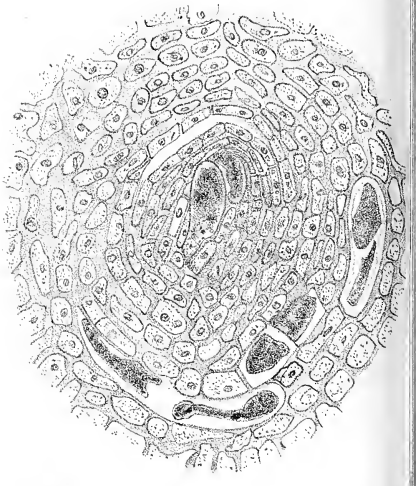

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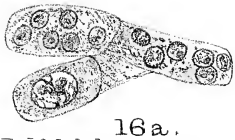

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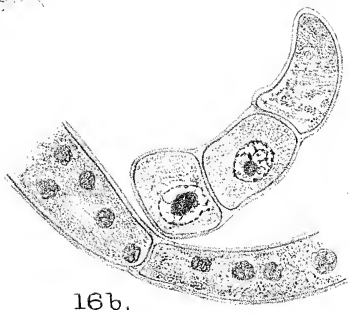

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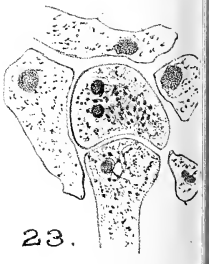

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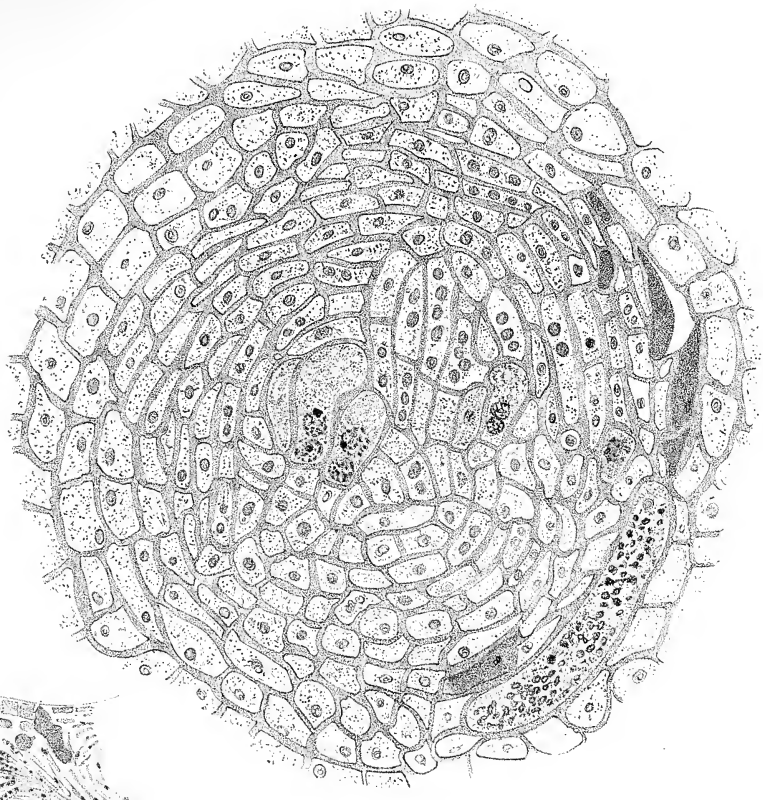
BLACKMAN & WELSFORD — POLYSTIGMA.



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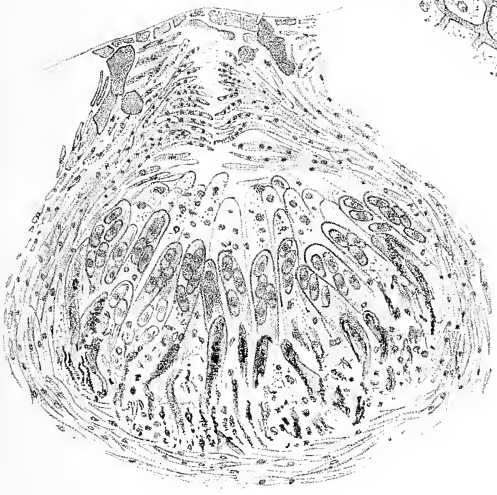

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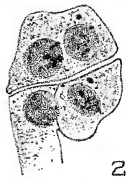

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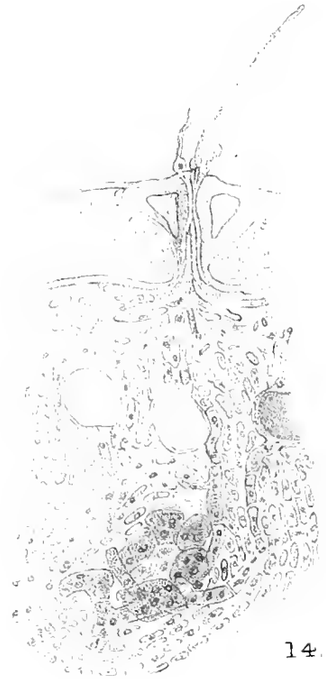

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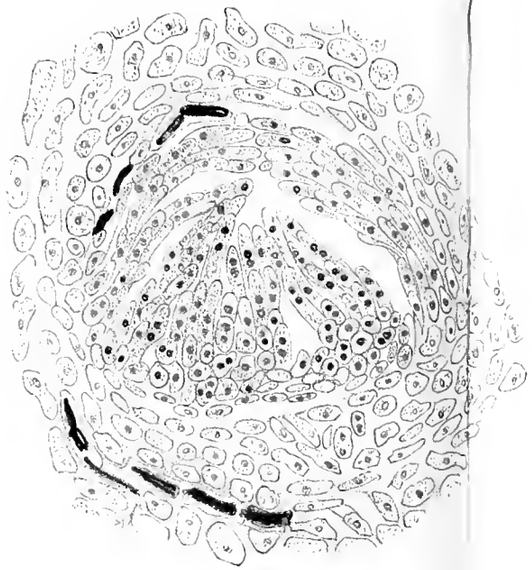

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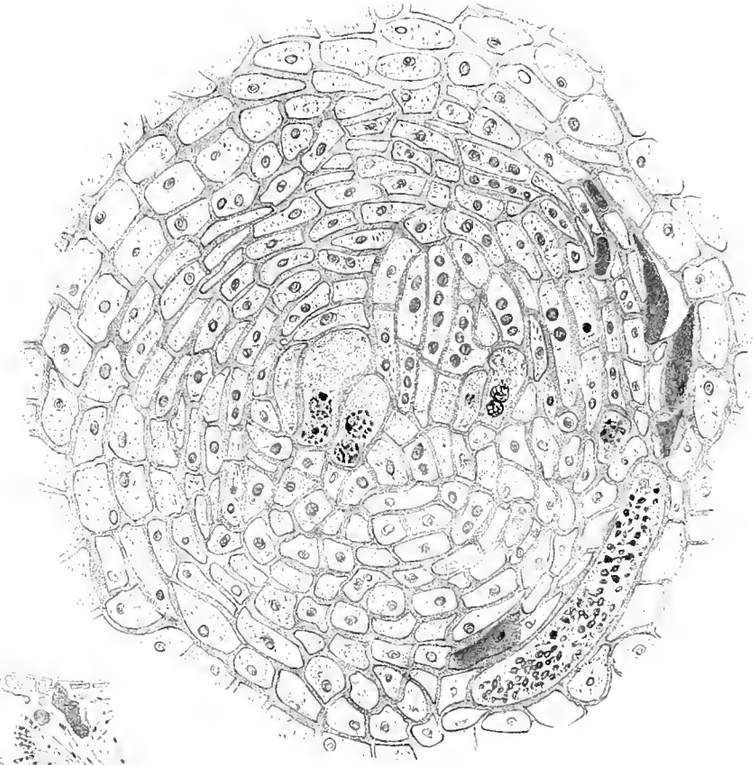

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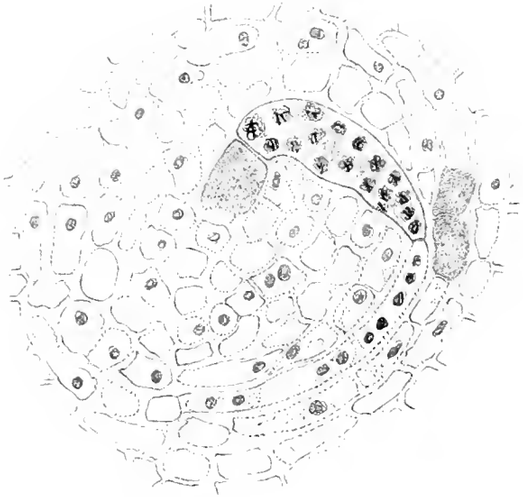

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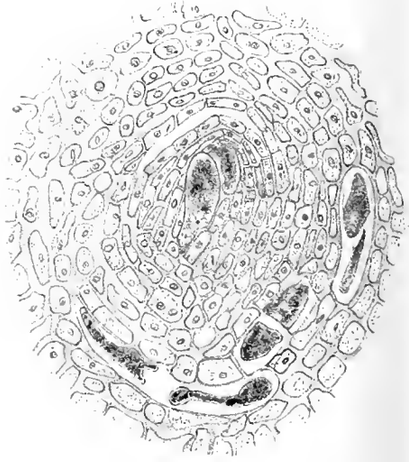

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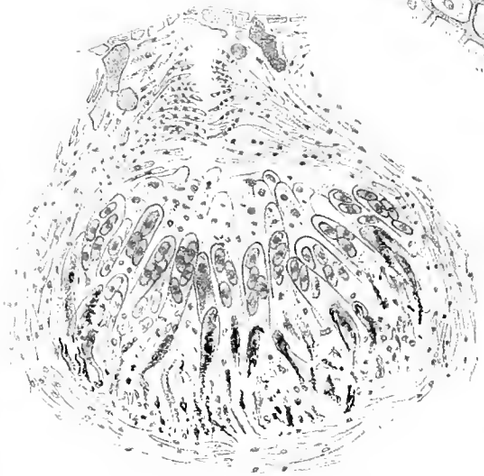

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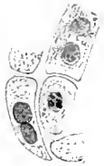

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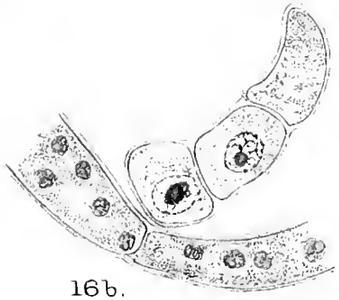

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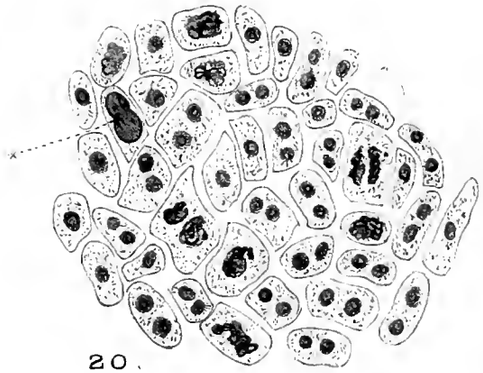

20.

E.J.W. del.

Hanh lith. et imp.

BLACKMAN & WELSFORD — POLYSTIGMA.

Observations on Asarum europaeum and its Mycorhiza.

BY

E. J. SCHWARTZ, M.A., B.Sc., F.L.S.

King's College, London.

With Plate LXXII.

ASARUM europaeum, the Asarabacca, is said to form our sole native representative of the Natural Order Aristolochiaceae; it is, however, questionable as to whether its claims to be indigenous are justifiable. The plant was formerly cultivated for medicinal purposes, a volatile oil, asarol, being extracted from it, and it is probable that the few plants found wild in this country are either 'escapes', or the descendants of 'escapes', from plants so cultivated. The plant, which in its wild state grows in woods and damp, shady places, consists of a branched rhizome which creeps along or near to the surface of the ground, each branch bearing annually two kidney-shaped green leaves with long petioles. These leaves are protected in the bud by a few scale leaves also borne by the rhizome. From the under surface of the rhizome spring a number of adventitious roots, which with their branch roots form the root-system of the plant. The roots penetrate the soil to a depth of from six to eight inches. The flowers resemble those of other members of the Aristolochiaceae in having a three-lobed calyx and twelve stamens, the latter being united to the style to form a gynostemium, thus showing some similarity to a monocotyledon, the similarity being heightened by the existence in the roots of an endotrophic mycorhiza, of a character somewhat resembling that described by Groom (2) as being found in the roots of *Thismia Aseroë*. The plant, however, is an undoubted dicotyledon, as is evident from the anatomy of its rhizome, and from the possession of two cotyledons by the seedling plant. The affinities of the order are, however, very obscure; it has been placed with the Santalaceae, Loranthaceae, and Rafflesiaceae to form the class Hysterophyta.

The material used in this investigation was obtained from a small group of plants growing in the garden of my home at Sevenoaks. These plants were uprooted in the early autumn of 1911, and portions of the roots

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and stems were fixed in Bouin's picro-formol solution. The plants were growing in dense shade, being overshadowed by taller herbaceous plants in the immediate vicinity. The soil was a loam in which clay predominated. In spite of the dry summer it had remained somewhat damp; it was not particularly rich in humus.

Microtome sections of the rhizome and root were cut, and were stained, some with Erlich's haematoxylin and orange G, others with Benda's iron-haematoxylin and orange G, and a few with anilin safranin.

Structure of the Rhizome. A transverse section of the rhizome showed the usual dicotyledonous structure. The ring of vascular bundles was of typical appearance, and both fascicular and interfascicular cambium were well differentiated; the hard bast was lacking. The cortical and pith cells were many of them filled with starch grains, although a few of the cells contained oil, presumably the 'asarol'.

No fungus was to be seen in any of the cells, nor were calcium oxalate crystals present either in the cells of the rhizome or of the leaf.

Structure of the Root and its Mycorhiza. In transverse section the root showed either a diarch or triarch stele; one such section with a diarch stele is shown in Pl. LXXII, Fig. 1. The endodermis had thickened walls, and showed the usual 'radial dot' on the side walls of its cells. The cortical cells of the root contained a lot of reserve starch, which, however, was lacking from the first two or three outer layers, and also from the innermost layers which formed the home of the 'mycorhizal' fungus. In roots free from the fungus, however, all the cells of the cortex contained starch grains except the few outer layers.

Although the cortex of roots containing the mycorhizal fungus may be said to consist of three differentiated portions outside the endodermis—viz. (1) the outer cell layers, two or three in number, free from starch; (2) the median three or four layers of starch-containing cells; and (3) the innermost two or three layers with the mycorhiza and containing but little starch—these portions are, however, not sufficiently well differentiated and separated to admit of division into exocortex, mediocortex, and endocortex.

In *Thismia Aseroë* these divisions are well differentiated, and Groom observed in the exocortex a number of sacs containing crystals of calcium oxalate in the form of raphides. Neither these nor any other form of calcium oxalate is to be seen in *Asarum*. Outside the endodermis the first two or three layers of cells are occupied by the mycorhizal fungus, the cells of the layer abutting on the endodermis containing irregular-shaped masses of fungoid matter as shown in Figs. 1, 2, and 3. These masses are highly refractive and are composed of dead portions of fungus, only slender portions of hyphae being as a rule distinguishable in them. The cells are mostly devoid of starch, and are of normal shape and appearance; their nuclei, when visible, show no signs either of hypertrophy or degeneration.

The appearance presented suggests that the fungus, on entering this layer of cells, has encountered some substance which has caused its death and disorganization, and probably partial digestion by the plant, the irregularly shaped mass left in the cells being composed of portions of fungus, which the plant is incapable of absorbing. Cells of this nature are to be seen in Figs. 2 and 3. Starch grains, when present in these or other cells of the plant, stain blue with iodine solution, and not red, as was the case in *Thismia*.

The cells of the two or three layers external to that described above are for the most part filled with the coiled hyphae of the fungus, as is shown in Figs. 2 and 3. The fungal hyphae mostly run in a longitudinal direction, as illustrated in Fig. 2, and coil round and round the cell, which finally becomes filled with fungus; the hyphae may infect the neighbouring cell by penetration of the cell-wall (see Fig. 6). Intercellular hyphae also occasionally occur; these run a straight course between the cells and send branches into them which give rise to the coils mentioned above.

The hyphae are very irregular in shape. Sometimes they are swollen out into bladder-like forms, as is shown in Fig. 4; at other times they resemble ordinary fungal hyphae. The bladder-like swellings occasionally found on them bear no relation to the cell nucleus, which when visible in mycorhizal cells is of normal size and appearance; on the other hand, the nuclei of cells in portions of roots which are free from the mycorhizal fungus have sometimes been observed to be considerably enlarged and of abnormal shape.

The fungal hyphae, which are densely filled with granular protoplasm, are non-septate and contain numerous small nuclei, which stain well with Benda's iron-haematoxylin.

The hyphae, unlike those of the fungus in *Thismia*, do not make straight for the cell nucleus, but, as stated above, form coils in the cells. The fungus is confined entirely to these few cortical layers in the vicinity of the stele, and shows no inclination to spread into the outer region of the cortex with its cells rich in reserve starch. Portions of fungal hyphae are shown in Figs. 4, 5, and 6.

On some hyphae may be seen intercalary or terminal swellings of a spherical or a pear shape. These swellings, which are thick-walled, contain dense granular protoplasm and are multinucleate. They doubtless, if belonging, as I believe they do, to the mycorhizal fungus, represent its resting or reproductive stage. They are not very commonly met with, and it is just possible that they belong to a parasitical fungus which has also obtained an entrance into the root. When met with, however, they are invariably found in the inner cortical layers with the mycorhiza, although the hyphae bearing them are somewhat different in appearance, owing to their staining at times rather more deeply than those of the undoubted

mycorhiza. These swellings are illustrated in Figs. 7 and 8. In the former figure the swelling is giving rise to a tube which is penetrating a neighbouring mycorhizal cell containing undigested fungal matter.

For the purpose of comparison, the roots of plants of *Asarum europæum* obtained from the Chelsea Physic Gardens and from the garden of Mr. E. M. Holmes, at Sevenoaks, were examined. But in both cases these were free from mycorhiza, as were also the roots of *Aristolochia Sipho* obtained from the same sources. It is, however, by no means unusual for a mycorhizal fungus to be absent at times from a root, the general rule being that when the plant is growing in a soil rich in humus the fungus will be present, and that when the soil is poor in humus it will be absent. It may be added that the roots of plants possessing the mycorhiza were found to possess comparatively few root-hairs, whereas in those which lacked the mycorhiza the root-hairs were more abundant.

Endotrophic and ectotrophic mycorhizas have long been known; the former are commonly to be found in the roots of the Orchidaceae, Ericaceae, and Epacridaceae, although the hyphae are never so deeply seated as in *Asarum europæum*. The ectotrophic forms are to be found in the Cupuliferae and many of the Gymnosperms, not to mention numerous other orders in plants of which they have been observed.

The mycorhiza of *Neottia Nidus-avis*, which bears points of similarity to that of *Asarum*, has been investigated by Magnus, in 1900 (3), who states that the fungus enters the root or rhizome, where it branches and fills up a series of concentric layers of cells. In some cells it kills the host protoplasm, while in others it is itself killed and partly digested by the plant, the indigestible residue aggregating into a ball in the centre of the cell and being covered by a membrane.

In 1904 Ternetz (6) isolated a fungus, which was presumably its mycorhiza, from the roots of some of the Ericaceae and stated that it was an active agent of nitrogen-fixation from the atmosphere.

In 1909 Osborn (8) described a fossil mycorhiza found in the lateral roots of *Amyelon radicans*. According to this authority, the hyphae are non-septate, and they are found in the inner cortical layers of the root, and are apparently very similar to those found in *Asarum*. He states that some of the cortical cells are filled with coils of fungal hyphae, whereas others, which he designates 'digestive cells', contain irregular masses of fungoid matter. The fungus, like that of *Asarum*, is limited to the inner cortical layers of branch roots, and is absent from the tissue of the main roots. On some of the hyphae thickened terminal dilatations were found, evidently representing a resting stage of the fungus. These dilatations correspond with the thick-walled swellings observed by me on some of the hyphae in *Asarum*, and lend support to the view that these swellings belong to the mycorhizal and not to some other parasitic fungus.

Weiss (5) has also described a mycorhiza from the Lower Coal Measures; it, however, does not bear so many points of resemblance to that of *Asarum* as does the one on *Amyelon radicans*.

SIGNIFICANCE OF THE MYCORHIZA. It is difficult, if not impossible, to assign the exact rôle played by a mycorhiza in the nutrition of the plant. It may be, as stated by Ternetz (6), that some mycorhizas have the power of fixing atmospheric nitrogen. Starting from the fact that plants possessing mycorhiza are to be commonly met with in soils rich in humus, Stahl (4) suggests that the fungus aids the plant in the absorption of the ash constituents from the soil. He thinks that the numerous Fungi to be found in a soil rich in humus are severe competitors with any Phanerogams for the available plant food in the soil, and is of opinion that the Phanerogam probably benefits in some way by association with a fungus which might yield up the ash constituents to it. This might conceivably apply to the case of plants possessing an ectotrophic mycorhiza, but certainly is no explanation of such a deeply seated mycorhiza as is found in the roots of *Asarum*. Moreover, the soil in which the *Asarum europaeum* was growing was not very rich in humus, nor was it crowded with Fungi.

Stahl also suggests that the fungus may prepare and transform the raw nutritive salts (as absorbed from the soil) into products of assimilation ready for the use of the plant, as for example asparagin. He notes the absence of any form of calcium oxalate, a substance which is usually associated with the assimilation of nutritive salts. As I have stated, calcium oxalate crystals are not found in *Asarum*. This hypothesis seems to fit in with the facts so far as they are known.

According to Gallaud (7) the Fungi of the endotrophic mycorhiza are 'saprophytes internes', which by means of highly developed haustoria borrow some non-living nutritive material from the cells in which they live. The plant cells finally kill the haustoria, digest, and partially absorb them. The cells, he thinks, defend themselves from the attack of the fungus by their digestive power, and consequently the fungus is but little harmful. Against this view, in the case of *Asarum*, is the fact of the fungus being confined to the innermost cortical layers and not straying from them. One would expect a parasitic fungus to attack the whole of the cortex and the soft bast of the stele. Gallaud doubts whether any true mycorhizal fungus has been grown isolated from the root of its host plant.

RELATIONSHIP BETWEEN THE PLANT AND THE FUNGUS. The question whether the fungus found in the roots of *Asarum* be a parasite, or whether the relationship existing between the plant and the fungus be a symbiotic one, is of considerable interest.

From a purely morphological point of view, the signs of parasitism may include destruction or hypertrophy of tissue, hypertrophy of cells or nuclei, degeneration of nuclei, unhealthy appearance and poor growth of

the plant, at times giving rise to its death, and a poor store of reserve food such as starch.

On the other hand, in the case of symbiosis, plants with a symbiotic partner ought to, and do, flourish better than those in similar situations without a symbiont. This is the case in the Leguminosae, in which plants bearing plenty of root nodules, containing the nitrogen-fixing Bacteria, thrive better than those with fewer or no nodules on their roots. Again, the host plant may provide a special home or region for the use of its symbiont; this, however, may likewise be the case in parasitism, as for example oak-galls. We may compare also the root nodules of the Leguminosae with those of the Juncaceae, the former being a case of symbiosis and the latter one of parasitism. With regard to *Asarum*, there is neither hypertrophy nor destruction of tissue, and the cell nuclei when visible in mycorhizal cells are in most cases of normal size and appearance. On the other hand, a special region of the root, viz. the innermost cortical layers abutting on to the stele, is set apart by the plant for the habitation of the fungus, which for its part makes no attempt to stray from this region. In the cell layer outside the endodermis, the cells were seen to be filled with dead fungoid matter, suggesting that either the fungus was providing food for the plant, and the cells contained the undigested residue left after the absorption of the digested portion into the stele, or that the fungus was a parasitic one which had been killed by this innermost cell layer to prevent its entrance into the stele. The position of the fungoid layers close to the conducting stele, the similarity of the fungus with its coiled hyphae in some cells and irregular dead masses in others to the mycorhizal Fungi of *Thismia Aseroë*, *Neottia Nidus-avis*, and other roots, are all facts which favour the former interpretation. The shade-loving nature of the plant, and the presence of humus in the soil, also point to the same conclusion.

It is evident from the deeply seated situation of the fungal home that the fungus obtains all its nutriment from the plant, although some of it may be in a raw state, unaltered by the plant since its entrance into the root from the soil. The carbohydrate is apparently supplied by the reserve starch of the root, which is mostly absent from the mycorhizal cell layers, or the fungus may feed on the sugars supplied by the green leaves to the roots, these sugars being converted into starch in those cells of the root which are free from fungus. As far as the facts observed warrant the expression of an opinion as to the function of deeply seated endotrophic mycorhiza, one is inclined to accept Stahl's hypothesis that the rôle of the fungus is to elaborate the raw salts absorbed from the soil into organic compounds such as asparagin, and in this connexion the lack of calcium oxalate crystals from *Asarum* roots is suggestive. The diminished numbers of root-hairs on mycorhizal roots is difficult to explain when we have such

a deeply seated mycorhiza as in *Asarum*. In cases of ectotrophic mycorhiza root-hairs may be absent altogether.

In conclusion, my thanks are due to my sister, Miss Alice M. Schwartz, for her kind assistance with the drawings.

Summary and Conclusions.

1. The roots of *Asarum europaeum* are inhabited by a fungus which is limited to the cortical region abutting on to the steles of young roots.

2. This fungus is very similar to those found in *Thismia Aseroë* and *Neottia Nidus-avis*.

3. Thick-walled swellings are to be found on some of the hyphae, representing a resting stage of the fungus. These correspond with those on the fossil fungus on roots of *Amyelon radicans*.

4. The fungus forms an endotrophic mycorhiza in most respects similar to those found in other roots.

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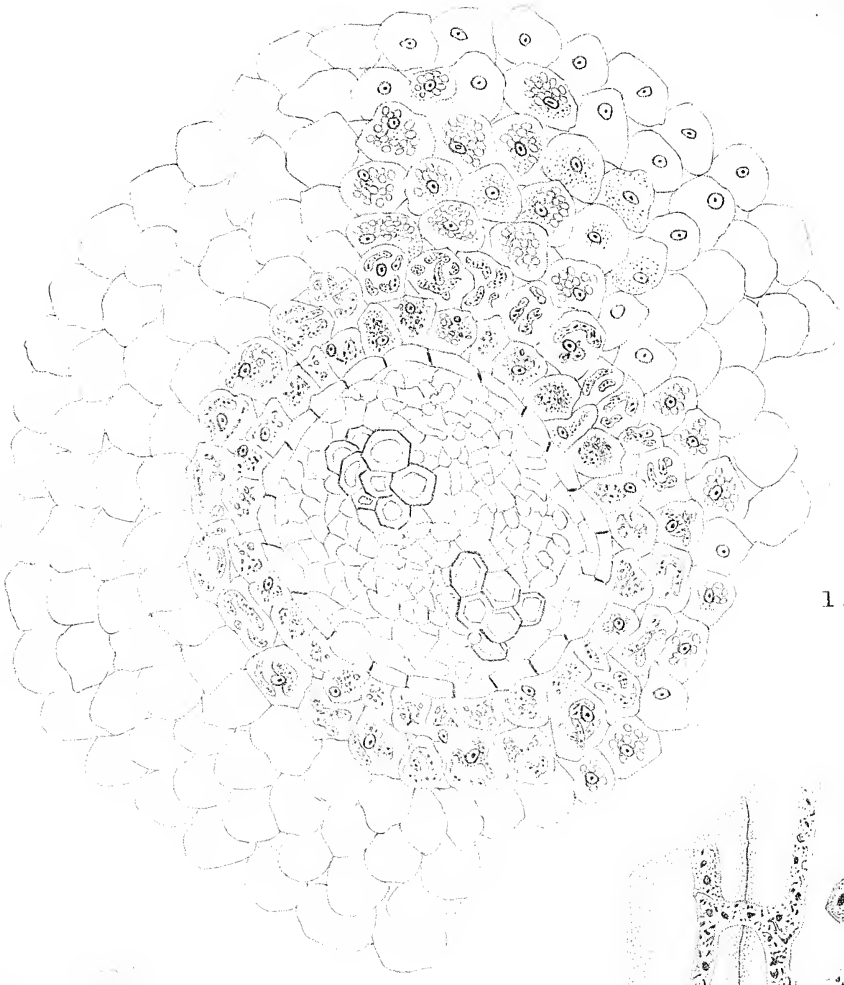
EXPLANATION OF PLATE LXXII.

Illustrating Mr. Schwartz's paper on *Asarum europaeum* and its Mycorhiza.

Fig. 1. Transverse section of root, showing position of fungal layers. × 325.
Fig. 2. Longitudinal section of root. × 325.
Fig. 3. Longitudinal section through fungoid layers. × 730.
Fig. 4. Bladder-like swelling on hypha. × 1,180.
Fig. 5. Hypha with nuclei. × 1,180.
Fig. 6. Hypha piercing cell-wall. × 730.
Fig. 7. Resting body of fungus, germinating. × 730.
Fig. 8. Terminal hyphal swelling forming resting body. × 730.



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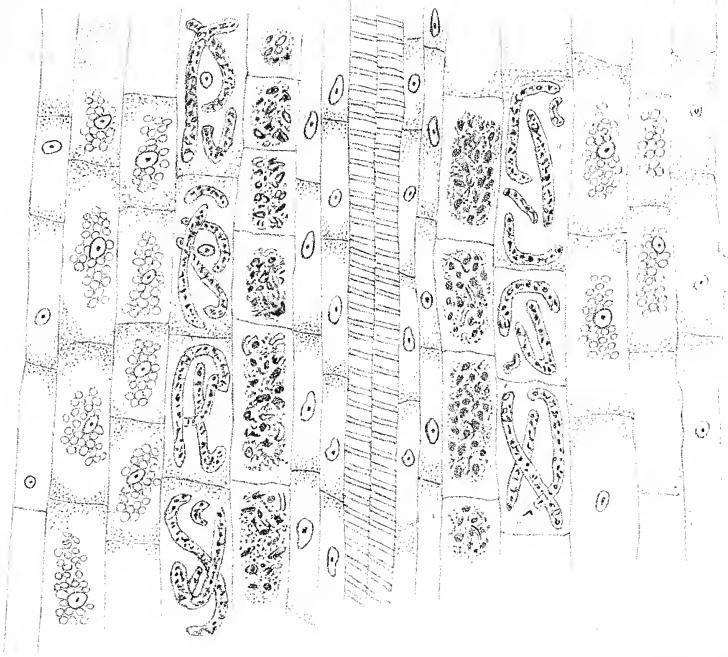
SCHWARTZ — ASARUM, MYCORHIZA.



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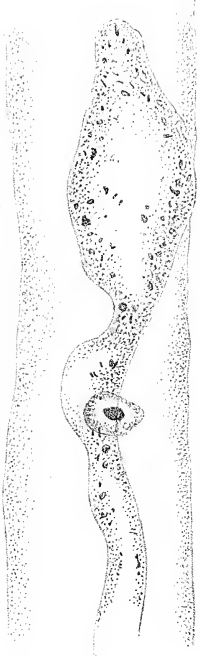

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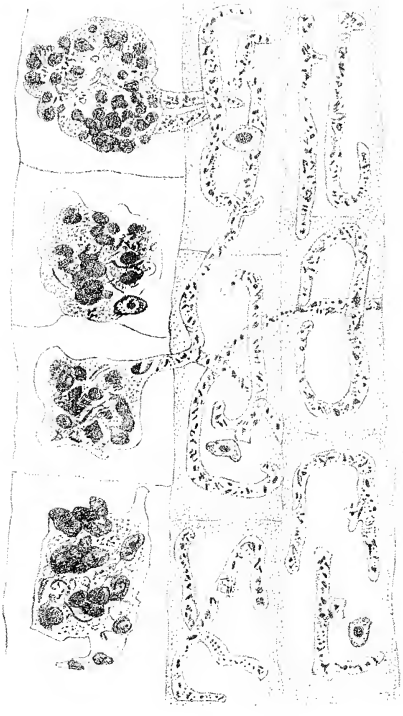

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Huth, lith et imp.

Some Wound Reactions in Filicinean Petioles.

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With Plates LXXIII and LXXIV and one Figure in the Text.

Introduction.

THE healing of wounds in phanerogamic tissues is normally accomplished by the production of either a callus or of a wound periderm. This subject has, on account of its economic value, long occupied a prominent place in botanical research, especially among experts in forestry and arboriculture, in whose province the question is naturally an all-important one. For similar reasons one finds that the occurrence of disease due to fungal agency has also received close attention, with the result that a considerable amount of literature has been produced bearing on these problems and their treatment, of which the works of Prillieux (23), Sorauer (28), Hartig (13), and Tubeuf (35), and more recently those of Duggar (9), Massee (17), and Smith (26), may be regarded as typical. In the case of the vascular cryptogams the investigation of wound response seems to have excited little interest, since any economic value is, in their case, absent.

The Bonn text-book (33) says of wound response in these forms, 'The wounded cells die, and become brown and dry, whilst the walls of the underlying, uninjured cells become lignified' (pp. 150–1); it also briefly touches upon the formation of periderm in *Ophioglossum* (p. 148), a feature first noted by Holle (16), whose observation is also quoted by de Bary (8), and upon the formation of a pseudoperiderm in the Marattiaceae.

Incidental reference to a case of periderm formation is made by Chandler (4) for *Polypodium aureum*, while Stopes (30) and Seward (24) also refer to the healing of deep wounds in Calamitean axes.

Wound reactions of a somewhat different character are recorded by Strasburger (32), who describes the formation of tyloses in the carinal canals of Equiseta when the upper portions have been cut away, and by

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Weiss (36), who regards the abnormal proliferation in a Stigmarian rootlet, described by him, as due to a fungal attack.

The writer was fortunate enough to discover a case of superficial wounding in a Medullosean petiole, which had been repaired by the production of a typical wound periderm (14), and this led to an investigation of the reactions of Filicinean petioles to wounding, with a view to ascertaining whether similar phenomena were exhibited by these.

An incidental study of two cases of surface wounds in Cycadean petioles, those of *Cycas circinalis* and *Bowenia serratula*, was also made in connexion with the present paper. These both showed the same type of response as the 'Myeloxylon' referred to, having to the outside a layer of dead, excised cortical tissue, immediately below which was a band of suberized cells, devoid of contents, and finally a mass of cambiform cells which merged into the typical cortical parenchyma (Pl. LXXIV, Fig. 21).

Methods.

The investigations were commenced in the summer of 1909 at Manchester, where, owing to the kindness of Mr. Garnett, of Whalley Range, I was enabled to perform a preliminary series of experiments in his fern-house. I should like to express here my appreciation of his unfailing courtesy, and my thanks for the facilities he granted me during the earlier portion of the work. Further series of experiments were performed during the spring and summer of 1910 and 1911, and a considerably increased number of forms was examined.

For purposes of investigation the petioles, where possible, were divided into three regions, as follows: (i) the curled apical portion, (ii) the region of pinna insertion, (iii) the region below pinna insertion; where special sterile and fertile segments occurred, as in *Osmunda regalis* and *Allosorus crispus*, both segments were examined.

It was thought that the nature of response would in all likelihood differ in the still actively growing apices and the maturer parts below, and, as the former did not appear to have been investigated in this connexion, would possibly reveal some points of interest.

The wounds were made with a sharp scalpel in each of the regions indicated, and were of a purely superficial nature, a thin shaving being removed which penetrated below the sub-epidermal layer of sclerenchyma characterizing most fern petioles, but did not seriously disturb the vascular supply. In those cases where the vascular supply was, accidentally, interfered with to any appreciable extent, it was found that various parasitic Fungi and Bacteria caused the complete rotting of the injured member, thus rendering it of little use for further study for this purpose.

The petioles thus wounded were allowed to continue growth for periods varying between a fortnight and fifteen weeks, and were then cut and at

once preserved in chromo-acetic fixative, Perenyi, or Farmer's fluid. The number of forms examined in this way included the following species: *Adiantum Capillus-veneris*, *A. Edgworthii*, *A. fulvum*, *Allosorus crispus*, *Asplenium Belangeri*, *A. bulbiferum*, *A. fontanum*, *A. viride*, *A. viviparum*, *Aspidium thelypteroides*, *Athyrium Filix-foemina*, *Blechnum brasiliense*, *Cystopteris bulbifera*, *C. fragilis*, *Dennstaedtia punctilobula*, *Davallia polyantha*, *Faydenia prolifera*, *Lastraea dilatata*, *L. Filix-mas*, *L. reflexa*, *L. thelypteris*, *Lomaria chilensis*, *Microlepia anthurifolia*, *Osmunda regalis*, *Phegopteris hexonaptera*, *Phlebodium* (*Polypodium*) *glaucum*, *Polystichum angulare*, *P. proliferum*, *P. falcatum*, *Pteris aquilina*, *P. cretica*, *Scolopendrium vulgare*, *Struthiopteris germanica*, *Woodwardia orientalis*, *W. radicans*, *W. virginica*.

A large proportion of these were found to be much too hard in texture to microtome successfully, and consequently only sections of the apices of such were so prepared ; some of the less resistant forms, however, offered little difficulty. The material was transferred from absolute alcohol, through pure cedarwood oil, to paraffin, as this method caused less shattering than when xylol was used. The harder forms were sectioned by hand. The best general results were obtained by double staining with carthamin and picric anilin blue, or safranin and Lichtgrün ; for nuclear studies Heidenhain's iron-haematoxylin with a light erythrosin counterstain was satisfactory.

The microchemical investigation of the cell-walls and contents involved the use of special reagents, which will be dealt with in detail later.

Results and Discussion.

The change in the external appearance of the affected surfaces, apart from a browning of the dead cells, was, as a rule, inconsiderable. In one or two cases, however, notably in *Pteris cretica*, the petiole became distorted and bent at the seat of injury (Pl. LXXIV, Fig. 23), and the explanation of this seems to be that the removal of tissue had caused a violent disturbance of the osmotic balance. This had resulted in the flexure of the petiolar strand and the consequent crushing of the cortical parenchyma on that side of the strand remote from the wound.

With regard to the details of internal modification a systematic survey of the results obtained serves to demonstrate that, although no rigid scheme of classification of the various types of wound response is possible, there are yet certain underlying broad similarities and differences, correlated with the region of the wound and the texture of the petiole. Thus an almost solid deposit of gummy matter in the cells at the seat of injury is of practically universal occurrence, whilst the more or less widespread thickening of the cortical tissue is also a very frequent feature.

It is a well-known fact that the tissues of many fern petioles are, even at maturity, of a comparatively delicate and parenchymatous character, a feature which is shared by all forms in their extreme apical portions, whilst others, such as *Osmunda regalis* and *Davallia polyantha*, are characterized, when mature, by an extremely tough, almost horny exterior, together with a highly developed sub-epidermal band of sclerized tissue, this rendering them very resistant. This tough character is doubtless a necessity in the large, upstanding forms, but is by no means confined to these, several of the small forms (e.g. *Adiantum* spp.) showing similar characters.

It appeared to the writer that, in this difference in the character of the more mature parts of the petiole, there occurred one of the features which lent itself to the grouping of the forms worked upon, and this proved to be the case, for it was only in what might be termed the softer bodied forms that any appreciable cell-growth, in response to injury of the maturer parts, occurred.

(i) Taking first the curled, apical region, it was found that practically the whole of the forms experimented upon showed attempts at cambium formation. This, in the most successful cases, resulted in the production of a complete wall of meristem in the injured area, and was found to occur in only a small number of the species examined, namely, *Asplenium Belangeri*, *A. bulbiferum*, *A. viviparum*, *Polystichum proliferum*, *Woodwardia orientalis*, and *W. radicans*. A reference to the figure (Pl. LXXIII, Fig. 1) illustrating the wound meristem produced in the case of *Polystichum proliferum* will serve to demonstrate the complete nature of the healing. Here the wounded area has been completely traversed by an entirely new growth, several cells in depth, of typically cambiform cells with delicately granular contents. This growth is due to the elongation and division of the layers of cortical parenchyma abutting upon the wound, and has, in a few cases, rendered the identification of these primary units difficult, though in most parts the outlines of the original parent cells can still be determined.

The result of this continued elongation and division has been, in favourable cases, to produce at the originally flat, or slightly concave wound surface, a distinct convexity or intumescence (Pl. LXXIV, Fig. 20). The mode of growth of the affected cortical cells is well shown in specimens which have been allowed to grow for some time (nine or ten weeks). In these it is clear that the elongation is accomplished by a process of sliding growth somewhat analogous to that obtaining in the development of prosenchymatous elements, and often results in the production of a row of cambiform cells, of which the terminal members are somewhat longer than their fellows, and are bluntly conical in shape, thus enabling them to dovetail into the spaces between the divergent ends of their neighbours (Pl. LXXIV, Fig. 19).

A peculiar feature with regard to the cambial cells which lie at the

actual surface of the injured area is that they show no traces of suberization upon their outer walls. They are, however, usually protected to a large extent by the scab-like remains of the cells originally injured when the wound was made, and it would appear that this has proved adequate. The brown appearance of the wound scar in most cases is due very largely to the remains of these tissues, though in the few petioles where suberization occurs the same effect is produced.

Immediately below the meristem proper there is a marked change in the character of the cells, which are much larger and possess abundant, coarsely granular contents. It will be seen that the most peripheral of these, that is, those which lie next to the cambial tissue, have also, in many cases, elongated somewhat, and have divided by a transverse wall suggesting an incipient attempt at cambium formation.

The features upon which I should like to lay emphasis in these specially active forms is, first, that there is an actual production of a complete pad of healing tissue, formed by the elongation and division of the cortical parenchyma, and second, that there is no evidence of the secretion of gummy matter in any of these species during the earlier stages of reaction.

It is a singular fact that the forms which exhibit the most successful attempts at the production of a healing tissue should be those in which the formation of bulbils for vegetative propagation is a characteristic feature.

It would seem that the production of these structures must necessitate the retention of a particularly mobile and responsive type of tissue, and this view receives strong support from the evidence afforded by the results obtained in other, non-bulbiferous, species of the same genera, in other soft-bodied forms, and by the nature of the response to injury in the maturer parts of the petioles of the same forms.

The young plantlets raised from the bulbils do not possess this power of regeneration to anything like so marked an extent as do the mature forms. In their case, in fact, an elongation of the cortical cells in the region affected, and the occasional division of these by a single transverse wall, suggesting cambial possibilities, seems to be the extent of their powers (Pl. LXXIII, Fig. 2).

This evident contrast with the bulbil-producing petioles also lends strong support to the view that the cambial activity and the formation of reproductive buds are correlated, since the immature forms lack this power.

The elongation of the cortical cells, and their subsequent division to produce two or three daughter cells, would seem to be the most general type of response, and is a feature of the majority of the remaining forms examined. Thus in *Lastraea Filix-mas*, in *Polypodium glaucum*, and in

Athyrium Filix-foemina there is a remarkable increase in the length and bulk of the cortical cells for a depth of from four to six cells, and these divide by one or more transverse walls, giving a distinctly meristematic character to the region, and affording a marked contrast to the cells which have not become secondarily active. Here, too, the suberization of the outer walls of the most peripheral cells has also taken place, thus affording adequate protection without interfering with their vitality (Pl. LXXIII, Fig. 3).

This stage of wound repair seems to be generally attained within three weeks of the infliction of the injury, but in the case of *Asplenium Belangeri* there was no trace of secondary activity in that period. Petioles of this species examined after three weeks showed a dry but in no way discoloured wound, and sections demonstrated that the collapse of the cells at the surface was the only response. In five weeks, however, the characteristic cambial development was perfectly obvious, and, apart from the slower rate of the early development, this species showed no striking points of difference from the remaining bulbiferous forms.

One form, *Cystopteris bulbifera*, however, appears to stand somewhat apart from the rest. The cortical cells of the petiole showed very little secondary activity either at the apex or below, but if a young, barely visible bulbil were cut off there was an appreciable cambial reaction. This is perhaps partially explicable in the comparative slenderness of the petiole, since this would be obviously more weakened by wounding than one of a more robust type, such as *Asplenium viviparum*.

The behaviour of *Scolopendrium vulgare* was also of some interest, as the cortical cells, for a much greater depth than the average, elongated, but on the other hand this was accompanied by a relative paucity of transverse divisions.

As the plant develops, and the originally curled portion becomes straight, secondary changes are involved. In one or two cases, however, plants of *Lastraea Filix-mas* which were wounded early, whilst they were still completely enveloped in ramenta, and collected eight weeks afterwards, before the spring period of rapid uncurling and growth had supervened, were found to show very little further modification. It is a noteworthy fact that all the hardy indigenous forms appear to show a more pronounced activity if wounded before the first great elongation of the petiole has taken place, the results with *Lastraea*, referred to above, with *Athyrium Filix-foemina*, and with *Pteris aquilina*, being specially marked (Pl. LXXIII, Fig. 3).

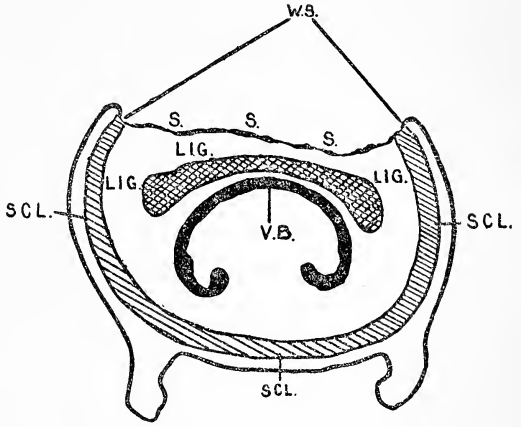
This second type of apical wound response, with its inadequate attempts at cambium formation and its inability to produce a really efficient cell-barrier, affords a striking contrast to the condition characterizing *Woodwardia radicans* and its allies.

A still further reduction is shown in the attempt at healing made by *Polystichum angulare*, *Pteris cretica*, *Lastraea reflexa*, and *Woodwardia*

virginica, in which only a single layer of cortical cells, namely, that consisting of the uninjured units immediately below the wound, exhibits any activity. This layer elongates quite appreciably, but generally shows no trace of cell-division (Pl. LXXIII, Fig. 4).

The superficial resemblance of these cells, both in *Polystichum angulare* and in the species which have previously been described, to the intumescences produced on the leaves of *Hibiscus* and other forms (Dale, 5, 6), led to a careful investigation of their cytology with a view to ascertaining whether there were any induced pathological nuclear phenomena. In a few cases cells were found to contain two nuclei, almost invariably in close proximity to one another, and in a considerable number of cells nuclei of abnormal shape, often showing a median constriction, were noticed (Pl. LXXIII, Fig. 10).

In *Osmunda regalis*, *Blechnum brasiliense*, and *Struthiopteris germanica*, which serve to illustrate the last gradation of wound response, there is very little or no elongation of the individual cells, and only very rarely a transverse division, but the outermost cells develop very early a strong suberization of their walls. These, and others of the hardest-bodied forms, also show a band of lignified tissue immediately above the vascular structures, which, though differing in structural detail from the sub-epidermal sclerenchyma, serves largely to replace this tissue as a protective sheath on the injured side (Text-figure).



TEXT-FIGURE. Transverse section, young petiole of *Osmunda regalis*. SCL., sub-peripheral sclerenchyma; V.B., vascular bundle; W. S., wound-surface; S. S., suberized cells; LIG., cortical cells, secondarily lignified.

From the foregoing examples it will be seen that a distinct and gradual series of forms is traceable, commencing with those producing a perfect pad of healing tissue such as *Asplenium Belangeri* and *Polystichum proliferum*, and passing through forms like *Lastraea Filix-mas*, with cell-elongation and the production of an incipient cambium, to forms like *Polystichum angulare*, with cell-elongation alone, and finally to types like *Osmunda regalis*, in which cell-elongation and division are very infrequent, and where these are replaced by an efficient suberization and lignification.

The secondary changes which occur in the wounded apical areas as these unfold and become mature were found to be extremely variable,

and to tend, in ultimate effect, towards the condition resulting from wounds of the second order, that is, those within the region of pinna-insertion.

In the bulbil-producing forms there were very marked changes: the cambiform cells had evidently ceased to divide and had become decidedly more thick-walled (Pl. LXXIV, Fig. 19), this thickening proving to be of a purely cellulose nature, whilst, in addition, the whole of the cortical tissues on that side of the stele adjacent to the affected surface had become filled with a gummy deposit (Pl. LXXIV, Fig. 20 *b*). This intracellular gum deposition was also a characteristic feature of all the remaining species, whether the cambial response had been still fairly strongly in evidence or not.

The gum, even in the fresh material before preservation, was extremely thick and viscous, especially in the outermost cells of the affected region, and on fixation and hardening in alcohol was found to have become quite solid. The slight shrinkage of the cell-contents had, in many cases, caused the solid gum to crack and furrow, so providing a ready means of recognition.

The process of secretion of this substance in the cells could be followed with ease, since the earlier stages were shown in the specimens which had only been allowed to grow for a comparatively brief period after wounding, and also in the deeper seated cells of the maturer specimens. From these it could be seen that the secretion was first produced as minute granules or globules in the cytoplasm, these gradually becoming larger and ultimately coalescing to produce an almost solid deposit.

This substance, which is presumably of a protective nature, gives the following reactions characteristic of wound gum:

1. It is insoluble in alcohol, chloroform, or benzol.
2. It is insoluble in hot or cold concentrated H_2SO_4.
3. It is insoluble in hot or cold concentrated solution of KOH.
4. It stains deeply with alkannin, and with ruthenium red.

Treatment with thymol, orcinol, and phloroglucin gave, however, entirely negative results. It differs from wound gum also in the fact that it is intracellular, and does not appear to be due to any tissue degeneration, since even the cells in which the deposition is most complete show no traces of disintegration, and the nuclei do not appear to be adversely affected.

The cell-walls in the gum-secreting zone invariably increase in thickness, but this, in different forms, and even different specimens of the same form, varies considerably in its microchemical reactions.

In practically all species the outermost cells gave a pure cellulose reaction with chlor-zinc-iodine, and absolutely negative results with phloroglucin and HCl, and other lignin-demonstrating reagents. This is easily understood in those petioles where the cells show elongation and division,

as lignification would obviously interfere with their growth, but where the elongation is slight in amount or absent, the explanation does not seem at all clear.

The inner layers were also occasionally of cellulose, but more commonly showed distinct evidences of lignification, giving a pink colour with phloroglucin after acidification with HCl. The depth of the coloration indicates a very imperfect lignification, the walls being of a ligno-cellulose rather than a true lignin nature. In other cases the lignification had become much more pronounced, giving the typical bright red phloroglucin coloration and in some of the smaller petioles extending throughout the cortex.

The modified cells, especially in the immediate vicinity of the wound, were coloured yellow or yellow-brown by the deposition of tannin-like substances in their walls, and, as this interfered to a certain extent with the action of the various reagents, it was, as a rule, removed by gently warming the sections with eau de Javelle. Zimmermann (37, p. 143) cites Mangin as stating that treatment with this liquid causes lignified elements to give the cellulose reactions, but in the experience of the writer, if reasonable care is observed, this is certainly not the case in the Filicineae.

As a test of the reliability of the phloroglucin reaction after treatment of the tissues with eau de Javelle, several series of microtome sections of a wounded petiole of *Lastraea reflexa* were employed.

Half of these were acidified with HCl and then treated with phloroglucin solution, whilst the remainder were first decolorized with eau de Javelle and then similarly treated. The coloration, apart from the yellowness of the walls of the most peripheral cells, was identical in each case, the xylem elements being bright red, whilst the imperfectly lignified cortical tissue was stained pink. The walls of the modified cells are abundantly pitted, though the pits themselves are not large; they are shown very clearly on swelling with caustic potash (Plate LXXIII, Fig. 8).

(ii) With regard to the reactions of the tissues in the regions below the apex, the same variability is noticeable in the different forms, but to a less degree.

A section of a wounded area produced just below the curled apex in *Asplenium bulbiferum* exhibited a slight elongation of the cortical cells, accompanied by a large number of cambial divisions, this resulting here and there in the production of a small tabulate cell. There was not, however, any attempt at the production of a complete new growth. The cells at the periphery showed what seemed to be slight traces of suberization, but the tests for suberin gave results which could only be described as extremely inconclusive (Pl. LXXIII, Fig. 5).

Similar cambial activity, to a perhaps slightly less extent, was perceptible in the middle of the fully unfolded pinna-bearing region of all the

bulbiferous species examined with the exception of *Cystopteris bulbifera* and *Adiantum Edgworthii*, but gum did not begin to make its appearance until the lapse of from four to five weeks (Pl. LXXIII, Fig. 6).

In one case, however, in which a plant of *Woodwardia radicans* had accidentally been kept without water, the gum deposit was abundant within a fortnight, and there were practically no traces of either cell-elongation or cambium formation.

Two specimens of *Woodwardia orientalis* were also somewhat exceptional in that for a considerable depth the affected tissues after division became suberized (Pl. LXXIII, Fig. 11).

That actual suberization and not merely a deposit of tannin in the tissues had occurred was amply demonstrated by the following reactions:

1. The cell-walls were insoluble in hot or cold concentrated H_2SO_4.

2. They were only dissolved with difficulty by a strong solution of chromic acid.

3. They stained readily with cyanin, with alkannin, and with fresh chlorophyll solution after removal of the tannin with eau de Javelle.

4. They stained brown-violet with chlor-zinc-iodide after treatment with KOH solution.

5. They were doubly refractive with polarized light.

It was at first a source of difficulty to understand how the suberization could be so complete, and the cells yet retain their contents after three months apparently but little affected.

Staining with cyanin, alkannin, and chlorophyll, however, showed the presence of abundant pits in the walls, these showing especially well where the walls had been cut tangentially, and doubtless serving to ensure protoplasmic continuity.

An abundant deposit of gum was a general feature in all the remaining cases, but there were certain differences apparent in the mode of repair.

In *Athyrium Filix-foemina*, *Lastraea reflexa*, and most of the remaining species there was a certain amount of cell-elongation, with an occasional transverse division in a few forms, but this was seldom pronounced. It was always accompanied by an increase in the thickness of the walls, this usually being of a ligno-cellulose character.

The species of a more horny nature, such as *Pteris cretica*, *Blechnum brasiliense*, and *Polypodium glaucum*, varied somewhat, specimens occasionally showing some elongation, but more commonly contenting themselves with a thickening of the walls and a dense aggregation of gum. The modified walls in these also were often slightly lignified, and in one or two instances the lignification was strongly marked. In *Lomaria chilensis* and *Phegopteris hexonaptera* the lignification extended throughout the petiole in the wound area. *Osmunda regalis* and *Struthiopteris germanica* as a rule behaved similarly to the above forms, but in one or two instances, in the

large mature petioles, the reaction was quite different, and was similar to that constantly resulting in *Davallia polyantha* and *Adiantum* spp. Here the peripheral cells became brown and enormously thick-walled with large pits for protoplasmic connexions, the thickening becoming less pronounced as the cells became further from the wound until they merged into the normal cortical parenchyma (Pl. LXXIV, Fig. 12).

Microchemical investigation demonstrated that this thickening was also peculiar in consisting throughout of pure cellulose. As in previous cases, the outermost cells showed a solid deposit of gum, whilst those near the exterior also showed large quantities of granular matter.

The reaction produced in *Scolopendrium vulgare*, which is soft bodied and possesses an undivided leaf, was exceptional. The parenchymatous cells extending through fully half the diameter of the cortex, and in some specimens even partially round the vascular bundle, had, under favourable circumstances, without actually dividing, elongated to a very considerable extent, producing exaggerated intercellular spaces owing to the partial separation of adjacent cells (Pl. LXXIII, Fig. 9). The result of this lengthening is that a slight intumescence is formed, comparable to those produced as a consequence of traumatic stimulus in the case of many roots (Nêmec (20), Bayliss (1), Davis (7)).

No amitotic nuclear divisions were observed, though the majority of those in the cells affected were spindle shaped, and some showed a median constriction (Pl. LXXIII, Fig. 10). The number of cells which were completely filled with gum was exceptionally large, these extending inwards to a depth of six or more, and, as in previous cases, the cells below also showed abundant granules.

From these facts it will be perceived that there is in the pinna-bearing region a distinct loss of cambial activity, the bulbil-producing species of *Asplenium*, *Polystichum*, and *Woodwardia* being the only ferns among those examined which still show any marked efforts in this direction.

The tissues developed during the formation of 'pneumathodes' which characterize the rhizome and stipules of the Marattiaceous ferns as figured by Hannig (12), offer a remarkable analogy to those produced in the wounded areas in the pinna-bearing parts of the bulbiferous species referred to.

In the remaining species examined the general mode of response takes the form of local thickening, either of cellulose, ligno-cellulose, or lignin, of the tissues at the seat of injury, this being accompanied by a greater or less amount of cell-elongation and division, and an abundant deposit of gummy matter.

(iii) In the petiole below the region of pinna-insertion, the tissues of which may be regarded as quite mature, there is an almost uniform response to wounding. Here there is no elongation of the cortical elements in normal cases, thickening and the production of gum in large quantities

being general. In one case only, and that of a decidedly exceptional nature, was any secondary growth observed. This exceptional case occurred in the basal part of the petiole of *Woodwardia orientalis*, and was apparently due to bacterial infection.

The petiole showed a pronounced local dilatation in the wound area (Pl. LXXIV, Fig. 13), and on microscopic examination it was found that the uninjured cortical cells at the wounded surface had proliferated in a peculiar, irregular manner, giving rise to a well-marked intumescence (Pl. LXXIV, Fig. 14).

The cells had, in some cases, especially at the margins of the affected area, grown very long without dividing, whilst the remainder had divided up by transverse walls at varying intervals, and the resultant daughter cells had also grown. The appearance of these cells was very different from that produced by ordinary cambial division. The whole of the cells were filled with cytoplasm of a cloudy nature, and in addition contained minute rods, which were presumably the cause of the growth. Cells containing two nuclei were not uncommon, and in one or two of the largest cells three nuclei were detected (Pl. LXXIII, Fig. 10).

Strasburger (34) records somewhat similar instances of cells containing several nuclei in the case of graftings where tissues have been injured.

Hypertrophy as a result of infection by Bacteria is not uncommon, the root-nodules produced in Leguminosae, *Alnus*, and *Myrica* being typical instances, whilst Smith (27) has also described local swellings produced on the olive due to similar causes.

Immediately below the meristematic zone there was a large patch of tissue several cells in diameter which had become strongly impregnated with tannin, this cutting off the infected cells from the general mass of cortical tissue (Pl. LXXIV, Fig. 14).

Appearances point to this being of the nature of a protective wall since the rodlets were not detected in any of the cells within this zone.

The results obtained in the basal petiolar region are precisely what might have been anticipated from a comparative study of the effects of wounding in the younger parts. As the petiole becomes absolutely set and mature the stimulus causing anything but the simplest form of response, that is, local thickening, would have to be of a very unusual character, a condition fulfilled in the abnormal case recorded.

The description of the various reactions recorded has been confined to the results produced in the general mass of cortical parenchyma, and as the remaining tissues appear to each show considerable uniformity it has been thought preferable to deal with them separately.

(*a*) *The epidermis* in the most freely reacting forms (e. g. *Asplenium Belangeri*) showed a certain amount of sliding growth in the wound area accompanied by one or more cell-divisions, this

resulting in a callus-like incurving at the margins somewhat similar in appearance to the early stages of repair seen in trees after a branch has been severed (Pl. LXXIV, Fig. 20). In the remainder the epidermal cells nearest the wound showed at the most an increase in general bulk rather than elongation in any particular direction, and were usually filled with gum (Pl. LXXIV, Fig. 16). The walls did not exhibit any additional growth in thickness in any case.

(*b*) *The vascular bundle and bundle-sheath.* Whenever a bundle was cut into to any great extent, degeneration of the tissues and a general deposit of coarse, brown, granular matter seemed invariably to result. Where the bundle-sheath was not actually cut away the cells on the side adjacent to the wound showed a growth in length and also division, this often leading, where the sheath did not show radial thickenings, to loss of identity as a definite layer. Where radial thickenings were present the daughter cells resulting from the division also showed these (Pl. LXXIV, Fig. 20).

The endodermal cells of some forms (e. g. *Davallia*) are characterized by heavy brown thickenings, but these do not seem to be in any way affected by wounds, the bundle-sheath of parts remote from the wound showing an amount of thickening equal to those nearer to it (Pl. LXXIV, Fig. 15). With regard to the phloem and xylem, where the differentiation of these elements is already complete no traumatic effects are perceptible, but where, as in the apical portions, the lignification of the later-formed xylem elements has not begun, both this and the phloem show distinct elongation towards the affected part. The cells of the phloem and of the bundle parenchyma often show both elongation and transverse division; in the case of the large xylem elements elongation alone occurs. That this elongation is of the nature of traumatic response, and is not due to the disturbance of the bundle symmetry by the departure of a leaf-trace, is strongly supported by the fact that specimens wounded on the abaxial side of the petiole show this feature very markedly (Pl. LXXIV, Fig. 17).

From the foregoing it will be perceived that the more specialized tissues, such as the epidermis, the endodermis, and the vascular elements, are all responsive to a greater or less extent to traumatic stimuli, but that, as might be expected, the modification is of a much less pronounced character than that obtaining in the comparatively simple cortical parenchyma.

A first series of experiments such as these are almost of necessity incomplete, since the aim has been to obtain a general idea of the mode of response to wounding in the Filicineae as a group, rather than a detailed knowledge of the behaviour of one or more species. As a result the effect of varying physiological conditions has not been studied, except incidentally.

With the exception of a few of the hardiest forms, such as *Pteris*

aquilina and *Lastraea Filix-mas*, the plants were grown under glass and under practically identical conditions. The writer hopes to make the physiological aspect of the paper the subject of a subsequent investigation.

Summary.

1. When a fern petiole is wounded in the still meristematic apical zone, the plant attempts to protect the injured area by the production of a pad of cambiform cells, which arise by the subdivision of the cortical parenchyma.

2. In the most successful cases the wound-cambium completely covers the affected part with a typically meristematic tissue, as in *Asplenium bulbiferum*, *A. Belangeri*, *Polystichum proliferum*, *Woodwardia orientalis* and *W. radicans*.

3. More usually the cambial response is imperfect, consisting of the elongation of the cells at or near the seat of injury, with a greater or less number of transverse divisions of these (e. g. *Lastraea Filix-mas*, *Polypodium glaucum*, *Scolopendrium vulgare*). In *Polystichum angulare*, *Pteris cretica*, *Lastraea reflexa*, and *Woodwardia virginica*, elongation of the outermost layer alone occurs.

4. The cambium, whether well developed or imperfect, is supplemented by the scab-like remains of dead cortical cells on the outside.

5. As the petiole develops secondary changes take place, resulting in the thickening of the cell-walls by deposits of cellulose, ligno-cellulose or lignin, and in the deposit of an almost solid mass of intracellular gum.

6. Petioles wounded in the more mature 'region of pinna-insertion' produce cambium less readily, only those producing bulbils, of the forms examined, showing this mode of response in any marked degree.

7. It is suggested that the production of bulbils may demand a more adaptable type of tissue, this accounting for the readiness with which cambium is produced.

8. In other forms, elongation of the outer cells, together with an abundant deposit of gum, is the most general type of wound response. Transverse divisions of the modified cells are few in number or do not occur.

9. Occasionally, as in *Davallia polyantha*, there may be an extremely thick deposit of cellulose on the walls of the cells at the seat of injury, resulting in the production of a very resistant tissue. Gum is deposited as in the previous cases.

10. Plants wounded in the basal part of the petiole generally show no cell-elongation, but thickening of varying character together with a constant deposit of gum were constant features.

11. In only one case, an abnormal one, in which the wound was infected by Bacteria, was any secondary activity evinced. This resulted in the local

dilatation of the petiole, and the formation of a distinct intumescence by the irregular division of the infected cells.

12. Where cell-elongation occurs, pathogenetic, amitotic nuclear division, either complete or incomplete, may accompany this.

13. The epidermis in the most active species may show sliding growth comparable to that exhibited by the cortical cells, but usually, beyond an abundance of gum and an increase in the size of the cells, little sign of activity is shown.

14. The cells of the endodermis either enlarge or elongate, and may divide so that their identity as a definite layer is lost on the side nearer the wound, when this approaches the bundle at all closely.

15. There is some evidence that the phloem and xylem, when young, are influenced by wounding, and elongate towards the seat of injury.

In conclusion, the author's thanks are due to Professor Weiss for much encouragement and help; to Professor Carr, in whose department the major portion of the work has been done, and who has permitted his plant-house to be used for the experimental work; to Mr. S. Garside, M.Sc., lately of the Victoria University, Manchester, who kindly collected and preserved part of the material used; and to Miss Bancroft, B.Sc., who performed a series of control experiments on a number of the hardier forms, whilst studying at the University College, Nottingham.

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EXPLANATION OF PLATES LXXIII AND LXXIV.

Illustrating Mr. Holden's paper on Wound Reactions in Filicinean Petioles.

PLATE LXXIII.

Fig. 1. *Polystichum proliferum*. Curled apex in transverse section, showing formation of a complete wall of cambiform tissue. × 250.

Fig. 2. *P. proliferum* (young). Curled apex in transverse section, showing elongation of the outer cortical cells, and occasional transverse divisions. Slightly diagrammatic. × 250.

Fig. 3. *Lastraea Filix-mas*. Curled apex in transverse section, showing elongation and division of the cortical cells. Slightly diagrammatic. × 250.

Fig. 4. *Polystichum angulare*. Curled apex, showing elongation of outermost cortical cells only, without division. Slightly diagrammatic. × 250.

Fig. 5. *Asplenium bulbiferum*. Transverse section of petiole just below curled apex. × 250.

Fig. 6. *A. bulbiferum*. Transverse section of petiole in region of pinna-insertion. × 250.

Fig. 7. *Lastraea Filix-mas*. Transverse section of petiole in region of pinna-insertion. One side shows cell-contents, the other the elongation and division of the cortical cells and the thickening of the walls. × 250.

Fig. 8. *L. Filix-mas.* Small portion of section, showing solid deposit of gum traversed by cracks, also neighbouring cells with granules; one cell shows the pits in the wall in surface view. × 600.

Fig. 9. *Scolopendrium vulgare.* Transverse section of petiole in region of pinna-insertion, showing cell elongation for an exceptional depth. × 250.

Fig. 10. Cells from various forms, showing pathological nuclear effects. *a*, *b*, *Lastraea Filix-mas*; *c*, *d*, *e*, *Scolopendrium vulgare* (*d* and *e* show gum deposits); *f*, *Woodwardia orientalis*, with Bacteria. *a-f*, × 750.

Fig. 11. *Woodwardia orientalis.* Transverse section of petiole in region of pinna-insertion, showing division and suberization of cortical cells. × 250.

PLATE LXXIV.

Fig. 12. *Davallia polyantha.* Transverse section of petiole below region of pinna-insertion. The cortical cells are heavily thickened, the outer ones contain abundant gum, and the connecting pits are well marked. × 250.

Fig. 13. *Woodwardia orientalis.* Petiole infected by Bacteria, showing local dilatation. × 1.

Fig. 14. *W. orientalis.* Transverse section of infected area of same with suberized layer below. × 250.

Fig. 15. *Davallia polyantha.* Small portion of petiole, showing difference in character of endodermal thickenings and those of other parts of the cortex. × 600.

Fig. 16. *Scolopendrium vulgare.* Small portion of epidermis to show slight growth of deposit of gum, due to wound stimulus. × 500.

Fig. 17. *Polystichum proliferum.* Single vascular bundle from extreme apex, showing effect of wounding on young phloem and xylem-elements. × 600.

Fig. 18. *Woodwardia orientalis.* Small portion of infected petiole in Fig. 13, showing edge of wounded area. × 400.

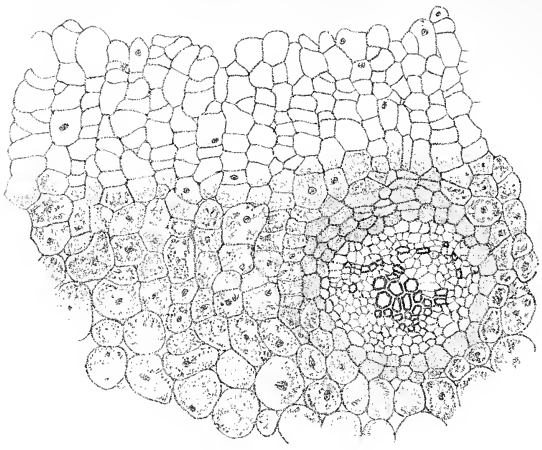
Fig. 19. *Asplenium Belangeri.* Small portion of cortical parenchyma of wounded apex, illustrating method of growth of secondarily active cells. The intercellular spaces are darkened. × 600.

Fig. 20. *A. Belangeri.* (*a*) Curled apex of petiole in transverse section to show division of cortical cells, epidermis, and endodermis. Slightly diagrammatic. × 300. (*b*) Diagram showing area (lightly shaded) containing gum deposit.

Fig. 21. *Bowenia serratula.* Portion of petiole in transverse section, showing true wound periderm. Specimen from Kew. × 120.

Fig. 22. *Pteris cretica.* Petiole, showing flexure due to wounding. × 1.

Fig. 23. *Woodwardia virginica.* Transverse section of petiole, showing cell-elongation and slight cellulose thickening. × 400.



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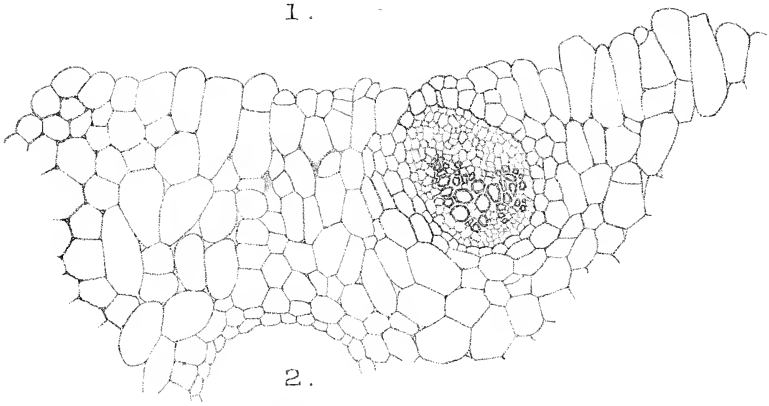

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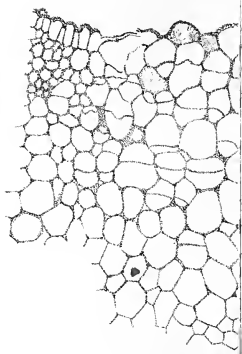

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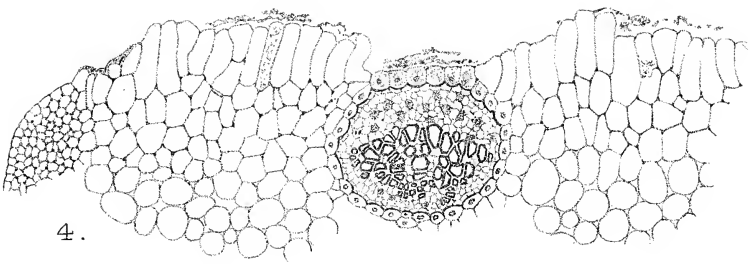

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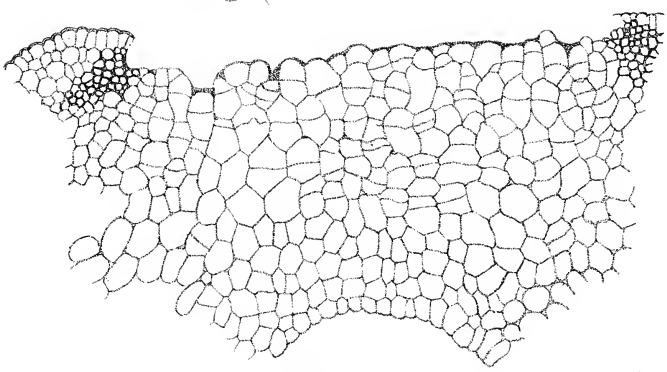

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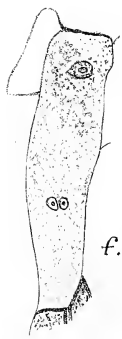

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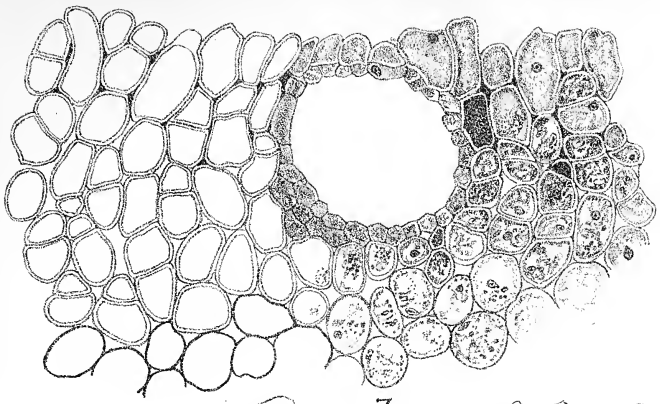
HOLDEN — FILICINEAN WOUND REACTIONS.



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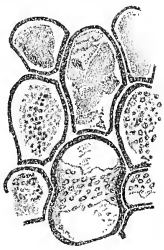

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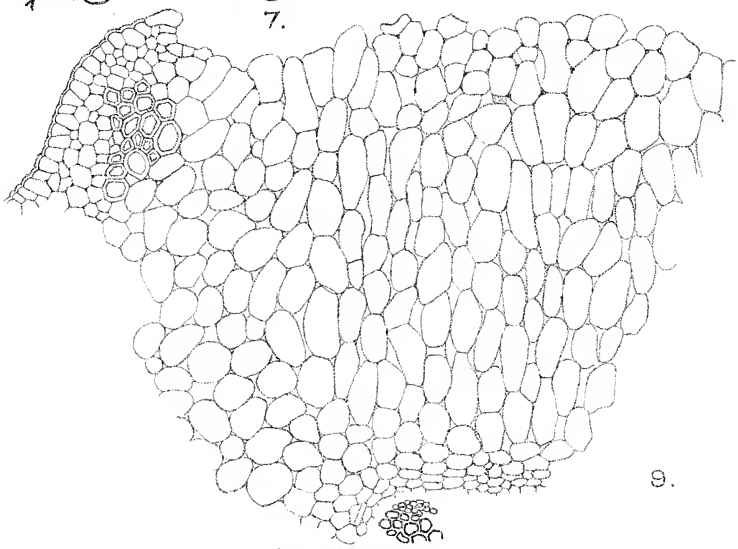

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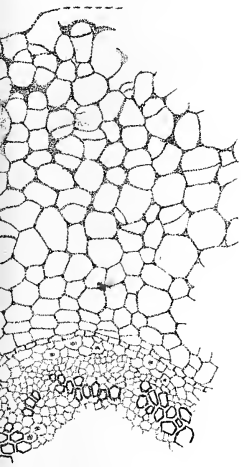

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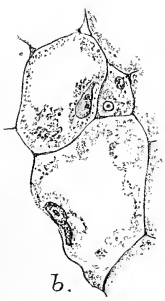

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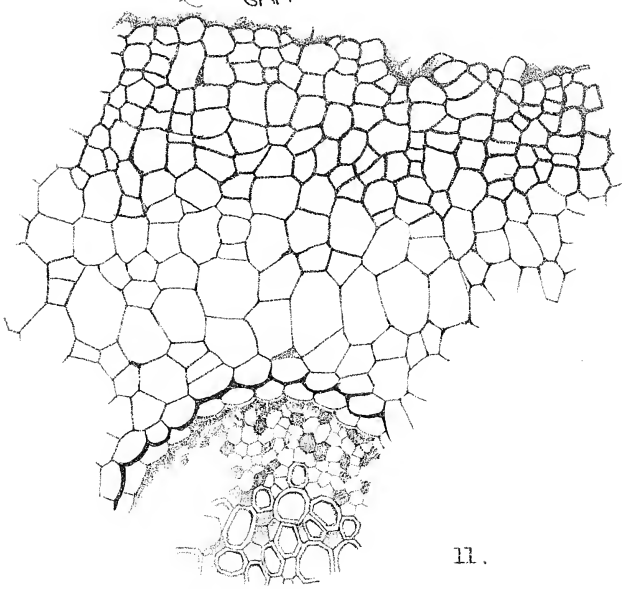

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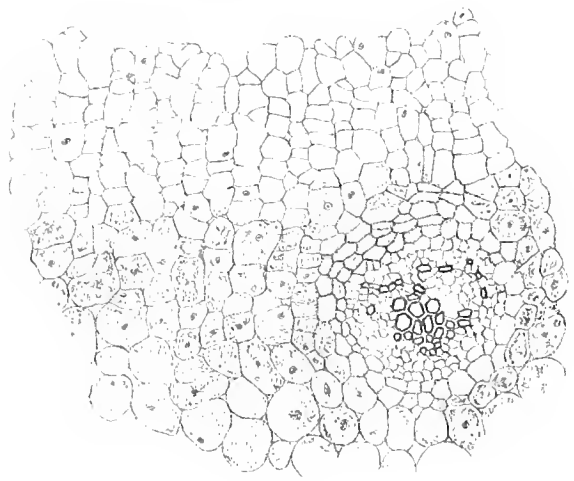
Huth, lith. et imp.



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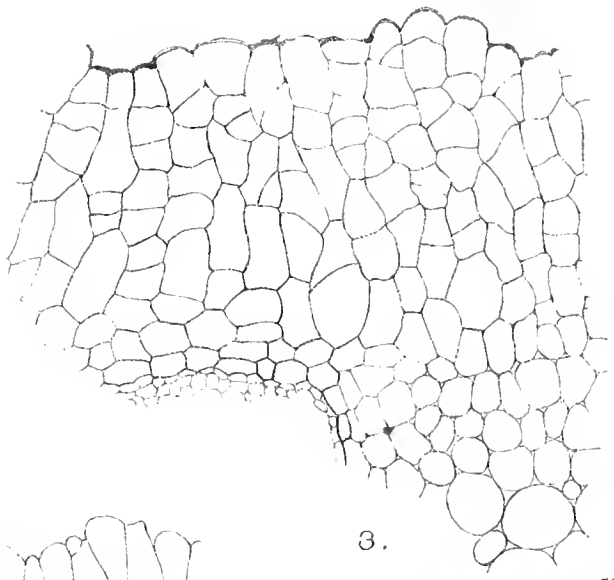

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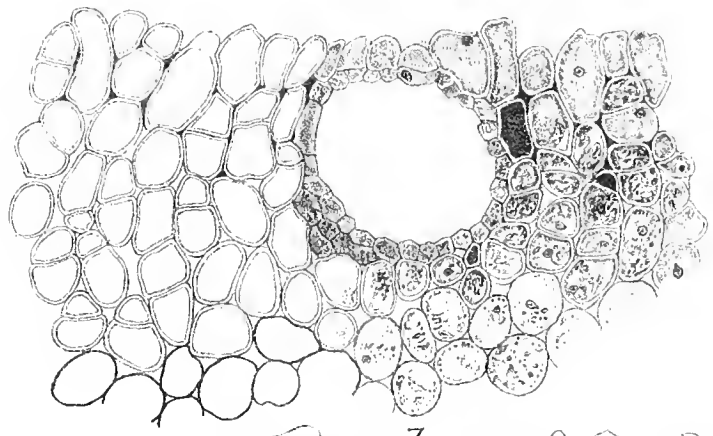

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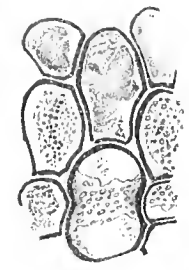

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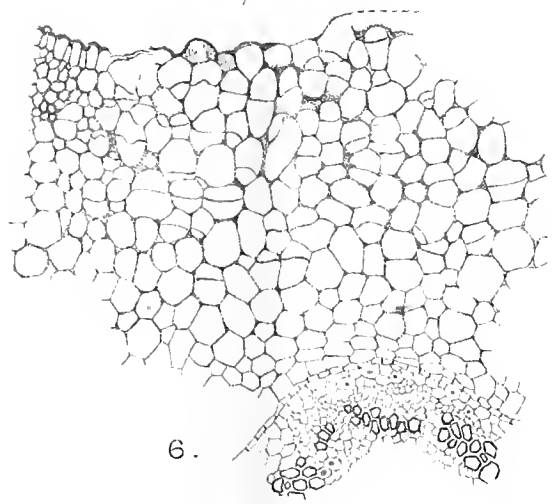

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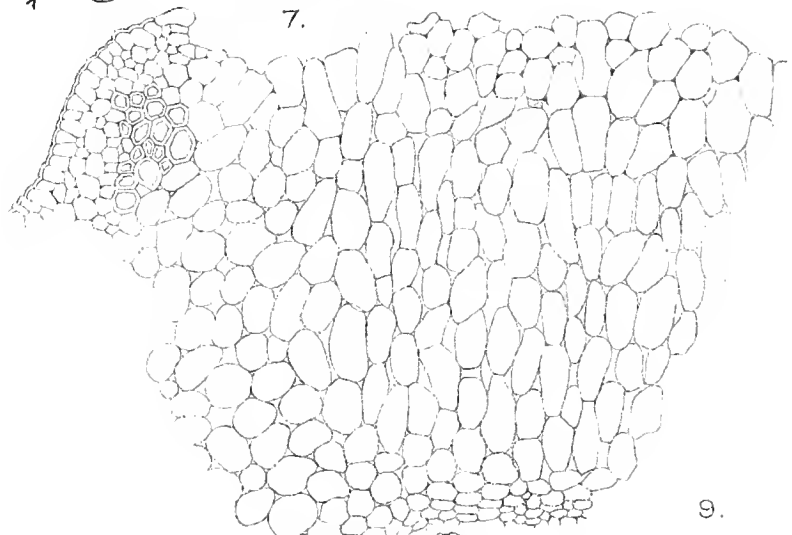

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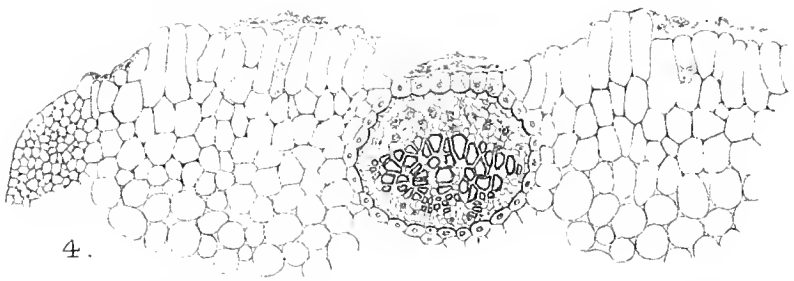

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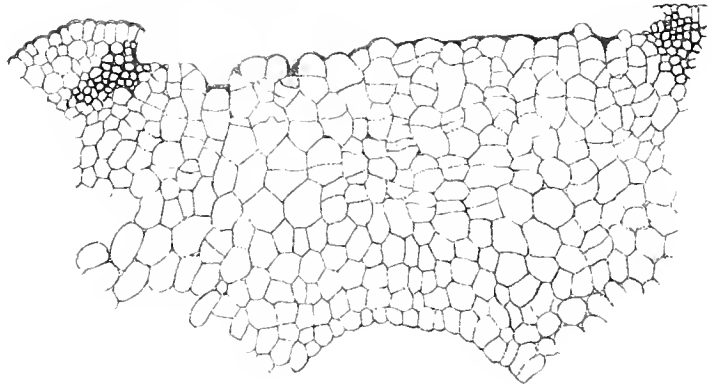

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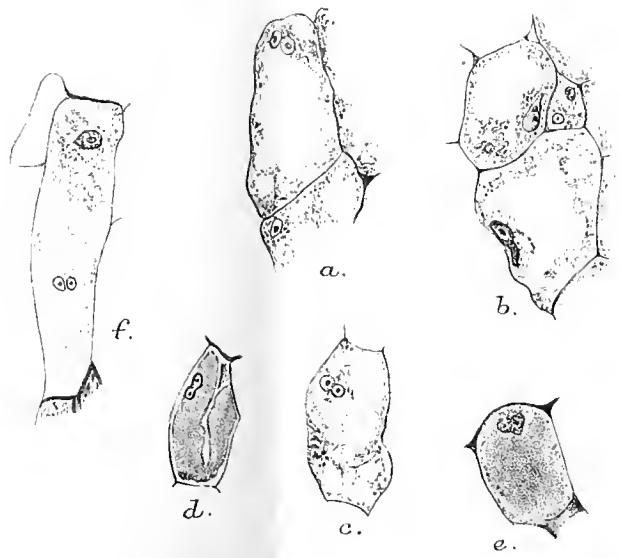

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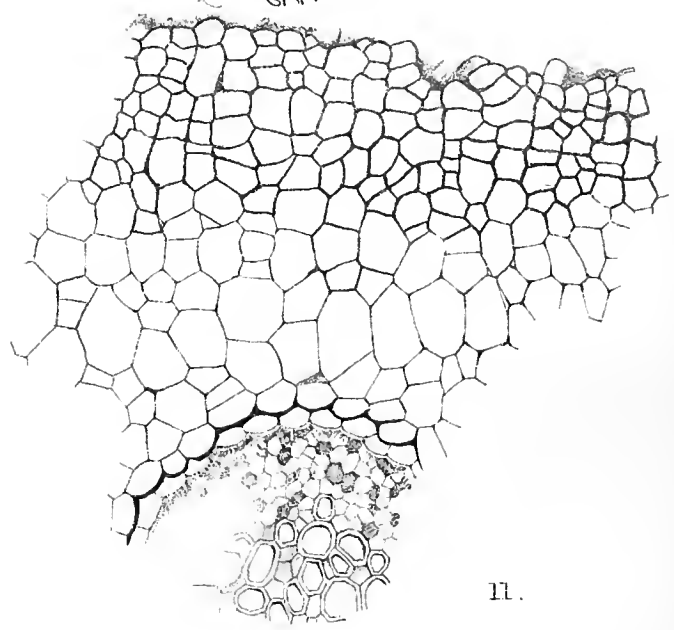
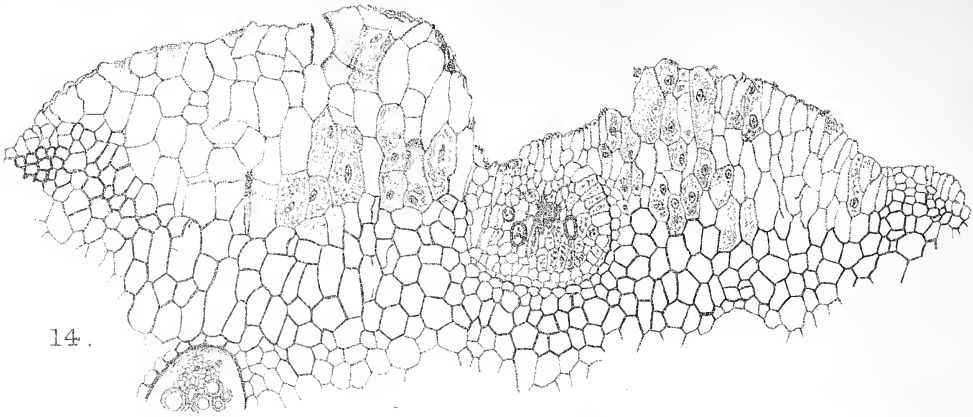


11.

H.S.H. del.

HOLDEN — FILICINEAN WOUND REACTIONS.

Huth,lith et imp.



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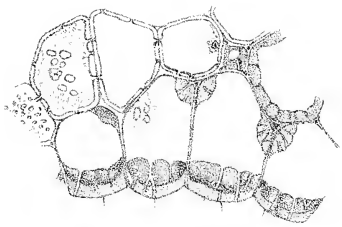

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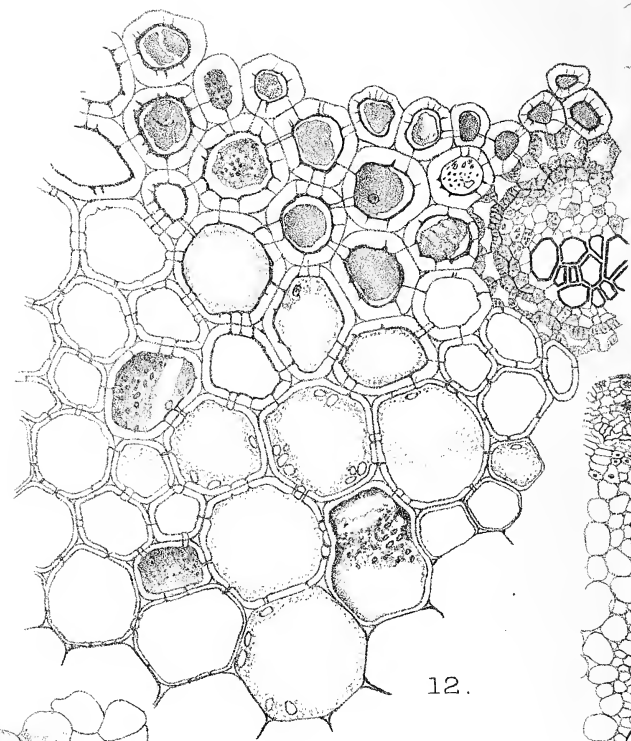

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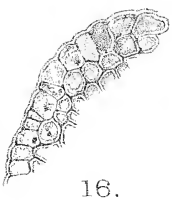

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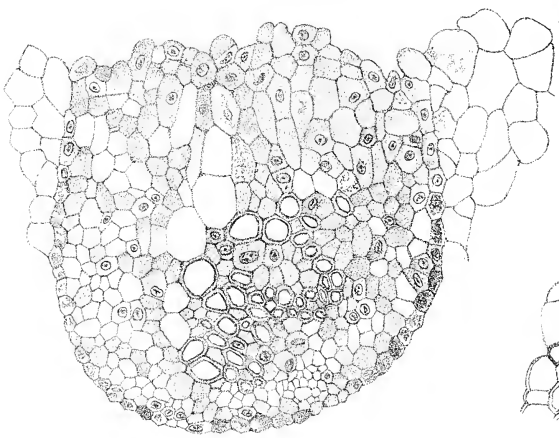

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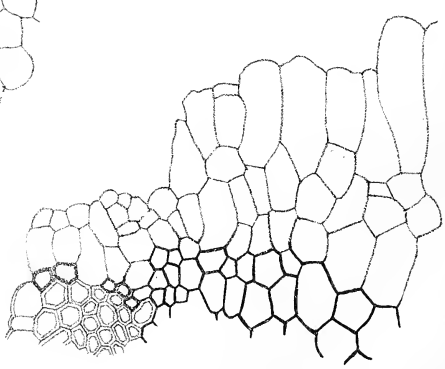

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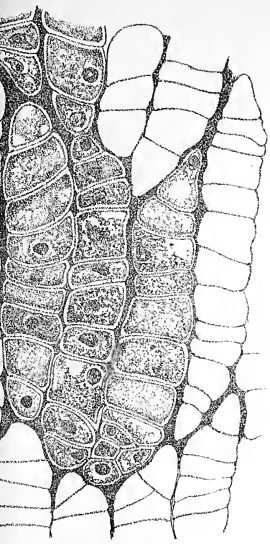
H.S.H. del.

HOLDEN — FILICINEAN WOUND REACTIONS.



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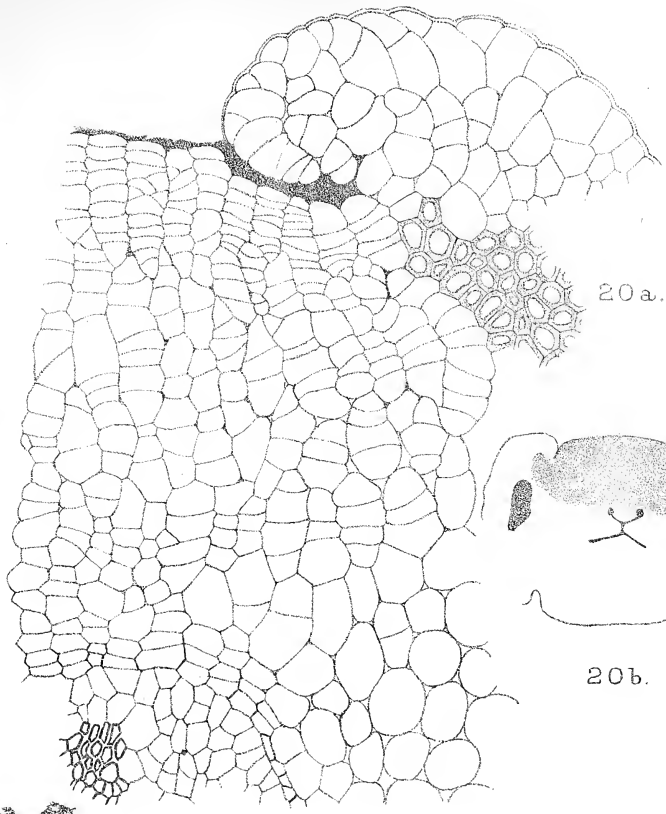

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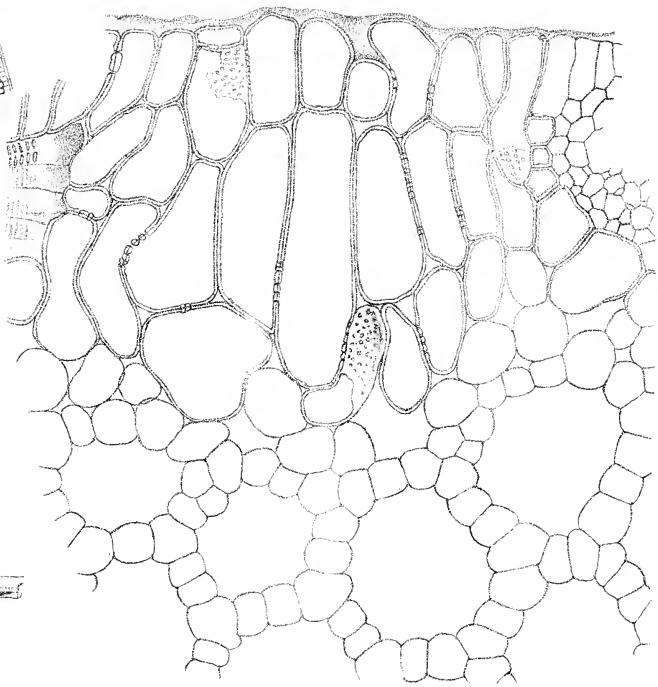

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Huth, lith. et imp.

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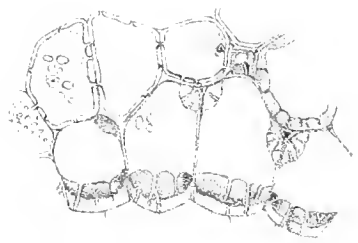

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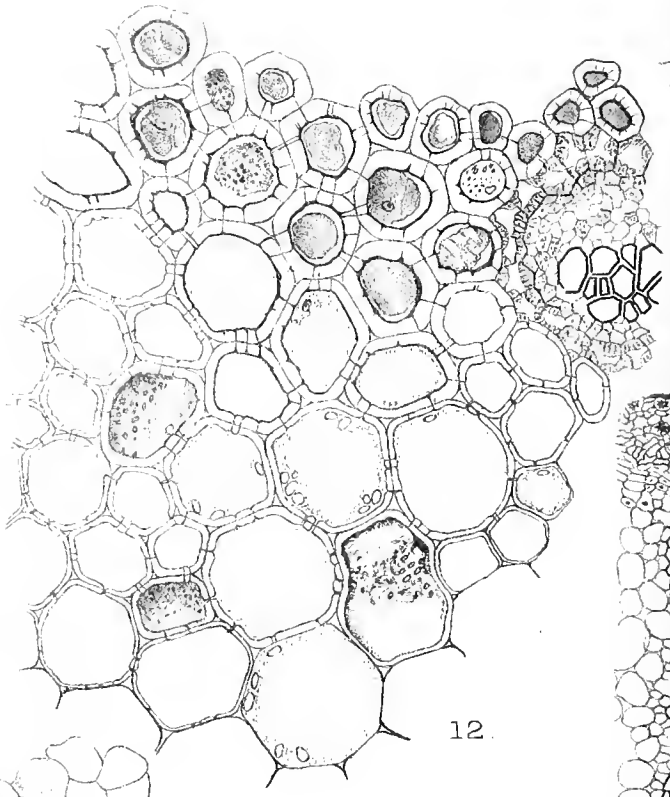

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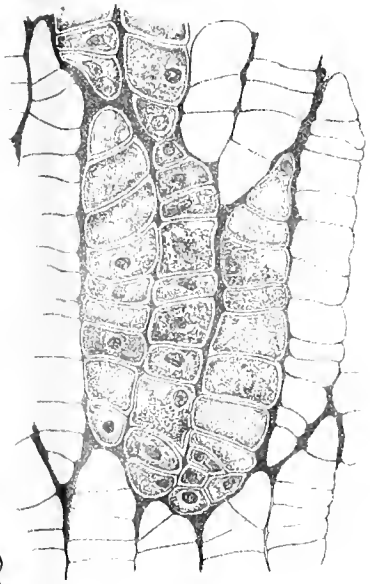

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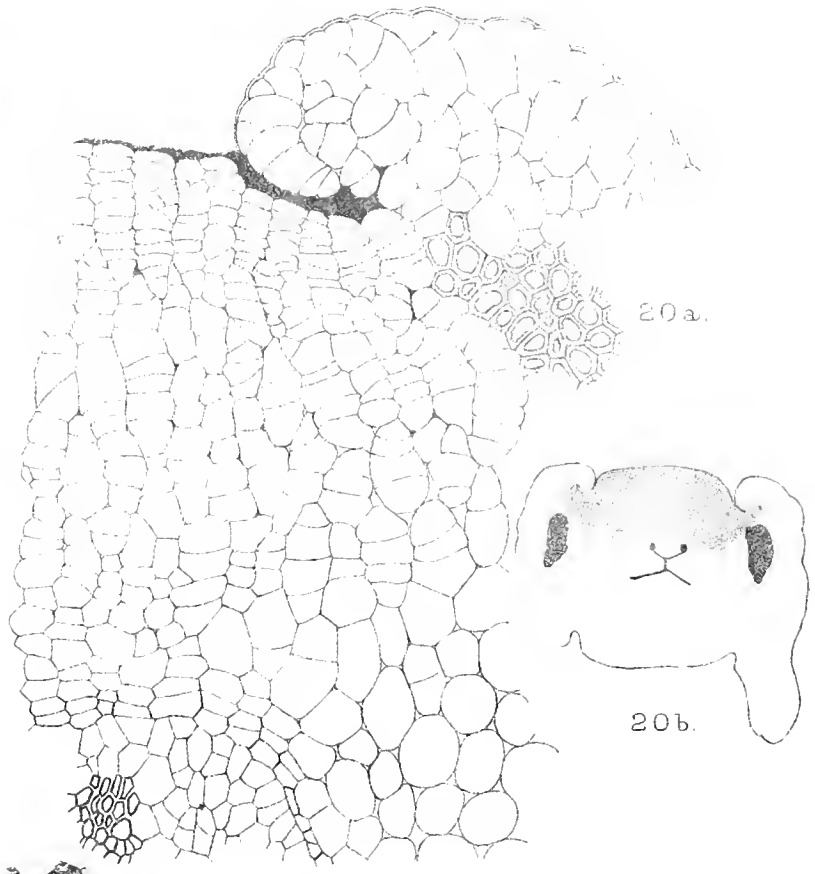

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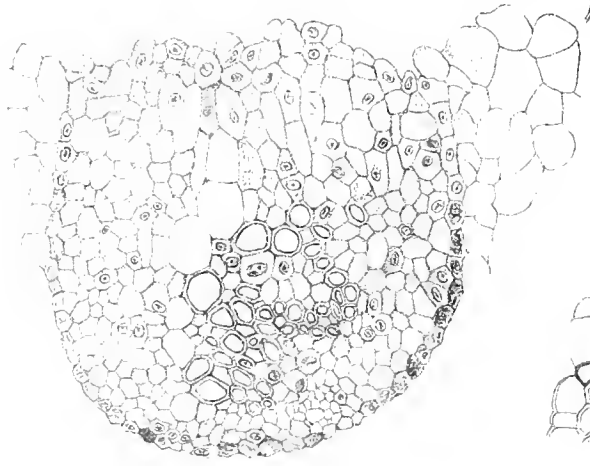

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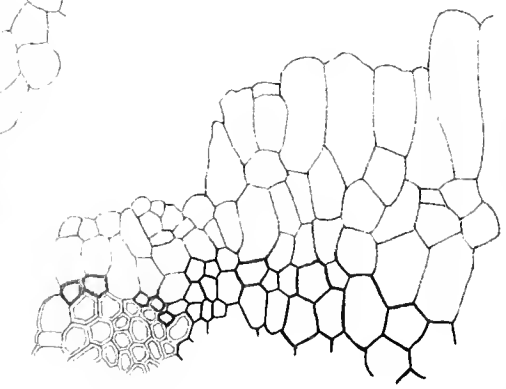

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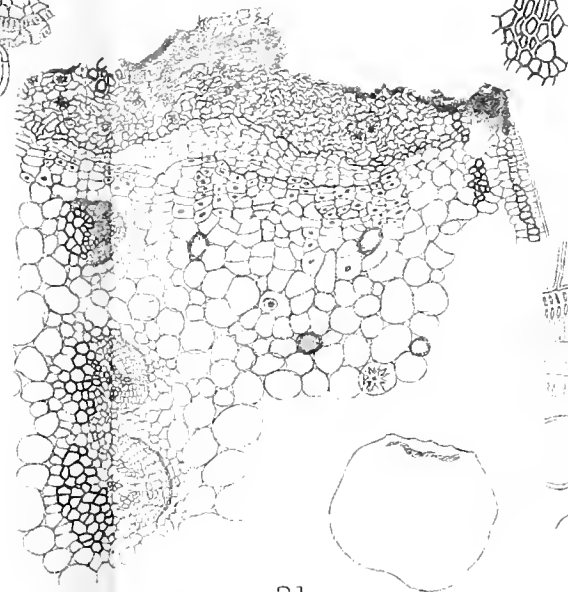

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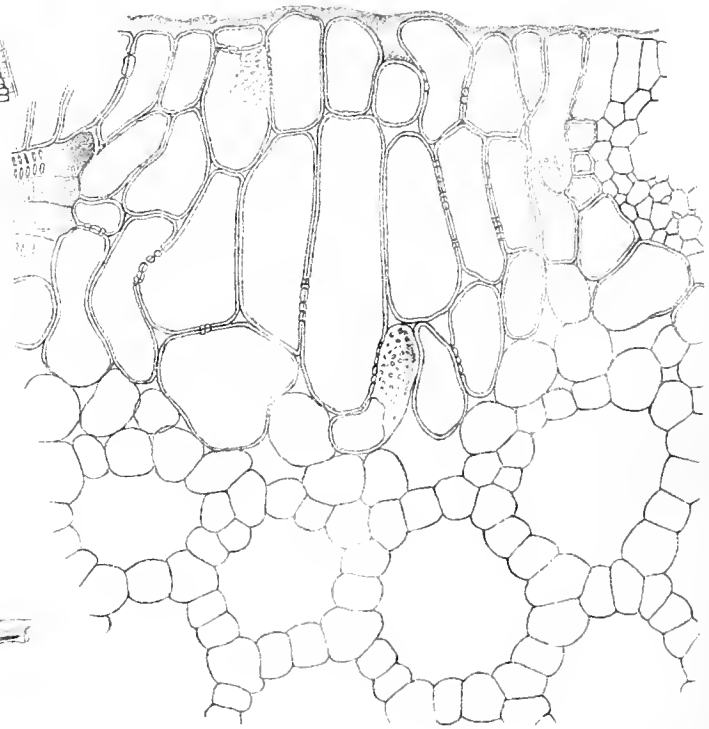

22.

H.S.H. del.

Huth, lith. et imp.

HOLDEN — FILICINEAN WOUND REACTIONS.

A Bee-hive Fungus, Pericystis alvei, Gen. et Sp. Nov.

BY

ANNIE D. BETTS, B.Sc.

With Plates LXXV and LXXVI.

THE bee-hive Fungi, as a class, have been studied but little, though bee-keepers have long been aware of the occurrence of 'mould' in their hives, and some of the diseases to which the honey bee is liable are known to be of a fungous nature. An investigation was begun with the object of filling this gap in our knowledge of the economy of the hive; in the course of it, the species which is the subject of the present paper was met with.

Pericystis alvei is a common fungus, being in fact the principal constituent of the 'pollen-mould' prevalent in hives during the winter and early spring. It grows, as this name indicates, on the pollen stored in the combs (Pl. LXXVI, Fig. 11), and is not found, so far as is known, on any other substratum. The contents of cells attacked by the fungus tend to dry up ultimately into hard plugs, which often split into layers (see Pl. LXXVI, Fig. 12); they are permeated by mycelium, which is, however, most plentiful on the surfaces and especially on the top (that is, the surface exposed when the plug is in the cell).

This species may be readily distinguished from all others found in the hive by the character of its mycelium. Many of the hyphae have a large proportion of their cells converted into chlamydospores; this is sometimes carried so far as to give the appearance of an oidium-hypha (Pl. LXXV, Fig. 2, *b*). The remaining cells tend to lose their protoplasmic contents, so that the hyphae when mature break up readily (Fig. 2, *a*). Other hyphae are more or less devoid of spores, or only bear occasional lateral or intercalary ones (Pl. LXXV, Fig. 1, *a*).

The hyphae are 2–6 μ in diameter. As a rule, they assume their characteristic appearance when quite young (Pl. LXXV, Fig. 3, *a*, where chlamydospore is shown in course of formation on a young hypha).

The chlamydospores are of various forms (Fig. 3). In position they are terminal, lateral, or intercalary, or are borne on short lateral processes (Pl. LXXV, Figs. 1, 2). Their dimensions vary from 4–5 μ to 9·5 × 7 μ or even

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larger; 8–6 × 7–5 μ are average measurements.[1] The walls of these spores are thicker than those of the ordinary hyphal cell; this, together with their frequently isolated position, is felt to justify the use of the term chlamydospore rather than that of oidium-cell, although they resemble the typical oidium-cell in their capacity for immediate germination.

If a comb on which *Pericystis alvei* is present be examined in the early spring,[2] some cells will be found in which the fungus is no longer white, but grey. In May it is not unusual to find specimens of a dark slate-grey colour. This change is due to the formation of the cysts. These are subglobose or irregularly oval objects 20 μ (occasionally less) to 40 × 30 μ (average about 30 μ in diameter),[3] and contain numerous spores. The cyst-wall is thin, smooth, and structureless, semi-transparent, and has a polished appearance when viewed by reflected direct sunlight. It is dark green in colour, appearing black by reflected light. Each cyst is borne on three to five short hyphal branches, also of a dark green colour. The general appearance of a culture with mature cysts is shown in Pl. LXXVI, Fig. 10.

The walls of the mature cyst and its supporting hyphae are not dissolved when placed in concentrated sulphuric acid on a slip, covered with a cover-glass, and heated till bubbles are expelled.

The cyst-spores are hyaline, spherical, and thick-walled (Pl. LXXV, Fig. 9); they are 3·7–4·7 μ in diameter, average 4·3 μ. The number present in a cyst is variable, depending on the dimensions of the cyst. So far they have not been observed to germinate. Possibly they require a resting period, or conditions different from those which are favourable to the germination of the chlamydospores.

The cyst arises as a swelling or lateral projection on a hypha. This puts out one or two (rarely three) processes, which fuse with a neighbouring hypha, usually bending through a right angle to do so (Pl. LXXV, Figs. 4, 5). The young cyst now increases in size, and its contents assume a vacuolated appearance (Pl. LXXV, Fig. 6). The wall presently begins to acquire a brownish tint; a culture at this stage is of a pale salmon or bone colour to the naked eye. The tint deepens, till finally the wall turns green; it almost attains its mature depth of colour before the spores begin to be visible within the cyst (Pl. LXXV, Fig. 7).

The fusing hyphae become septate at an early stage. Inspection of stained material reveals that there are three septa; one at the junction with the cyst, and two others close together further along the hypha (Pl. LXXV, Fig. 7). The contents of the young cyst are highly granular, and the septa are in consequence frequently invisible in unstained material.

[1] Measurements taken from honey-gelatine culture material.

[2] Possibly in mid-winter also. Grey specimens were plentiful on some material in January; but these combs were from hives where the bees had died, so that the conditions were not normal.

[3] Measurements taken from honey-gelatine culture material.

The green colour, in the mature cyst, extends as far as the third septum; the fusing hyphae can therefore be easily distinguished from the hypha bearing the cyst, by the septum near their distal ends (Pl. LXXV, Figs. 7, 8).

When several cysts form on one hypha, their processes often all fuse with the same neighbouring hypha (Pl. LXXV, Fig. 5). It has not been ascertained that this is invariably the case, so that any deductions from it as to a possible differentiation of the hyphae would be premature.

The cyst is indehiscent; the spores are presumably set free by the breaking of the wall.

Pericystis alvei is not very easy to cultivate. It will germinate readily on most media, and produce mycelium and chlamydospores; but cysts, if formed at all, are usually empty. The only satisfactory medium was found to be honey-gelatine.[1] On this the fungus developed normally and cysts containing spores were produced.

Cultures of the fungus have a distinctly alcoholic odour. Gelatine is liquefied, but slowly; the process is often one of softening rather than of liquefaction.

Some rough experiments were made to test the effect of different temperatures on the germination of the chlamydospores. The results indicate that:

(1) The chlamydospores will not germinate at 26°–38° C., but do so when transferred from this to 15°–18° C., so are not killed. The cultures were kept at 26°–38° for 13–16 days.

(2) They germinate at 15°–18° C. in 1–5 days.

(3) They germinate under outdoor conditions (mild winter weather) in about 11 days.

These facts are of interest in view of the probable life-history of this species. In the spring the bees clean out their combs, throwing the plugs of mouldy pollen out of the hive. Some of the spores will, however, be left in the hive, and will remain there during the summer. When the stock swarms, the bees of the swarm probably take with them some spores adhering to their bodies, and so transfer the fungus to the new colony. If this be so, we can see that the inability to germinate at high temperatures is of use to this species. The temperature of the bee-hive when the bees are active is 32°–34° C.; were the spores to germinate under these circumstances, the bees would remove the fungus from the combs as fast as it grew. In the winter, on the other hand, the temperature does not usually exceed 12° C.;[2] and the fungus is able to establish itself unmolested by the bees, which are hibernating in a cluster in the central part of the hive.

[1] Diluted honey (three or four parts of water to one of honey), 100 c.c.; gelatine (Gold Label), 10 grm. The medium was usually left acid.

[2] The consensus of opinion seems to point to this as the temperature of the cluster when quiescent. The outer combs, where the fungus usually grows, are of course colder.

Attempts to cultivate *Pericystis alvei* on bouillon, bee-pupa agar, &c., were not very successful ; and there is no reason to suppose that this species is ever pathogenic in the bee. It is, in fact, present alike in healthy and in diseased stocks ; if occasionally more plentiful in the latter, this is due to some secondary cause such as the weakness of the colony rather than to any direct connexion with disease.

As this fungus is undoubtedly a normal inmate of the healthy bee-hive, and is, so far as is known, confined to that habitat, I propose for it the name *Pericystis alvei*.

Pericystis, gen. nov. Hyphis repentibus vel suberectis, contextis, ramosis, septatis, multis cellulis in chlamydosporas se mutantibus, tandem saepe se dissolventibus. Chlamydosporis terminalibus lateralibus intercalariisque, subglobosis vel irregulariter ovalibus, membrana crassa, lēvi. Cystis subglobosis vel ovoidibus, tribus ad quinque ramis hyphalibus ferentibus, sporis numerosis repletis. Membrana cysti membranacea, simplice, lēvi, tandem fusca. Sporis hyalinis, sphericis, membrana crassa, lēvi.

P. alvei, sp. nov. Characteres ut supra. Mycelium album. Hyphis 2–6 μ (saepissime 5 μ diam.). Chlamydosporis 9·5–4·5 × 7–4·5 μ. Cystis 40–20 × 30–20 μ; membrana cysti tandem atroviridi. Sporis 3·7–4·7 μ (saepissime 4·3 μ diam.).

Hab. ad pollinem in favis *Apis mellificae*.

Pericystis, gen. nov. Hyphae creeping or suberect, interwoven, branched, septate, having many cells converted into chlamydospores, ultimately breaking up. Chlamydospores terminal, lateral and intercalary, subglobose or irregularly oval, thick-walled, smooth. Cysts subglobose or ovoid, borne on three to five hyphal branches, containing numerous spores. Cyst-wall membranaceous, structureless, smooth, ultimately dark-coloured. Spores hyaline, spherical, thick-walled, smooth.

P. alvei, sp. nov. Characters of the genus. Mycelium white. Hyphae 2–6 μ (average 5 μ diam.). Chlamydospores 9·5–4·5 × 7–4·5 μ. Cysts 40–20 × 30–20 μ; cyst-wall ultimately dark green. Spores 3·7–4·7 μ (average 4·3 μ diam.).

Hab. on pollen in the combs of the honey bee.

In conclusion, I wish to express my thanks to Miss A. Lorrain Smith for her kindness in giving me much helpful advice.

Summary.

A new fungus, *Pericystis alvei*, prevalent in bee-hives, is described, and its probable life-history discussed.

EXPLANATION OF PLATES LXXV AND LXXVI.

Illustrating Miss Betts's paper on *Pericystis alvei*.

The magnification is uniform for Plate LXXV, and is approximately × 700. The figures were drawn with the aid of a camera lucida from unstained material. All the specimens (with the exception of those figured in Plate LXXV, Fig. 2, *a*, and Plate LXXVI, Figs. 11 and 12) were taken from cultures on honey-gelatine.

PLATE LXXV.

Fig. 1. Hyphae and chlamydospores from a young culture. *a*, hypha with few spores; *b*, spores on lateral processes.

Fig. 2. Hyphae and chlamydospores from an older culture. *a*, an old hypha, cells between spores have lost their contents; *b*, an oidium-hypha (from comb).

Fig. 3. Chlamydospores. *a*. spore germinating, and a chlamydospore forming in the resulting hypha.

Fig. 4. Young cyst; early stage, before fusion.

Fig. 5. Two young cysts on one hypha, after fusion.

Fig. 6. Young cyst; shortly before wall changes colour.

Fig. 7. Cyst; wall has turned green, but spores not yet visible.

Fig. 8. Mature cyst.

Fig. 9. Cyst-spores.

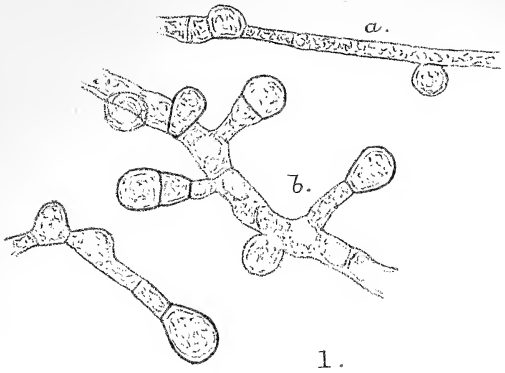
PLATE LXXVI.

Fig. 10. Mycelium and cysts. × 50.

Fig. 11. *P. alvei* growing on pollen in comb.

Fig. 12. Pollen from cells, attacked by *P. alvei*, and converted into hard plugs

(Figs. 11 and 12 are slightly more than twice natural size.)



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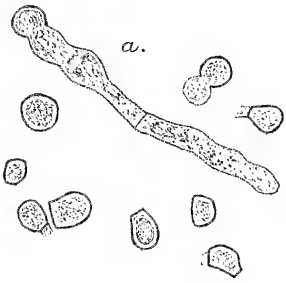

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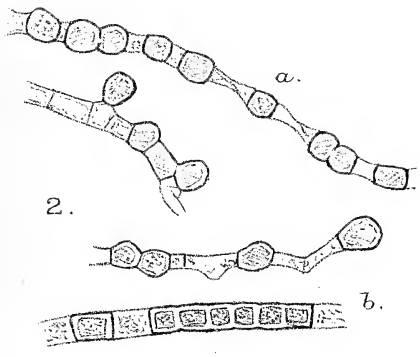

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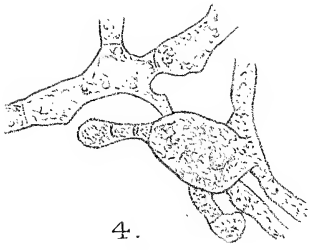

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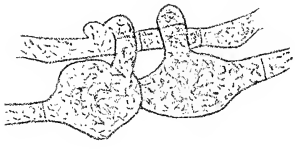

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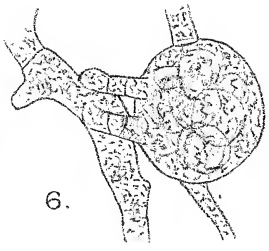

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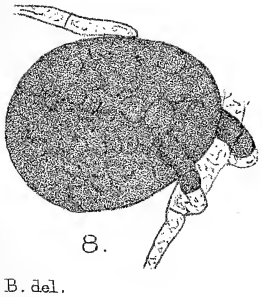

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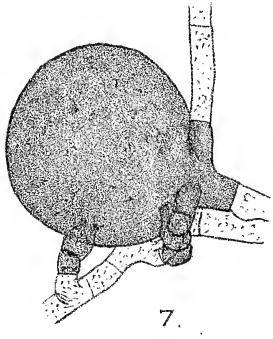

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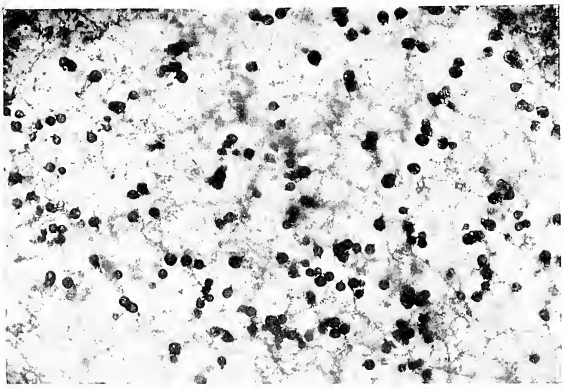
BETTS — PERICYSTIS.



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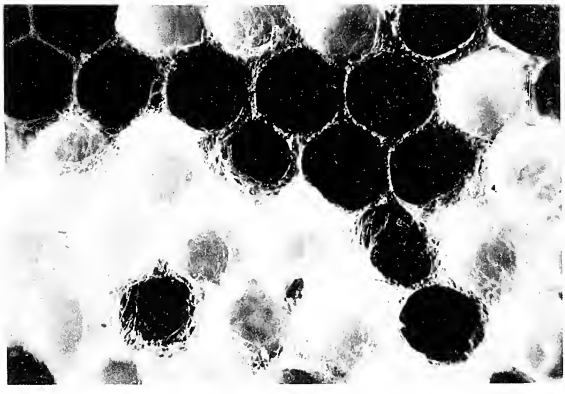

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BETTS — PERICYSTIS.

The Formation and Physiological Significance of Root Nodules in the Podocarpineae.

BY

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With Plates LXXVII–LXXX.

THE assimilation of atmospheric nitrogen by plants, belonging to families other than the Leguminosae, which possess structures on their roots known as nodules, has long been recognized in the Cycadaceae, Elaeagnaceae, *Alnus*, and *Podocarpus*. Quite recently, too, Bottomley has shown that the root nodules of *Myrica Gale* are intimately associated with the fixation of free nitrogen.

Nobbe and Hiltner, in 1899, demonstrated that the nodules of *Podocarpus* were active agents in the utilization of atmospheric nitrogen. In their experiments it was found impossible to cultivate plants of *Podocarpus* in the absence of the fungus which caused the nodule formation, but plants possessing nodules were grown for five years in quartz sand from which nitrogen was entirely absent, and during this period they remained perfectly healthy and grew well.

The earlier observers failed to demonstrate the presence of Bacteria in the nodules of these non-leguminous plants, but they found numerous hyphal-like structures in them, which they considered to be the hyphae of a mycorhizal fungus forming a symbiotic association with *Podocarpus*. Shibata described a mycelium, composed of non-septate hyphae possessing numerous nuclei, as being present in the cortical cells of the nodule. His investigations showed that these hyphae are eventually disorganized and absorbed, through the agency of a proteolytic ferment, by the host cells. These and similar observations in connexion with *Alnus*, *Elaeagnus*, and *Myrica* led to the idea that the formation of the nodules of non-leguminous plants was caused by the presence of an endophytic mycorhiza. More recent investigations, however, have shown this idea to be erroneous in some cases. Bottomley, in 1909, isolated and grew the nitrogen-fixing organisms *Pseudomonas radicicola* and *Azotobacter* from *Cycas* tubercles,

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and, in 1912, he has shown the nodules of *Myrica Gale* to be bacterial in origin. The author has described the root tubercles of *Alnus* and *Elaeagnus* as being caused by the infection of the root with a species of *Pseudomonas radicicola*, which afterwards flourishes in the cortical cells of the nodule.

The first part of the present series of investigations was carried out with material of *Podocarpus Totara*, *Podocarpus elongata*, and *Podocarpus chilina* obtained from the Royal Botanical Gardens, Kew, at various seasons of the year. At the suggestion of Professor Bottomley, however, the observations were extended to comprise the whole of the group Podocarpineae; and through the kindness of the Director of the Royal Botanical Gardens, Kew, roots of *Microcachrys tetragona*, *Dacrydium Franklini*, *Saxegothaea conspicua*, and *Phyllocladus trichomanoides* were obtained.

In all the genera of the Podocarpineae numerous nodules are present on the small roots, along the sides of which they are arranged in two very definite rows, thus producing a beaded appearance (Pl. LXXVII, Figs. 1–5). In the four species of *Podocarpus* examined, *P. Totara*, *P. elongata*, *P. chilina*, and *P. alpina*, the nodules are almost spherical in shape, attaining a diameter of 0·8 to 1 mm., and are very symmetrically disposed along the two sides of the root (Fig. 1). The nodules are very numerous, occurring quite close to one another along the whole length of the roots, which branch relatively infrequently. In general appearance the roots of *Dacrydium Franklini* resemble those of *Podocarpus*, but they branch somewhat more frequently; the nodules are smaller, only attaining a diameter of 0·54 to 0·68 mm., and although they occur in two rows along the sides of the roots they are less regularly arranged (Fig. 2). The roots of *Microcachrys tetragona* are very fibrous, numerous very fine branches being produced, and along two sides of all these numerous small rootlets, minute nodules, with a diameter of 0·25 to 0·35 mm., are arranged side by side in close proximity with one another (Fig. 5). In *Saxegothaea conspicua* the roots present a coralline appearance, being quite small, but so repeatedly branched that dense clusters are produced, and the roots bear two very closely packed rows of nodules, which are 0·4 to 0·5 mm. across (Fig. 3). *Phyllocladus trichomanoides* has a much more straggling root, and the nodules are less numerous than in the other genera, but are present upon the small roots, where there are two rows, thus presenting the characteristic beaded appearance. In this genus they appear to be more widely separated from one another, and attain a diameter of about 0·5 mm. (Fig. 4).

The roots and nodules of all these plants being quite small, it was found convenient to mount some of them whole for examination under the low power of the microscope, which immediately revealed the fact that the nodules in all the genera are endogenous in origin and each possesses a small but well-defined vascular strand, which is connected with the root-

stele. In the material of *Podocarpus* obtained during the autumn and winter the surface of the nodules was always quite smooth, whilst in *P. chilina*, *P. elongata*, and *P. Totara* obtained in the spring the epidermal cells in a few nodules were growing out into hairs, and a few were covered with numerous fine non-septate hairs about the same length as the nodule. In *P. alpina* gathered in February a very large number of the nodules were clothed with a thick mantle of long hairs, some of which were quite three times as long as the nodules. The hairs were most abundant on nodules which were one year old, and which were, in many cases, attached to the roots in, or near, the regions where they themselves were producing root-hairs, but they were also sometimes present on the nodules, which were two, three, or even four years old. In *Microcachrys tetragona* the surface of the nodules was generally quite smooth (Fig. 8), but occasionally small protuberances from the surface cells were visible. In *Dacrydium Franklini*, *Saxegothaea conspicua*, and *Phyllocladus trichomanoides* the production of hairs, in the spring, is a constant feature of all nodules which have completely emerged from the cortex of the root. It is, therefore, usually in the beginning of the second season's growth that practically all the surface cells of the nodules are capable of growing out to produce hairs, which radiate in all directions from the nodule (Figs. 6 and 7). In *Dacrydium* they attain a length about equal to that of the nodule in many cases, but sometimes they are longer; in *Saxegothaea* a thick mass of relatively long hairs covers the nodules, whilst in *Phyllocladus* they are very long and straggling.

These hairs are evidently comparable to root-hairs; they are non-septate structures produced by the epidermal cells of the nodules, which are themselves modified roots. Like other root-hairs they are produced abundantly in the spring, whilst later in the year, in the autumn and winter, they collapse and eventually disappear. Any nodule which has reached maturity seems to be capable of producing these structures, but since they persist for very varying periods of time, nodules of diverse ages may be seen to possess hairs on their surface. When the nodule is more than two years old, however, they can distinctly be seen to be portions of the old hairs, which have remained attached to the nodules. They are evidently produced in response to certain environmental conditions in *Podocarpus* and *Microcachrys*, where it is only some of the nodules which produce these outgrowths, but in *Dacrydium*, *Saxegothaea*, and *Phyllocladus* there is a much greater tendency for the formation of hairs, their presence in the spring being characteristic of these genera. The frequent presence of root-hairs, not only on the roots but also on the nodules in all the genera of the Podocarpineae, precludes their association with a mycorhizal fungus.

In order to ascertain whether there were any Bacteria present in the nodules, some were removed from the roots, carefully washed with distilled

water, then crushed on a slide, and the material so obtained allowed to dry. This was then stained with Ziel's carbol fuchsin or aniline gentian violet, and subsequent microscopical examination revealed the presence of numerous small rod-shaped Bacteria. These organisms were apparently identical with the *Pseudomonas radicicola* found in the root nodules of leguminous plants and also the Cycadaceae, Elaeagnaceae, *Alnus*, and *Myrica*. Many of them contained two or three minute, spherical, densely staining bodies, which are so characteristic of that organism.

For further investigation with regard to the nature and growth of this organism, the exterior of some nodules from the *Podocarpus* roots was sterilized by placing them for about two minutes in a sterilizing fluid of the following composition:

2·5 c.c. concentrated hydrochloric acid,
1 grm. mercuric chloride,
500 c.c. water.

They were then removed and thoroughly washed in distilled water, precautions being taken that all the instruments used were quite sterile. The nodules were then crushed, and their contents placed in a nutrient medium of the following composition:

1 grm. saccharose or glucose,
0·5 grm. acid potassium phosphate,
0·02 grm. magnesium sulphate,
0·1 grm. calcium carbonate,
100 c.c. distilled water.,

The solution was then incubated at a temperature of 28° C. for two days, at the end of which period it had become quite cloudy owing to the rapid development of the rod-shaped Bacteria.

Pure cultures of this organism were also obtained on solid media, prepared by adding 2 per cent. agar-agar to the above nutrient solution. The colonies produced, after two days' incubation at 28° C., were ovoid to circular in shape, entire, raised, shining, and viscous, with a diameter of 0·75 to 1 mm. They were apparently characteristic colonies of *Pseudomonas radicicola*.

Since this organism so closely resembled, in its appearance and growth on nutrient media, species obtained from the nodules of legumes and the non-leguminous plants mentioned above, which are known to assimilate atmospheric nitrogen, 100 c.c. of the above nutrient solution containing saccharose was placed in each of four Erlenmeyer flasks, 300 c.c. capacity. To two of them, the controls, was added 2 c.c. of a liquid culture of the organism, obtained, as described above, from *Podocarpus* nodules. All the flasks were then sterilized by autoclaving, at a temperature of 140° C. and

a pressure of two and a half atmospheres, for ten minutes. After cooling, 2 c.c. of the same culture as had been added to the controls was used to inoculate each of the other two flasks. All were then incubated at 28° C. for ten days. During this period the contents of the control flasks remained quite clear, whilst the others had become cloudy owing to the presence of a growth of a very slimy nature. The nitrogen content of the flasks was determined by the Kjeldahl method of analysis, with the following results:

Nitrogen found in the control.	*Nitrogen found in the culture.*	*Gain in nitrogen due to organism.*
1·06 m.grm.	3·72 m.grm.	2·66 m.grm.
0·93 m.grm.	3·46 m.grm.	2·53 m.grm.

The increased nitrogen content of the solution in which growth took place can only have been produced by the activity of the living organisms obtained from the *Podocarpus* nodules and introduced into the media, and must have been caused by the utilization of the free nitrogen of the atmosphere by these organisms during the processes of metabolism. Hence it becomes evident why Hiltner was able to grow *Podocarpus* plants in pure sand, containing no nitrogen, provided nodules were present on the roots. The Bacteria in the nodules assimilate atmospheric nitrogen, and thus render it available to the *Podocarpus* plants.

Material used for sectionizing was fixed in either Flemming's or Bouin's fixative, or absolute alcohol. Microtomed sections were stained with Flemming's triple, Heidenhain's iron-haematoxylin, Ziel's carbol fuchsin, or Kiskalt's amyl gram stain with very satisfactory results.

The nodules of *Podocarpus*, *Microcachrys*, *Dacrydium*, *Saxegothaea*, and *Phyllocladus*, as of other non-leguminous plants, are modified lateral roots, being developed from the pericycle immediately opposite the protoxylem of the diarch root (Pl. LXXVIII, Figs. 10 and 11), and possessing a central stele (Pl. LXXX, Fig. 17). Owing to some cells of the pericycle becoming meristematic (Pl. LXXVIII, Fig. 10, *a*, *b*), a small swelling is produced beneath the endodermis of the root. These cells continue to develop, and as the protuberance increases in size a plerome becomes differentiated (Fig. 10, *c* and *d*), which later produces a vascular strand directly connected with the vascular cylinder of the root (Pl. LXXVIII, Figs. 11 and 12, and Pl. LXXIX, Figs. 13 and 14). This structure gradually pushes its way through the cortex of the root and may become a lateral root, but very frequently, as indicated by the enormous number of nodules present on the roots, its growth is arrested, with the result that it produces a small almost spherical structure known as a nodule. This arrest in its development is caused by the infection of the young cells with the nitrogen-fixing organism *Pseudomonas radicicola*, before it emerges from the cortex of the root (Fig. 10).

The Bacteria enter the cortex of the root by means of the root-hairs, in which they have frequently been seen, particularly in some young roots of *Phyllocladus* (Fig. 9). Having once penetrated the cell-wall and entered the hair, they multiply rapidly, producing a zooglea thread, exactly comparable to the infection threads of the Leguminoseae. The thread passes into a neighbouring cell, and subsequently numerous zooglea threads are produced which pass from cell to cell of the cortex (Figs. 9 and 16). When, however, a meristematic region arises in the neighbourhood of the Bacteria, they appear to be stimulated to further and more rapid development, and chemotactically attracted by the meristematic tissue, so that some of the infection threads penetrate into the young tissue (Fig. 10). Having once entered this new structure the Bacteria divide repeatedly, and the zooglea threads so produced ramify through the young cortical cells, causing their expansion, but arresting their further division, and consequently the immediate growth of the nodule.

A mature nodule possesses a small stele (Fig. 17), surrounded by a definite endodermis, the cells of which are slightly thickened on the outer side, although there is no evident radial dot, and many of them, as in the endodermis of the root, contain a deposit of tannin which renders them more apparent. In all the species of *Podocarpus*, where the nodules attain the greatest size in the group Podocarpineae, the nodular stele becomes distinctly diarch (Fig. 17); so also in *Saxegothaea* it usually becomes thus differentiated, but in *Microcachrys* and *Phyllocladus* the vascular strand is frequently very rudimentary, the xylem only consisting of two or three tracheides, around which are two or three layers of parenchymatous cells, and then an endodermis. The stele is continuous with that of the root and traverses about half the length of the nodule. The remaining nodular tissue is composed of parenchymatous cells, in which there is no differentiation of a meristematic zone, all the cells are similar, but the outermost layers after the nodule has emerged from the root for some time become slightly more compressed, and their walls become a little thicker, the outer ones eventually becoming slightly suberized (Figs. 11 and 17). The outermost layer of cells of a mature nodule, like the epidermal cells of the roots, have the capacity of growing out to form hairs (Figs. 6 and 7). This they characteristically do in *Dacrydium*, *Saxegothaea*, and *Phyllocladus* in the spring of their second year, and it sometimes also occurs in *Podocarpus* and *Microcachrys*.

The cortical cells of both roots and nodules in all the genera appear to be traversed by numerous very narrow filaments. These, however, when stained with Kiskalt's amyl gram stain are seen to be composed of numerous rod-shaped Bacteria, obviously *Pseudomonas radicicola*, which are embedded in a mass of slime, forming a zooglea, and thus giving the appearance of a network of hyphae (Figs. 15 and 16). These threads are capable of pene-

trating the cell-walls of the host tissue, and where the zooglea thread comes in contact with the cell-wall it widens out somewhat before passing through into the neighbouring cell. Expansion in this area is characteristic of infection threads produced by *Pseudomonas radicicola* in leguminous nodules. In the interior of the host cell some of the Bacteria become isolated from the infection threads and divide independently, forming groups of organisms. The threads vary considerably in width, even in the same nodule, sometimes being only a single chain of organisms, at others, especially in *Podocarpus*, as many as five, six, or even more are arranged across side by side, so as to form apparently one thread. The Bacteria are quite evident in the cells when the sections are treated with Flemming's triple stain or Heidenhain's iron-haematoxylin. They also respond, in the manner described by Harrison and Barlow as being characteristic of *Pseudomonas radicicola*, to treatment with alcohol after Gram's method of staining, that is, the aniline gentian violet is rapidly removed by ethyl alcohol, but not by amyl alcohol.

The zooglea threads are undoubtedly analogous to those present in leguminous nodules, and like them they appear to have an affinity for the nucleus of the host cell. Shibata described the nuclei of the cortical cells of *Podocarpus* nodules, which he said contained mycorhizal hyphae, as assuming an amoeboid form, and subsequently dividing directly until as many as eight nuclei were present in a single cell. The nuclei appear to be stimulated to activity when they become surrounded by a large number of Bacteria, for many instances have been seen, especially in *Podocarpus*, where more than one nucleus has been present in the cell containing Bacteria. The increased number is evidently produced by amitosis, no karyokinetic figures having been observed in such cells, but numerous elongated nuclei, and others in which the elongated structure is constricted in the centre and about to form two nuclei (Fig. 15). This effect of the presence of Bacteria around the nucleus of the host cell is quite comparable with that seen in the root-nodules of *Elaeagnus*, where the nuclei have been described by the author as becoming amoeboid and very irregular in shape, and then in some cases disintegrating.

As the season advances towards autumn further changes occur in the cortical cells of both root and nodules in all the genera. The cytoplasm and nuclei are gradually used up in the deposition of numerous cellulose bars on the walls of the cells, which gives them a scalariformly striated appearance, and assists in keeping them distended. The Bacteria tend to migrate from these cells, which appear first in isolated positions and thus become quite devoid of living contents. Gradually, however, almost the whole of the parenchymatous cortex, except the two or three outermost layers, becomes transformed into this water-storage tissue, and here the Bacteria continue to live in a quiescent state through the winter, until

environmental conditions are again favourable for their activity, and also that of the host plant (Figs. 11, 12, and 16).

The following spring, in the nodules of all the genera of the Podocarpineae examined, some of the cells immediately below the endodermis at the apex of the vascular strand of the nodule become meristematic (Fig. 12). A rapid formation of new tissue ensues in the centre of the nodule, pushing out and crushing the old water-storage tissue, which now readily collapses. The formation of new tissue beneath the endodermis causes the rupture of the latter, but later, when the rapid cell division ceases, a new endodermis is differentiated around the apex of the stele (Fig. 13). The Bacteria resume their activity and are stimulated to produce numerous infection threads, which penetrate into the newly formed cortical cells (Fig. 13). In this way living, active cortical cells are produced inside the old tissue of the nodule, which now forms a protective covering around the new nodule. This formation of a meristematic zone at the apex of the vascular cylinder, and the consequent production of new internal tissue, recurs year after year, so that a number of successive protective layers are formed around the active cortical cells by the annual displacement and collapse of the tissue formed the previous year (Fig. 14). The outermost zone in all the nodules always remains intact, being composed of a few layers of cells which are part of the first year's growth. It is this zone which has the capacity for producing hairs if occasion arises. The tissue produced in succeeding years becomes very crushed, and often, as in *Microcachrys* and *Saxegothaea*, almost obliterated, except at the base, just around the vascular strand, where the annual additions are always apparent.

In *Saxegothaea conspicua*, where the branching of the root is extremely prolific and the nodules are produced side by side in very close proximity to one another, the nodules have frequently been observed to branch (Figs. 7, 18, and 19). In the spring, when the meristematic zone arises at the apex of the vascular strand, bifurcation of the zone takes place (Fig. 18), with the result that the nodule at first appears to have a branched stele; but later a distinct zone of cortical cells is produced by the apex of each branch of the stele. In this way two new cortical zones centred round a portion of the stele are produced inside the original nodule (Fig. 19), and these, at first, compress the tissue formed the previous year or years, and by their united activities eventually break through it. The tissue of the original nodule thus becomes ruptured and later is cast off; the two nodules produced in its place are each complete, independent structures, which subsequently develop according to the usual method described above. The capacity which the nodules in this genus have for branching gives the root its characteristic coralline, rather than beaded, appearance, a very large number of nodules being produced around the root eventually by their

bifurcation. The rupture and subsequent shedding of the old outer nodular tissue when two nodules are produced in place of the old ones also adds to the possibilities of hair production by the surface cells. The old protecting layers having disappeared, the outer cells of the new nodules can, on reaching maturity, grow out into these structures. This explains the apparently more constant appearance of hairs on the nodules of *Saxegothaea* than on those of the other genera, because, on any portion, this method of branching makes it probable that there will be many nodules present which are only one year old, and which will probably be sending out hairs.

In their method of growth the nodules of the Podocarpineae differ from all other root nodules with which they are associated. The leguminous nodules are annual structures and are not modified roots, and the non-leguminous nodules of the Cycadaceae, Elaeagnaceae, *Alnus*, and *Myrica* are modified roots with a perennial habit, each possessing an apical meristematic region, by means of which their growth is continued from year to year. The nodules of the Podocarpineae, although perennial modified roots like the latter, are unique in having no persistent meristematic zone, the tissue definitely functioning for one year only. The formation of new tissue is always endogenous in this group, a new nodule being formed inside the old one year after year, whilst in the other non-leguminous nodules the growth is continued at the apex.

The nodules of the Podocarpineae are typically simple structures, whilst those of other non-leguminous plants are characteristically branched. The bifurcation of the nodule which occurs in *Saxegothaea*, as described above, recalls the branched nodules of the Cycadaceae, Elaeagnaceae, and *Alnus*, but in these there is no breaking through of old tissue ; this latter occurs in *Myrica*, when the stele continues its growth and eventually penetrates the nodule, emerging as a small root. There is, however, no other known case where the original nodule, by branching, becomes replaced, as it does in *Saxegothaea*, by two apparently simple nodules.

The outer zones of dead empty cells in the old nodules provide a suitable substratum for the development of various Fungi, and in *Podocarpus* one sometimes finds true fungal hyphae in these cells. Some of these have probably been described by other observers, and may, indeed, as they suggest, be of a mycorhizal nature, forming a symbiotic association with the roots of the plant, but they are evidently not concerned with the formation of the nodules. This conclusion is supported by the production of root-hairs on the nodules at the beginning of their second year's growth, which sometimes occurs in *Podocarpus Totara*, *Podocarpus elongata*, *Podocarpus Chilina*, and *Microcachrys tetragona*, usually occurs in *Podocarpus alpina*, and always occurs in *Dacrydium Franklini*, *Saxegothaea conspicua*, and *Phyllocladus trichomanoides*. Shibata, however, does not suggest that the fungus he describes causes the formation of the nodules, because he remarks

that they may attain their full size even when they are not infected with it.

In the old roots there is a formation of periderm (Fig. 14), the phellogen arising immediately outside the endodermis, so that when this takes place the whole of the cortex is thrown off, amongst which are some cells containing Bacteria. This, however, does not usually occur until the root is two or three years old, by which time the formation of new nodules has ceased in this region, and the old ones already have a supply of organisms for the infection of their new cells.

These investigations entirely support the theory that the root nodules of *Podocarpus* are actively concerned in the assimilation of atmospheric nitrogen, not, however, primarily owing to the presence of a mycorhizal fungus, but to their symbiotic association with a nitrogen-fixing bacterium. This not only occurs in all the species of *Podocarpus* examined, but also in *Microcachrys*, *Dacrydium*, *Saxegothaea*, and *Phyllocladus*, four other genera of the Podocarpineae. The production of nodules on the roots is thus a constant feature throughout the Podocarpineae, their development and morphology being in every case of the same characteristic type, and they are always inhabited by apparently the same organism. This organism is morphologically and physiologically identical with *Pseudomonas radicicola* found in the nodules of the Leguminosae, Cycadaceae, Elaeagnaceae, *Alnus*, and *Myrica*. This organism enters the roots of plants of the Podocarpineae, and the infection of the meristematic cells of a young lateral root causes its transformation into the structure known as a nodule. In this tissue the Bacteria multiply very rapidly, and in their growth they have been shown to utilize the free nitrogen of the atmosphere, so that by their development in the nodules attached to the plant they are rendering that vast store of food material available, and enabling the plant to grow even under circumstances which exclude all other nitrogen supply.

The division of the Coniferales known as the Podocarpineae are inhabitants of the southern hemisphere, principally Australia. The dominant genus *Podocarpus* has been considered related to the Araucarineae, through *Dacrydium* and *Saxegothaea*, on certain morphological grounds, and this association is supported by the common and peculiar geographical distribution of the two groups. Tison supports this idea, from the behaviour of the two systems of vascular bundles found in the megasporophyll, but he also says that *Podocarpus* and *Saxegothaea* are very definitely related through *Microcachrys*. There are many arguments against a union of the Podocarpineae and the Araucarineae, but all the morphological work on the various parts of the plants indicates that *Saxegothaea* and *Podocarpus* are the most widely separated of all the genera placed in the Podocarpineae, and *Dacrydium* and *Microcachrys* form connecting links between them. This conclusion is supported by the morphology of the root nodules in this

group, those of *Podocarpus* and *Microcachrys* being most nearly alike, differing only in size, whilst *Dacrydium* resembles *Saxegothaea* in the characteristic prolific formation of hairs, and *Saxegothaea* differs from all the rest in the extremely frequent branching not only of the root but also of the nodules.

The genus *Phyllocladus* has led to considerable discussion as to its relationships, since it possesses some of the characters of both groups of the Taxaceae, namely, the Taxineae and the Podocarpineae, and consequently may belong to either of these groups, or it may be an intermediate group between them. Young has summarized the characters which it possesses that are common to these groups respectively, and has decided that the features which relate it to the Podocarpineae are more fundamental than those relating it to the Taxineae, and are also too fundamental to admit of its being placed in an intermediate group. The presence of root nodules, indicating the capability of the root to form a symbiotic association with the nitrogen-fixing organism *Pseudomonas radicicola,* in *Phyllocladus*, as well as in all the other genera of the Podocarpineae, is an additional character relating *Phyllocladus* with this group.

In conclusion, I wish to thank the Director of the Royal Botanic Gardens, Kew, for supplying the material necessary for my work, and also Professor W. B. Bottomley for his suggestions and kindness during the progress of these investigations, which have been carried out in his laboratory at King's College, London.

Summary.

1. Root nodules are present in all the genera of the Podocarpineae examined, namely, *Podocarpus*, *Microcachrys*, *Dacrydium*, *Saxegothaea,* and *Phyllocladus.*

2. The nodules are modified lateral roots. They are perennial unbranched structures, except in *Saxegothaea,* where bifurcation frequently occurs.

3. The outer layer of cells in the nodules is capable of producing non-septate hairs at the beginning of the second year's growth in all the genera, but their presence is characteristic of the nodules *Dacrydium*, *Saxegothaea*, and *Phyllocladus.*

4. The nitrogen-fixing organism *Pseudomonas radicicola* penetrates a root-hair and from thence enters the cortex of the root, where it propagates itself.

5. The nodules, in all the genera, are produced by the infection of the meristematic tissue of the young root, before it emerges from the cortex of the parent root, by *Pseudomonas radicicola.*

6. A mature nodule is traversed for about half its length by a small

stele. In *Podocarpus* and *Saxegothaea* the stele is diarch; but it may remain more rudimentary, as in the small nodules of *Microcachrys*, where frequently only two or three tracheides are present. There is always an endodermis.

7. There is no differentiation of a meristematic zone in the cortical tissue. In this respect the nodules of the Podocarpineae differ from the nodules of all the other non-leguminous plants with which they associated.

8. The majority of the cortical cells of the root and nodules eventually become water-storage cells, and in some of these the Bacteria remain quiescent during the winter.

9. The Bacteria produce a very definite zooglea in the cells, and in these slime threads pass from cell to cell.

10. The nuclei of the host cells, in *Podocarpus*, are stimulated by the presence of the zooglea around them to elongate and then divide amitotically, so that several nuclei are frequently present in one cell.

11. In the spring the cells immediately below the endodermis at the apex of the nodular stele become meristematic and produce new cortical cells in the interior of the old nodule.

12. Successive zones of collapsed tissue surround the new cortical cells of the nodule year by year.

13. In *Saxegothaea* the meristematic tissue at the apex of the nodular stele frequently bifurcates before producing the new cortical cells, with the result that there are two centres of tissue formation and two new nodules are produced inside the old one, which is eventually ruptured and cast off.

14. The outer empty cells in *Podocarpus* are sometimes inhabited by fungal hyphae, which may be of a mycorhizal nature.

15. The Bacteria isolated from the nodules are found to be identical in structure, and growth in pure cultures, with *Pseudomonas radicicola* obtained from the root nodules of the Leguminosae, Cycadaceae, Elaeagnaceae, *Alnus*, and *Myrica*.

16. When isolated from the nodule the organism is capable of assimilating atmospheric nitrogen when grown on suitable media, and consequently its presence is undoubtedly beneficial to the plants of the Podocarpineae, with which it is associated.

17. The morphology of the nodules supports the theory that *Podocarpus* and *Saxegothaea* are the most widely divergent of the genera in the Podocarpineae, and that they are connected through *Microcachrys* and *Dacrydium*.

18. The presence of root nodules in *Phyllocladus* is additional evidence for regarding it as a member of the Podocarpineae rather than of the Taxineae, or a group intermediate between these two.

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DESCRIPTION OF PLATES LXXVII–LXXX.

Illustrating Miss Spratt's paper on the Root-nodules of the Podocarpineae.

In Figs. 6-8, *r* = root, *n* = nodule, *st* = stele, *h* = hairs, *a* = young nodule, *b* = nodule one year old, *c* = nodule two years old, and *d* = nodule several years old.

In Figs. 10-19, *x* = xylem, *ph* = phloem, *p* = pericycle, *e* = endodermis, *c* = cortex, *b* = Bacteria.

PLATE LXXVII.

Fig. 1. Root of *Podocarpus Chilina* with nodules. × 2.
Fig. 2. Root of *Dacrydium Franklini* with nodules. × 2.
Fig. 3. Root of *Saxegothaea conspicua* with nodules. × 2.
Fig. 4. Root of *Phyllocladus trichomanoides* with nodules. × 2.
Fig. 5. Root of *Microcachrys tetragona* with nodules. × 2.
Fig. 6. Root and nodules of *Dacrydium Franklini*. × 60.
Fig. 7. Root and nodules of *Saxegothaea conspicua*. *e* = branched nodule. × 60.
Fig. 8. Root and nodules of *Microcachrys tetragona*. × 60.

PLATE LXXVIII.

Fig. 9. Root-hair and cortical cells of *Phyllocladus trichomanoides*, showing the infection with *Pseudomonas radicicola*. *b* = Bacteria, *i.t.* = infection thread, *r.h.* = root hair, *e* = epidermal cell of root. × 730.

Fig. 10. Longitudinal section of a root of *Podocarpus Totara* with young nodules A, B, C, and D. *i.t.* = infection thread, *pl* = plerome, *w* = water-storage cell. × 105.

Fig. 11. Transverse section of a root of *Podocarpus chilina* with a mature nodule attached. *p.x.* = protoxylem, *s.x.* = secondary xylem. × 105.

Fig. 12. Longitudinal section of a root of *Podocarpus elongata* with a nodule at the beginning of its second year, showing the meristematic zone, *m.z.*, which will produce the new cortical tissue of the nodule. × 105.

PLATE LXXIX.

Fig. 13. Longitudinal section of a root of *Podocarpus elongata* with a nodule in the second year of its growth, when the new cortex has been formed. *n.c.* = newly formed cortex, *o.c.* = old cortex. × 105.

Fig. 14. Longitudinal section of a root of *Podocarpus chilina* with a nodule several years old. 1 = new tissue, 2, 3, 4, 5 = layers of tissue which formed the cortex of the nodule in preceding years, *c* = cork. × 105.

Fig. 15. Cortical cells of the nodule of *Podocarpus Totara* with Bacteria. *i.t.* = infection threads, *n* = nucleus of the host cell; *a*, *c*, and *d* are nuclei in stages of direct division. × 730.

Fig. 16. Cortical cells of the root of *Podocarpus Totara* with Bacteria. *i.t.* = infection threads, *n* = nucleus of host cell, *w* = water-storage cell. × 730.

PLATE LXXX.

Fig. 17. Transverse section of the root nodule of *Podocarpus elongata*. × 120.

Fig. 18. Longitudinal section of the root nodule of *Saxegothaea conspicua*, showing the branching of the meristematic zone. *c* = cortex formed last year, *a* = tissue formed the first year, *st* = stele, *m.z.* = meristematic zone, *h* = hair. × 120.

Fig. 19. Longitudinal section of the root nodule of *Saxegothaea conspicua*, showing two new nodules produced as the result of the branching of the meristem formed in the original nodule. *n* = cortex of the new nodule, *st* = stele, *a* = old nodular tissue being cast off. × 120.

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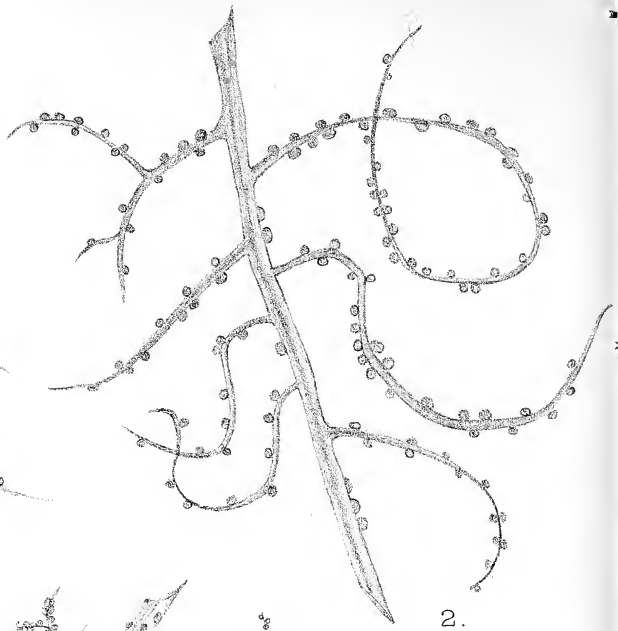

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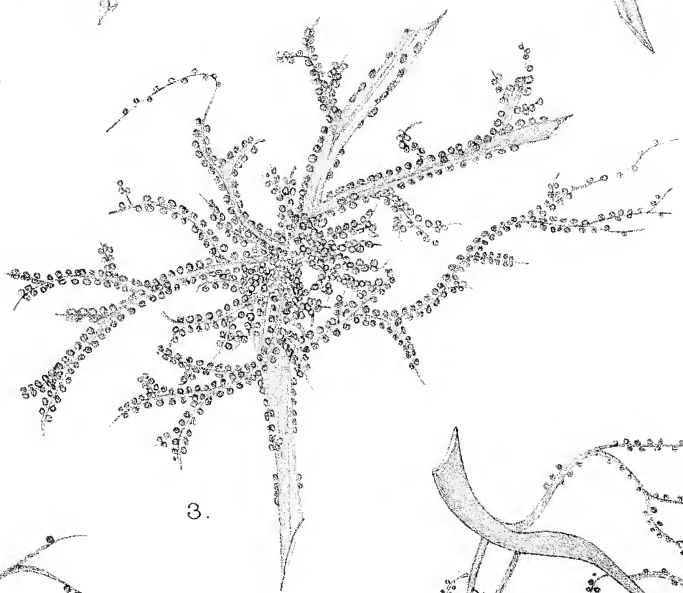

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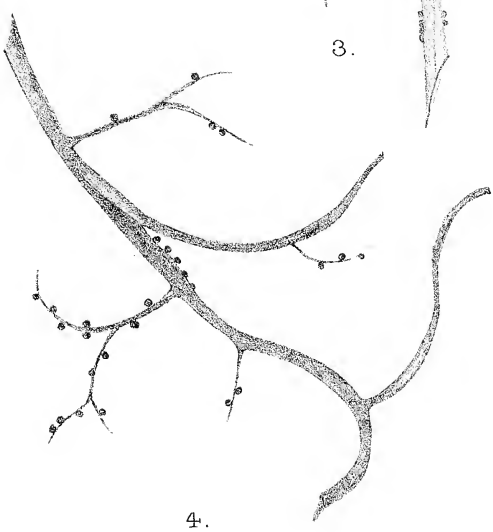

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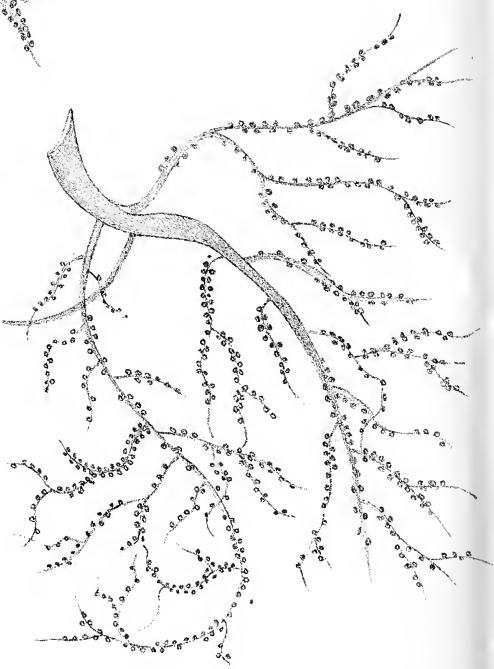

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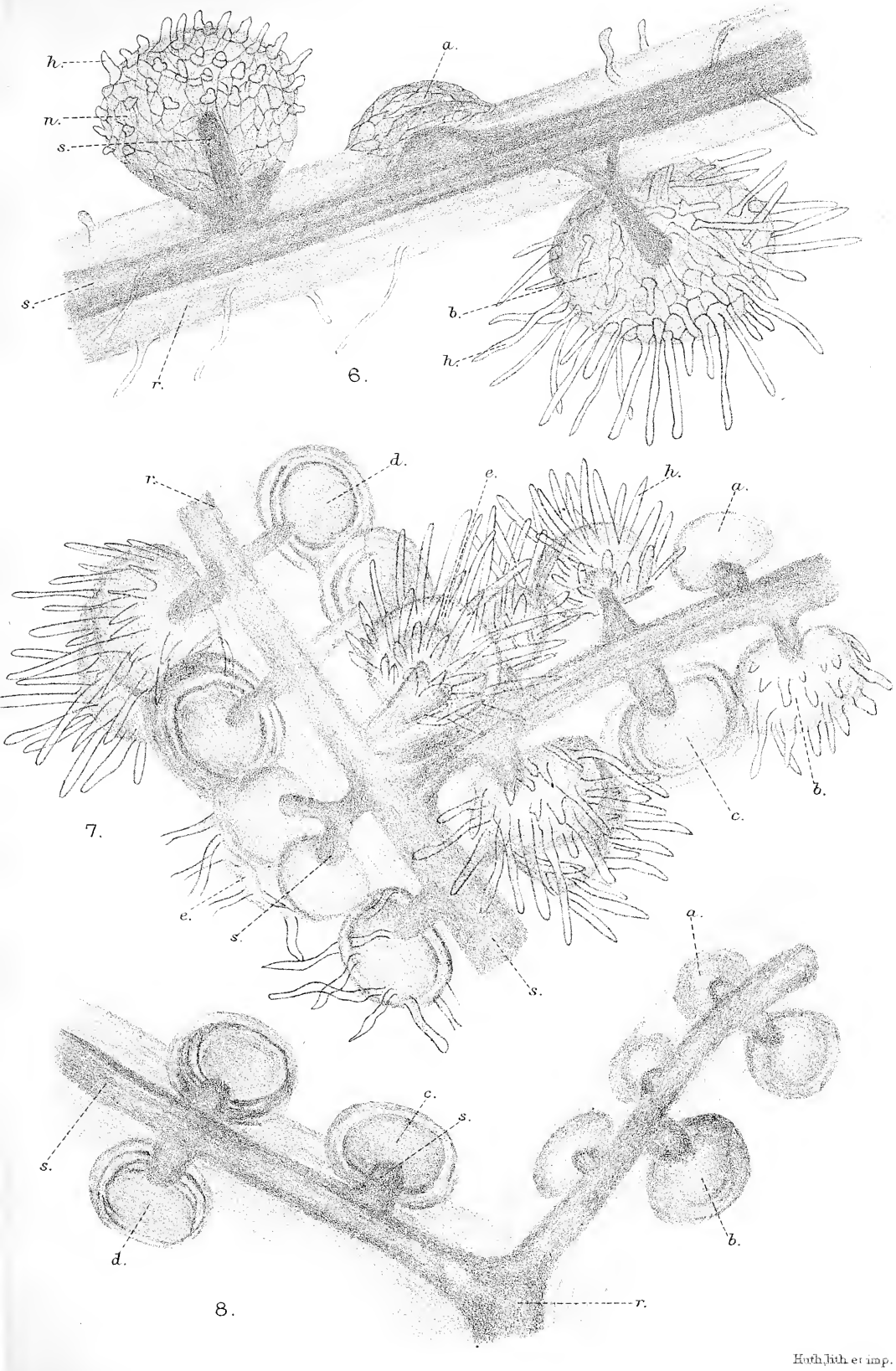

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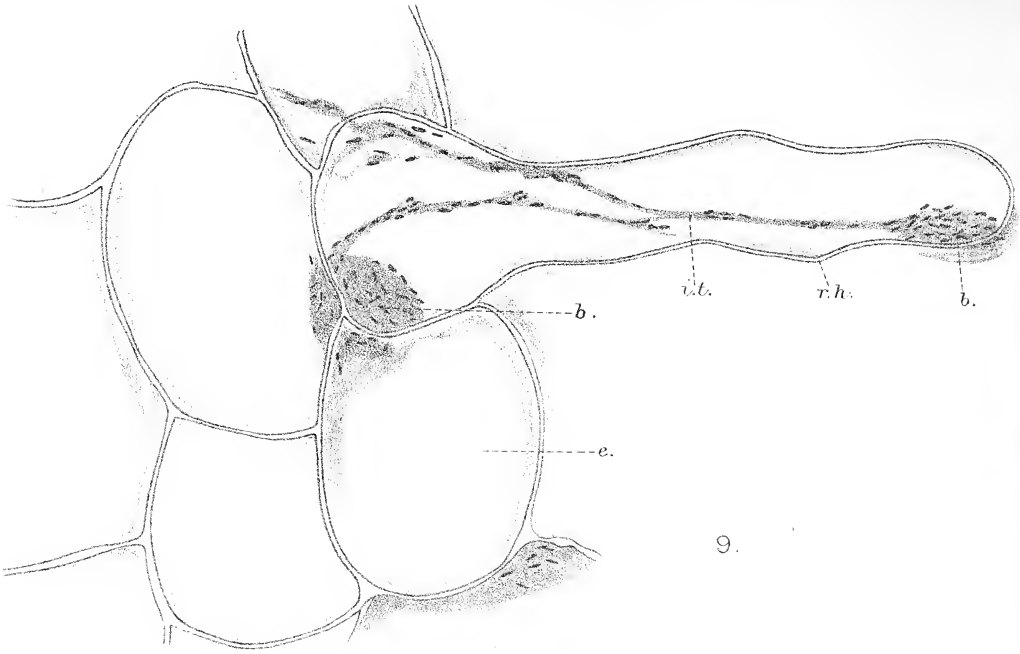

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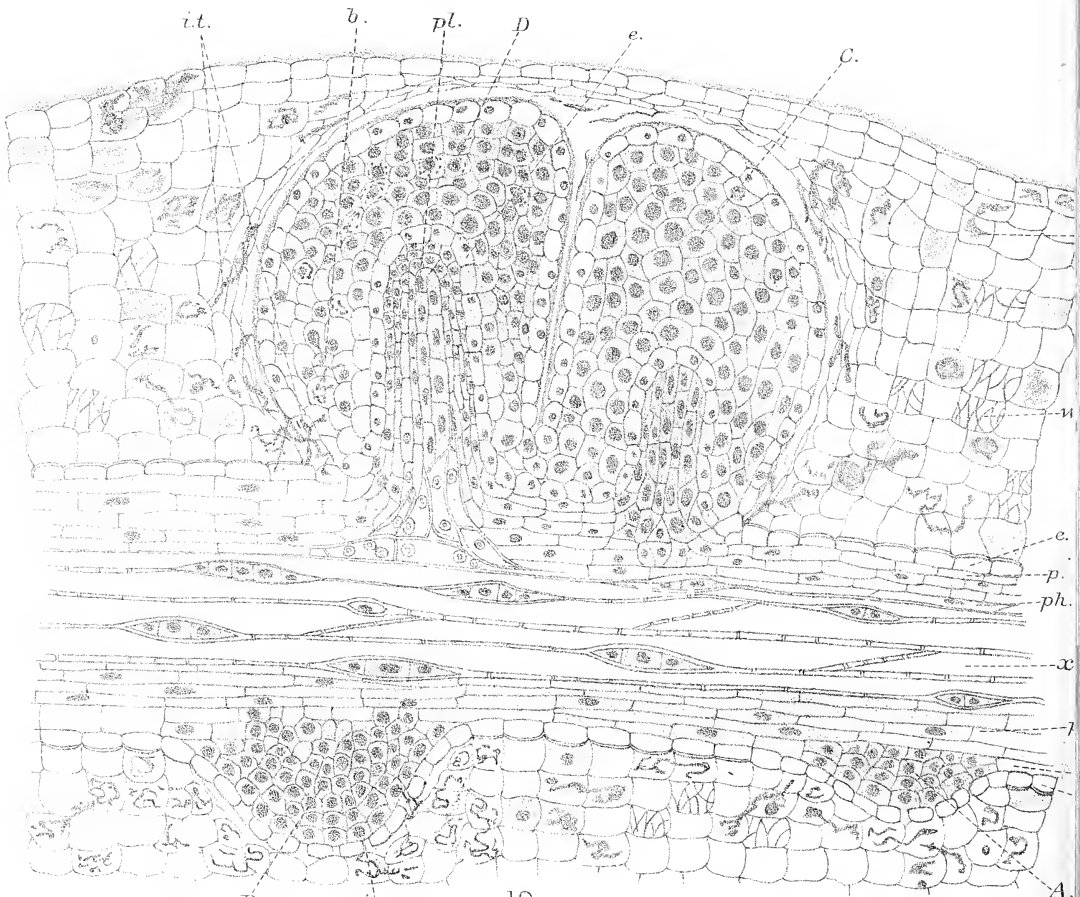

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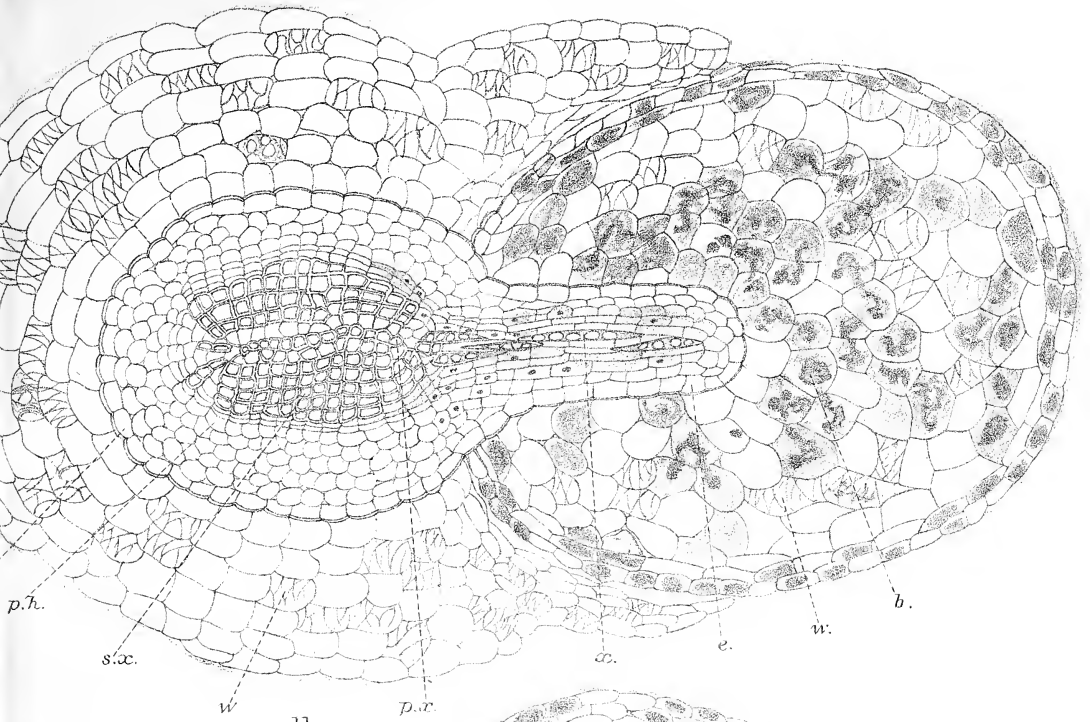

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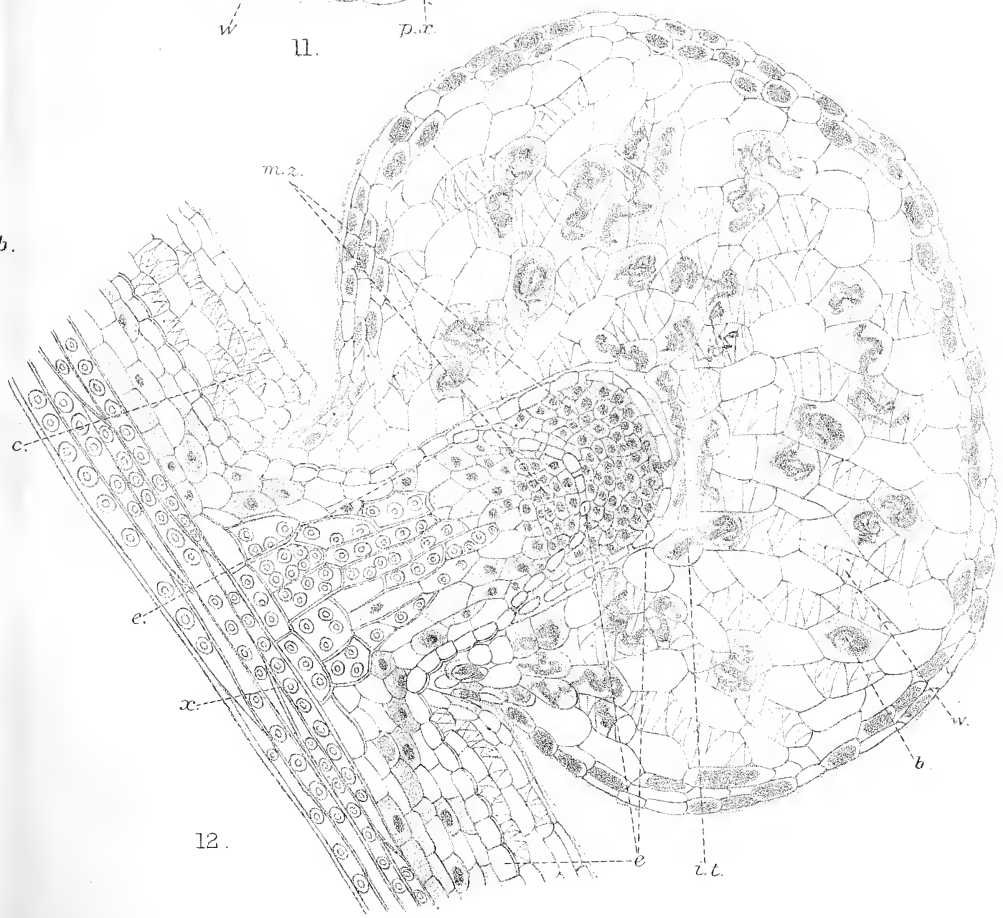

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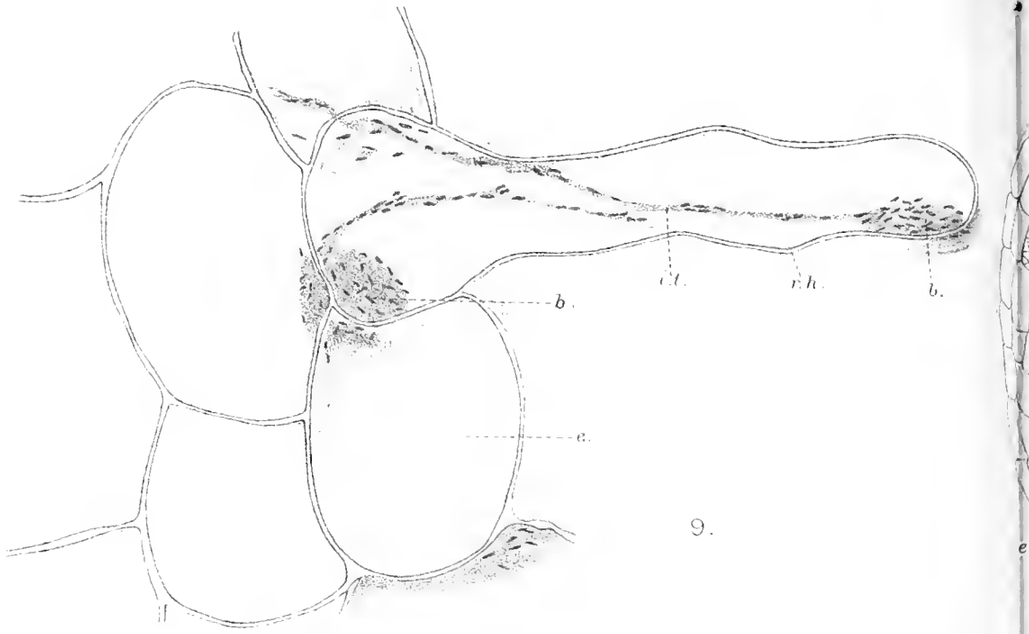

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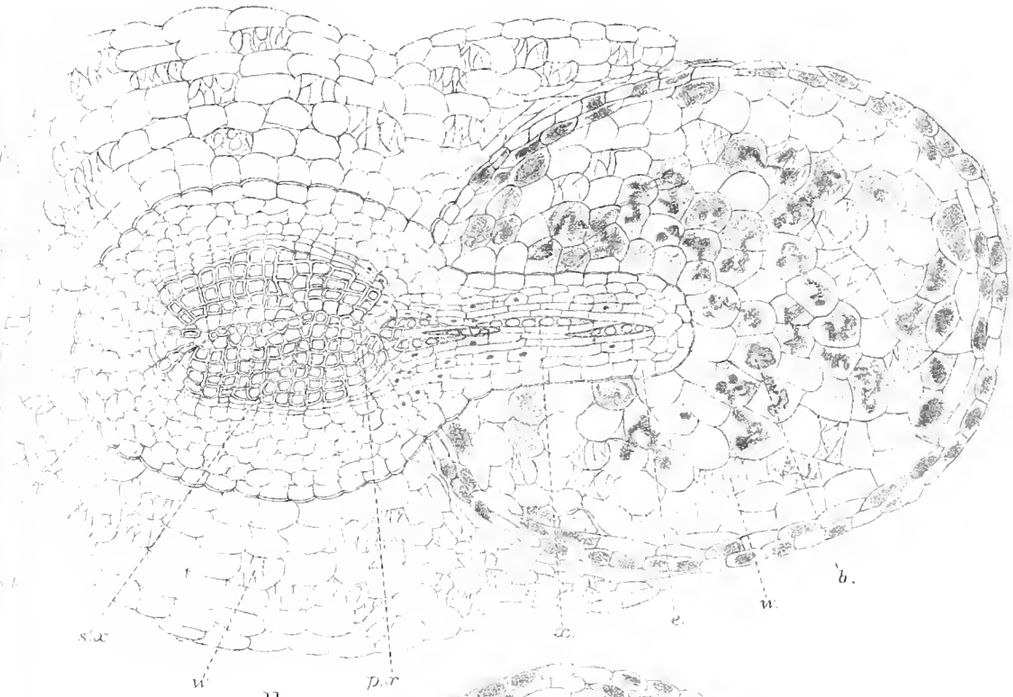

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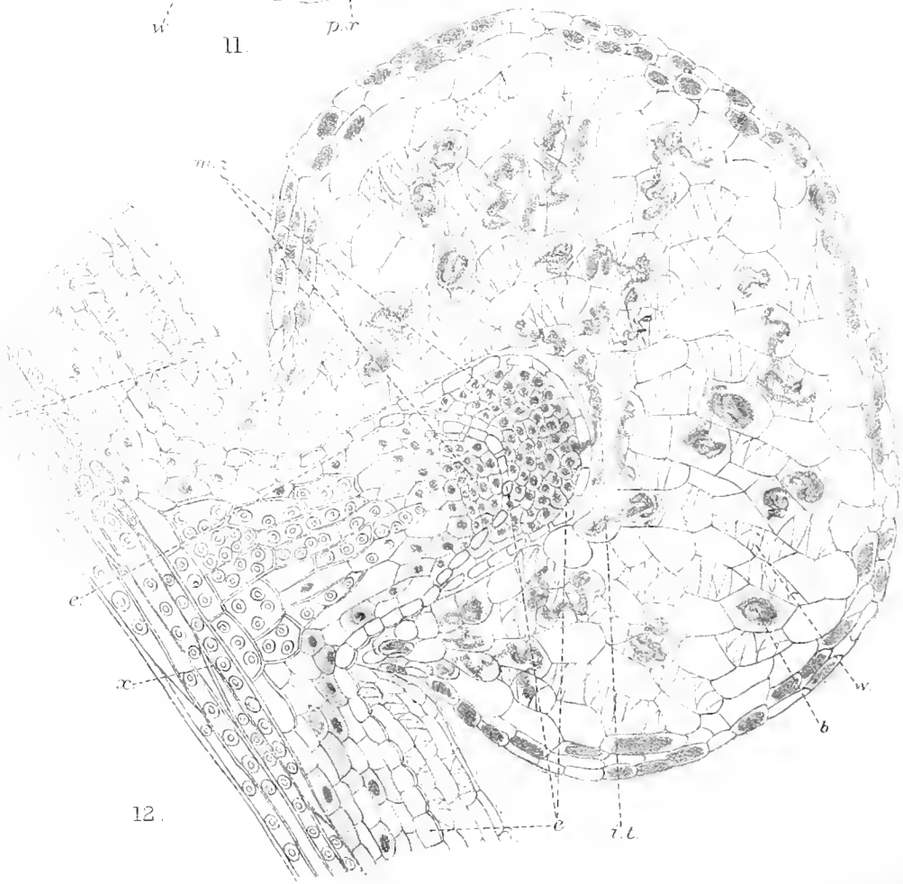

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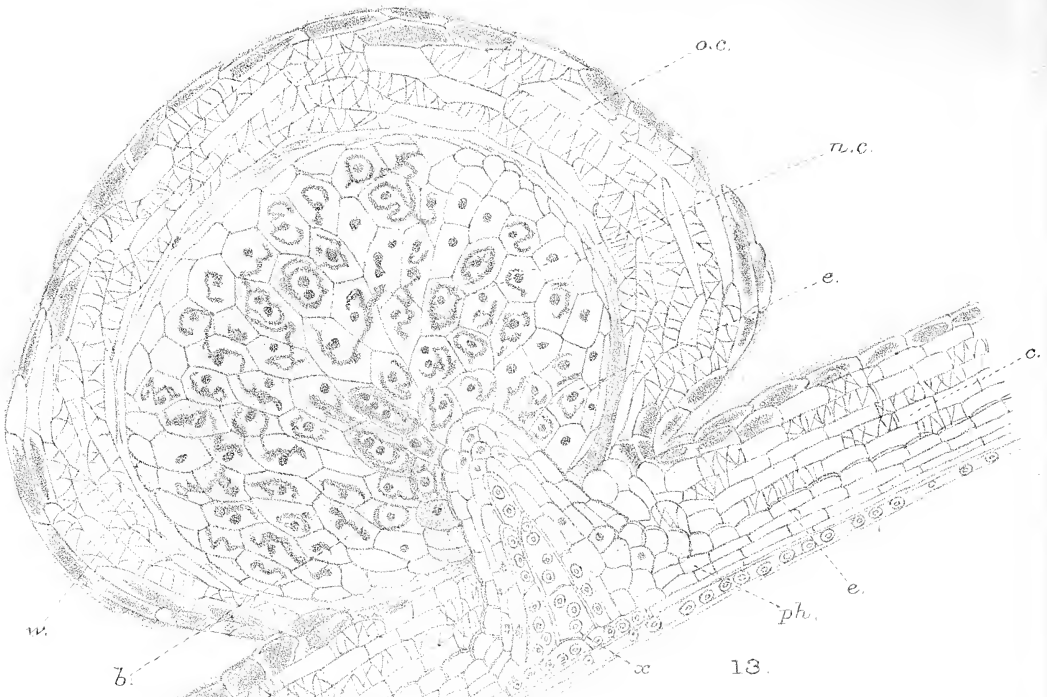

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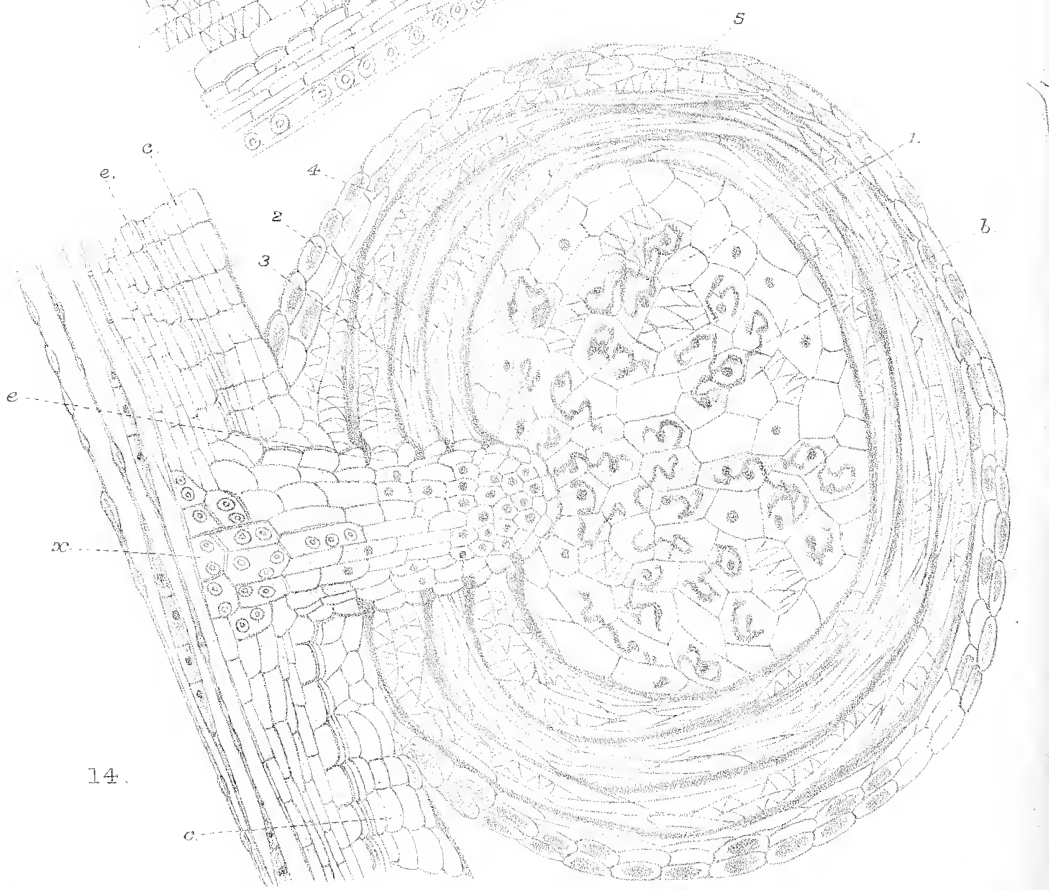

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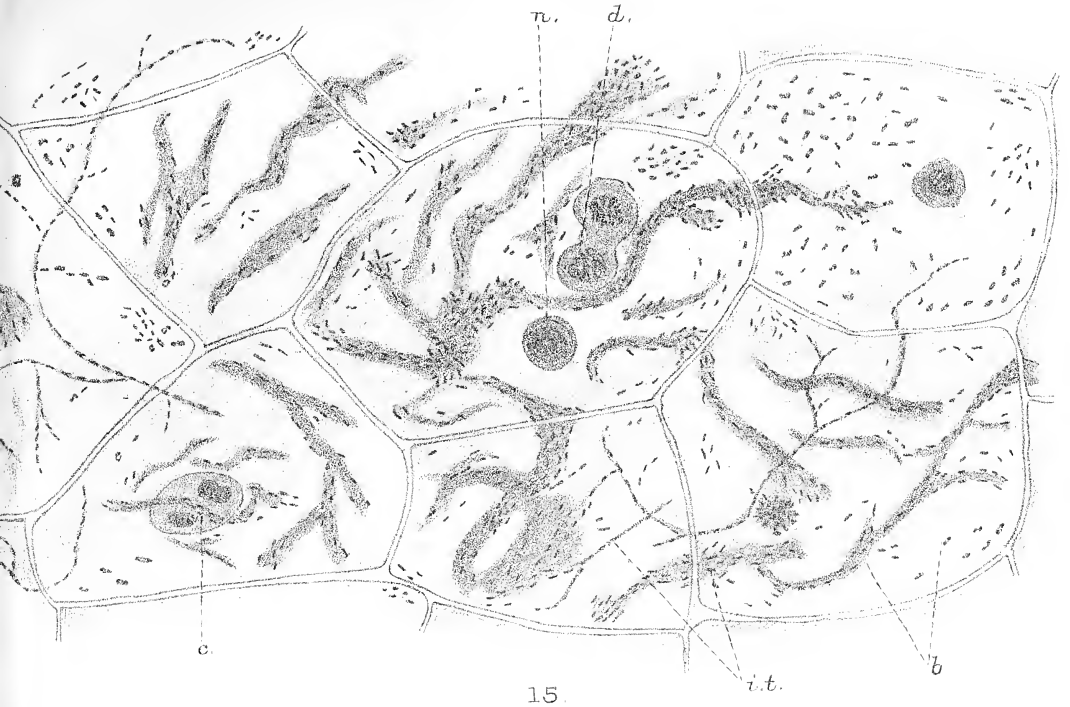

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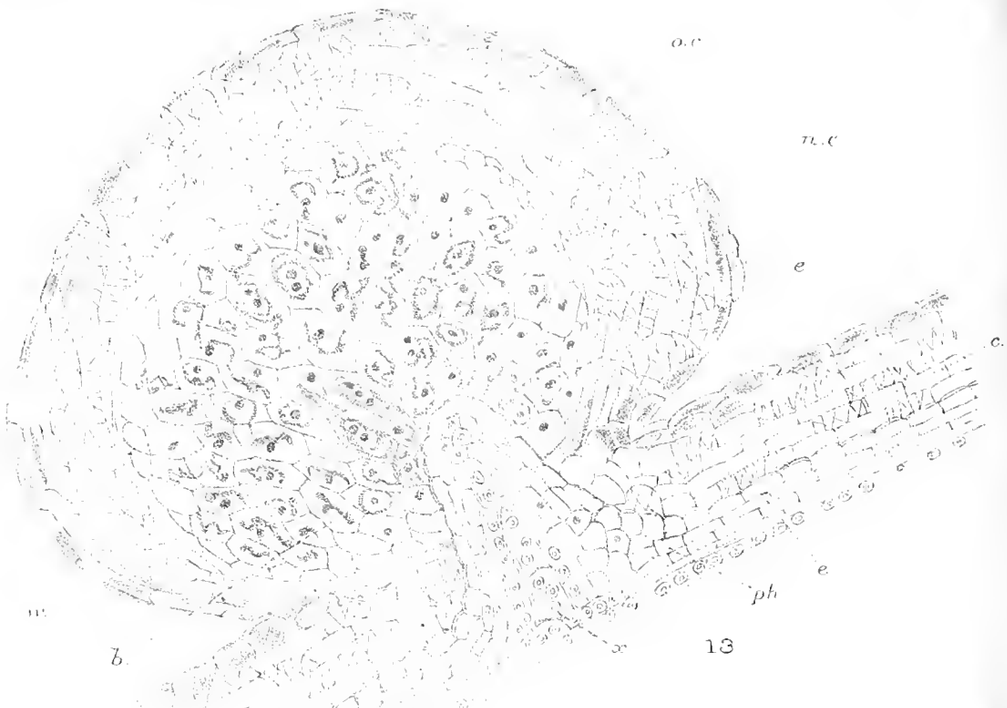

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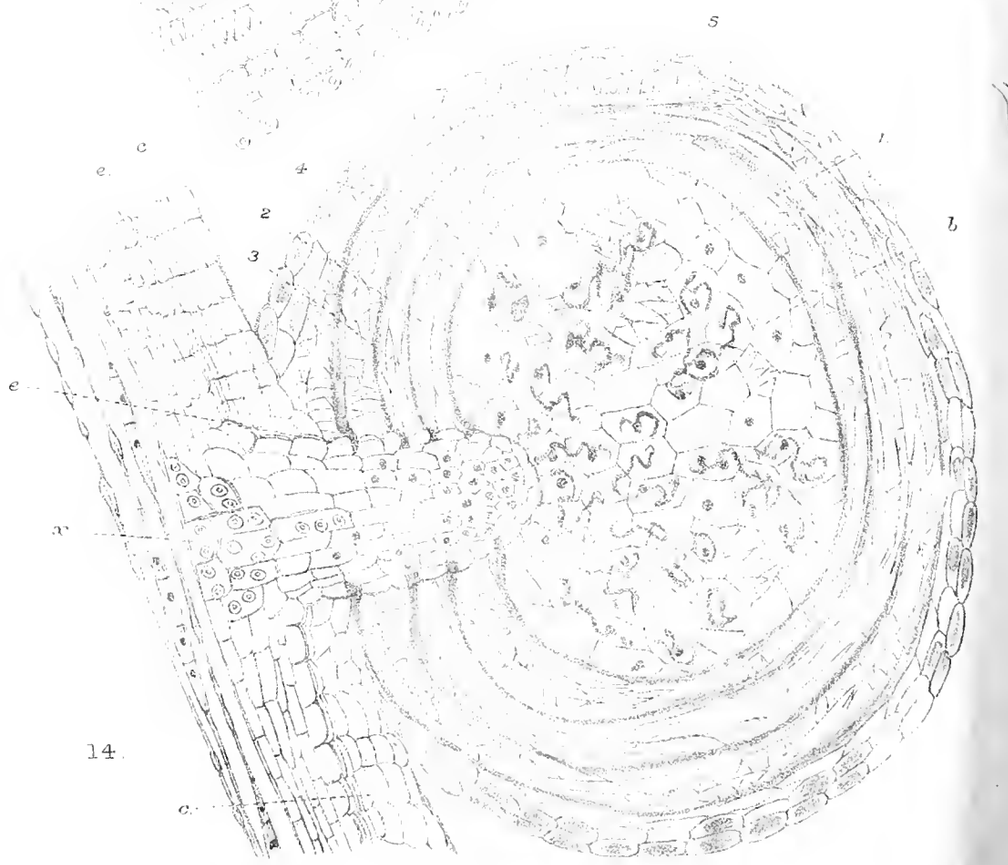

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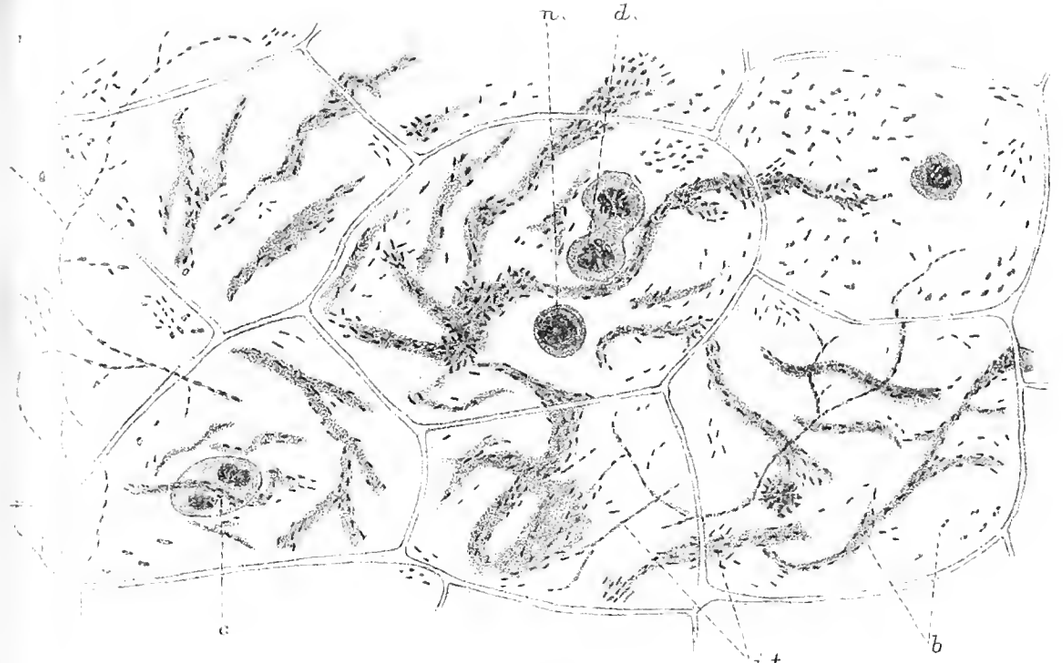

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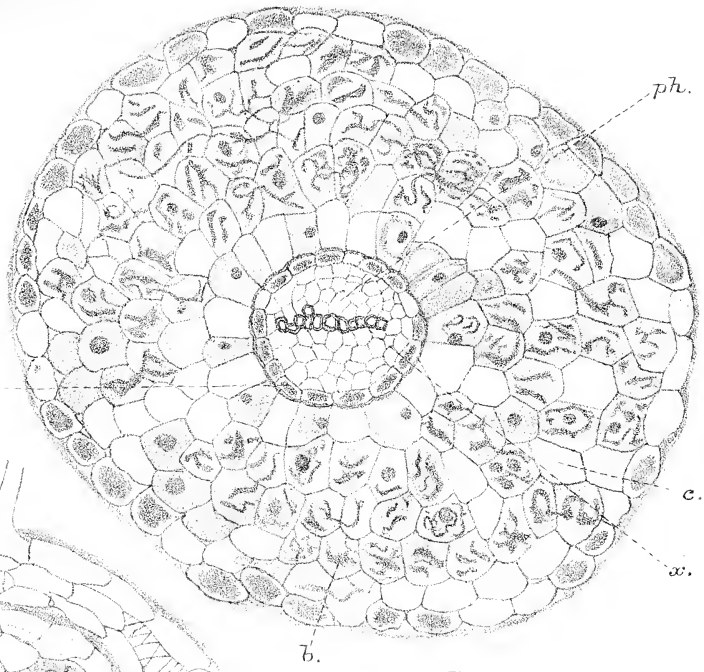

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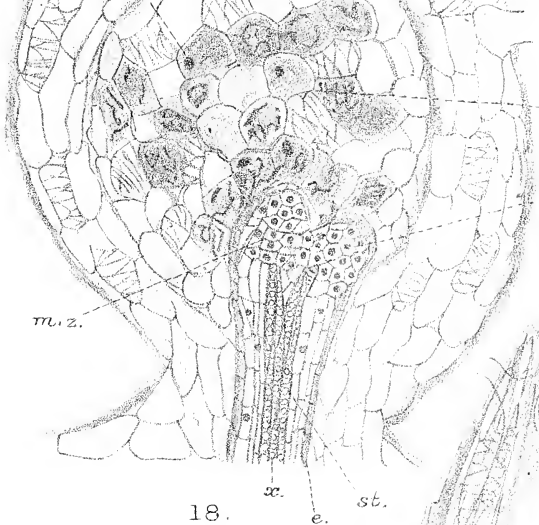

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SPRATT — NODULES OF PODOCARPINEAE.

Spiraea Ulmaria, L., and its Bearing on the Problem of Xeromorphy in Marsh Plants.

BY

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With Plates LXXXI–LXXXIII and eleven Figures in the Text.

CONTENTS.

§ 1. INTRODUCTION.

IN Warming's 'Pflanzengeographie', published in 1896, occurs the following passage[1]: 'Das Ideal der wissenschaftlichen Behandlung der einzelnen Vereine muss der wissenschaftliche Nachweis dafür sein, wie jedes einzelne seiner Mitglieder (Lebensformen) im morphologischen, im anatomischen und im physiologischen Einklange mit den verschiedenen ökonomischen und geselligen Verhältnissen, worunter es lebt, ist.' We are as yet far from the attainment of this ideal in respect of even a single plant-

[1] Warming ('96), p. 119.

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association. But in the meantime it is probable that light may be thrown on many ecological problems by the intensive study of the ecology of selected species.

The present paper, which is intended as a further contribution to our knowledge of 'swamp xerophytes',[1] is an attempt to see how far the study of a single species may help towards an explanation of xeromorphy in marsh plants. The species chosen for this purpose is the common Meadow-sweet, *Spiraea Ulmaria*, Linn., the leaves of which, as is well known, are covered beneath by a dense white felt of hairs. In this paper special attention is devoted to this characteristic pubescence, which is usually regarded as a device for depressing transpiration.

§ 2. Hairiness in Marsh Plants.

Though aquatic phanerogams, as a class, are remarkable in being for the most part devoid of hairs, the latter are by no means uncommon on the leaves or stems of marsh plants. Indeed, I have noted the presence of a greater or less degree of hairiness in upwards of thirty of the commoner British species of marsh plants. No doubt even this list could be considerably augmented. In some cases the aerial parts are only slightly hairy, e. g. in *Angelica sylvestris*, *Hydrocotyle vulgaris*, *Valeriana sambucifolia*, *Phragmites communis*, *Molinia coerulea*, &c. In others the hairiness is more pronounced, as in *Epilobium hirsutum*, *Cnicus pratensis*, *Lysimachia vulgaris*, *Mentha aquatica*, &c. In a few cases the lower surface of the leaves is covered with a dense felt of hairs: such is the case in *Spiraea Ulmaria*, *Salix* spp., &c.

It may be remarked here that hairiness occurs most frequently in those species of marsh plants which either grow in the drier parts of marshes,[2] or else have their aerial parts more or less exposed to the effects of drying winds.[3]

§ 3. Distribution of Certain Hairy Species of *Spiraea*.

The genus *Spiraea*[4] comprises some 127 species, which are spread over the temperate and colder regions of the northern hemisphere. Of these, the majority appear to be glabrous or nearly so, though others are hairy to a greater or less degree. A few species have dense pubescence on the under surface of their leaves; these fall naturally into two groups. In the first, which belongs to the § *Ulmaria*, are *Spiraea Ulmaria*, L., *S. palmata*, Thunb., and *S. vestita*, Wall. These are closely allied Old

[1] Cf. Yapp ('08, two papers), ('09), and ('10).

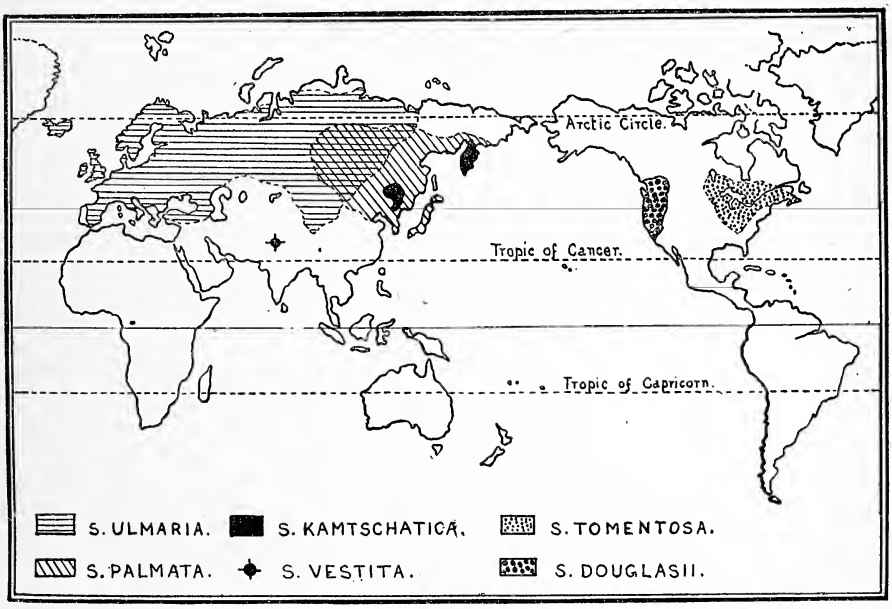
[2] e. g. *Spiraea Ulmaria*.

[3] e. g. *Phragmites communis*, *Viburnum Opulus*, *Salix* spp., &c.

[4] In the sense used by Linnaeus, Bentham and Hooker, and others, cf. Wenzig ('88). Other authors, probably rightly, separate the several sections of *Spiraea* as distinct genera, e. g. Focke ('94).

World forms, with pinnate leaves and exactly the same type of pubescence, i. e. their leaves are 'subtus albo-tomentosa'. The second group includes several N. American species with entire leaves, belonging to the § *Euspiraea*. Their pubescence is more or less similar to that of the first-named species,[1] e. g. *S. Douglasii*, Hook., *S. tomentosa*, L., &c.

It is interesting to note that most of these densely pubescent species have closely allied glabrous forms. Thus *S. Ulmaria* has the nearly glabrous variety *denudata*;[2] *S. palmata* has the varieties *tomentosa* and *glabra*; and *S. vestita* is practically indistinguishable, except in respect of



TEXT-FIG. 1. Map showing distribution of certain species of *Spiraea*.

hairiness, from the glabrous *S. Kamtschatica*, Pallas. Again, the two N. American species mentioned above are allied not only to each other, but also to the widely distributed glabrous *S. salicifolia*, L. For the general distribution of these species see Text-fig. 1.

§ 4. HABIT AND HABITATS OF *SPIRAEA ULMARIA*.

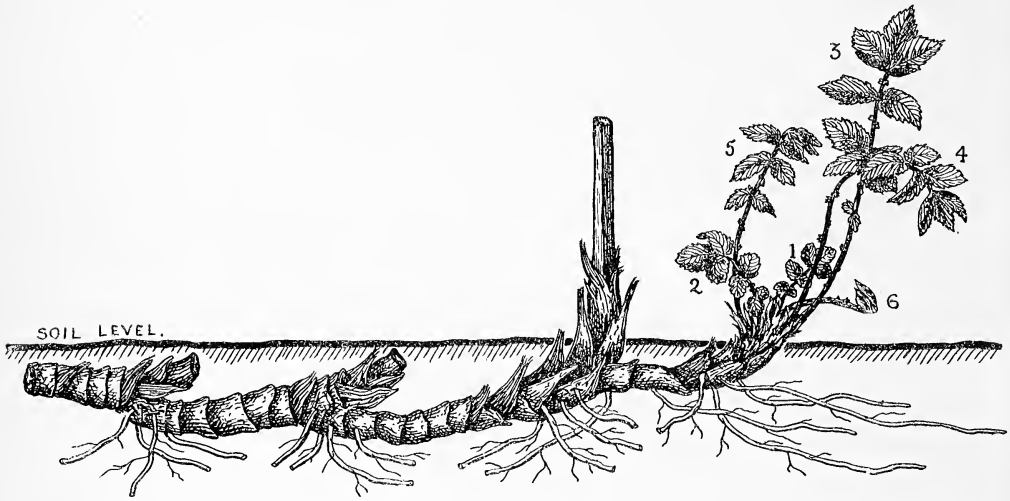
S. Ulmaria (= *Filipendula Ulmaria*, Max.) is a perennial herb with a sympodial rhizome which creeps on or just below the surface of the soil (Text-fig. 2). The aerial parts die back in autumn, leaving the persistent

[1] Densely downy leaves, similar to those of the species mentioned above, are apparently not very common in the Rosaceae, though they are met with in various genera, e. g. *Rubus*, *Prunus*, *Couepia*, &c.

[2] In Rouy et Camus ('00), p. 151, var. γ, *S. unicolor*, Nob. (= *S. denudata*, Presl.), is stated to be glabrous, except for pubescence on the veins below; while var. δ, *glaberrima*, Nob., is entirely glabrous.

rhizome with its winter buds. The plant thus belongs to Raunkiaer's class of 'hemicryptophytes'.[1] The winter buds begin to unfold during March, and each produces several radical leaves. In a vigorous adult plant such a bud may subsequently form an erect flowering shoot, with cauline leaves. In young plants, or buds of less vigour, however, the shoot does not elongate, but remains prostrate. In the latter case it continues to form radical leaves till September. These two kinds of shoot will be referred to in this paper as the *erect flowering shoots* and the *non-flowering shoots* respectively.

Text-fig. 1 shows that *S. Ulmaria* is widely spread over the north temperate and colder regions of the Old World. In Great Britain it is a com-



TEXT-FIG. 2. Sympodial rhizome of *Spiraea Ulmaria*, showing four years' growth. The winter bud has unfolded (April), but has not yet formed the erect flowering shoot. Leaves are numbered in order of age. About $\frac{1}{3}$ natural size.

mon plant, being found in the whole of Watson's 112 counties,[2] whilst its altitudinal range is from the coast to some 2,700 feet above sea-level.[3]

Itself a characteristic plant of the drier parts of marshes and fens,[4] the habitat of *Spiraea Ulmaria* is usually where the water content of the soil is considerable rather than excessive. It prefers soils with a high water capacity, such as peat or clay. Provided that its needs in respect to soil moisture are satisfied, it will grow almost anywhere: at the edges of marshes and rivers; in the damper parts of meadows; in roadside ditches; on the sea-coast;[5] in damp hollows between sand-dunes;[6] and even in woods, if

[1] Raunkiaer ('07), p. 49.
[2] London Catalogue ('08).
[3] Watson ('47), p. 334.
[4] Yapp ('08, second paper), pp. 70 and 72.
[5] In 1910 my friend Professor Weiss sent me some specimens collected close by the sea, growing practically in brackish water.
[6] Moss ('07), p. 16.

the shade is not too dense.[1] It readily colonizes moist ground which has been recently disturbed; and hence frequently establishes itself at the foot of railway embankments, &c.[2]

§ 5. The Distribution of Pubescence on the Leaves.

The various British Floras state that the type form of *Spiraea Ulmaria* has pinnate leaves covered with white down beneath. Consequently, in September of 1906 I was surprised to find that the Spiraeas at Wicken Fen varied markedly in this respect. Some leaves were completely hairy on the lower surface; others were glabrous, while all possible transitions were to be found (see Pl. LXXXII). The reasons for this extreme variability were by no means obvious, so a large number of specimens were grown for some years. It was hoped that if any explanation for such a marked phenomenon were forthcoming, it might throw light on the general problem of xeromorphy in marsh plants.

The experiments (confirmed by many field observations) revealed the existence of a regular periodicity in the production of hairs. The rules governing hair formation may now be stated. It should be borne in mind that all the following descriptions refer to the hairy type form of *S. Ulmaria*, and not to its glabrous variety, *denudata*.

(*a*) *Seedlings.* In all cases the seedlings are glabrous. This applies to every leaf formed during the first year. The plant, if small and weak, may remain in this juvenile glabrous condition for two or more years; though if under favourable conditions, it may form normal hairy shoots in the second year.

(*b*) *Erect flowering shoots of adult plants.* When a winter bud opens early in the year (about March), the first leaves unfolded are juvenile in type, i. e. they are small and glabrous. After a variable number (usually from 3 to 5[3]) of these glabrous radical leaves have unfolded, they are followed, about the end of April or the beginning of May, by one or two leaves which are partly hairy. The latter are in turn succeeded, as the flowering shoot elongates and becomes erect, by leaves which are completely covered with hairs on the lower surface.[4] The pubescence becomes more dense as the erect stem is ascended. (Pl. LXXXI shows all the successive leaves formed on one flowering shoot. Leaves 1–7 are radical, and leaves 8–16 cauline. Hairy parts show white, and glabrous dark.) The plant

[1] Adamson ('12), pp. 358, &c., gives a good account of various *S. Ulmaria* societies in a Cambridgeshire woodland.

[2] At least some of the other species of *Spiraea* mentioned in § 3 are also swamp plants: e. g. *S. salicifolia* and *S. tomentosa*. See Britton and Brown ('97), p. 196.

[3] I have seen as many as twelve, but this is quite exceptional.

[4] The flowering shoot usually begins to elongate about the middle of May, by which time most plants already show some hairy leaves.

generally flowers towards the end of July (or earlier on drier soils), by which time all the leaves on the shoot are unfolded.

Thus the leaves of the erect flowering shoots vary markedly in hairiness, according to both their time of unfolding and their position on the stem.[1]

(*c*) *The non-flowering shoots of adult plants* exhibit an even more remarkable periodicity in hair formation. They commence about March by unfolding glabrous leaves in precisely the same way as the erect flowering shoots. Similarly, too, as the season advances, the successive leaves are *increasingly hairy*. This goes on till about midsummer (the actual time appears to vary, possibly with the kind of season, from about the middle of June to the middle of July), when the process is reversed. The new leaves unfolded are now *decreasingly hairy*, until finally glabrous leaves are once more produced on the approach of autumn. The actual number of hairy or partly hairy summer leaves formed on a given shoot appears to depend on its size and vigour. If a shoot is fairly large and vigorous, it may form only a few glabrous leaves, but a number of nearly or completely hairy ones. On the other hand, a small, weak shoot may produce only one or two slightly hairy leaves; all the rest formed during the year being glabrous.[2]

To sum up, the successive leaves on an erect flowering shoot exhibit a continually rising curve of hairiness, the spring leaves being glabrous and the summer leaves hairy. On the other hand, the non-flowering shoots have glabrous spring and autumn leaves, and (if the plants are sufficiently vigorous) more or less hairy summer ones. In other words, the curve of hairiness rises from zero in spring-time to a maximum about midsummer, once again falling to zero towards autumn.

(*d*) *Distribution of hairs on the partly hairy leaves.* The series of leaves shown on Pl. LXXXII and LXXXIII, selected from various non-flowering shoots, serves to illustrate the rules governing the localization of hairs on leaves which are not completely hairy. In each leaf, taken as a whole, it is readily seen that *hairiness decreases from above downwards.* Out of many hundreds of leaves examined, I have only met with one or two partial exceptions to this rule. If individual partly hairy leaflets are considered, it is equally obvious that the distribution takes the form of *a marginal band of hairs* (Pl. LXXXII, leaves 2, 3, 5, 7, 11, &c., and Pl. LXXXIII, leaves 1 and 2); with sometimes, but by no means always,

[1] Ascherson and Graebner ('00–'05), p. 438, state that the radical leaves are less hairy than the cauline in certain varieties of *S. Ulmaria*.

[2] Curious cases are sometimes met with in which a fairly vigorous shoot shows no sign of elongating to a flowering shoot at the usual time. It proceeds to behave like a non-flowering shoot, showing first increasing, then decreasing, hairiness. A few weeks later it suddenly alters its behaviour again; the leaves once more exhibit increasing hairiness, and a belated flowering shoot is formed.

additional bands running inwards from the margin, between the main veins (Pl. LXXXII, leaves 4, 6, 9; also Pl. LXXXIII, leaf 3).[1] The partly hairy leaves on the erect flowering shoots also follow the same rules (Pl. LXXXI, leaves 6, 7, and 8). All the leaves photographed (Pl. LXXXI–LXXXIII) are mature. The hairs would not spread subsequently from the margin over the entire surface.

Broadly speaking, the rule followed in every case is that when a leaf or leaflet is only partially hairy, the hairs are localized principally at those points which are most remote from the main water supply. This point will be dealt with later.

§ 6. Comparison with other Hairy Plants.

Spiraea Ulmaria is not unique in this curious periodic production of hairs, though it is the most striking case I have seen. Specimens of *S. vestita*, Wall.,[2] a species quite distinct from *S. Ulmaria*, though closely allied to it, were examined in the Cambridge University and Kew Herbaria. The upper leaves are downy, but in both herbaria specimens exist which show that the lower leaves are only partly hairy. In one or two cases the lowest leaf of all is glabrous. Thus this species apparently follows much the same rules as *S. Ulmaria*. I have also grown *Spiraea tomentosa*, L., from specimens kindly sent me by Professor H. C. Cowles, of Chicago, and Professor J. B. Pollock, of Michigan. This is a shrubby form, with small downy leaves. Here, too, as the buds open in the spring-time, there is a similar succession of glabrous, partly hairy, and hairy leaves.

A number of other marsh herbs resemble these species of *Spiraea* in forming small glabrous leaves in spring-time, followed, as the season advances and the erect flowering shoots are formed, by a steadily increasing production of hairs on the later leaves.[3] Such are *Epilobium hirsutum*, *Lysimachia vulgaris*,[4] *Mentha aquatica*, *Valeriana sambucifolia*, *Scabiosa succisa*, *Eupatorium cannabinum*, *Lycopus europaeus*, &c.

But the phenomenon is not confined to marsh plants. I noticed a specimen of *Rubus discolor*, W. and N., growing partly shaded by trees in the New Forest, in which the proximal leaves on the lateral shoots were invariably much less hairy than the distal ones. Again, I examined the cultivated Raspberry (*Rubus idaeus*, L.), without finding any trace of

[1] The distribution is not always quite so regular as shown in the series of leaves photographed; though it is so in most cases. Sometimes an interveinal band is incomplete: in this case its innermost portion is often present as a group of hairs in a fork where two veins meet (Pl. LXXXIII, leaf 2). It will be shown later that hairs are developed also on the main veins themselves. Cf. Renner ('08), p. 128, who states that in many leaves in which the lamina is folded in the bud, both on the midrib and lateral veins, hairs are developed especially on the veins and the edges of the leaf, e. g. *Fagus sylvatica* and *Carpinus betulus*.

[2] *S. vestita*, Wall. MSS. = *S. Kamtschatica*, var. *himalensis*, Lindl. Cf. Lindley ('41), t. iv, and Hooker ('79), p. 323.

[3] Yapp ('08), p. 691.

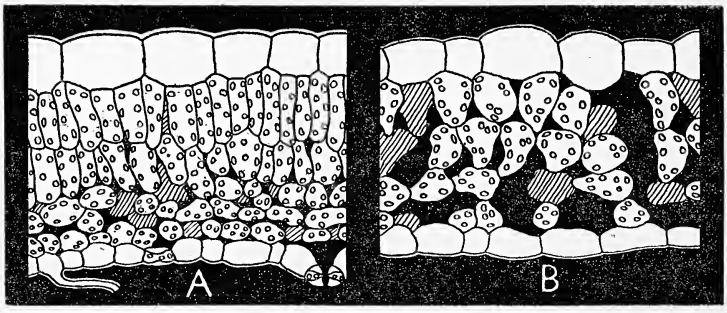
[4] Yapp ('09), Text-fig. 1, p. 278.

diminished hairiness in the lower leaves. But Mr. E. J. Salisbury informs me that in the wild Raspberry, when the canes are growing in thick clumps, the lower leaves are considerably less hairy. Specimens kindly sent by Mr. Salisbury confirm his observation.

Thus we are evidently dealing with a widespread phenomenon.

§ 7. Seasonal Differences in Leaf Structure.

Various authors have called attention to the periodicity of growth exhibited by shoots.[1] The leaves increase in size and the internodes in length (and also in the length of their cells) from below upwards. Such increase is followed by a corresponding decrease after the maximum is reached (cf. Pl. LXXXI and Text-fig. 5). It has been shown above that a similar periodicity of hairiness obtains in the non-flowering shoots of *Spiraea*



Text-fig. 3. Sections of (A) a 'sun' leaf, and (B) a 'shade' leaf of *Spiraea Ulmaria*. × 350.

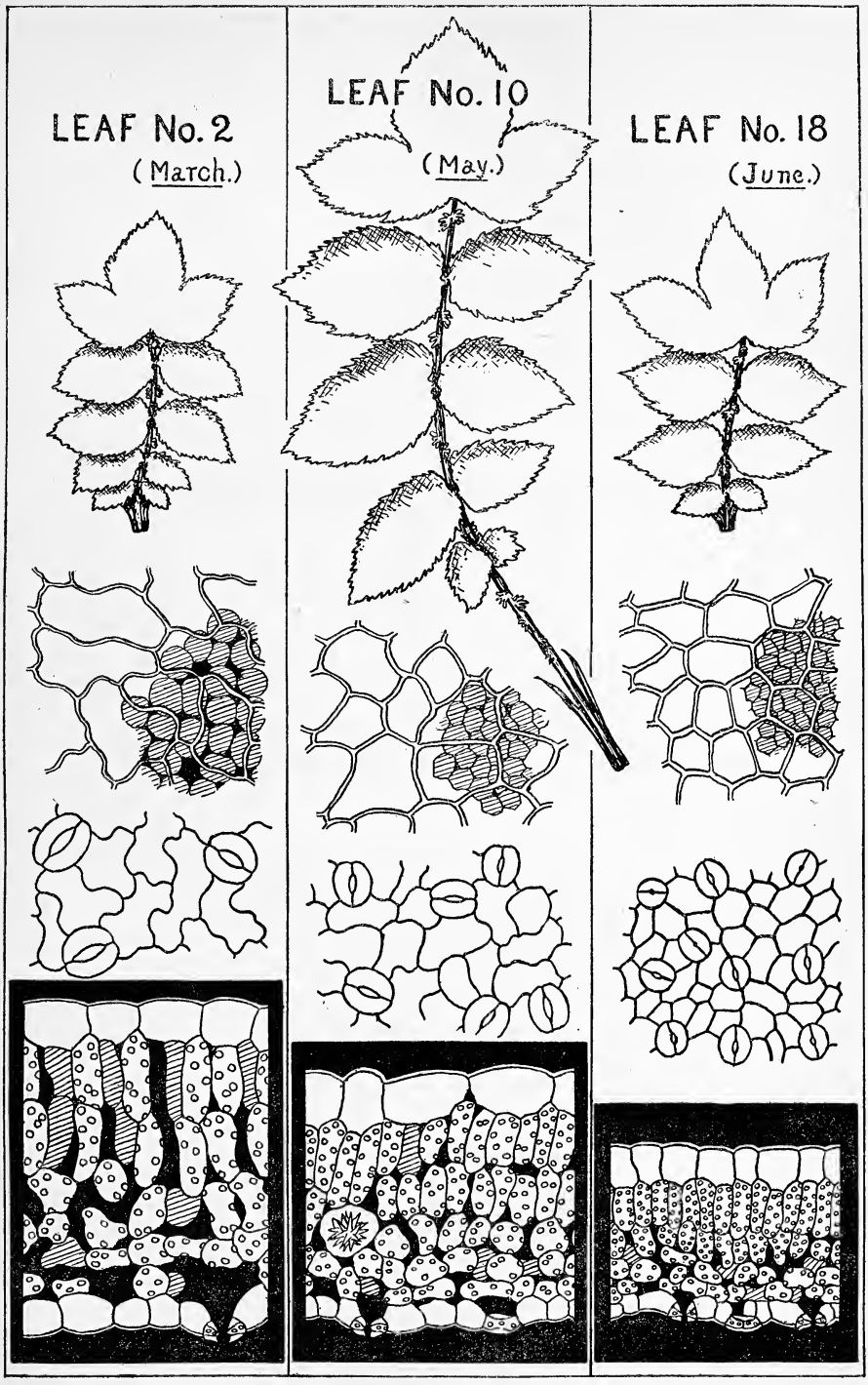
Ulmaria. The leaves of the erect flowering shoots, on the other hand, exhibit a more or less continually rising curve of hairiness. It will now be shown that corresponding seasonal differences of structure occur in the successive leaves formed on these erect flowering shoots.[2]

The possible range of structure may first be indicated. Text-fig. 3 shows sections taken through extreme 'sun' and 'shade' leaves. A is from a cauline leaf of a fully exposed plant, and B from a radical leaf of a plant grown in deep shade and a constantly humid atmosphere (under a bell-glass).[3]

[1] Moll ('76), Pfeffer ('03), Tammes ('03), Groom ('08), &c.

[2] The method of investigation was as follows: The successive leaves on selected shoots were preserved in alcohol, care being taken not to remove any leaf from the plant till it was fully mature and several later leaves had unfolded. Sections were taken from corresponding portions of the terminal leaflet. Another portion of the same leaflet was cleared in eau de Javelle and mounted in water as a transparent object. In this state the size and relations of the epidermal, palisade, and other cells could be studied, and the number and size of the stomata ascertained. The cells remain of approximately the same size as during life. The latter point was determined by taking a contact print of a living leaf on photographic paper. The leaf was then treated as above, when it was still found to correspond in size with the photographic print. Thus the number of stomata per unit area given later may be taken as practically the same as in a living leaf.

[3] Cf. the figures given in Hesselman ('04), p. 419.



TEXT-FIG. 4. Three leaves from one erect flowering shoot of *S. Ulmaria*, var. *denudata*. The months indicate time of unfolding. Below each leaf are shown successively (*a*) part of its upper epidermis, superposed on palisade cells, (*b*) part of lower epidermis, with stomata, and (*c*) section of leaf lamina. Intercellular spaces are black. Shaded cells are at a somewhat different level to the others. Leaf outlines × about $\frac{3}{5}$; all other drawings × 350.

We have seen that two types of leaf (glabrous and hairy), with intermediate transitions, occur on each shoot. As was perhaps to be expected, the glabrous low-growing leaves were found to possess a more or less definite 'shade' structure; while the upper, exposed hairy leaves approximated to the 'sun' type. There is a gradual transition (often fluctuating as regards individual leaves) in structure between the two extremes.

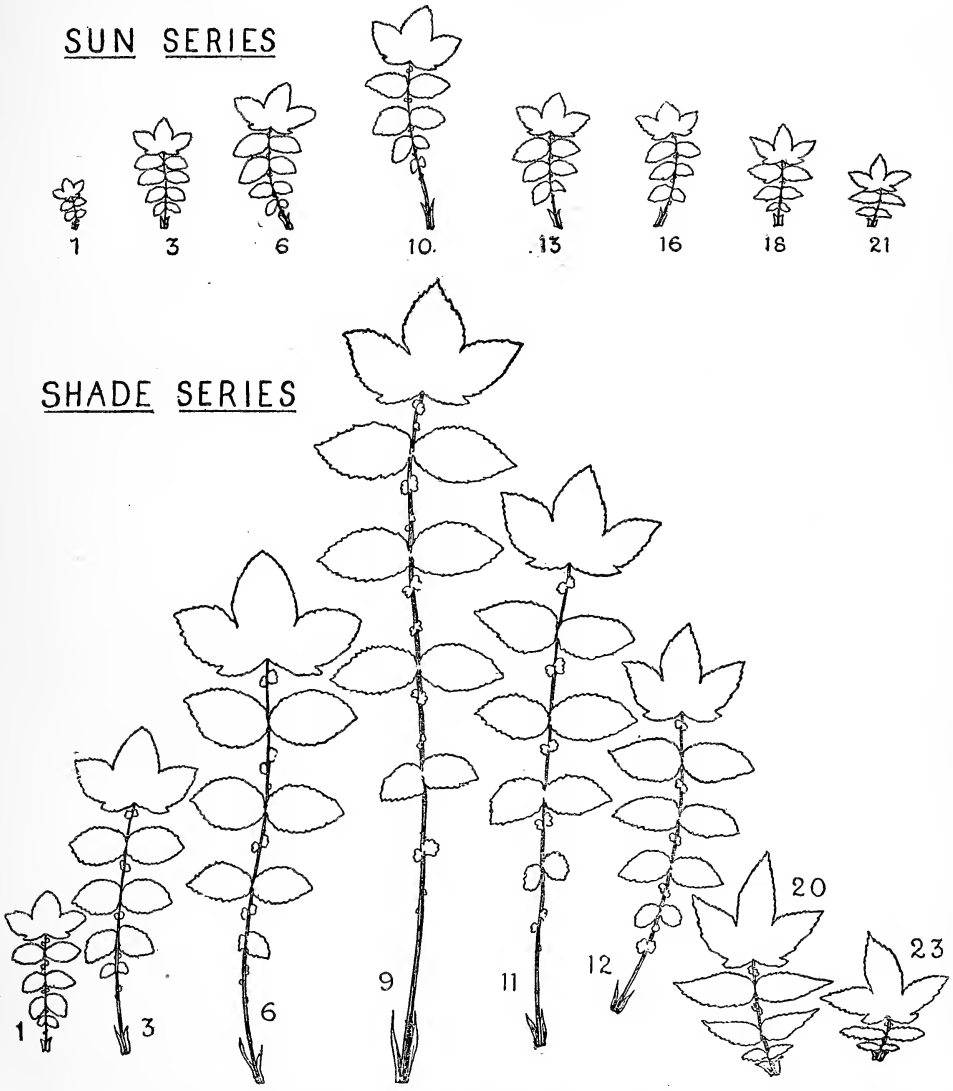
The glabrous radical leaves, which are unfolded about March, have large epidermal cells, with more or less sinuous lateral walls (especially those of the lower epidermis). The cuticle is thin. The stomata are large and relatively few in number. The mesophyll is composed of large, loosely arranged cells, with large and abundant intercellular spaces (Text-fig. 4, leaf 2). As more leaves are unfolded the following progressive changes may be observed. The epidermal cells (both upper and lower) of the successive leaves become gradually reduced in size, and their lateral walls less sinuous. The cuticle of the upper epidermal cells is thicker, though in this plant it is always fairly thin. The stomata become progressively smaller, and at the same time considerably more numerous per unit area.[1] The lower surface of the leaves is more and more hairy. The mesophyll is more compact (especially the upper palisade layer), its cells are smaller, and the intercellular spaces are gradually reduced to a minimum (Text-fig. 4, leaves 10 and 18).

The general question of relation of structure to environment will be discussed later. It may, however, be remarked here, that while the above relative differences were found in the successive leaves of each shoot examined, the actual structure of any given leaf depends partly on its position on the shoot, and partly on the general nature of the habitat. Thus the most lacunar leaves, with the fewest stomata, &c., are the radical leaves of plants grown under conditions of extreme humidity and shade. The converse also holds true (cf. Text-fig. 3). Again, the observed differences depend only to a certain extent on the actual size of the leaf lamina. Thus, a 'shade' leaf has not only a larger surface area, but also larger epidermal cells, fewer stomata per unit area, &c., than *a corresponding leaf* from a plant grown under fully insolated conditions (see Text-fig. 5). On the other hand, if the leaves formed on a single shoot are considered, a curve showing the periodicity in size of lamina would exhibit a very distinct rise and subsequent fall (both sides of this curve are steeper in shade than sun plants, cf. Text-fig. 5). But a curve showing the progressive changes of structure would be a continually rising one. Thus two leaves from opposite ends of a shoot may be approximately equal in size, yet possess a totally different structure (Text-fig. 4).

[1] Dufour ('87), p. 407, states that stomata are more numerous on leaves grown in sun than in shade, while Eberhardt ('03), pp. 151–2, says that in humid air the size of epidermal cells is increased, and the number of stomata decreased, as compared with the effect of dry air; cf. Fig. 4 p. 81.

Some of the more important features may now be dealt with in detail.

(*a*) *The stomata.* These are slightly raised, even in the more exposed leaves of *S. Ulmaria*, var. *denudata* (Text-figs. 3 and 4). They are for the



TEXT-FIG. 5. Selected leaves from two flowering shoots, to show (*a*) periodicity of leaf size, (*b*) difference in actual size of 'sun' and 'shade' leaves, and (*c*) narrowing of leaflets of upper leaves. Certain leaves of each series have been omitted (see numbers of those selected). In each case the largest and smallest are shown. All × $\frac{1}{5}$.

most part confined to the lower surface of the leaf. The only exceptions to this are (1) a few at the sides of the larger veins, and (2) groups of very small stomata, which are crowded on the upper surface of the leaf-teeth, immediately above the endings of the more important veins. On determin-

ing the numbers of stomata per unit area, it was at once apparent that an increase occurred in successive leaves. Unfortunately, while the numbers on the glabrous leaves are readily ascertainable, the dense pubescence of the majority of leaves renders accurate counting impossible. Many methods of surmounting this difficulty were tried, but none proved really successful.[1] Finally, the var. *denudata* was used, though this is also hairy to a certain extent. It is from this plant that the only complete series of counts were obtained. A few actual examples may be given.

SHOOT A[2] (Type form). A 'shade' plant, i. e. grown in ordinary garden soil, under currant bushes, and sheltered by a high wall on the south side. The shoot produced a total of 24 leaves. Of these, the first two were quite glabrous. A few extremely short hairs appeared on the main veins of the *third* leaf. The hairs gradually increased in number and length until the eighth leaf, when it was difficult to count the stomata; above this it was impossible.

Leaf No.	1	2	3	4	5	6	7	8
No. of stomata per sq. mm.[3]	221	221	224	226	240	255	302	about 348

SHOOT B (Type form). Grown under distinctly drier and sunnier conditions than A. In all, 14 leaves were produced. Very short hairs again began on the *third* leaf. The stomata could not be counted beyond the sixth leaf.

Leaf No.	1	2	3	4	5	6
No. of stomata per sq. mm.	239	283	273	350	360	372

SHOOT C (var. *denudata*).[4] Grown in a garden, fully exposed to sun. In all, 21 leaves were produced. A very few short, straight hairs appeared on the main veins of the *fifth* leaf. These slowly increased in number and length on the remaining leaves. In this variety, however, the leaves never become densely hairy, though in a few cases stomata counting was somewhat difficult.

Leaf No.	1	2	3	4	5	6	7	8	9	10	11
No. of stomata per sq. mm.	393	303	313	322	313	352	482	440	543	678	807

Leaf No.	12	13	14	15	16	17	18	19	20	21
No. of stomata per sq. mm.	776	856	945	934	928	1095	1188	1293	1083	1090

Thus there is a gradual though fluctuating rise in the number of stomata through the successive leaves of the entire shoot. The numbers themselves are worthy of comment. Not only are the absolute numbers of

[1] Hesselman ('04), p. 419, states that he, too, was similarly baffled by this same species.

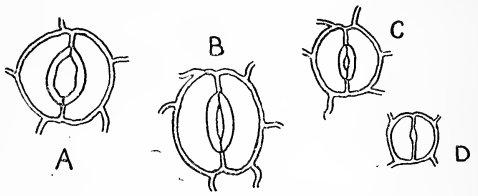
[2] The same shoots will also be referred to later as Shoots A, B, and C.

[3] The stomata counts, in this and the following cases, were taken from the terminal leaflet, cleared as previously described. As a rule, the numbers represent the mean of ten counts taken from different parts of the leaflet (lower surface only). The larger, and as far as possible even the smaller, veins were avoided.

[4] The leaves used for Text-fig. 4 are from this shoot.

stomata on the upper leaves extraordinarily high,[1] but the range from about 300 to nearly 1,300 per sq. mm. for different leaves of the same shoot is equally remarkable. It may be that *Spiraea Ulmaria* is exceptional in the latter respect. But it would be worth while comparing the successive leaves of a number of other plants, for if the phenomenon is at all general, the numbers already published for a large number of species[2] might need considerable revision. The highest number previously recorded appears to be 1,000 per sq. mm. in *Psidium Guyava*, which Parkin and Pearson[3] mention as being very remarkable in this respect.

The glabrous autumn leaves resemble the glabrous spring leaves in structure as well as appearance. Thus the number of stomata in the former is usually between 200 and 300 per sq. mm. Further, in the case of tall radical leaves, there is often considerable difference between the upper and lower leaflets, the latter possessing a more pronounced 'shade' structure than the former.[4] Thus the lower leaflets may be less hairy, and have fewer stomata and more lacunar mesophyll. E. g. the sixth leaf of Shoot A had 255 stomata per sq. mm. on the terminal leaflet, and only 213 on the lowest leaflet. Again, in a partly hairy leaf (the fourth on the shoot) collected at Wicken Fen, the terminal leaflet had 395 stomata per sq. mm.; the second pair of leaflets, 333; the fourth pair, 269, and the lowest pair of all, only 176. In other cases, however, the differences were less than these.



TEXT-FIG. 6. Stomata from different leaves. For description see text. × 535.

But the number of stomata is not the only thing to be considered. From the point of view of transpiration, the size of the stomata, and more particularly of their pores, is as important as their number. Text-fig. 6 shows stomata taken from three different leaves. A is from a radical leaf of a plant grown in deep shade, under a bell-glass, i. e. in saturated air and weak illumination. The pore is widely open. The leaf had an average of 255 stomata per sq. mm. B is from the second leaf of Shoot C, with 303 stomata per sq. mm. Here the pore is smaller, but still much larger than that of C, which is taken from the eighteenth leaf of the same shoot (C),

[1] Shoot C was not unique in possessing such numerous stomata. In all, three shoots of var. *denudata* were examined, and in each case the stomata on the upper leaves exceeded 1,000 per sq. mm. In one shoot 1,252 were recorded, and in another, grown in shade, but otherwise exposed, 1,112.

[2] Cf. Morren ('63), Weiss ('65), and other authors. Miss Delf ('11), p. 500, has recently recorded a similar rise (from 97·5 to 236·25 per sq. mm.) in the number of stomata on successive internodes of *Salicornia annua*.

[3] Parkin and Pearson ('03), p. 443. Jost ('07), p. 38, states that in extreme cases there may be as many as 625 (*Olea*) or 716 (*Brassica rapa*) per sq. mm.

[4] Boodle ('04), pp. 663–4, records an instance in which the upper, exposed pinnae of a leaf of *Pteris aquilina* had marked xerophytic characters, which were absent in the lower, sheltered pinnae.

with 1,188 stomata (cf. Text-fig. 4). Many of the stomata on the upper leaves had even smaller pores than C. Moreover, abortive stomata, or what appear to be such (Text-fig. 6, D), with no actual pore at all, are very frequently found. These are much more common on the leaves with numerous stomata than on the lower leaves, and have been included in the countings given above.

The question at once arises, so far as the stomata alone are concerned, will the transpiration from the numerous small stomata of the upper leaves be greater or less, per unit area of leaf surface, than that from the fewer but larger stomata of the lower leaves? If very much greater, it would seem possible that any economy of water effected by the production of hairs and other xerophytic devices in the upper leaves might be rendered nugatory by the numerical increase of stomata. Appended below are rough calculations,[1]

[1] Unfortunately, the only measurements available at the time of writing this paper are taken from the stomata of leaves fixed and cleared in the manner described above. As, however, the several leaves were treated in exactly the same way, it is probable that the respective sizes of the stomatal pores bear much the same relations to each other as when fully open in nature. Still, the measurements, &c., given must be taken as approximations only.

Regarding the stomatal pores as ellipses, the areas of the pores shown in Text-fig. 6, B and C (i. e. from the second and eighteenth leaves), are 0·0000136 sq. mm. and 0·0000036 sq. mm. respectively. As the second leaf has 303 stomata per sq. mm. of leaf surface, the total area of the stomatal openings amounts to about 0·00412 sq. mm., i. e. 0·41 % of the whole lower surface of the leaf. In the eighteenth leaf, however, which has 1,188 stomata per sq. mm., a considerable number of the stomata are abortive. Moreover, the pores of many of the remainder are smaller than the one figured (see Text-fig. 6, C and D). It has been estimated (roughly) that the number of effective stomata on this leaf may amount to about 80 % of the whole. On this assumption, the total area of stomatal openings would be about 0·00342 sq. mm., or 0·34 % of the lower leaf surface; that is, actually less than in leaf 2.

For calculating the amount of water vapour which could be transpired through the stomata of the two leaves respectively, the formula given by Brown and Escombe ('00, p. 276) for the absorption of CO_2 may be used. It is assumed that the air in the intercellular spaces of the leaves is saturated with water vapour, and the free air outside the leaves two-thirds saturated. The measurements are calculated at 18° C. and 760 mm. The formula in question is:

$$Q = \frac{k\rho \,.\, A \,.\, y \,.\, 3{,}600}{l + x}$$

where Q = the amount of water vapour (in c.c.) transpired per hour per sq. cm. of lower leaf-surface, and

(1) in the case of leaf 2:

k = diffusivity of H_2O vapour at 18° C. in C.G.S. units = 0·248.

ρ = difference of pressure of H_2O vapour inside and outside leaf, in atmospheres = 0·0067.

A = area of pore of stoma = $1{\cdot}36 \times 10^{-7}$ sq. cm.

y = number of stomata per sq. cm. = 30,300.

l = length of stomatic tube = 0·0010 cm.

$x = \frac{1}{8}\pi \times$ diameter of circle of same area as elliptical pore of stoma = 0·00016 cm.

Hence, Q = 21·2 c.c. of water-vapour transpired per hour per sq. cm. of lower leaf surface.

(2) For leaf 18, the differences will be:

$A = 3{\cdot}6 \times 10^{-8}$ sq. cm.

y (reckoning 80 % of the total number as effective) = 95,000.

l = 0·000714 cm.

x = 0·000084 cm.

In this case, Q = 25·4 c.c. of water vapour transpired per hour per sq. cm. of leaf surface.

It will be seen that there are several possible sources of error in such a calculation as the above.

from which it seems probable that the difference is not great. Thus, *considering the stomata alone*, and under certain specified conditions (the same for the two cases), it is calculated that for Shoot C transpiration from the second leaf (Text-figs. 4 and 6, B) might amount to 21·2 c.c. per hour per sq. cm. of leaf surface. From the eighteenth leaf (Text-figs. 4 and 6, C) the corresponding amount would be 25·4 c.c. per hour per sq. cm. The difference is not very pronounced; so as the upper leaves possess a dense covering of hairs, more compact mesophyll, &c., it may safely be asserted that, in spite of the numerous stomata, they are, on the whole, distinctly more xerophytic than the lower leaves (see § 13). The argument here is from leaf structure alone; the ultimate test is of course the measurement of actual transpiration (see § 12).

(*b*) *Thickness of leaves*, &c. The leaves of *Spiraea Ulmaria* are fairly thin, varying in total thickness (excluding the hairs) from about 78 to 140 μ. Curiously enough, the thickest leaves on a shoot are invariably the glabrous spring leaves. In these the individual cells are large and loosely arranged. On passing up the stem, the successive leaves show a gradual shrinkage in the size of these cells,[1] which at the same time become more tightly packed together (see Text-fig. 4). The leaves thus exhibit a progressive reduction in thickness. The upper leaves, in fact, may almost be said to be shrunken miniatures of the lower. This of course applies to structure, not to surface area of the lamina. It is chiefly in this variation in size of the individual cells that the plasticity of the leaves of *Spiraea* consists, rather than in any marked change in the number of layers comprising the leaf, or the proportions assumed by the various tissues. There are, however, some changes in these proportions, which may now be instanced, and a few average measurements given (in μ).

	Total thickness of leaf, between veins.	*Depth of cells of upper epidermis.*	*Depth of cells of lower epidermis.*	*Depth of palisade.*	*Depth of spongy mesophyll.*	*Ratio of palisade to spongy mesophyll.*
SHOOT A (referred to above)—grown in shade:						
Leaf No. 2	138·0 μ	22·0 μ	15·5 μ	54·5 μ	46·0 μ	1·2 : 1·0
„ 18	78·0	15·5	8·0	31·0	23·5	1·3 : 1·0
SHOOT B—grown in light of moderate intensity:						
Leaf No. 2	140·0	19·5	15·5	60·0	45·0	1·3 : 1·0
„ 12	104·0	18·0	8·0	47·0	31·0	1·5 : 1·0
SHOOT C—fully insolated:						
Leaf No. 2	135·0	19·5	15·5	57·0	43·0	1·3 : 1·0
„ 18	80·5	17·0	8·0	31·0	24·5	1·3 : 1·0

These figures illustrate the general shrinkage referred to above. Further, the lower epidermal cells decrease in depth in the upper leaves,

But it is probable that the difference in transpiration could not be very much greater, and might even be considerably less than that calculated here.

I am indebted to Professor G. A. Schott for assistance in making this calculation.

[1] This has already been noted for the epidermal cells and stomata.

much more than those of the upper epidermis (cf. also the sections and surface views in Text-fig. 4). The latter cells remain relatively large in the upper, exposed leaves, especially at the leaf margins.

(*c*) *Palisade tissue.* The above table shows that the ratio of the total depth of the palisade *tissue* to that of the spongy mesophyll is remarkably constant. The variation, however, in the ratio of the length of a palisade *cell* to its breadth is considerably greater. As one would expect, the palisade cells of 'shade' plants tend to be relatively shorter and broader than those of 'sun' plants. In addition to this, in an individual leaf, those palisade cells which are most remote from the larger veins are relatively longer and narrower than those situated near to the veins. Again, taking a lower and an upper leaf from each of the three shoots previously used, we get the following average measurements (expressed in μ):

	Length of palisade cell.	*Breadth of palisade cell.*	*Ratio of length to breadth.*
Shoot A—shade:			
Leaf 2 (*a*) near vein . . .	30·0 μ	15·5 μ	1·9 : 1·0
(*b*) remote from vein	32·0	11·5	2·8 : 1·0
Leaf 22 (*a*) near vein . . .	19·0	9·5	2·0 : 1·0
(*b*) remote from vein	18·5	6·5	2·8 : 1·0
Shoot B—moderate sun:			
Leaf 4 (*a*) near vein . . .	27·5	9·5	2·9 : 1·0
(*b*) remote from vein	35·5	8·0	4·4 : 1·0
Leaf 10 (*a*) near vein . . .	24·0	8·5	2·8 : 1·0
(*b*) remote from vein	31·0	7·0	4·4 : 1·0
Shoot C—full sun:			
Leaf 2 (*a*) near vein . . .	30·0	12·5	2·4 : 1·0
(*b*) remote from vein	36·5	10·0	3·6 : 1·0
Leaf 18 (*a*) near vein . . .	20·0	9·5	2·1 : 1·0
(*b*) remote from vein	24·0	7·5	3·2 : 1·0

This table shows clearly (1) that the actual dimensions of the palisade cells are less in the upper than the lower leaves; (2) that the palisade character is best developed at some distance from the larger veins;[1] and (3) that the palisade character is least marked in shoots grown under shade conditions.

§ 8. Development of the Leaves, and Time of Origin of the Hairs, Palisade Cells, &c.

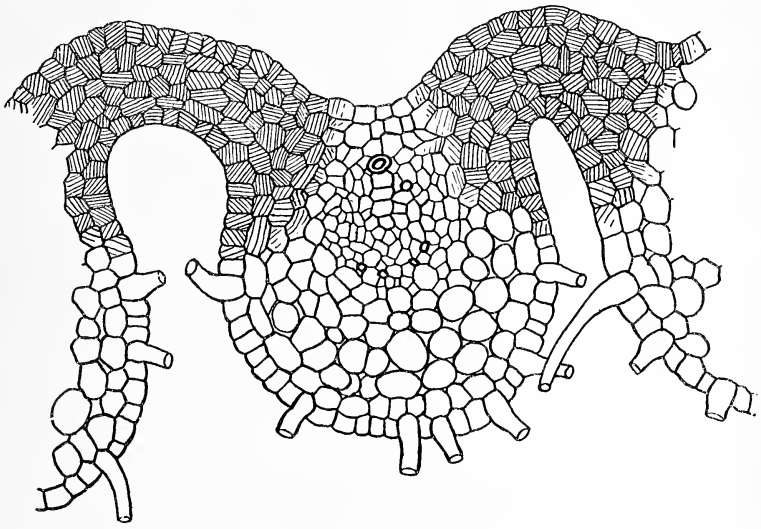
In order to appreciate the effect of environment on the production of hairs, it is necessary to follow to some extent the development of the leaves themselves. Comparatively little appears to have been written on the later stages of leaf development.[2] A brief account will therefore be

[1] Stahl ('83), p. 35, records the same fact for the sun leaves of the Beech and the Bilberry. This is analogous to the development of the interveinal bands of hairs in partially hairy leaflets.

[2] Goebel ('05), p. 302 et seq., and others have described the distribution of growth during development, &c. The former points out (p. 305) that 'in general, parts which have the earlier functions to perform appear the earliest'. Mer ('83), p. 111, has given a very brief account, unaccompanied by figures, of the order of development of the tissues of the lamina itself.

given of the order and time of appearance of certain of the tissues of which the lamina is composed.

If a winter bud of *Spiraea Ulmaria* be examined about November, it will be found that the stipular bud-scales are well developed. The most advanced foliage leaves (the glabrous ones of the following spring), however, have only reached a stage approximately equivalent to that shown in Text-fig. 7 (this figure is taken from a hairy leaf). The leaves remain practically in this condition throughout the winter, and are little further developed by the following March, when the bud begins to expand. Multicellular mucilage hairs are abundant, especially on the upper surfaces of the veins. But the long cutinized hairs[1] which form the characteristic pubescence of the later leaves are at this stage absent from the entire bud. It is



TEXT-FIG. 7. Section through part ot a young developing leaf, while in the bud. The still meristematic portions (i. e. the interveinal parts of lamina) are shaded. × 360.

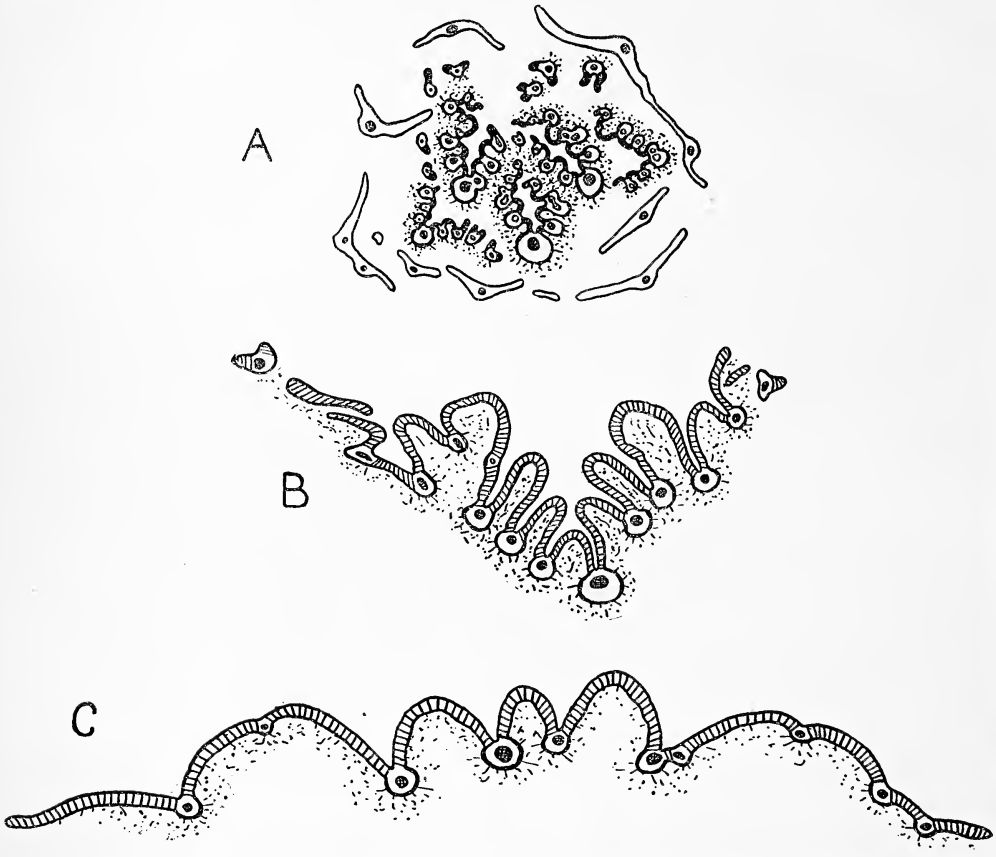
important to notice that *few, if any, of these cutinized hairs are developed in the bud until after the first foliage leaves have expanded and become functional.*

The development of one of the hairy leaves may now be followed. In every case the larger veins are the first parts of the lamina to develop, and are relatively large and well differentiated at a time when the rest of the lamina is still meristematic (see Text-fig. 7). On a leaf such as this, which is destined to become hairy, the first hairs are formed early. They appear

[1] At least three kinds of hairs occur on the leaves of *Spiraea Ulmaria* : (1) capitate mucilage hairs, which appear to function mainly in the bud (in *Spiraea* they wither soon after the leaf expands); (2) short bristly hairs found scattered on the upper surface and at the margins; and (3) the long, unicellular, twisted, cutinized hairs, of which the pubescence clothing the lower surface of the leaves is composed. It is the last kind which is to be understood when 'hairs' are referred to in the remainder of this paper.

on the larger veins at approximately the same time as the protoxylem, and while the leaf is still completely enclosed in the bud (Text-fig. 7). At this time the remainder of the lamina is meristematic, though the upper and lower epidermis and several layers of mesophyll are distinguishable.

A somewhat later stage is diagrammatically represented in Text-fig. 8, A. The first seven leaves of the shoot had expanded, but the leaf figured was still completely enclosed in the bud. The interveinal parts of the lamina



TEXT-FIG. 8. A, transverse section through part of an apical bud, showing several leaflets of a single developing leaf. Meristematic portions shaded black. B, a single leaflet just emerged from the bud. C, a still later stage. All × 16.

are developing, and in consequence assuming a looped appearance. They are still meristematic, and as yet bear no hairs. The mesophyll is undifferentiated, and there are neither stomata nor intercellular spaces. The smaller veins have not yet been formed. In the main veins the protoxylem alone is lignified, but the veinal hairs are now so numerous that they form a fairly dense packing below and between the veins.

Text-fig. 8, B is taken from the terminal leaflet of the ninth leaf of the

same shoot as 8, A. This leaf had emerged from the bud, and so was in contact with the outer air. It was a little further expanded than leaf 6 in Text-fig. 2. By this time more xylem tracheae have matured, and many of the finer veins, also bearing hairs like the main veins, have been differentiated. Many stomata have appeared, and also intercellular spaces in the mesophyll, but the latter are small, and few of the former quite mature. The uppermost layer of mesophyll has begun to take on the character of palisade tissue (this is most marked at the tops of the loops), and in places even a second row of palisade cells has begun to form. Thus at this stage, i.e. very soon after the leaf emerges from the bud, and before it is fully expanded, all the chief tissues are recognizable. But the leaf has not as yet attained either its full size or thickness.

A still later stage is seen in Text-fig. 8, C, which represents a section through the lowest leaflet of the same leaf as 8, B. (N.B.—The leaflets, like the leaves, develop and unfold in regular acropetal succession ; see Text-fig. 2, leaf 5.) The leaf has now nearly reached its final thickness. The smallest veins are present, also the cuticle and two layers of palisade. The stomata are more mature and the intercellular spaces larger.

The ultimate degree of expansion of the cells and tissues, and also the question of whether the lamina shall become flat or remain more or less crinkled or folded, depends chiefly on the external conditions.

The general distribution of pubescence on the leaves has been discussed earlier in this paper. It is now clear that the earliest hairs appear on the main veins, while the leaf is still in the bud. Subsequently, when the leaf is emerging, or has just emerged from the bud, the next hairs to be formed are those on the finer veins at the leaf margins or between the main veins ; that is, on those parts most remote from the main veins, and which are the first to be exposed to the air as the leaf unfolds.[1] Development of the pubescence may now be arrested, as in the partly hairy leaves or leaflets; or it may proceed further until even the finest veins are covered with hairs. In many cases hairs are even formed on the ultimate parenchyma 'islands' bounded by the finest veinlets.

To sum up, the main veins of the lamina, together with the hairs they bear, reach a high state of development in the bud. On the other hand, the hairs on the finer veins, and those which occur over the parenchyma 'islands', only appear when the leaf has emerged from the bud ; i.e. when some parts of it are already in direct contact with the outer air. This is also true of the palisade tissue, the intercellular spaces of the mesophyll, the cuticle, and the stomata.

We are now in a position to discuss :

[1] In the case of the interveinal bands of hairs, it is the *upper* surface which is most exposed, while the hairs are actually developed on the *lower*. Cf. Text-fig. 8, B.

§ 9. The Effect of Environment on the Production of Hairs and Palisade Cells.

(*a*) *Historical.* Many of the older observers noticed that plants not infrequently varied in hairiness according to habitat. Thus Linnaeus[1] mentioned *Plantago Coronopus* as being 'in humido foliis glabris integris, in sicco hirsutis dentatis'.[1] De Candolle[2] said that lymphatic (i. e. non-glandular) hairs 'are in general rare in plants which grow in the shade, or in rich and moist places'; while 'they are more abundant, generally, in those which grow in warm and dry places, much exposed to the sun'. Kraus[3] instanced potato shoots as being less hairy or even glabrous when grown in very moist air than in dry air.

During the last thirty years a considerable literature has arisen, dealing with the influence of external factors, not only on the development of epidermal structures such as hairs, stomata, &c., but also on the differentiation of the mesophyll, and especially of the palisade tissue. One of the earlier works of this period was the classic paper by Stahl,[4] published in 1883, in which the action of light is regarded as the factor determining the structure of 'sun' and 'shade' leaves.

In the same year Mer[5] found that light and also dry air favour the production of palisade tissue, while shade and humidity produce the reverse effect. The same is true also of hairs on aerial stems.[6] A well-watered soil hindered the formation of both stem- and root-hairs, while both were produced if the soil were drier.

Vesque and Viet[7] proved that light, accompanied by more or less dry air, increases the development of palisade tissue, and the number and length of hairs on leaves. In a later paper, Vesque[8] says that the result of a large number of cultural experiments shows that a marked development of palisade tissue can always be referred to the action of transpiration.

Kohl[9] stated that changes of relative humidity in the atmosphere surrounding growing organs are capable of inducing the formation or suppression (as the case may be) of palisade cells. Again, he found[10] that in the case of hairy species of Compositae or Labiatae, not only the number of hairs, but also their length, is determined to a great extent by the amount of transpiration from the organs themselves. The less the transpiration, the fewer and shorter the hairs.

Dufour[11] found that the dimensions of leaves developed in light of high intensity are greater in all respects (and the palisade tissue is more pronounced) than those of leaves grown in shade.

[1] Linnaeus ('63), p. 219.
[2] De Candolle ('41), p. 98.
[3] Kraus ('76), p. 153.
[4] Stahl ('83).
[5] Mer ('83), p. 112.
[6] Mer, l. c., p. 117.
[7] Vesque and Viet ('81), p. 176.
[8] Vesque ('83), p. 489.
[9] Kohl ('86), p. 93.
[10] Kohl, l. c., p. 103.
[11] Dufour ('87), pp. 406–8.

Eberhardt [1] showed that plants grown in dry air tend to be more hairy, and their leaves thicker (in consequence of the great development of palisade), than those grown either in normal or in very humid air.

Zinger,[2] in an interesting paper on some European species of *Camelina*, shows that in *C. microcarpa* and the different varieties of *C. sativa*, a gradual transition from a xerophytic to a hygrophytic type can be traced, with marked differences in respect of palisade tissue, hairiness, &c. These differences are strictly correlated with the conditions of illumination and moisture in the respective natural habitats.

Lesage [3] found that when plants were grown in a saline soil, or solutions in which the salts reached a certain concentration, they developed more palisade than when grown in ordinary soil or very dilute salt solutions. Later [4] he subjected growing plants to periodic variations of atmospheric pressure. Those which had been exposed to the periodic lowering of pressure had (if leaves of the same age, &c., were compared) more marked palisade than the control plants grown under ordinary air pressure.

Similar results to those of the above-mentioned authors have been obtained by other experimenters.[5]

Although the question of root-hairs scarcely comes within the scope of this paper, it may be mentioned that their formation also is largely dependent on the water supply. Schwarz [6] found that no root-hairs were formed in very wet soil. Further, that as the water content of the soil decreased, root-hairs increased correspondingly. Finally, however, when the soil attained a certain degree of dryness, root-hairs were again suppressed.

If the above results be analysed, it will be noticed [7] that *in general*,[8] *both palisade cells and the epidermal hairs of aerial shoots* (*and to some extent at least, root-hairs*) *are most fully developed under conditions which either promote transpiration or hinder absorption*; namely, under conditions which impose on the plant some necessity for increased economy of water.

The results of the writer's own work on *Spiraea Ulmaria* and other marsh plants may now be given.

(*b*) *Field observations on Spiraea*, &c. It has been stated above that even the type-form of *Spiraea Ulmaria* produces both glabrous and hairy

[1] Eberhardt ('03), pp. 149–52. [2] Zinger ('09), pp. 340–9.

[3] Lesage ('89), p. 204, and ('90), p. 170. Cf. also Boodle ('04, second paper), p. 41.

[4] Lesage ('94), p. 255.

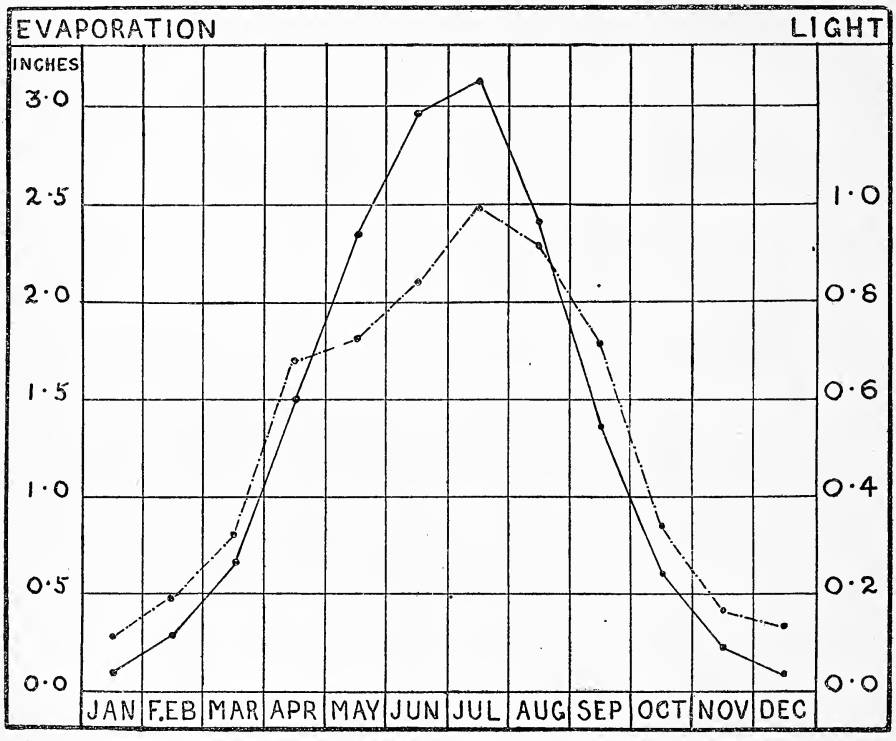
[5] e. g. Géneau de Lamarlière ('92), p. 483 et seq., Lothelier ('93), p. 520, &c. Cf. also Burgerstein ('04), pp. 47–53, and the additional literature cited by him.

[6] Schwarz ('83), p. 14 et seq. Cf. also Sorauer ('86), pp. 95–6, and Mer ('83), p. 117.

[7] Cf. Lesage ('94), and Burgerstein ('04), pp. 51 and 53.

[8] Of course plants vary much in plasticity. Stahl ('83), pp. 5–10, instances cases of pronounced shade and sun plants which have little or no power of adapting themselves to sunny and shady habitats respectively.

leaves. As a rule, these occupy in nature the more shady and humid, and the sunnier and drier positions respectively. This is true as regards both time and space; i. e. whether we consider the leaves in relation to the annual march of the seasons, or the vertical distribution of humidity and light in the vegetation. The curves in Text-fig. 9 represent the mean evaporation and light intensity for several years. Both curves rise to a maximum in July and then fall steadily towards autumn. Further, I have shown that in marshes there is a rapid rise in the average evaporation as the



TEXT-FIG. 9. Curves of mean evaporation [continuous line] and light-intensity [broken line]. The former shows the mean monthly evaporation for twenty-five years in London [see Mill ('10), p. 56]. The latter represents the mean maximum light-intensity at Vienna for three years only [taken from Wiesner ('07), p. 52].

successive strata of the vegetation are ascended.[1] Lately, I have also determined by experiment that the same is true of light-intensity.

If the degree of hairiness could be conveniently represented by means of curves, it would be found that the curves of actual hairiness in *Spiraea Ulmaria* follow very closely the mean curves of evaporation and light-intensity, both in space and time. Thus, (1) in the erect flowering shoots, the glabrous spring leaves are formed near the ground, at a time of

[1] Yapp ('09), p. 287 et seq. Dachnowski ('11), p. 146, has recently confirmed my evaporation results, in the case of the bog vegetation of Cranberry Island.

year (about March) when the average evaporation is slight and the light-intensity low. As the season progresses and the curves of evaporation and light rise steeply (Text-fig. 9), and further, as the shoot itself grows upwards into drier and lighter strata, the leaves exhibit continually increasing hairiness (Pl. LXXXI, and Text-fig. 10). (2) The curve of hairiness of non-flowering shoots rises steadily to June or July; subsequently falling till glabrous leaves are once more reached in autumn. This corresponds very closely indeed to the curves in Text-fig. 9. Finally, (3) the same general relations obtain even in the case of individual partly hairy leaves. Just as

TEXT-FIG. 10. Diagrammatic section through a group of plants of *Spiraea Ulmaria*, showing relation of glabrous and hairy leaves to strata of varying humidity. Glabrous leaves, black with white veins; hairy leaves, white with black veins. (June.) × about $\frac{1}{7}$.

successive leaflets occupy higher and less humid levels, so they exhibit increasing hairiness from below upwards (Pl. LXXXII and LXXXIII, and Text-fig. 10).

The same relations are broadly true of other hairy marsh plants,[1] and also for the structural variations described earlier in this paper. These field observations are at once seen to agree with the general experimental results quoted above. They suggest considerable responsiveness, on the part of *S. Ulmaria* and other plants, to even slight variations in the environment.

[1] See § 6; also Yapp ('08), p. 691.

(*c*) *Experiments on Spiraea*, &c. In the first instance I was easily able to confirm the researches of Vesque and Viet, Kohl, and others,[1] on the effect of humidity on hair production in certain plants. E. g. in *Epilobium hirsutum* and *Mentha aquatica*, the formation of hairs was completely inhibited by growing the plants in a damp atmosphere; the converse being true of a dry atmosphere. The case of *S. Ulmaria*, however, proved to be somewhat different. A large number of plants of this species were grown, some at the Cambridge Botanic Garden (through the kindness of Mr. R. I. Lynch), and others at Aberystwyth. A wide range of habitats was secured by varying the following factors: (1) soil, (2) soil-moisture, (3) humidity of air, (4) light-intensity, and (5) degree of exposure or shelter.[2]

The experiments extended over a period of four years, so that while the same individual plants were used, several generations of shoots were observed.

On the whole, the effect of varying habitats on the hairiness of *Spiraea Ulmaria* was less than had been expected, the character being apparently one of a certain degree of fixity. Differences of soil, soil-moisture, light-intensity, and exposure produced no effect whatever on the normal periodicity of hair formation. Under no conditions could the normally glabrous spring or autumn leaves be induced to become hairy,[3] while in all cases the upper leaves on the flowering shoots produced hairs.

But though external conditions had comparatively little controlling effect on the actual formation of hairs, in certain instances they exercised considerable influence on the frequency, and especially on the length of the hairs. This was the case with shoots grown under glass shades, i. e. in still, humid air. When (*a*) these were exposed to full sunlight,[4] the effect was

[1] Vesque and Viet ('81), Kohl ('86), &c.

[2] *At Cambridge* the plants were potted in either (*a*) pure peat, (*b*) loam, or (*c*) a mixture of peat, loam, and sand. The pots were then divided into two sets. One set was placed in a fully insolated part of the garden, the other in a shady spot, never reached by the direct rays of the sun. Soil-moisture was varied by submerging (partly or completely) certain pots in water. Others had good drainage, but were copiously watered, while a third set were only watered at infrequent intervals. A final differentiation of habitat consisted in placing glass shades over some of the drained and also undrained pots. The aerial shoots were thus developed in a still, humid atmosphere, under varying conditions of light, soil-moisture, &c.

At Aberystwyth plants were grown in various situations in a garden, some in deep shade, others in the open, &c. Others were kept in the laboratories, and also on the roof of the College, which is situated close to the sea, and exposed to great gales of wind.

[3] Cf. Vesque ('84), p. 21, who says that he had never succeeded in inducing a single hair to form on a really glabrous plant: though theoretically he thought it should be possible, as 'les plantes glabres ont dû précéder historiquement'.

[4] As determined by experiment:—

(1) The shade plants (*b*) were grown under light-intensity = 1·0
(2) ,, sun ,, (*a*) ,, ,, ,, ,, = 4·5
(3) In the free air outside the glass shade (*a*) ,, = 4·9

Thus, a little light is cut off by the glass shade, or by the moisture which condenses on its

comparatively slight; the leaves appearing to be nearly as hairy as those grown in the open. The hairs, however, were distinctly shorter. *The greatest diminution of hairiness was induced when low light-intensity was superadded to stillness and humidity of the air.* Thus (*b*) flowering shoots grown in weak light under glass shades still produced more or less hairy leaves, but the hairs were straight and very short. The lower cauline leaves appeared to be more easily influenced than the upper.

A curious inhibition of hairs occurred on plants which had been protected from cold, &c., during the winter. A number of plants were kept indoors continuously for some years, in the Cool Fern House and the Cool Pit at Cambridge, and the Laboratories at Aberystwyth. In all cases the rhizomes of these plants remained horizontal, and each year produced a crop of radical leaves of the glabrous spring or autumn type.[1] In a few instances patches of hairs occurred on a leaf, but for the most part the leaves were entirely glabrous. On the other hand, plants brought indoors towards the close of the winter produced normal erect flowering shoots with hairy leaves.

Another experiment which resulted in decreased hair formation is recorded on p. 847.

Spiraea Ulmaria, var. *denudata*, Boenn., like the type form, commences in spring-time by forming glabrous leaves. These are followed by increasingly hairy leaves as the erect flowering stem elongates. But here the hairiness begins somewhat later, and is chiefly confined to the larger veins of the leaves; the general pubescence, so characteristic of the type, being absent. In nature I have found many transitional forms intermediate between *S. Ulmaria* and its variety *denudata*.[2] Thus individuals are sometimes found with an unusual number of glabrous radical leaves. In others, one, two, or even more of the cauline leaves may also be glabrous. Finally, plants occur in which only the leaves at the extreme top of the flowering shoot are markedly hairy. But in all cases, whether in the type, or its var. *denudata*, or in any of the numerous intermediate forms, the lower leaves are glabrous. On the other hand, the cauline leaves are (in varying degrees) increasingly hairy as the flowering shoots are ascended.

inner surface. The method used was based on the following: If a solution of potassium iodide be mixed with dilute sulphuric acid, and exposed to light in the presence of oxygen, the iodine liberated is a measure of the total light received during the time of exposure (see Manchester ('93), p. 88 et seq.). This method, to which Professor Weiss called my attention, has the advantage of recording the *total light received*, and not merely the intensity at a given moment. Like other photometric methods, it has the disadvantage of recording only the chemically active rays. For a discussion of the utility of such methods for botanical researches, see Rübel ('08), pp. 5–7.

[1] Gates ('10), p. 677, similarly found that the internodes of Oenotheras grown in a tropical greenhouse did not elongate, the plants thus remaining (for the entire time of the experiment, i. e. twenty months) in the rosette condition. Cf. also the experiments of Klebs ('10), p. 552 et seq., on *Sempervivum*.

[2] Trow ('11), p. 58, also states that *S. denudata* occurs in Glamorganshire, together with many intermediate forms connecting it with the type.

The var. *denudata*, as well as several intermediate forms, has been grown for some years. The results show that the individual, in its successive generations of shoots, retains to a considerable extent its peculiar characteristics, even under varying habitat conditions. For example, *S. denudata* has never been induced to become really hairy, even when grown in dry, sunny, exposed situations.

The results recorded in this section show that (1) the production of glabrous and hairy leaves in *Spiraea Ulmaria* and certain other marsh plants closely follows in nature the mean curves of evaporation and light-intensity; but that (2) *S. Ulmaria* possesses a more limited plasticity, in respect to hairiness, than that exhibited by such species as *Mentha aquatica*, &c.

§ 10. The Localization of Withering in Leaves exposed to Wind, etc.

Before discussing the causes of hair formation, certain parallel phenomena must be mentioned. As stated above (p. 820), the partially hairy leaves and leaflets of *Spiraea* exhibit a definite localization of the hairs (cf. Pl. LXXXII and LXXXIII). It was early noticed[1] that when *Spiraea* plants with glabrous leaves were exposed to winds sufficiently strong to cause partial withering, the distribution of the withered parts coincided very nearly with the distribution of hairs on the partly hairy leaves of the same species. Exposure withering thus begins round the margins of the leaflet, and sometimes, though not always, extends inwards in the form of bands between the main veins. Subsequently observations were made on the localization of withering in the leaves of many other plants. These, which for the most part confirm the results of Schröder,[2] published in 1909, may now be summarized (cf. Text-fig. 11).

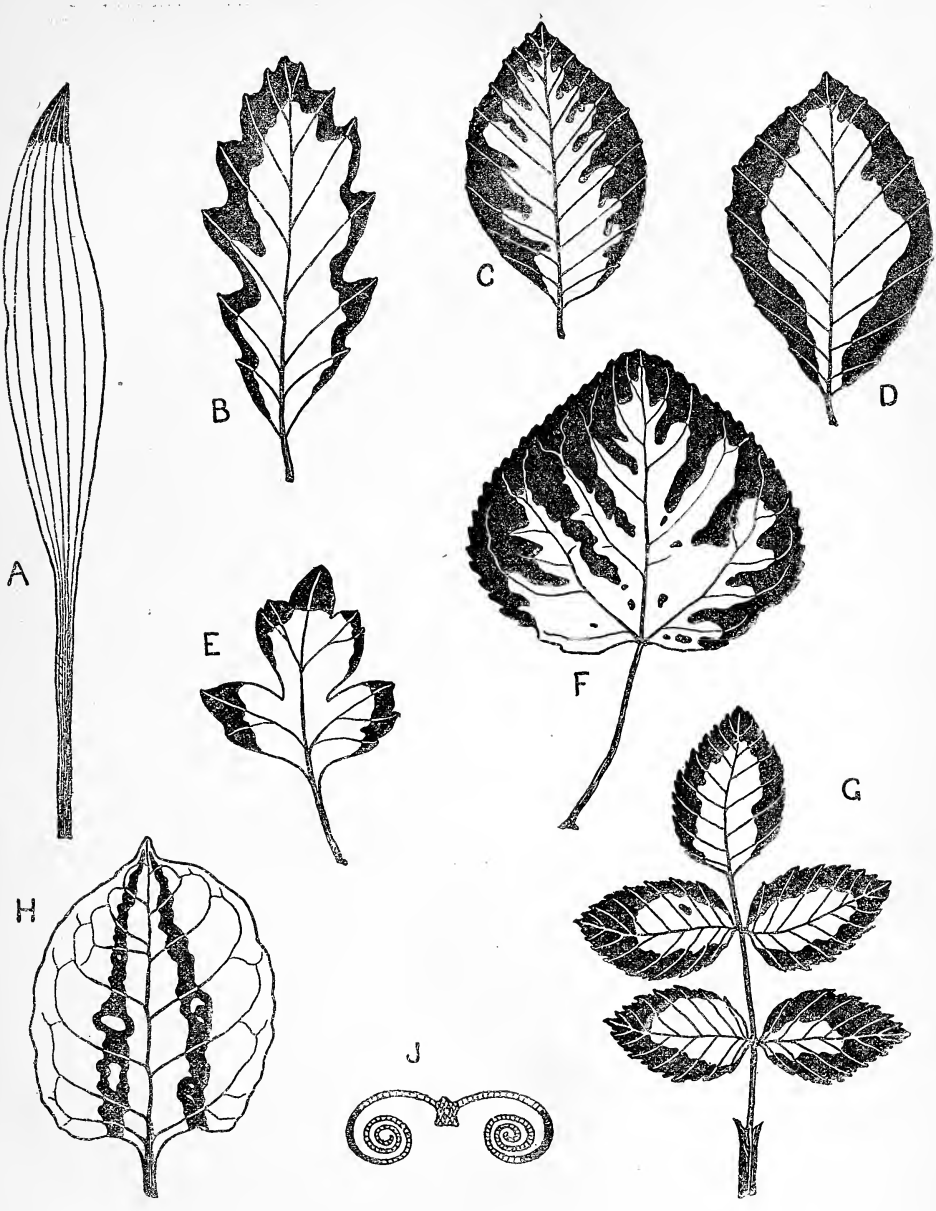
Leaves which are allowed to wither naturally in still air lose their colour gradually, and either more or less uniformly or else irregularly. On the other hand, the effect of exposure to strong winds, &c., is to cause first a marked withering of projecting apices, e. g. the apex in linear leaves,[3] &c., leaf-teeth, or the various tips of lobed leaves (see Text-fig. 11, A, B, and E). Next, in other than linear leaves, the withering extends round the margin of the leaf, and sometimes, though by no means always (even the same species may vary in this respect, cf. Text-fig. 11, C and D), between the larger veins (Text-fig. 11, B–G).[4] The process may now stop, or it may

[1] Yapp ('08), p. 691.

[2] Schröder ('09), pp. 85, &c. Apart from this paper the literature on the withering of leaves is scanty. Hansen ('01), p. 32, and ('04), p. 34, calls attention to the withering of leaf apices and margins through wind. Hasenclever ('79), and Oliver ('93), p. 22, show that acid gases will cause discoloration of the apices and margins of leaves. Cf. also Stahl ('09), pp. 134–5.

[3] Cf. Yapp ('08, second paper), p. 65.

[4] My attention was called by Professor F. W. Oliver to the exceptional case of *Polygonum*



TEXT-FIG. 11. To illustrate localization of withering in leaves exposed to wind, &c. Withered portions, black with white veins; uninjured parts, white with black veins. A, *Plantago lanceolata*; B, *Quercus Robur*; C and D, *Fagus sylvatica*; E, *Crataegus Oxyacantha*; F, *Populus* sp.; G, *Rosa* sp.; H and J, *Polygonum cuspidatum*. J is a section through an unfolding leaf: the black parts here are not yet withered, though they may wither later. A–H, × $\frac{2}{3}$; J, × $4\frac{1}{2}$.

continue until only the tissue immediately surrounding the larger veins remains green;[1] finally this too may wither and die.

Further, a similar localization is seen in other connexions. Thus anthocyan in young leaves in spring-time is frequently confined to the tissues at the apex of the leaf, round the margins, and between the larger veins.

Lidforss[2] figures leaves of *Viburnum Tinus*, in which certain portions have been killed by frost. These portions are the margins, and to some extent the interveinal regions. On one occasion the present author noticed an autumn leaf of *S. Ulmaria* which had been injured by frost. Not only did the frozen portions show a similar localization in the individual leaflets, but the different leaflets exhibited decreasing injury from above downwards (cf. the hairiness described on p. 820).

Again, Professor T. H. Macbride, of Iowa, told me in 1909 that Myxomycetes often confine their attacks to just these same regions of a leaf.

It is obvious that those regions of the leaf lamina which show a special tendency to form hairs (in *Spiraea*), to wither on exposure to strong winds, &c., &c., are physiologically different from the remaining parts. So far as their water supply is concerned, it may be pointed out that they are usually thinner and more exposed than the parts immediately surrounding the larger veins. They are thus more liable to lose water. Further, they are themselves just the regions of the lamina which are most remote from the water supply coming up from below and passing along the main veins.

§ 11. The Causes of the Formation of Hairs and Palisade Cells.[3]

Hairs on aerial shoots and palisade cells have a good deal in common. Both are elongated in a direction more or less at right angles to the surface of the organ which bears them. The production of both is favoured (at least in many cases) by the same external conditions (see § 9). Root-hairs

cuspidatum. If in exposed situations, the leaves of this plant are often marked in the spring-time with red bands, parallel to the midrib, and therefore crossing the larger lateral veins. Later, these red bands may disappear, or, if windy weather ensues, the tissue may wither and actual lesions occur (Text-fig. 11, H). This and other Polygonaceae (e.g. *Rumex* spp.) are the only instances I have seen in which the withered areas cross the larger veins, instead of lying between them. It is to be noted that the injury occurs in those parts which are exposed during the unfolding of the revolute leaves (Text-fig. 11, J). If withering is delayed until the leaf is mature, it is then localized round the margins in the normal manner.

[1] In certain cases, e. g. leaves of *Acer, Populus*, &c., fallen leaves may be found with the larger veins dead and brown, and the interveinal tissues green. This is the exact converse of exposure withering. It is due, so Mr. G. Massee informs me, to the action of certain parasitic Fungi, which attack only the leaf veins.

[2] Lidforss ('07), Taf. I.

[3] A short paper on this subject was read at the Portsmouth meeting of the British Association, Yapp ('12), p. 565.

also may to some extent be included in the parallel. Probably all three elements play an important part in relation to the water supply of the plant (cf. § 12). They may therefore be considered together, though the main concern of this paper is with the leaf-hairs of *Spiraea*.

In the first place, in so far as the plant is plastic, is it possible to determine the nature of the stimulus to which the plant responds by the development of these special elongated cells?

Kraus [1] explained the formation of hairs on potato stems in dry air by supposing that decreasing humidity hindered growth in length. In consequence, said he, turgor increases in the transverse direction, and the epidermal cells grow out in the form of hairs.

Mer [2] supposed that the appearance of both stem- and root-hairs is due to the accumulation of nutritive substances caused by a slowing down of growth. Vesque [3] adopted Mer's view, though he thought it complicated matters to include root-hairs in the hypothesis.

Jungner [4] suggested a theory respecting the formation of hairs on certain xerophytes of alpine regions, extending it to include those of deserts and salt steppes. Although acknowledging that the plants are exposed to strong evaporation, he assumed that transpiration is depressed during the early stages of leaf development. Consequently the pressure of turgor in the cells is high, and, the air outside being rarified, the turgid cells grow out in the direction of least resistance.

Turning now to theories of palisade formation, Stahl [5] supposed that intense light retarded the growth in length of the veins of developing leaves. The young assimilating cells then expand in the only direction possible (i.e. that perpendicular to the leaf surface), and in consequence assume the form of palisade cells.

Mer [6] attributed a marked development of palisade cells to active nutrition resulting from the action of light in dry air.

Vesque [7] regarded transpiration as the chief factor inducing the formation of palisade tissue. Kohl,[8] who held the same view (and applied it also to hair formation), further pointed out that transpiration affects turgescence, and turgescence regulates the growth of cell membranes.

Of these theories, that of Kohl (and of Vesque so far as palisade cells are concerned) agrees best with the known facts (cf. § 9). All the evidence available suggests that any marked development of these peculiar elongated elements is in some way associated with *decreased turgor* rather than the *increased turgor* postulated by Kraus and Jungner. This evidence is as follows:

1. *The experiments of many observers*, which show that in general these

[1] Kraus ('76), p. 153.
[2] Mer ('83), p. 115.
[3] Vesque ('83), p. 495.
[4] Jungner ('94), pp. 233–4.
[5] Stahl ('83), p. 34.
[6] Mer, l. c., p. 112.
[7] Vesque, l. c., p. 489.
[8] Kohl ('86), pp. 95 and 103.

elements are developed especially under conditions which either promote transpiration or hinder absorption (see p. 835). For *S. Ulmaria*, &c, in particular, we have seen that in nature the curves of hairiness follow very closely the mean curves of evaporation, &c. It is obvious that such external conditions can only act by inducing internal changes in the cells themselves. Kohl (l. c.) has already suggested that changes of turgor may be important in this connexion.

2. *What may be termed topographical evidence.* It has been shown (§§ 5 (*d*) and 10) that under certain conditions the margins of leaves and the thinner areas between the main veins exhibit phenomena which the other parts of the leaf do not. Also that these parts are not only especially liable to lose water, but are situated furthest from the water supply coming up from below. Thus it is easy to understand that any shortage in the supply of water to the leaf will affect these regions first and most severely. That such is the case is evidenced by the localization of withering from exposure to wind.[1] These considerations suggest that the marked localization of hairs (and to some extent palisade cells, see p. 830) in the same regions of the leaf may also be connected with a diminished water supply. This is, of course, especially so in view of the known effects of certain external factors (see 1 above).

3. *Evidence derived from leaf-development in Spiraea.* As regards the hairs, we must distinguish between those formed in the bud and those formed during the unfolding of the leaf (see § 8).

It has been shown that, while the earliest leaves are entirely glabrous, the later ones become progressively more hairy, the hairs on the larger veins being formed while the leaf is still in the bud (see Text-figs. 7 and 8). This increasing hairiness of the larger veins of the developing leaves occurs *pari passu* with the general increase of transpiration from the shoot. The latter is of course due partly to the increase of transpiring surface as more leaves are unfolded, and partly to the march of the seasons. Knowing the effect in general of a dry habitat on hairiness, it is difficult to avoid the suggestion that this increase of hairiness may be connected with a diminution of the water supply to the apical bud. This may readily be brought about by a partial deflexion of the water current into the continually increasing number of mature leaves. In this way the cells of the young developing leaves will find it increasingly difficult to maintain themselves in a complete and constant state of turgor. If this be so, it may be regarded as another case of what Wiesner[2] has called 'correlative transpiration'. Under this term he includes all cases in which strongly transpiring organs deprive others (which transpire little or not at all) of water. Such displacements of water not infrequently bring about striking results. Thus

[1] Hansen ('04), p. 43, &c., discusses the withering of leaves as a result of withdrawal of water by wind.

[2] Wiesner ('05), p. 477 et seq.

Wiesner (l. c.) observed the production of anisophylly in *Aesculus* under the influence of correlative transpiration. Again, Schimper,[1] Haberlandt,[2] and others found that the lower leaves of certain plants yielded up their water to the younger leaves when subjected to drought. Two cases which approach that of *Spiraea* very nearly are recorded by Smith and Balls. Smith[3] produced evidence to show that during the day-time transpiration from adult bamboo culms may be so great as to draw off the supply of water from the growing culms, thus slowing down their rate of growth. Similarly, Balls[4] found that in summer-time in Egypt the growth of the stem of cotton plants and others (e. g. *Helianthus*) was completely arrested whenever direct sun was shining on the plant. This he showed to be due to the consumption of all the available water in transpiration from the already unfolded leaves. The water supply was thus the limiting factor in stem growth. These instances add to the probability that a similar disturbance of the supply of water to the apical bud takes place in *Spiraea Ulmaria*, and that this disturbance affects the production of hairs in the bud.

But most of the leaf hairs, as well as the palisade cells, actually elongate after the leaf emerges from the bud, and while it is unfolding in direct contact with the outer air (p. 833). At this stage cuticular transpiration must be an important factor.[5] Owing to its vernation, the parts directly exposed to atmospheric influences when the leaf first emerges from the bud are the larger veins (which are already covered with hairs) and the delicate leaf margins (cf. Text-figs. 8 and 2, leaf 6). Naturally, therefore, the loss of water by cuticular transpiration will be most severely felt by the latter. As the leaf still further unfolds, and if the transpiration is sufficient, the next parts to experience a shortage of water will probably be the interveinal areas, because it is here less easily replenished. It is precisely in these regions that the hairs are localized in the partly hairy leaves. Finally, when the water supply is still further diminished, the lower surfaces of the unfolding leaves become completely covered with a dense pubescence.

Thus the formation of both the hairs on the larger veins, as well as those on the smaller veins and other parts of the lamina, may be correlated with a diminished water supply to the developing leaves. In the former it seems probable that correlative transpiration plays the most important part. In the latter, loss of water by cuticular transpiration comes into play, coupled with (as before) a strictly limited supply of water from below.

Further, the fact that all the cells of the upper leaves are much smaller than those of the lower (cf. Text-fig. 4) suggests development under conditions in which there is a partial deficiency of water. In other words, it is

[1] Schimper ('88), pp. 37–8.
[2] Haberlandt ('09), p. 368.
[3] Smith, A. M. ('06), p. 325.
[4] Balls ('10), p. 6, and ('11), pp. 229–30.
[5] F. von Hoenel ('78) showed that very young developing leaves actually transpire more than mature ones. This he attributed entirely to cuticular transpiration.

probable that the upper part of a normal *Spiraea* shoot has, during development, a less constantly maintained turgor than the lower,[1] and that the water supply is the limiting factor in the growth in size of the cells.

But now arises a difficulty. Although turgor is no longer regarded as the direct cause of the stretching growth of cell-walls,[2] it is none the less a condition of such growth.[3] It is difficult to conceive how either hairs or palisade cells can elongate unless they are turgid. And yet we have seen that they are most strikingly developed under conditions when turgor may be expected to be at a minimum. The difficulty, however, is more apparent than real. In nature the external conditions are never constant. No matter how active transpiration may be at certain times, periods frequently recur (e.g. at night-time or on still, humid days) when turgor is fully restored.

The course of events then is probably somewhat as follows: transpiration during the day-time, increasing with the number of leaves unfolded, and (at least until midsummer) with the march of the seasons, gives rise to a diminished turgor in the cells of the developing leaves. This disturbance of the water supply to the yet plastic cells acts to certain of them as a stimulus (merely, of course, an initial or proximate one), in response to which the cells assume an elongated form.[4] But the actual stretching growth of the cells can only take place when they once more become turgid. This occurs when transpiration falls at night-time, &c.[5]

Boodle,[6] in his suggestive paper on the Bracken Fern, has made a similar suggestion with regard to the factors determining leaf structure. He says 'it may possibly be some such factor as strong periodic fluctuations in the turgescence of the cells of the leaf (due to scarcity of water when transpiration is most active) which determines in the immature leaf of the Bracken whether the leaf shall be xerophytic or not; and the same may apply to other plastic species'.

The case seems clearly one of stimulus and response, and the somewhat mechanical theories referred to above appear to be inadequate.

[1] Cf. Pfeffer ('03), p. 120. Miss Delf ('11), p. 501, states that the upper internodes of *Salicornia* had not only more numerous stomata than the lower, but also much smaller epidermal cells. This she regards as indicating that neither they nor the guard cells had attained their full size; and this notwithstanding the fact that the stomata were fully formed and open. Whatever the case may have been in *Salicornia*, the upper leaves described for *Spiraea* were certainly mature, in spite of the small size of their cells.

[2] As Wortmann ('89), p. 293, &c., and others supposed it to be. Cf. the discussion of this question in Sokolowa ('98), p. 179 et seq.

[3] Cf. Pfeffer ('03), p. 118 et seq.; also Sachs ('87), p. 565.

[4] In this case the stimulus appears to induce the cells to assume a special *form*. In many of the cells of these upper leaves it is chiefly the *size* of the cell which is affected, owing to the stretching growth of the cell-walls being less.

[5] It seems certain that such fluctuations in the moisture content of leaves do actually occur, at least in mature leaves. Cf. Thoday ('09), p. 19 et seq., also Livingston and Brown ('12).

[6] Boodle ('03), pp. 665–7.

There yet remains, however, one factor to be briefly considered, i. e. *light*. We have seen that in *Spiraea* a humid atmosphere has relatively little effect in preventing hair formation unless coupled with light of low intensity. Many observers have regarded light as the determining factor, especially in the formation of palisade tissue. In practice it is extremely difficult to satisfactorily control habitats in such a way as to distinguish clearly between the effects of light and relative humidity: two factors very closely associated in nature. Critical researches in this direction are much needed. In the absence of these it is impossible to say anything definite as to the mode of action of light. Failing evidence to the contrary, the writer somewhat inclines to the view of Vesque and Viet:[1] i. e. that light acts by influencing transpiration. Even in a still, humid atmosphere, intense light would certainly tend to increase transpiration, by reason of its heating effect. This view makes it less difficult to understand why the combined effect of two such apparently different factors should be greater than when either acts alone. At present, however, the possibility cannot be excluded that light may act either as a direct, independent stimulus, or that we may be dealing with a case of 'associated stimuli'.[2]

So far the case has been made out most clearly for hair production in *Spiraea Ulmaria*. But in the light of our knowledge of the conditions which favour the formation of hairs (including root-hairs) and palisade cells in other plants (cf. §§ 6 and 9), it would seem not improbable that similar factors are at work in these also. In other words, that we are dealing with a more or less general phenomenon.[3]

An interesting apparent exception to what seems to be the general rule regarding the hairiness of successive leaves of a shoot is the case of certain South-west African plants recorded by Schinz.[4] In a number of species of *Pseudobarleria*, &c., the first-formed leaves are markedly hairy; the later ones, however, are much less hairy. This is the converse of *Spiraea*. But the more hairy leaves are apparently unfolded towards the close of the hot, dry season; the less hairy ones during the cooler period of rains. So that here, too, hair production shows a dependence on external factors similar to the cases described in this paper.

4. Acting on the assumption that hair formation in *Spiraea* is affected by the amount of water supplied to the apical bud, some experiments were tried on growing shoots. When a number of leaves had been unfolded and were vigorously transpiring they were all cut off, subsequent leaves being also removed as soon as mature. It was thought that the removal of the transpiring surface, after a vigorous water current from the roots had been

[1] Vesque and Viet ('81), p. 175. [2] Cf. Darwin ('06).

[3] It is of course possible that in special cases the stimulus may be different, either in nature or degree: e. g. when palisade cells are formed in aquatic plants.

[4] Schinz ('94), pp. 67–70.

established, might result in at least a temporary increase in the supply of water to the apical bud. This, by preventing the stimulus of decreased turgor, might perhaps bring about the formation of glabrous leaves. The results were not uniform, but this was to be expected, as we are dealing with a complex of variable factors. In each experiment, however, one or more glabrous leaves were sooner or later produced high up on an erect flowering shoot, where normally only hairy leaves are found.

One example may be given. A normal shoot was selected, which on May 21, 1907, possessed seven unfolded leaves. Of these, leaves 1–3 were glabrous, leaves 4 and 5 partly hairy, and leaves 6 and 7 completely hairy. These seven leaves were then removed from the plant. At this date leaf 8 (a hairy one) was unfolding. Subsequently, leaves 9 and 10 showed decreasing hairiness, while leaf 11 was quite glabrous. Leaf 12 had marginal hairs, leaves 13 and 14 exhibited increasing hairiness, while leaves 15–17 (the last produced on this shoot) were hairy, though not densely so.

It is difficult to say exactly what the reason is for this abnormal production of glabrous leaves. It may be that it is partly due to nutritive difficulties, for cutting off the leaves removes the assimilating as well as the transpiring surface. But if so, one would scarcely expect the return of hairiness described above. Taken in conjunction with the evidence previously given, the experiments, so far as they go, certainly suggest that the explanation may be, in part at least, the one already offered.[1] Some additional support of this view (i. e. that cutting off the transpiring surface may increase the supply of water to the apical bud) is furnished by an experiment of Balls's.[2] This author's observations on the cessation of growth of the cotton plant during sunshine have been already cited. He further found that removal of about one-third of the total leaf area in full sunshine caused the terminal shoot to resume growth at once, owing apparently to the decreased loss of water from the plant.

The causes which determine the presence or absence of hairs in *Spiraea Ulmaria* may now be summarized as a whole. Several distinct factors appear to be involved, i. e.:

(*a*) *Heredity.* It is difficult to assign exact limits to the part played by this. The inherent tendency to form hairs in the first instance is certainly hereditary. But how far the degree of hairiness in var. *denudata* and the

[1] Although the experiments were undertaken more or less with the expectation of arriving at the results actually obtained, there is reason for caution in interpreting these results. On several occasions I found plants in the field, the stems of which had been injured an inch or two above the ground, apparently at an early age. In each case two or three of the cauline leaves immediately above the injury were less hairy than the normal, and in some instances even glabrous. Thus it would seem possible that the abnormal production of glabrous leaves described in the text is in some way connected with the injury caused by the removal of the leaves, rather than with the water supply as suggested.

[2] Balls ('10), p. 9.

intermediate forms is determined by heredity, or how far it is attributable to individual variation, is at present problematical.

Goebel, in his interesting researches on the various forms of leaves in *Campanula rotundifolia*,[1] found that leaf form depended largely on illumination. In nature the seedling leaves, as well as the first leaves formed on the lateral runners, are always of a petiolate, rotund type. Linear leaves, on the other hand, are only formed on the erect flowering shoots. By lowering the light-intensity, rotund leaves could be induced to form on the flowering shoots in the place of linear ones. But the converse did not hold. Even the strongest illumination, whether continuous or intermittent, was powerless to hinder the formation of rotund leaves on either the seedlings or the runners. Goebel regarded the rotund form of the leaf in these cases as hereditarily fixed.[2]

Now the glabrous leaves of *Spiraea Ulmaria* are formed in nature under precisely the same conditions as the rotund leaves of *C. rotundifolia*, and the hairy ones correspond in position, &c., to the linear leaves of *Campanula*.[3] Further, all attempts to induce the formation of hairs either on seedling *Spiraeas* or on the early spring leaves of mature plants have been unsuccessful. It is therefore probable that glabrousness is a character hereditarily fixed in these leaves, just as Goebel supposes roundness to be in the corresponding leaves of *Campanula*.

(*b*) *Individual variation.* One of the most obvious expressions of this is seen in the variable number of glabrous leaves formed on a shoot. Usually the number varies from three to five, though in exceptional cases as many as twelve glabrous radical leaves have been observed. Again, in most cases one or more hairy radical leaves are produced before the erect flowering shoot begins to elongate. But individuals are sometimes found (see p. 839) in which even the lowest one or two cauline leaves are glabrous or nearly so.[4] As these plants occur side by side with normal ones, it seems probable that it is simply a case of individual variation.

(*c*) *Effect of external conditions.* This has already been discussed at length, and the conclusion arrived at that hair production is promoted by marked periodic fluctuations in the turgor of the hair-producing cells. It has also been shown that *Spiraea* is plastic only to a limited extent in regard to hairiness. Marked changes in the external conditions are required before any visible response can be obtained. This plasticity is less than that of *Campanula* in respect of leaf form, or than the plasticity with regard to hairiness in such plants as *Mentha aquatica*, &c. The stimulus, then, which

[1] Goebel ('96), ('08), and other papers. [2] Goebel ('96), pp. 8–9.

[3] There is frequently a slight approach even in the matter of form; see Text-fig. 5 and Pl. LXXXI, which show the tendency in *Spiraea* to form rounder leaflets below and narrower ones above. This is a common phenomenon in plants.

[4] And this in localities in which var. *denudata* does not occur, so that it can scarcely be a case of hybridization.

decreased turgor appears to afford to the hair-producing cells is probably more of the nature of an accelerating stimulus than an actually determining one.

Further, different parts of the plant vary in plasticity. As mentioned above, the lowest leaves are not plastic, but are persistently glabrous. The upper leaves on the erect flowering shoots, on the other hand, are fairly persistently hairy. The most plastic leaves of all appear to be those occupying an intermediate position, i. e. the lower hairy ones. Many plants are more plastic than *Spiraea* in regard to hairiness; but in all cases hairiness appears to be associated with relatively dry, and glabrousness with relatively humid conditions. Taking these facts into account, it would seem that *Spiraea Ulmaria* is retaining a certain degree of plasticity in regard to hairiness. But at the same time it is tending to become fixed in the direction of response to the average conditions, to which the upper and lower leaves of the species, respectively, have been exposed in nature for many generations.

§ 12. The Physiological Effect of the Hairs, etc.

Most authors agree that a dense covering of dead, cutinized hairs depresses transpiration. This is said to be due to the retarding of gaseous interchange on the transpiring surface, and also, in some cases, to the hairs acting as a screen against intense insolation. According to the distribution of the hairs on the respective surfaces of the leaf, one or other effect will be more marked. But, as Haberlandt[1] has pointed out, there is an absence of critical researches on the subject. At first sight, *Spiraea Ulmaria*, with its glabrous and hairy leaves, would seem to be admirably adapted for such critical researches. But, as we have seen, the progress towards hairiness in the leaves of this species is accompanied by other structural changes. These also cannot but exert an influence on transpiration. The whole question of the relation between leaf structure and transpiration is in a somewhat chaotic condition, different experimenters having obtained diametrically opposed results. Thus, von Hoenel[2] found that under similar conditions 'shade leaves' transpired more strongly than 'sun leaves'. Géneau de Lamarlière,[3] on the other hand, obtained exactly opposite results. The question is a complicated one, and the author hopes to deal with it more fully and critically in a future paper. For this reason no attempt will be made at the present time to do more than put forward certain *a priori* considerations, and to refer briefly to the work of other botanists.

[1] Haberlandt ('09), p. 116. This author himself performed some experiments on the effect of hairs on transpiration (l. c., p. 138).

[2] Von Hoenel ('79), quoted by Burgerstein ('04).

[3] Géneau de Lamarlière ('92), p. 542. Cf. also the literature cited in Burgerstein ('04), p. 55 et seq. Also Hesselman ('04), p. 402 et seq.

Wiegand [1] has recently experimented with various materials, so arranged as to simulate the hairy and cutinized coverings of plants, and determined the extent to which they hindered evaporation. From his experiments he concluded that hairy coverings (even when of a thin, strigose nature) markedly retard transpiration in wind, but have comparatively little effect in still air. They also have a marked retarding effect in sunshine. Further, that a waxy (i. e. cutinized) covering is more efficient in reducing transpiration than a hairy one.[2]

Wiegand's results confirm the previously expressed opinions of Goebel [3] and Haberlandt,[4] that a dense covering of hairs may be especially effective in reducing transpiration when the plant is exposed to strong winds. Haberlandt also refers to their similar action under strong insolation. On theoretical grounds it is to be expected that the hairs would have this effect. Thus, when the leaf is exposed to wind, the effect of the hairs would be to diminish the gradient of density of water vapour outside the leaf, by hindering the removal of the humid air from its surface. The distance between the absorbing surface (the free air outside) and the supply is thus increased, and the rate of flow of water vapour outwards correspondingly diminished.[5] On the other hand, if the air outside the leaf be perfectly still, the gradient of density would be much the same whether the hairs were present or not. Further, wind passing rapidly over the unprotected surface of a leaf would reduce the pressure just at the entrances of the stomatal apertures, and so tend to suck air (and consequently water vapour) out of them.[6] This effect also would be prevented by the hairs. Again, when exposed to direct insolation, white, air-containing hairs, such as those of *Spiraea*, must reflect an appreciable amount of light. In consequence, less heat is absorbed, and the transpiration again diminished.

In the case of *Spiraea Ulmaria*, it has been shown that its characteristic pubescence is localized precisely in those parts of the plant which in nature are most exposed to the drying effects of wind, sun, &c. Conversely, the glabrous leaves are those found under conditions where transpiration and the effect of wind are at a minimum. The same is also true of the majority of plants mentioned in § 6. Perhaps the most striking instance of all is

[1] Wiegand ('10), p. 430 et seq. He used blotting-paper as the evaporating surface, laying over this various cotton materials to represent hairy coverings, and bees-wax for cutin.

[2] Probably Wiegand has exaggerated the differences between the two kinds of covering, as in his experiments the layer of wax would have nothing to represent stomata, while all the cotton fabrics would be abundantly furnished with small apertures.

[3] Goebel ('91), p. 18.

[4] Haberlandt ('09), p. 116.

[5] Brown and Escombe ('00), footnote to p. 276, suggest that epidermal hairs may lessen the amount of CO_2 absorbed by the leaf when the air is in motion, by preventing air currents from sweeping away the external density 'shells'. The same must apply equally to the transpiration of water vapour.

[6] This action is similar, as Dr. Schott suggests, to that of a hair-dresser's sprayer, or of a Gifford injector in sucking water into a boiler.

afforded by the intermediate, partially hairy leaves of *Spiraea*. In these the leaflets, and even the portions of leaflets, which are most exposed to the danger of excessive loss of water, are provided with a dense covering of hairs (see Pl. LXXXII and LXXXIII).

Bearing in mind all the facts adduced in this paper, it is difficult to avoid the conclusion that the production of hairs in *Spiraea* and certain other plants, under the stimulus of drought, is of the nature of an advantageous adaptive response. The most important physiological effect, then, of this response would seem to be to prevent undue acceleration of transpiration by air movements and, though to a less extent, insolation.

Turning once again to the question of root-hairs and palisade cells, the chief rôle of the former (i. e. to increase the absorbing surface of the root) seems clear. The functions of palisade cells, on the other hand, have been much disputed. Probably they perform several important physiological functions. The only view which need be mentioned here is that of Areschoug,[1] who regards a well-developed palisade tissue as of importance in reducing transpiration. Areschoug's view has been accepted by many authors, though so far direct experimental evidence is lacking.[2]

Granted the foregoing, it appears that the stimulus of a decreased supply of water promotes the formation of three different kinds of elongated cells, each of which is physiologically important (at least in certain cases) in connexion with the plant's water supply. At one end of the plant the root-hairs increase the intake of water: at the other the hairs and palisade cells assist in reducing the output of water from the leaves. There would thus seem to be in these cases, as in many others, a more or less definite connexion between the nature of the stimulus and the physiological advantage of the response.

§ 13. The Xeromorphy of *Spiraea Ulmaria*.

Spiraea Ulmaria is far from being a pronounced xerophyte. Its leaves are thin and frequently of considerable size. They have a fairly thin cuticle, and generally wilt readily when gathered. Nevertheless the plant exhibits certain xerophytic characters which are practically confined to the upper, more exposed parts of the shoots. These characters are as follows:

1. The lower stomatal surface is covered by a dense pubescence.

2. The upper epidermal cells have a better developed cuticle than those of the lower leaves.

3. The mesophyll is much more compact; the internal transpiring surface being thereby considerably lessened.

[1] Areschoug ('82), p. 520, and ('06), p. 329 et seq.

[2] Hesselman ('04), p. 428, however, as a result of certain field experiments, is of opinion that palisade tissue does not markedly depress transpiration. Some of his experiments were actually carried out on *Spiraea Ulmaria*. Areschoug has discussed Hesselman's objections in his 1906 paper,

4. The leaf surface is much reduced. It is of course a very common thing to find the upper leaves of the shoots of herbaceous plants considerably smaller than those lower on the stem. But undoubtedly the effect of this diminution in size of the upper, more exposed leaves will be to reduce considerably the output of water from the terminal portions of the shoot. It has indeed been suggested above that this reduction in size of the leaf (or at least of its component cells) is in part due to development under conditions which strictly limit the available supply of water. In *Spiraea*, the leaves which present the greatest transpiring surface are placed in relatively sheltered, humid strata of the vegetation.[1] Though all shoots exhibit periodicity in the size of leaves, the actual sizes of the latter vary greatly according to the conditions under which they are grown (see Text-fig. 5).

5. When *Spiraea* leaves are exposed to strong insolation, they tend to assume the profile position. This is taken up, not by the leaf or leaflet as a whole, but by the two halves of a leaflet separately. A marked ventral curvature of the leaflet is thus produced. This will probably tend to a further reduction of transpiration. The phenomenon is a frequent one in marsh plants as well as others, but need not be fully discussed here, as it will form the subject of a forthcoming paper.

At first sight the more numerous stomata of the upper leaves suggest that they may counterbalance the characters which would otherwise reduce transpiration. But it has been shown (p. 828) that in all probability the possible transpiration per unit area of leaf surface, if the stomata alone are considered, would be little if any more from the upper than from the lower leaves. The balance of characters, then, shows that the upper parts of the shoots of *S. Ulmaria* are distinctly more xerophytic than the lower. But in any case the degree of xeromorphy is slight. The question now arises, is this degree of xeromorphy necessary or even advantageous to the plant?[2]

It may be regarded as an axiom that where functions such as transpiration are concerned, the need for special regulatory devices in a given species will be determined, not by the average conditions, but by what may be termed the normal extremes which the species is called upon to face in nature. In other words, to be successful, a plant must always be prepared for such emergencies as it may occasionally have to meet during the vegetative season.[3]

[1] Yapp ('09), p. 281.

[2] And if a dense pubescence is necessary to the type-form, why is it not equally so to the less hairy variety *denudata*? On this point all that can be said (though it is not advanced as being a complete answer) is that var. *denudata* appears to be a less successful form. It is rare in nature, while the type is common. So far as my experience goes, it usually occurs only in sheltered spots. Culture experiments on the roof of the College at Aberystwyth show that, especially if the soil is wet, it is much less able to endure the effect of wind than the hairy form. Moreover, after all, the hairiness of the two forms is merely a matter of degree.

[3] A. R. Wallace ('10), p. 258, goes even further than this. He says: 'A dominant species has

Considering hairiness alone, we have seen that its distribution on the plant is correlated in the most minute way with differences of external conditions; that those parts of the plant which would, if the conditions were severe, tend to lose water most readily, are those which are furnished with this hairy covering; and further, that the hairy type is more successful than the less hairy var. *denudata*. It may therefore be concluded that hairiness in this species is a positive advantage to the plant, and in all probability actually necessary whenever transpiration conditions are unusually severe, e. g. in the event of strong drying winds or prolonged insolation. The same may be said of the other xerophytic devices enumerated above. In other words, *Spiraea Ulmaria* is structurally in complete harmony with its environment.

But so far only the aerial organs have been considered, and it may well be asked, why does a plant rooted in damp soil require these regulatory devices at all? And further, even if the necessity be proved for *Spiraea*, how far does this particular instance afford a clue to the problem of xeromorphy in marsh and bog plants in general? An attempt will be made in the next section to give a provisional answer to these questions.

§ 14. The Present Position of our Knowledge of 'Swamp Xerophytes', and a Tentative Hypothesis.

Xeromorphy in plants growing on wet, peaty soils occurs all the world over.[1] A full historical account of the various theories put forward to account for the existence of these 'swamp xerophytes' need not be given here; but the positions taken up by certain authors will be briefly discussed.

Some writers maintain that the xeromorphic structures in question are, and others that they are not, present-day necessities to the plants which possess them.

The latter point of view may be dealt with first. Schwendener,[2] Clements,[3] and others believe that these structures are primitive characters which have persisted in certain stable species (especially Monocotyledones), in spite of a fundamental change of habitat conditions. Perhaps the most important evidence for this view is the fact that hydrophytic species frequently grow side by side in nature with those possessing xerophytic characters. Even Warming, who believes that the xeromorphy is adap-

become so because it is sufficiently adapted to its *whole* environment, not only at any one time or to any average of conditions, but to the most extreme adverse conditions which have occurred during the thousands or millions of years of its existence as a species.'

[1] Warming ('09), p. 194.

[2] Schwendener ('89), p. 73.

[3] Clements ('05), p. 126.

tive, says in this connexion: 'Finally, it may be noted that there are not only moors inclining to xerophily, but also others leaning rather to hydrophily, and that, in addition to the structural types and forms of leaves mentioned, there are others which apparently show no signs of xerophily, *and cannot be shown to be in harmony with this habitat.*'[1]

Several *a priori* arguments may be urged against the view advocated by Schwendener and Clements. The widespread occurrence of the phenomenon; the existence of varying degrees of xeromorphy in different types of marsh and bog vegetation, these being correlated with marked differences of soil, &c.; the fact that many of these species 'can grow both on extremely dry, warm soil, and on extremely cold, wet soil';[2] and finally, the probability that xeromorphy, unless advantageous, would actually handicap marsh plants in their competition with other species—all suggest that such xerophytic devices are in some way related to a need imposed on the plant by the nature of the habitat. Further, the argument from proximity of position, which has been so frequently employed, depends on the tacit assumption that plants growing together are necessarily under the same conditions. This is most certainly not the case. Indeed, the present author believes himself to have shown that, so far as the water supply of marsh plants is concerned, few of the species have to face precisely the same set of physiological problems.[3]

Various authors have discussed the question of whether a bog is to be regarded as a hygrophytic or xerophytic habitat. In this connexion it may be pointed out that only in the more extreme cases, and particularly (so far as xerophytes are concerned) those in which the plant associations are open, can habitats[4] as such be said to be markedly hygrophytic or xerophytic. In the great majority of closed, mixed associations, the mutual relations of the various species are such as to profoundly modify the general character of the habitat for each other; some plants being exposed, others sheltered, and so on.[5] In this way, according to the growth forms of the species, and in varying degrees according to the general characters of the habitat and of the vegetation, the same habitat may be xerophytic to one species and hygrophytic to another. This is probably to some extent what Warming had in mind when he wrote the passage quoted in the introduction to this paper. The emphasis here laid upon this point of view is not out of place, for there is some danger that it may occasionally be lost sight of in the comparative study of vegetation in its broader aspects. This is especially so in view of the stress laid on the generally accepted ecological classification of plants into xerophytes, mesophytes, hydrophytes,

[1] Warming, l. c., p. 196. The italics are mine.

[2] Warming, l. c., p. 194.

[3] Yapp ('09), pp. 306–8.

[4] Using the term in its broad sense, i. e. to indicate the general physical environment of plant associations, rather than merely of individual plants.

[5] Cf. Yapp, l. c., p. 279.

&c. Such a classification is admittedly useful, but it should not be forgotten that it is all a matter of degree, and that communities composed entirely of marked xerophytes, &c., are probably comparatively rare. Thus, though climatic and edaphic factors are the most important, in that they determine the general character of the vegetation, the inter-relationships of the various species also play a definite, if less conspicuous part, even in the case of relatively dwarf vegetation. But all this does not explain the paradox that certain of the plants rooted in the wet soil of marshes and bogs apparently need to exercise economy in respect to their water supply. We must therefore consider the theories put forward by authors who regard the xeromorphy of these plants as induced by the nature of the habitat.

It is at once obvious that either atmospheric or edaphic factors, or both, may be concerned. The present writer's researches have dealt mainly with the former, while most other observers have confined their attention to the latter.

i. *Edaphic factors* may be taken first, the following comprising the more important suggestions put forward:

1. *Actual physical drought.* Volkens[1] thought the important factor was the drying of the superficial layers of peat during summer. He pointed out that in some moorland and marsh habitats the soil is wet all the year round, while in others the upper layers become dry in summer. Studying the Carices in particular, he concluded that the more xerophytic species are largely confined to the second class of habitats. Davis[2] and Burns[3] have come to a similar conclusion from their observations on the lowering of the water table during droughts in North American peat-bogs.

2. *The low temperature of wet soils* may, it is suggested, check root activity, and so reduce the absorption of water. Kihlman[4] regarded this factor as of great importance in northern regions, especially when coupled with drying winds. Goebel[5] came to the same conclusion with regard to the mountainous parts of Venezuela. Transeau[6] found, as the result of laboratory experiments, that a cold substratum caused a reduction in size of both roots and leaves, and induced the formation of xerophytic characters in the latter. The effect was more marked when, in addition, the soil was badly aerated. He is of opinion that substratum temperatures are of prime importance in northern latitudes, but that they are inadequate to account for xerophytic structures in bog plants in Southern Michigan.[7]

3. *Paucity of oxygen in the soil.* Transeau's experiments showing the effect of a deficiency of oxygen have already been quoted. Again,

[1] Volkens ('84), p. 24.
[2] Davis ('06), p. 160.
[3] Burns ('11), p. 119.
[4] Kihlman ('90), p. 107 et seq.
[5] Goebel ('91), Teil ii, p. 11.
[6] Transeau ('06), p. 22 et seq.
[7] Transeau, l. c., p. 36.

Warming[1] remarks that an insufficient supply of oxygen may obstruct respiration, and so depress the functional activity of the roots. The researches of Kosaroff,[2] too, show that the absorption of water is retarded by suppressing the supply of oxygen to the roots. In this connexion it may be noted that the roots of many 'dry-marsh plants' tend to assume a markedly horizontal position, especially when growing in wet peat. This is very striking in the case of the tap-roots of certain Umbelliferae. The phenomenon may well be correlated with the paucity in oxygen of the water-logged soil.[3]

4. *The presence of free humus acids.* Schimper[4] attributed the need for xeromorphy in the plants of peat-moors to the action of humus acids in impeding the absorption of water by the roots. This was accepted by Warming and others as 'the weightiest cause of the physiological dryness of the soil'.[5] But the importance of free humus acids has been disputed by Livingston,[6] Transeau,[7] and Dachnowski.[8] Indeed, according to the recent important researches of Baumann and Gully,[9] it is extremely doubtful whether, in the so-called free humus acids of sour peat soils, we are dealing with organic acids at all. These authors attribute the acid character of such soils to adsorption by the colloidal cell-walls of plants, especially *Sphagnum*. They produce evidence to show that the cell-walls bring about decomposition of salts in solution, a greater proportion of the base than of the acid being adsorbed. The acid reaction, then, is due to the presence of free hydrogen ions yielded by the acid after the removal of the base.

5. *The presence of bog toxins*, which act on the roots of plants much as do drying media. The existence of such substances in bog water and bog soils has been rendered probable by the work of Livingston,[10] and later of Dachnowski.[11] These authors published a series of suggestive papers from 1904 onwards. They conducted many careful culture experiments, using polymorphic lower plants as indicators. They both conclude that inhibition from bogs of plants other than those possessing xerophytic characters is largely due to the presence of these injurious substances, which are soluble in water. Dachnowski[12] states that the toxicity of bog water can be corrected by various means: e. g. by the addition of certain adsorbing agents, and also by aeration. The latter acts, according to him, by slowly oxidizing the toxic substances, and not directly on the living roots, as supposed by other authors.

6. *The great water-retaining power of peat.* Of all soils peat has

[1] Warming ('09), p. 195.
[2] Kosaroff ('97), quoted by Jost ('07), p. 32.
[3] Yapp ('08), pp. 74–5.
[4] Schimper ('03), pp. 15 and 111.
[5] Warming, l. c.
[6] Livingston ('05), p. 351.
[7] Transeau ('06), p. 25.
[8] Dachnowski ('10), p. 327.
[9] Baumann ('09), and Baumann and Gully ('10).
[10] Livingston, l. c.
[11] Dachnowski ('08, '09, and '10).
[12] Dachnowski ('08), p. 134, &c.

the greatest water capacity. It would therefore seem probable that an appreciable amount of the water actually present will not be available for absorption by the plant. With a view of determining what the actual percentage of non-available water may be in moorland soils, Crump[1] has conducted a number of interesting wilting experiments. He calculates that certain plants (e. g. *Eriophorum angustifolium*) are unable to absorb more than about 48 per cent. of the total water present.

The above are some of the more important suggestions that have been made with respect to edaphic factors. But, as both Livingston and Dachnowski point out, our knowledge of the chemistry of bog water and bog soils is still very incomplete.

ii. *Atmospheric factors.* Certain authors deny the importance of these. E. g. Dachnowski,[2] referring to the xerophytic characters of bog plants, says, 'None of these features are correlated with atmospheric influences.' Elsewhere, however, he seems to admit that they may play a part.

On the other hand, both Kihlman and Goebel (see 2 above) emphasize the importance of drying winds, especially when accompanied by coldness of soil.

The present author[3] has shown that even in the comparatively dwarf vegetation of a marsh, great and constant differences of relative humidity exist in the different aerial strata. Thus the leaves of plants which occupy the lower strata are, on e. g. dry and windy days, exposed to transpiration conditions far less severe than leaves growing in the upper strata of the vegetation. More recently Dachnowski[4] has obtained similar results in the bog vegetation of Cranberry Island. The position, then, of the transpiring organs of the various plants must be of considerable importance.[5] Indeed, a slow absorption of water can only be prejudicial in so far as it is liable to be accompanied by excessive transpiration from the aerial parts.

Another factor that has to be taken into account in this connexion is the length of the vegetative period of the different species. So far, for instance, as the danger arising from limitation of absorption by reason of low soil temperatures is concerned, it is obvious that, in temperate latitudes, this will be experienced to the greatest extent by evergreen plants during the winter or spring time. Schimper[6] was the first to emphasize the connexion between the coldness of the soil and the xerophytic structure of the leaves of evergreen trees and shrubs in temperate regions. Früh and Schröter[7] similarly attribute the need for such structure in the plants

[1] Crump ('12).

[2] Dachnowski ('11, second paper), p. 34. Cf. also ('10), p. 338.

[3] Yapp ('09).

[4] Dachnowski ('11), p. 146.

[5] Kearney ('01), p. 442, in his survey of the Dismal Swamp Region, points out that the tall, reed-like plants of freshwater marshes protect the smaller, more hygrophytic forms against excessive transpiration.

[6] Schimper ('90), pp. 15–17.

[7] Früh and Schröter ('04), p. 15.

of a *Hochmoor* partly to difficulties of absorption, and partly to the prevalence of the evergreen habit.

A Tentative Hypothesis.

Much remains to be done before the causes which have contributed to the production of xeromorphy in bog and marsh plants can be stated with exactitude. In particular, our knowledge of the physical chemistry of peat soils is still very incomplete. It is also uncertain as yet to what extent the stomata of marsh plants are capable of normal movement.[1] Again, the osmotic properties of the cell-sap of marsh and bog plants would be worth investigating. But the problem is gradually becoming less obscure. As seen above, many possible factors have been suggested by different authors, each factor in turn being assigned a position of paramount importance.

Probably the truth is that a number of factors, both edaphic and atmospheric, are operative. Further, it is probable that while some factors are of greater importance than others, the importance of any given factor will vary according to circumstances. As regards edaphic factors, the general trend of evidence, a good deal of it experimental, renders it increasingly probable that the soils of peat-marshes and bogs are to a certain extent, if not physically, at least physiologically dry to plants. But the factor, or combination of factors, which renders absorption difficult is not the same in all cases. For example, low soil-temperatures probably play a part in far northern latitudes, at high altitudes, and in the case of evergreen plants during the winter-time. But the same factor may be entirely inoperative in the case of plants which only produce leaves during the summer-time; and especially in the bogs and marshes of warmer latitudes. Again, the presence of toxic substances or the lack of oxygen (whether this acts directly, or merely by oxidizing injurious compounds) may possibly affect chiefly the more deeply rooted plants.[2] On the other hand, plants rooted on or near the surface may secure abundant oxygen, but are exposed to the danger of physical drought if the water table is lowered to any considerable extent. Further, it is probable that chemical, and perhaps also physical, differences between the soils of bogs and marshes (i. e. *Hochmoore und Flachmoore*) respectively, are largely responsible for the fact that xeromorphy is more prevalent in the plants of the former than in those of the latter (but see below). Doubtless in many cases two or more soil factors, often in different combinations, co-operate.[3] At the same time, we know little of the ultimate causes which limit the absorption of water by the roots.

But edaphic factors alone cannot suffice to account for the fact that xerophytic and hygrophytic species so frequently occur side by side in

[1] Cf. Darwin ('98), p. 552, and the literature there cited. Also Früh and Schröter ('04), p. 14.
[2] Cf. Yapp ('08), pp. 74–5.
[3] Cf. Warming ('09), p. 195.

nature. It is here that atmospheric factors become important. Granted that the characters of the substratum are such that the rate of absorption of water is slow, then the atmosphere will play a differentiating rôle. A reduction in the amount of water absorbed does not injuriously affect the plant, provided that transpiration is reduced in the same ratio. The latter is effected in one of two ways, according to the position of the aerial parts of the plants. (1) For the taller plants, the shoots of which occupy the upper strata of the vegetation, and are therefore liable to exposure to strong winds, insolation, &c., xeromorphy may well be a necessity; and even more so if the plants are evergreen. (2) On the other hand, the leaves of the smaller plants occupy the lower strata, where they are protected from wind, and transpire into a constantly humid atmosphere. As is to be expected, these lower strata do actually contain the more hygrophytic species, e. g. *Hydrocotyle vulgaris*, &c., and also the hygrophytic seedlings and lower leaves of other species. Conversely, the more xerophytic species, e. g. *Cladium Mariscus*, or parts of shoots (cf. *Spiraea Ulmaria*), are those occupying the most exposed positions.

So far as atmospheric factors are concerned, the less dense the vegetation, and the larger the proportion of plants of an evergreen habit, the more xerophytic may the vegetation be expected to be. In this country these two conditions obtain to a greater extent in bog than in marsh vegetation. It is therefore not surprising, seeing that atmospheric and (probably) also edaphic factors are in general more favourable, that the vegetation of peat-marshes should contain a larger proportion of hygrophytic species than that of bogs; and, conversely, that the latter should support a greater number of xerophytic types.

The general nature of the habitat is of course important, but having made due allowance for that, the whole matter really resolves itself into a consideration of the different problems of individual species.[1] And if this be so in any given marsh or bog, much more is it true when we come to compare different types of swamp vegetation and different latitudes and climates.

As an example, two species of marsh plants may be briefly compared:

Spiraea Ulmaria is a plant of the drier parts of marshes. Its roots grow in moist but not water-logged soil. Probably they rarely experience lack of oxygen, and absorption in the well-aerated soil is unlikely to be much impeded by toxic substances. The vegetative period is comparatively short (about March to October), so that coldness of soil will scarcely play a very important part in these latitudes. On the other hand, it is liable to suffer from actual drought through lowering of the water table. The aerial parts have been discussed at length in this paper, and it has been shown that the exposed leaves exhibit a definite if slight degree of xeromorphy,

[1] Cf. Yapp ('09), pp. 306–8.

while the sheltered leaves are more hygrophytic in character. Probably the chief danger to this species is the drying effect of wind, especially when coupled with a temporary desiccation of the surface layers of soil.

Cladium Mariscus presents somewhat of a contrast. It is a wet-marsh plant, which is more pronouncedly xerophytic than *Spiraea*. Its roots grow in water-logged soil, and are indeed frequently entirely submerged. It is a relatively tall plant, and not only are the upper parts of its leaves exposed to wind and sun, but they are evergreen. Absorption, then, may possibly be impeded by such factors as lack of oxygen, toxic substances, &c., in the summer time, and by low soil temperatures in winter and spring. At the same time the leaves may be exposed to unduly severe transpiration conditions at any time of the year. Hence it is not surprising that, as is actually the case, *Cladium* should be more decidedly xerophytic than *Spiraea*. The comparison could readily be extended to include such diverse types as *Hydrocotyle vulgaris*, *Andromeda polifolia*, &c., &c.

To sum up, there is every reason to suppose that associations of bog and marsh plants are as much in harmony with their environment as are those occupying other distinctive habitats, such as the various woodland, desert, and other plant associations. But to arrive at this conclusion, it is necessary to take many factors into consideration. In all probability edaphic factors, climatic factors, the growth forms of the plants, with the resulting differences of exposure and shelter, the duration of the vegetative period, and possibly other factors as well, have all contributed to the production of a series of physiologically intricate, and at first sight paradoxical types of vegetation.

Finally, it may be once more emphasized that the xeromorphic structures in question are required to meet, not so much the everyday needs, as the extremes, possibly even only the occasional extremes, which the plants possessing them are called upon to face.

Summary of Results.

1. The present paper is an experiment in what may be called species-ecology. It is an attempt to see how far the intensive study of a single species, supplemented by observations on others, may help towards an explanation of the problem of xeromorphy in marsh plants.

2. The species selected (*Spiraea Ulmaria*) is a 'dry-marsh' plant, the leaves of which are densely pubescent on the lower surface. This pubescence is subject to a kind of periodicity, and appears only under certain definite conditions. The chief rules governing the appearance of the hairs are as follows: (*a*) The seedlings, also all leaves formed during the first year, are glabrous. (*b*) On the erect flowering shoots of adult plants, there is a regular succession of glabrous, partially hairy, and completely hairy

leaves. The earliest radical spring leaves are glabrous, the cauline leaves hairy (see Pl. LXXXI). (*c*) The non-flowering shoots of adult plants produce only radical leaves. The earliest of these are glabrous as in (*b*). Subsequently, the successive leaves exhibit increasing hairiness up to June or July, after which they are decreasingly hairy, till finally glabrous leaves are once again produced in autumn. (*d*) The distribution of pubescence on the partially hairy leaves is interesting. The terminal leaflet is invariably the most hairy, and there is a regular decrease in hairiness from above downwards. Individual partly hairy leaflets generally possess a marginal band of hairs, with sometimes additional bands running inwards between the main veins (see Pl. LXXXII and LXXXIII).

3. In reality this seasonal periodicity in the production of hairs is a widespread phenomenon. *Spiraea Ulmaria* was the most striking case observed, but many other marsh, and also certain land, plants exhibit the same general succession of glabrous, partly hairy, and more or less completely hairy leaves.

4. Similarly, there are marked seasonal differences in leaf structure. The lower glabrous leaves have on the whole a more or less definite 'shade' structure, while the upper, more exposed, hairy leaves approximate to the 'sun' type. The former have large epidermal cells, with sinuous lateral walls, and few but relatively large stomata. The mesophyll is loose, with large intercellular spaces. On passing up the erect stem, the following progressive changes in leaf structure may be observed: the leaf becomes thinner, but the cuticle thicker; the epidermal cells smaller, and their walls less sinuous. The stomata are more numerous but smaller, and the hairs much more abundant. The mesophyll is more compact, and the intercellular spaces are gradually reduced to a minimum (see Text-fig. 4). Speaking generally, the upper leaves show a distinctly greater degree of xeromorphy than the lower. These changes are independent of the actual size of the respective leaves.

5. The variations in the number of stomata are remarkable. There is a gradual though fluctuating rise in the number of stomata through the successive leaves of the entire shoot. In one shoot, for instance, the numbers varied from about 300 to nearly 1,300 per sq. mm. of lower leaf surface. The latter number is the highest yet recorded for any species. At first sight it might appear that these numerous stomata of the upper leaves would neutralize the effect of the xeromorphy referred to above (4). But the stomata of the lower leaves have larger pores, and it is calculated that under similar conditions, and *considering stomata alone*, the possible transpiration from the upper leaves could be but little, if any more, per unit area of leaf surface, than that from the lower.

6. The effect of environment on the development of certain leaf tissues, especially hairs and palisade cells, is considered. The results of many

investigators show that in general these are most fully developed under conditions which impose on the plant some necessity for increased economy of water, i. e. under conditions which either promote transpiration or hinder absorption. *Spiraea Ulmaria* is found to be less plastic than many other species in respect to hairiness. Many experiments show that it is difficult to inhibit the formation of hairs on leaves which are normally hairy, though their number and length can be readily influenced. At the same time, the distribution of pubescence on the plant (described in 2 above) corresponds in the most striking manner with the average physical conditions of the natural environment. Thus the curves of hairiness in *Spiraea* follow very closely the mean curves of evaporation and light-intensity. This is true whether the vertical changes in these factors (due to the density of the vegetation) or the annual march of evaporation and light-intensity are considered (cf. Pl. LXXXI and Text-figs. 9 and 10).

7. The localization of hairs on the partially hairy leaves of *Spiraea* (in particular the marginal and interveinal bands of hairs) shows a marked correspondence with the localization of withering in leaves in general, when such withering is due to the effect of wind, &c. (cf. Pl. LXXXII and LXXXIII, and Text-fig. 11). Anthocyan in young leaves in spring-time, the local effect of freezing, &c., often show a similar distribution. As regards their water supply, these regions of the leaf-lamina (i. e. the margins and interveinal parts) are physiologically different to the remaining parts. They are more liable to lose water, and are also the most remote from the water supply coming up from below.

8. The development of the leaves is described, and it is shown that no hairs are developed in the bud until the first foliage leaves have expanded and become functional. Hairs then appear on the main veins of the developing leaves, and the hairiness of these veins (while still in the bud) increases *pari passu* with the general increase of transpiration from the shoot. But the hairs on the finer veins, and those which occur over the parenchyma 'islands', only appear when the leaf has emerged from the bud; i. e. when some parts of it are already in contact with the outer air (Text-figs. 7 and 8). It is these later formed hairs which are most conspicuous in the mature leaves (see 2 above).

9. The causes of the formation of the hairs, &c., are discussed, and it is shown that a parallel may be drawn between the epidermal hairs of leaves and stems, the palisade cells of leaves, and to some extent root-hairs as well. In so far as the development of these special elongated elements is determined or accelerated by external conditions, it would appear that similar factors are usually operative in all three cases.

Hair formation in *Spiraea Ulmaria* is considered in detail. In this case the factors which determine the presence or absence of hairs on the leaves are probably the following: (*a*) heredity, (*b*) individual variation,

and (*c*) external conditions. It is shown that evidence of many kinds points to hairiness being associated with a diminished, and glabrousness with an abundant supply of water (see 6 and 7 above). In so far then as *Spiraea* is plastic in respect to hairiness, the course of events would seem to be somewhat as follows. As transpiration increases with the unfolding of the leaves in spring-time, the amount of water available for the apical bud becomes diminished. Certain cells of the developing leaves are thereupon stimulated to form hairs. This is a case of what Wiesner has called 'correlative transpiration'. When the partially developed leaf emerges from the bud, its supply of water is still further lessened by cuticular transpiration from its surface (cf. 8 above). The initial stimulus which leads to hair formation is thus afforded by diminished turgor; the actual growth in length of the hair cells, however, cannot take place till turgor is once more restored. This takes place at more or less regularly recurring intervals, especially at night-time. Thus hair production is promoted by marked periodic fluctuations in the turgor of the hair-producing cells. *Spiraea Ulmaria* possesses only a limited degree of plasticity in regard to hairiness. It is suggested that the species is tending to become fixed, in the direction of response to the different conditions to which the upper and lower leaves respectively have been exposed in nature for many generations.

10. With regard to the physiological effect of the hairs, &c., it is concluded that the pubescence prevents undue acceleration of transpiration, and especially that due to air movements and, to a less extent, insolation. It is probable that the stimulus of a decreased supply of water during development promotes the formation of three special kinds of elongated cells, each of which is physiologically important in connexion with the problems of water supply. At one end of the plant the root-hairs increase the intake of water, and at the other the hairs and (probably) the palisade cells assist in reducing the output of water from the leaves.

But the experimental treatment of the subject of the effect of leaf structure on transpiration is reserved for a future paper.

11. Structurally, the leaves of *Spiraea Ulmaria* show considerable differences according to position and time of development. The lower sheltered leaves are hygrophytic, while those which are exposed to wind, strong insolation, &c., are distinctly more xerophytic in character. It is concluded that this degree of xeromorphy of the upper leaves (hairiness, &c.) is a positive advantage to the plant, and probably actually necessary, at least under unusually severe transpiration conditions.

12. The whole position of our knowledge of 'swamp xerophytes' is reviewed, and the conclusion arrived at that all the evidence points to the various bog and marsh associations being as much in harmony with their environment as are those (e. g. woodland, desert associations, &c.)

occupying other distinctive habitats. In other words, xeromorphy is of physiological utility to those bog and marsh plants which exhibit it. It may be regarded as directly related to the needs imposed on the plant by the special nature of the habitat. But in each case the special regulatory devices are required to meet, not so much the everyday needs, as the extremes, possibly even only the occasional extremes which the species has to face in nature.

The environmental factors of a bog or marsh habitat are complicated. No single factor can be regarded as of sole importance in determining the need for xeromorphy. The hypothesis is put forward that, as suggested by so many authors, the soil is to some extent physiologically dry. The degree of physiological drought varies in different cases (e.g. bog soil is doubtless drier to plants than marsh soil), as do the factors or combination of factors which occasion it. But edaphic factors alone cannot account for the fact that hygrophytic and xerophytic species often live side by side in nature. Here atmospheric factors become important. Granted that the characters of the substratum are such that absorption of water is slow, then the atmosphere will play a differentiating rôle. A retardation of absorption is only prejudicial when accompanied by an excess of transpiration. On the whole the more hygrophytic species, or the more hygrophytic stages or portions of other species (e.g. seedlings, or the lowest leaves of herbaceous plants), occupy the lower humid strata of the vegetation. On the other hand, the more xerophytic species, or the more xerophytic parts of others, are those which grow under more severe transpiration conditions; e.g. evergreen plants, and those occupying the drier, more exposed strata of the vegetation.

Thus, in all probability, edaphic and climatic factors, the growth forms of the various species, with the resulting differences of exposure and shelter, the duration of the vegetative period, and possibly other factors, all play their part in determining the need or otherwise of special devices for regulating transpiration in swamp plants. In short, after making due allowances for the general nature of the habitat, the whole matter really resolves itself into a consideration of the different problems of individual species.

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EXPLANATION OF PLATES LXXXI–LXXXIII.

Illustrating Professor Yapp's paper on *Spiraea Ulmaria*, L.

The plates consist of photographs taken from dried and pressed leaves of *Spiraea Ulmaria*, L., to illustrate the distribution of hairiness. In each case, only the lower surface of the leaf is shown. *Hairy parts are white, glabrous parts dark.*

PLATE LXXXI.

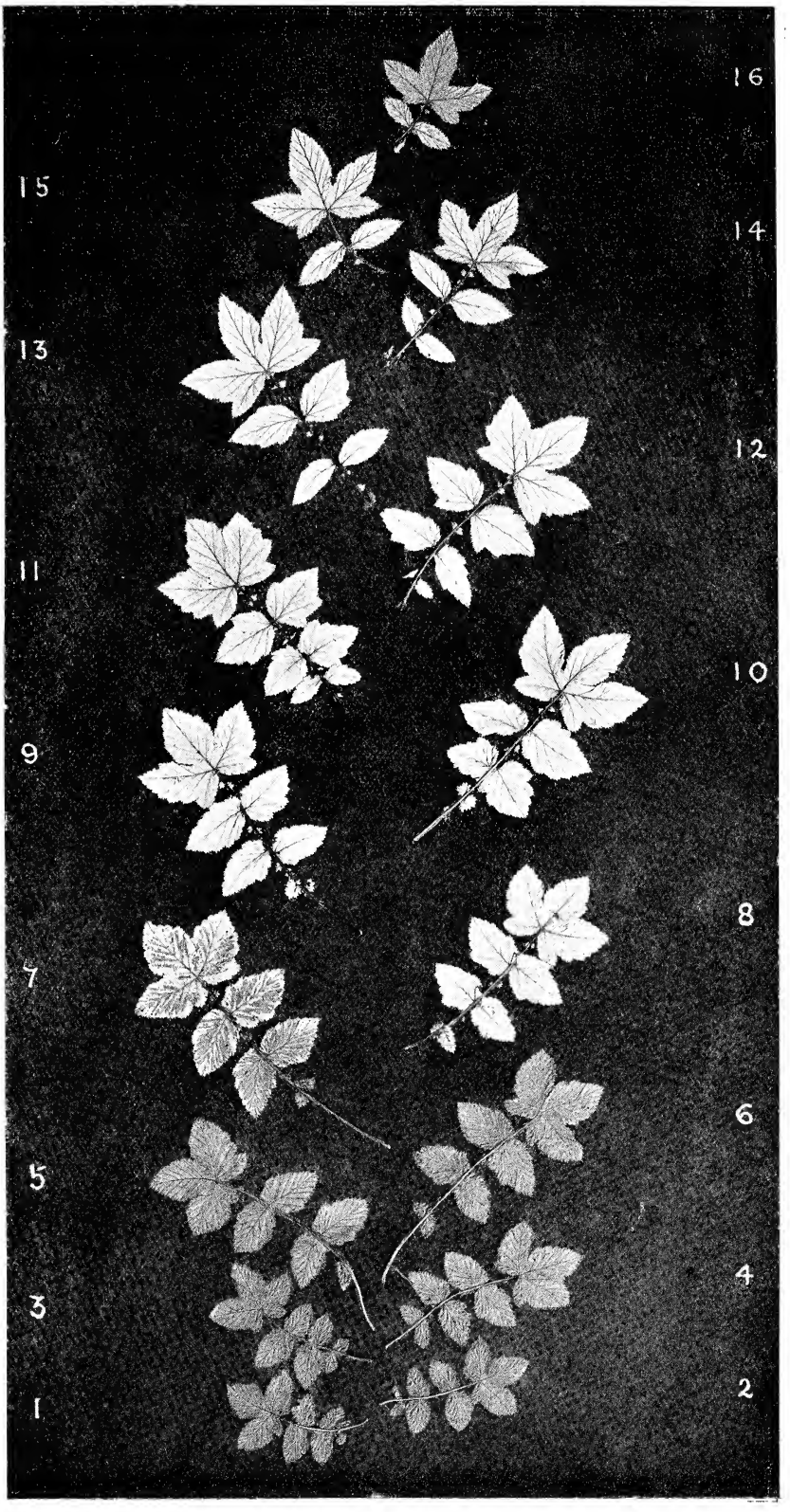
Shows all the leaves formed during one season on an erect flowering shoot. The leaves are arranged and numbered in order of development. Leaves 1–7 are radical, and 8–16 cauline. There is (as always) a gradual transition from glabrous to hairy leaves. The shoot was grown under fully insolated conditions. Note the narrowing of the leaflets in the upper leaves (cf. Text-fig. 5).

PLATE LXXXII.

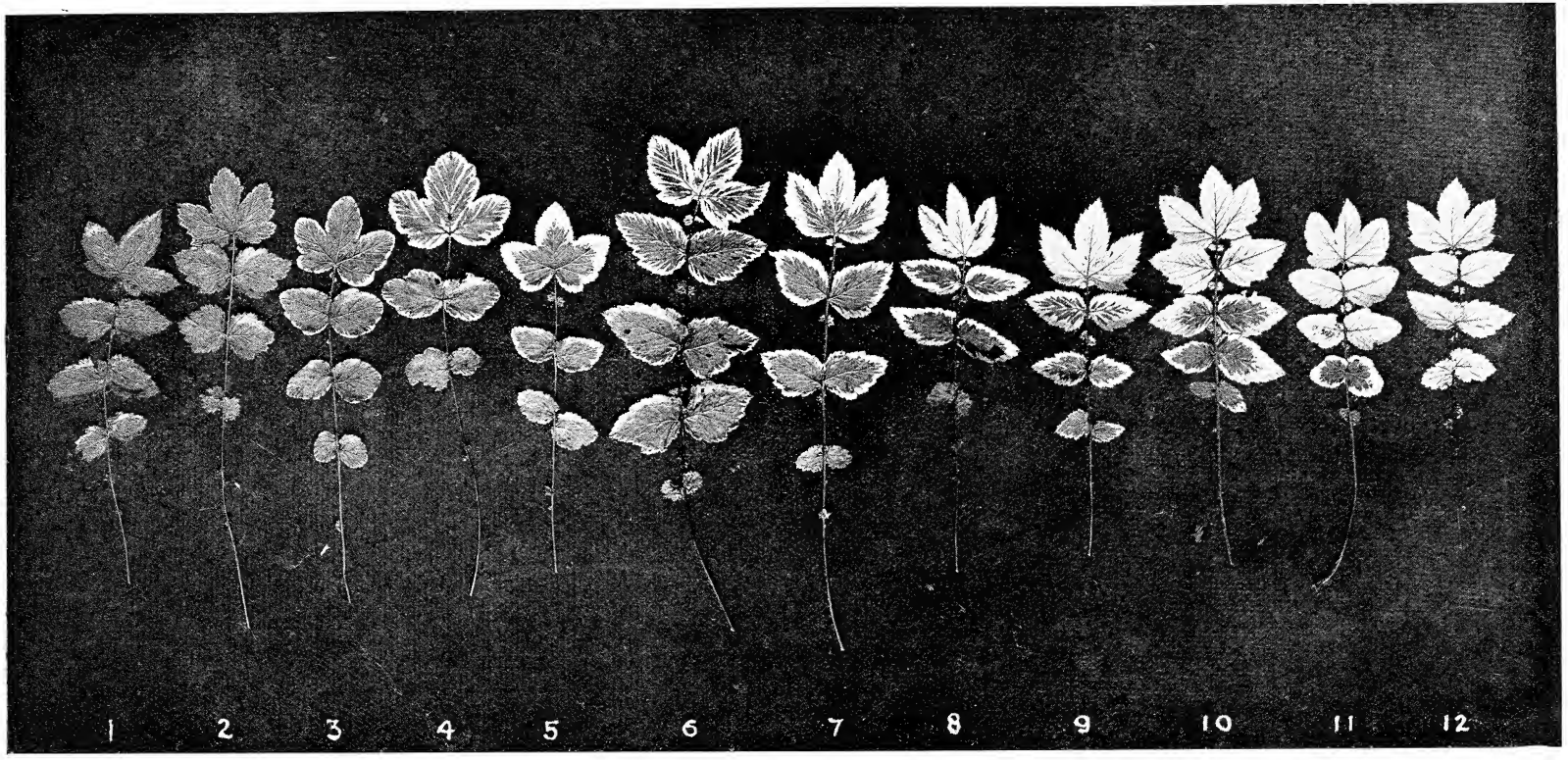
A selected series of radical leaves. N.B. These were taken from a number of different non-flowering shoots. This series is arranged to illustrate the distribution of hairiness on partially hairy leaves. Note the increase in hairiness of successive leaflets as the individual leaf is ascended: the terminal leaflet is invariably the most hairy. Compare the increase in hairiness of successive leaves as the entire plant (flowering shoot) is ascended—see Pl. LXXXI. In individual partially hairy leaflets the hairs usually form a marginal band, with sometimes (e. g. Nos. 4, 6, and 9) extensions in the form of bands between the main veins (cf. the localization of exposure withering—Text-fig. 11).

PLATE LXXXIII.

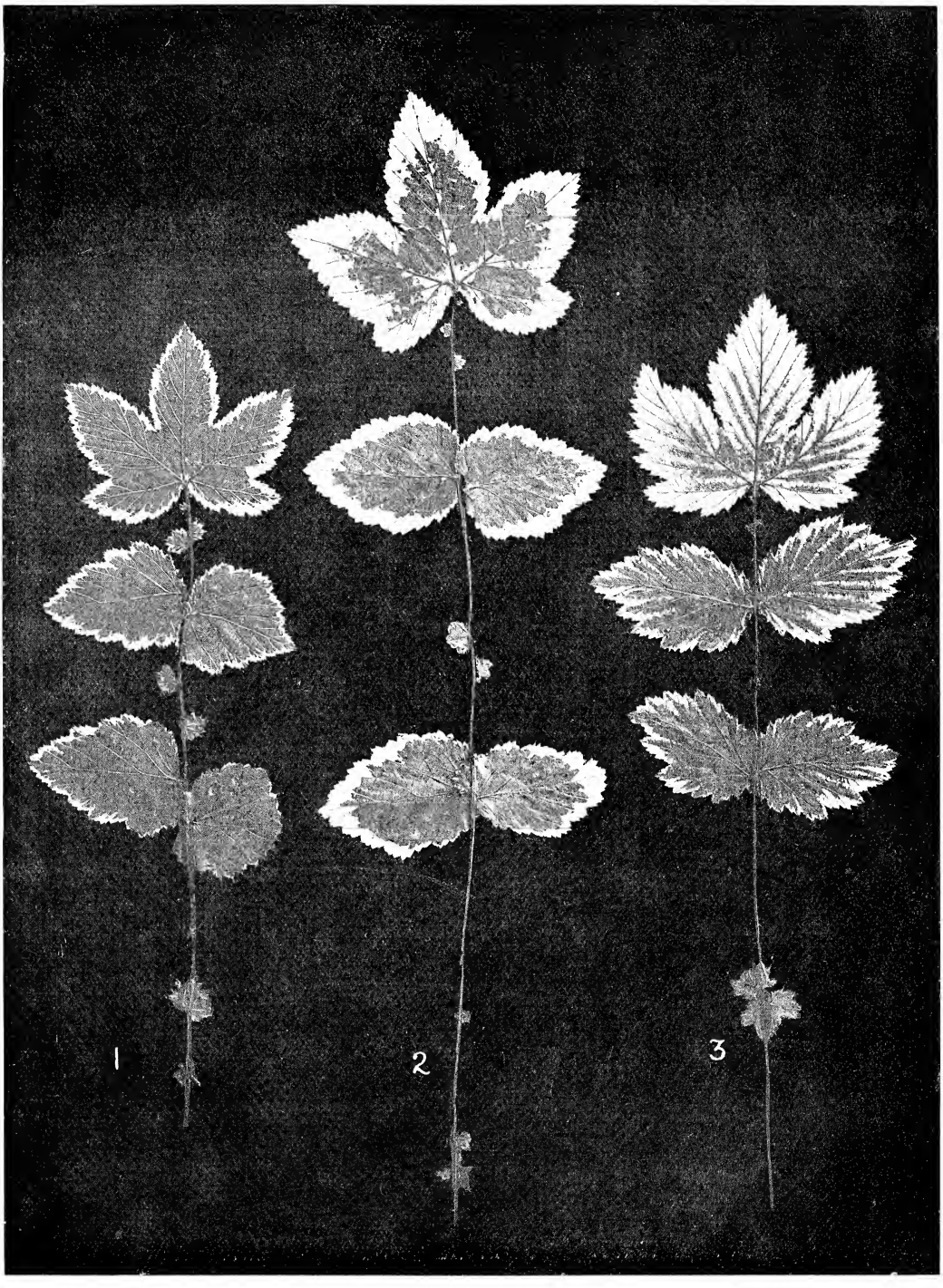
Three partially hairy leaves. No. 1 shows the marginal bands of hair; No. 3 the additional interveinal bands. The terminal leaflet of No. 2 illustrates a not infrequent case in which only the innermost portions of the interveinal bands are developed. These now show, in addition to the marginal bands, patches of hairs occupying the forks of the veins.



YAPP.—LEAVES OF SPIRAEA.



YAPP.—LEAVES OF SPIRAEA.



YAPP.——LEAVES OF SPIRAEA.

Some Conditions influencing the Fixation of Nitrogen by Azotobacter and the Growth of the Organism.

BY

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DURING the progress of some research upon the utilization of various organic substances as food material by *Azotobacter*, of which an account will appear in due course, considerable difficulty was experienced in obtaining and keeping pure liquid cultures of the organism. Other investigators appear to have encountered a similar difficulty, for many different nutrient media and methods of culture are recommended by various authors. Most of the solutions advocated by them were tested in turn, with such unsatisfactory results that it was at last deemed advisable to make a thorough examination of the behaviour of the organism in these different culture solutions, both in respect of the rapidity and purity of the growth, and the extent of fixation of nitrogen obtained per unit of the carbohydrate consumed. The work was undertaken in the first place solely for the author's convenience, in order to clear away these preliminary difficulties as a preparation for further research, but the results appear to be of sufficient importance to warrant publication, and are embodied in the following account, in the hope that they may be of use to other workers on the subject.

Undoubtedly the best method of obtaining a luxuriant growth in a short space of time is by the use of plate cultures in the manner advocated by Hoffmann and Hammer, in which the surface of a mannite-agar plate is covered with 5 or 10 c.c. of a suspension of *Azotobacter* in 0·9 per cent. sodium-chloride solution. By this means a prolific growth is obtained uniformly over the whole surface of the plate, and, if the medium is sterile in the first place, no difficulty is experienced in keeping pure vigorous cultures on these agar plates. The trouble arose when liquid cultures were required, and it was necessary, in the first place, to arrive at some conclusion as to what are the optimum conditions with regard to alkalinity; and, in the second place, to decide which are the inorganic constituents of the nutrient medium which are necessary and beneficial to the organism, the

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best organic constituent having been accepted for the time being as mannite. Mme Krzemieniewski has shown that potassium, calcium, sulphur, magnesium, and phosphorus are all essential to the development of *Azotobacter*. Recent work by Omeliansky and Ssewerowa, and also by Remy and Rösing, has brought out the importance of iron, while Kaserer lays stress on the need for aluminium and silicon also; but further reference will be made to this work later.

The solutions, apart from those containing iron, aluminium, and silicon, which had been used with more or less success, practically resolve themselves into two classes: firstly, those in which neutralization of the acid potassium phosphate had been effected simply by the addition of excess of calcium carbonate; and, secondly, those in which the acidity had been neutralized by the addition of dilute sodium-hydrate solution, either to the whole culture solution, or to a solution of the phosphate made separately and afterwards added to the main bulk, the necessary calcium being supplied to the medium in the form of a small amount of a salt other than the carbonate. A combination of the two methods is found in the medium used by Ashby, who dissolves the phosphate separately, just neutralizes it with sodium hydrate, and, after the addition of this to the main portion of the solution, adds an excess of calcium carbonate. The solution used by Beijerinck consisted of:

Tap water	1,000 c.c.
Mannite	20 grm.
Di-potassium phosphate . .	0·2 grm.

and, having used this for purposes of experiment, he claims that *Azotobacter* will not, in pure culture, fix nitrogen. Chester points out that the medium is at fault, since no attempt is made to correct the acidity of the phosphate, and he reports that a good fixation of nitrogen has been obtained at the New Jersey Experiment Station on a medium consisting of the following:

Tap water	1,000 c.c.
Mannite	15 grm.
Di-potassium phosphate . .	0·5 grm.
Magnesium sulphate . .	0·2 grm.
Calcium chloride . . .	0·02 grm.

the whole being rendered alkaline by means of sodium hydrate. Many attempts have been made by the writer to obtain growths of *Azotobacter chroococcum* on this medium, with very little success; and when a surface film has developed at all, it has been very scanty, the best having been obtained in solutions which had been rendered just neutral by sodium hydrate, the presence of any excess, even in very small amounts, of the latter proving fatal to development. No determinations of the nitrogen-

fixation upon this medium were made, for failure to obtain any growth at all occurred so frequently as to prove that this solution was far from favourable for the development of the particular strain of Azotobacters concerned in this investigation. No good results could be obtained in any medium which did not contain an excess of calcium or magnesium carbonate ; and, this having been decided, the chief remaining point to be considered in this connexion was the question as to whether the addition of sodium, either as the hydrate or in any other form, is *beneficial* to the organism ; the fact that *Azotobacter* will grow and fix nitrogen without it proving that it is not *necessary*. Since the isolation of the organism from the soil in 1901 by Beijerinck, many workers have devoted a considerable amount of time to a determination of the conditions under which the most luxuriant growth of vigorous Azotobacters can be obtained ; for the importance, from a practical, agricultural point of view, of a precise knowledge of these conditions cannot be over-estimated, and the more simple the culture solution which can be used, the better. As regards the elements supplied, apart from the sodium and the neutralizing agents, the solutions used, except for the recent work above mentioned, are all practically similar, so that the liquid medium hitherto used by Professor Bottomley in the Botanical Laboratory at King's College was tested as a type of those without sodium, against Ashby's solution representing those containing sodium salts. At the suggestion of Professor Bottomley, an investigation was also made to obtain some idea of the efficiency of basic slag as a substitute for calcium carbonate, of which further details will be given later.

When the questions of the relative merits of Bottomley's and Ashby's solutions, and of calcium carbonate and basic slag as neutralizing agents, were settled, there arose the problem as to what is the best quantity of nutrient solution to supply to ensure maximum fixation. The practical worker requires a maximum yield of combined nitrogen for the minimum expenditure of carbohydrate ; and it has been suggested that the activity of the organism varies inversely, to a certain extent, with the quantity of food material supplied, i. e. that when provided with a very small portion of food, the *Azotobacter* economizes, using the carbohydrate to the best advantage ; so that under these conditions fixation is relatively greater than in a liberal supply of food material. Closely connected with this is the question as to whether the organism is able to utilize the last traces of carbohydrate in the medium as a source of energy for the fixation of nitrogen, or whether, as has also been suggested by various workers, after a certain period of time, while some of the carbohydrate originally supplied is still present in the solution, a process of auto-digestion sets in, nitrogen-fixation coming to an end for the time being. In the experiments described by different authors, amounts of nutrient solution ranging from 10 to 1,000 c.c. have been employed, with concentrations of carbohydrate ranging from 0·1 to 2

and even 5 per cent., whilst the cultures have been allowed to grow for widely divergent periods of time before analysis has been made of their contents. The results reported by different workers as to the amounts of nitrogen fixed appear to be somewhat conflicting, but they are not strictly comparable one with another, since Gerlach and Vogel have shown that the activity of the organism varies with the concentration of the carbohydrate, and with the age of the culture, which latter statement is borne out by the results obtained in the course of the present work.

Some attempt having been made to solve the foregoing problems, some consideration was given to the question of aeration. Considerable attention has been directed by various investigators to the discovery of some means of improving the aeration of organisms grown in liquid cultures. Freudenreich more particularly advocates the method of using gypsum plates, placed in Petri dishes and soaked in nutrient solution, and Krainski reports that he obtained the characteristic browning in from two to four days by the adoption of this method; while strips of gypsum placed in test-tubes containing some of the solution are favoured by other workers. For solid media, Koch recommends the use of large Petri dishes containing a thin layer of mannite-agar, and this method has been universally adopted in cases where it is suited to the special requirements of the occasion. Whatever be the vessels used for the production of liquid cultures, the use of a layer of nutrient solution as thin as is consistent with convenience is always recommended; and some workers, notably Hoffmann and Hammer, advocate the use of white quartz sand, which is shaken to one side of the flask, and forms a slope which rises well above the surface of the liquid. The use of these sand slopes proved to be by far the most efficient and convenient method of improving the aeration of the organisms in the course of the present work, for a suspension of the Bacteria could readily be obtained by adding water, well shaking the vessel, and allowing the sand to subside; while when required for analysis, the whole of the contents of the flask could most easily be transferred to a Kjeldahl flask by means of a jet of distilled water from a wash-bottle. Accordingly some attempt was made to obtain an idea of the extent of the superiority of this method over that of the ordinary liquid culture, as far as the fixation of nitrogen per unit of carbohydrate consumed was concerned.

In order to ascertain the best medium, as regards the inorganic constituents, for the growth of *Azotobacter*, a pure culture of the organism was first obtained on a mannite-agar plate. A colony was transferred by means of a sterilized platinum wire to about 30 c.c. of sterile water contained in a large test-tube, which was then well shaken, to ensure a uniform distribution of the Bacteria. One cubic-centimetre of this suspension was transferred by means of a sterile pipette to each of the flasks to be used for the experiment, for the purposes of which three nutrient solutions were

used. The first was that which had hitherto been used for the isolation and growth of the organism by Professor Bottomley in the Botanical Laboratory at King's College, and consisted of:

Mannite	10 grm.
Di-potassium phosphate .	2 grm.
Magnesium sulphate . .	0·2 grm.
Calcium carbonate . .	2 grm.
Distilled water . . .	1,000 c.c.

50 c.c. of this solution were put into each of seven 300 c.c. Jena glass Erlenmeyer flasks, which had previously been sterilized. The contents of the flasks were then boiled, 50 grm. of quartz sand, which had been previously washed, dried, screened, and recently ignited, were added, and each flask was plugged and allowed to cool, when the liquids were inoculated as described above. These flasks were numbered from 1 to 7.

The second solution employed was a modification of the first, suggested by Professor Bottomley, in which 0·2 per cent. of basic slag replaced the 0·2 per cent. of calcium carbonate. The acidity of the phosphate was neutralized by the lime in the slag, which, in addition, contained small amounts of the oxides of iron, manganese, magnesium, silicon, and aluminium, as shown in the analysis of the slag given later. The solution was thus made up as follows:

Mannite	10 grm.
Di-potassium phosphate .	2 grm.
Magnesium sulphate . .	0·2 grm.
Basic slag	2 grm.
Distilled water . . .	1,000 c.c.

50 c.c. of this solution were then put into each of seven flasks, which were provided with sand slopes and otherwise treated in a manner exactly similar to that adopted with the first seven. These were numbered from 8 to 14 inclusive.

The third solution tried was that used by Ashby, but only 10 grm. of mannite were used per litre, instead of 12 to 20 grm. as advised by Ashby, the alteration being made in order to render the solution comparable as regards the concentration of the carbohydrate with the preceding ones. The solution consisted of

Mannite	10 grm.
Mono-potassium phosphate .	0·2 grm.
Magnesium sulphate . .	0·2 grm.
Sodium chloride . . .	0·2 grm.
Calcium sulphate . . .	0·1 grm.
Calcium carbonate . .	5·0 grm.
Distilled water . . .	1,000 c.c.

The acid phosphate was dissolved separately and just neutralized by means of a decinormal sodium-hydrate solution before being added to the main bulk. The seven flasks used in this case were numbered from 15 to 21 inclusive. The whole series of 21 flasks was inoculated at the same time with the same suspension of *Azotobacter*, in order that the conditions should be as uniform as possible, and Nos. 1, 8, and 15 were sterilized, after inoculation, in the autoclave for ten minutes at a temperature of 135° C. These served as controls, one for each of the solutions used, and by inoculating the flasks before sterilizing a knowledge of the actual fixation of nitrogen was arrived at, and not the nitrogen fixed plus the small amount of nitrogen supplied in the bacterial cells. The twenty-one flasks were then incubated at a temperature of 28° C. for seven days, at the end of which period the contents of each were transferred by means of distilled water from a wash-bottle to round-bottomed flasks, to undergo analysis by the Kjeldahl process for their nitrogen content. The results of these analyses are given in the following table:

TABLE I.

Flask No.	*Culture Solution.*	*Nitrogen Content.*	*Gain in Nitrogen on ½ grm. Mannite.*	*Nitrogen-fixation on 1 grm. Mannite.*	*Average Nitrogen-fixation.*
1.	Bottomley's Solution (I)[1]—sterile .	0·14 mg.			
2.	,, with *Azotobacter*	4·78 mg.	4·64 mg.	9·28 mg.	9·70 mg.
3.	,, ,,	5·48 mg.	5·34 mg.	10·68 mg	
4.	,, ,,	4·92 mg.	4·78 mg.	9·56 mg.	
5.	,, ,,	5·20 mg.	5·06 mg.	10·12 mg.	
6.	,, ,,	4·64 mg.	4·50 mg.	9·00 mg.	
7.	,, ,,	4·92 mg.	4·78 mg.	9·56 mg.	
8.	,, (II)[1]—sterile .	0·14 mg.			
9.	,, with *Azotobacter*	6·18 mg.	6·04 mg.	12·08 mg.	12·04 mg.
10.	,, ,,	6·33 mg.	6·19 mg.	12·38 mg.	
11.	,, ,,	6·61 mg.	6·47 mg.	12·94 mg.	
12.	,, ,,	5·76 mg.	5·62 mg.	11·24 mg.	
13.	,, ,,	5·90 mg.	5·76 mg.	11·52 mg.	
14.	,, ,,	6·18 mg.	6·04 mg.	12·08 mg.	
15.	Ashby's solution—sterile	0·11 mg.			
16.	,, with *Azotobacter* .	3·10 mg.	2·99 mg.	5·98 mg.	6·08 mg.
17.	,, ,, .	3·30 mg.	3·19 mg.	6·38 mg.	
18.	,, ,, .	2·81 mg.	2·70 mg.	5·40 mg.	
19.	,, ,, .	3·09 mg.	2·98 mg.	5·96 mg.	
20.	,, ,, .	3·23 mg.	3·12 mg.	6·24 mg.	
21.	,, ,, .	3·37 mg.	3·26 mg.	6·52 mg.	

While the results show a very decided fixation of nitrogen in all the media, yet both of Bottomley's solutions appear to be better than Ashby's medium, and the presence of basic slag seems to have a beneficial effect. The fixations in each series appear to be fairly uniform among themselves, and are consistent with other results which have been obtained, so that, as a result of these determinations, Bottomley's solution (II),

[1] 'Bottomley's Solution (I)' is the solution containing calcium carbonate, while 'Bottomley's Solution (II)' is that containing basic slag.

which contains basic slag as the neutralizing agent, has since been used in the Botanical Laboratory of King's College for isolation and growth of Azotobacters. This nutrient medium is one of the simplest which have been used, and by the addition of 2 per cent. of agar-agar, answers equally well for plate cultures.

One further point in connexion with the nutrient solution remained to be determined; i. e. whether, after complete neutralization of the acidity of the solution due to the phosphate, any further addition of basic slag produced a beneficial effect. Hoffmann and Hammer have shown that, so long as the liquid is neutral, increase in nitrogen-fixation does not follow upon increase of calcium carbonate in the medium; but it seemed probable, since the presence of the slag appeared to give a distinct impetus to the fixation of nitrogen, that one or more of the constituents of the slag other than lime might have a tonic effect on the organism, which effect might be increased by the addition of a greater proportion of slag to the medium. The presence of 0·2 per cent. of the slag was sufficient to render the liquid neutral to litmus-paper, so five solutions were made up, each containing 1 per cent. mannite, 0·2 per cent. di-potassium phosphate, 0·02 per cent. magnesium sulphate, with the addition of 0·2, 0·4, 0·6, 0·8, and 1·0 per cent. of basic slag respectively. 50 c.c. of the solution containing 0·2 per cent. of slag were put into each of three flasks, which were provided with sand slopes and otherwise treated exactly as in the foregoing experiment. These flasks were numbered from 1 to 3, and, similarly, three flasks were used for each of the other four liquids, the total number being thus fifteen. Each one was then inoculated with the suspension of *Azotobacter*, and Nos. 1, 4, 7, 10, and 13 were sterilized to serve as controls. The whole series was incubated for seven days at a temperature of 28° C., and at the expiration of this time the contents of each flask were transferred to a Kjeldahl flask and analysed for their nitrogen content, with the following results:

TABLE II.

Flask No.	*Proportion of Slag.*	*Nitrogen Content.*	*Fixation on ½ grm. Mannite.*	*Fixation on 1 grm. Mannite.*	*Average Fixation.*
1.	0·2 %	0·55 mg.			
2.	0·2 %	6·54 mg.	5·99 mg.	11·98 mg.	12·30 mg.
3.	0·2 %	6·86 mg.	6·31 mg.	12·62 mg.	
4.	0·4 %	0·70 mg.			
5.	0·4 %	7·56 mg.	6·86 mg.	13·72 mg.	14·28 mg.
6.	0·4 %	8·12 mg.	7·42 mg.	14·84 mg.	
7.	0·6 %	0·77 mg.			
8.	0·6 %	6·58 mg.	5·81 mg.	11·62 mg.	11·34 mg.
9.	0·6 %	6·30 mg.	5·53 mg.	11·06 mg.	
10.	0·8 %	0·84 mg.			
11.	0·8 %	6·02 mg.	5·18 mg.	10·36 mg.	10·78 mg.
12.	0·8 %	6·44 mg.	5·60 mg.	11·20 mg.	
13.	1·0 %	0·98 mg.			
14.	1·0 %	7·56 mg.	6·58 mg.	13·16 mg.	13·02 mg.
15.	1·0 %	7·42 mg.	6·44 mg	12·88 mg.	

It appears from these figures that while a good fixation is obtained with 0·2 per cent. of slag, an even better one results from the use of 0·4 per cent. The addition of more than this, however, produces a perceptible, though not very considerable deterioration in nitrogen-fixing power, which effect increases with increase in the proportion of slag until 1 per cent. is reached, when the presence of this quantity appears to stimulate the organisms to greater activity than does the use of 0·6 or 0·8 per cent. The proportion of 0·4 per cent., however, obviously produces the most beneficial effects, probably on account of the stimulative effect of certain constituents of the slag; the addition of these substances in larger quantities than are contained in 0·4 per cent. being not only unnecessary, but harmful to the organism. The results obtained by the use of 1 per cent. of slag occasioned considerable surprise, but it seems possible that in this quantity just sufficient of another constituent, present in the slag in lesser proportion, has been added, to exert a second tonic effect, and overcome, to a certain extent, the depressing influence of over-doses of those elements to which the first stimulation was due. In order to test the truth of this hypothesis and to ascertain the effect of still larger proportions of slag, further series of experiments are now in progress, the results of which will be given as soon as possible.[1] It is clear, however, that the effect of the slag is not limited, as is that of calcium carbonate, to a simple neutralization of acidity, but that certain constituents, though not essential to the growth of and fixation of nitrogen by *Azotobacter*, yet when added in the correct proportions, exert a beneficial influence on the activity of the organisms.

The question of the best liquid nutrient medium having been thus far settled, the next point to be ascertained was whether the Bacteria are able to utilize the last traces of carbohydrate food material present in the culture solution, or whether, when the solution of the nutrient has become sufficiently dilute, the process of auto-digestion before mentioned sets in, no further increase in the nitrogen content taking place. It is generally recognized that the carbohydrate in 50 c.c. of 1 per cent. solution is practically used in seven days, so two series, each consisting of six flasks, were taken. These were numbered from 1 to 6 and from 7 to 12 inclusive. Each of the first six contained 50 c.c. of 1 per cent. Bottomley's solution, and each of the second six was supplied with 50 c.c. of 1 per cent. Ashby's solution, every one being provided with 50 grm. of sand to form a slope, and all being inoculated with 1 c.c. of the suspension of *Azotobacter* in sterile water. Nos. 1 and 7 were autoclaved at a temperature of 135° C., and the whole series was incubated at 28° C. The object of the experiment was to determine the fixation in each solution at the end of seven days, and also the fixation consequent on the consumption of the whole of the carbo-

[1] Since the completion of this paper, these results have been obtained, and are given on p. 885.

hydrate. In order to prevent any vitiation of results by the abstraction of small quantities of the solution for examination for the presence of carbohydrate, the following method was adopted. One flask more than was required for analysis was included in each series, and since all the flasks had been supplied with exactly the same quantity of culture solution, and inoculated with the same amount of bacterial suspension, it was assumed that growth would take place at the same rate in each, and when the mannite in the extra flask had been consumed, it would also have disappeared from the others. Nos. 6 and 12 were used as these extra flasks, and at the end of the seventh day Nos. 1, 2, 3, 7, 8, and 9 were analysed for their nitrogen content, while a drop was extracted from each of Nos. 6 and 12, evaporated to dryness on a glass slide, and examined microscopically for the crystals of mannite. Since the latter appeared to be present in both cases, Nos. 4, 5, 6, 10, 11, and 12 were re-incubated for another day, when Nos. 6 and 12 were again examined. This time no crystals appeared in the drop extracted from No. 6, so Nos. 4 and 5 were analysed, but No. 12 still contained traces of the food-stuff. The remaining three of the series were accordingly re-incubated, and this process of testing No. 12 each day for the mannite repeated until all the latter was consumed, when Nos. 10 and 11 were subjected to analysis. In this way some idea was obtained of the relation between the fixation in seven days upon 50 c.c. of the solution containing $\frac{1}{2}$ grm. of carbohydrate, and that upon the total $\frac{1}{2}$ grm. The results are shown in the following table:

TABLE III.

Flask No.	*Culture Solution.*	*Time taken.*	*Nitrogen Content.*	*Gain in Nitrogen.*	*Average gain in Nitrogen.*
1.	50 c.c. Bottomley's solution—sterile . . .		0·41 mg.		
2.	,, with *Azotobacter*	7 days	5·67 mg.	5·26 mg.	5·40 mg.
3.	,, ,,	7 ,,	5·95 mg.	5·54 mg.	
4.	,, ,,	8 ,,	6·22 mg.	5·81 mg.	5·85 mg.
5.	,, ,,	8 ,,	6·31 mg.	5·90 mg.	
7.	50 c.c. Ashby's solution—sterile		0·55 mg.		
8.	,, with *Azotobacter* . .	7 ,,	4·39 mg.	3·84 mg.	3·70 mg.
9.	,, ,, . .	7 ,,	4·12 mg.	3·57 mg.	
10.	,, ,, . .	18 ,,	4·27 mg.	3·72 mg.	3·79 mg.
11.	,, ,, . .	18 ,,	4·42 mg.	3·87 mg.	

It is obvious that nitrogen-fixation was almost complete at the end of seven days in both series. In Bottomley's solution, however, the presence of mannite was manifested for only one day after this period, and the increase in nitrogen content for this day was nearly a half-milligramme; whilst in Ashby's solution, although the mannite was not completely used for eighteen days, yet the increase in nitrogen for the last eleven days of that period was scarcely beyond the range of experimental error in analysis. It is clear, how-

ever, that even after the expiration of eighteen days there is no *loss* of nitrogen, and the evidence goes to show that the organisms are capable of utilizing the last traces of carbohydrate supplied to them, and, under the most favourable conditions, are able to continue the process of nitrogen-fixation until the food supply is exhausted. Exactly why the organisms should not have shown greater activity as regards the fixation of nitrogen in the cultures in Ashby's medium which had been left to grow for eighteen days is not clear, unless it be that the accumulation of certain products, either of partial decomposition of the mannite, or of excretion of the *Azotobacter*, or both, may have, in this rather less favourable medium, put an end to the nitrogen-fixing activity of the organism for the time being. Upon inoculation of a fresh portion of the culture solution with *Azotobacter* from flask No. 12 a good and strong growth was obtained, so that evidently the organisms were in a perfectly healthy condition. Since these results have been obtained, an account of an investigation conducted by Koch and Leydell has come to hand, in which they state that nitrogen-fixation comes to an end after five to eight days, the remainder of the carbohydrate being utilized for respiration and other purposes. This would account for the presence of carbohydrate for eighteen days, during the last eleven of which no fixation of nitrogen took place in Ashby's medium, but under the best conditions *Azotobacter* appears to fix nitrogen vigorously while the carbohydrate lasts.

Three series of cultures were taken in order to determine whether, in the same percentage solution, the fixation of nitrogen is strictly proportional to the amount of carbohydrate consumed; i. e. whether the increase in nitrogen consequent upon the consumption of 1 grm. of mannite is essentially twice that on $\frac{1}{2}$ grm., or four times that on $\frac{1}{4}$ grm. Each series consisted of four cultures; the flasks of the first series, numbering from 1 to 4, each containing 100 c.c. of 1 per cent. Bottomley's solution and 100 grm. of sand to form a slope; those of the second, numbering from 5 to 8, each containing 50 c.c. of the same solution and 50 grm. of sand; while those of the third, numbering from 9 to 12, each contained 25 c.c. of solution and 25 grm. of sand. When all had been inoculated, and Nos. 1, 5, and 9 afterwards sterilized in the autoclave in order to serve as controls, all were incubated at 28° C. Nos. 4, 8, and 12 were tested from time to time for the presence of mannite, by evaporating a drop to dryness and examining for the crystals, and when one of these cultures no longer showed evidence of the presence of carbohydrate, the contents of the three remaining flasks of the series were analysed for their combined nitrogen, with the following results:

TABLE IV.

Flask No.	*Culture Solution.*	*Time taken.*	*Nitrogen Content.*	*Gain in Nitrogen.*	*Average Gain in Nitrogen.*	*Relative Gain on 1 grm.*
1.	100 c.c. 1 % solution—sterile		0·69 mg.			
2.	,, with *Azotobacter*	10 days	9·21 mg.	8·52 mg.	8·38 mg.	8 38 mg.
3.	,, ,,	10 ,,	8·93 mg.	8·24 mg.		
5.	50 c.c. 1 % solution—sterile		0·71 mg.			
6.	,, with *Azotobacter*	8 ,,	5·27 mg.	4·56 mg.	4·48 mg.	4·48 × 2 = 8·98 mg.
7.	,, ,,	8 ,,	5·12 mg.	4·41 mg.		
9.	25 c.c. 1 % solution—sterile		0·71 mg.			
10.	,, with *Azotobacter*	7 ,,	1·84 mg.	1·13 mg.	1·13 mg.	1·13 × 4 = 4·52 mg.
11.	,, ,,	7 ,,	1·84 mg.	1·13 mg.		

The flasks used were all of the same size, namely Erlenmeyer flasks of 300 c.c. capacity; hence the depth of the liquid, and consequently the aeration, varied with the quantity of solution supplied. It follows that the organisms in the 50 c.c. cultures obtained a better aeration than those in the 100 c.c., and this fact probably accounts for the slightly greater nitrogen-fixation, relatively, in the former than in the latter. Directly opposed to this is the comparatively small increase in the 25 c.c. cultures, though the organisms in this case possessed the greatest advantage as regards aeration. It is a well-known fact, however, that when supplied with soluble nitrogen, *Azotobacter* does not fix until this available nitrogen has been consumed, so the very small fixation in the 25 c.c. cultures is probably explained by the assumption that the Bacteria first of all utilize the small amount of nitrogen present in the medium, as shown by the control, using at the same time a portion of the mannite as a source of energy. When the process of nitrogen-fixation began, a relatively much smaller amount of mannite would be available as a source of energy in these cultures than in the 50 and 100 c.c. ones, and hence a smaller gain in nitrogen would be expected. All the materials used were supposed to be chemically pure, but every control so far examined shows the presence of nitrogen. The growths were apparently perfectly healthy, but it is fairly evident that the organisms used throughout the series were more or less weakened by continued growth under artificial conditions, since the fixation per gramme of carbohydrate consumed amounted to only between 8 and 9 mg., while in the earlier part of the work the organisms originally obtained from the same source gave fixations amounting to 14 mg. However, Hoffmann and Hammer obtained good fixations in 25 c.c. of 1 per cent. solution, so that, when multiplied by four, their results gave fixations of 14·40 mg. on 1 grm. carbohydrate used—results which are not comparable with any of those obtained in 25 c.c. cultures in the course of the present work.

In connexion with the question of aeration, some experiments were started to obtain some idea of the extent of the superiority of the sand-slope method over that of ordinary liquid cultures as regards the yield of nitrogen per unit of carbohydrate used. Two series of six flasks each were employed, the first series, numbering from 1 to 6, containing Bottomley's solution, and the second, numbering from 7 to 12, being supplied with Ashby's solution. 50 c.c. of 1 per cent. solution were put into each flask, and 50 grm. of sand to form a slope were added to Nos. 4, 5, 6, 10, 11, and 12. The process of inoculation was carried out in exactly the same manner as before, with the suspension of *Azotobacter* in sterile water, and Nos. 1, 4, 7, and 10 were sterilized in the autoclave to act as controls, after which all were incubated at 28° C. for seven days. At the end of this period the contents of each vessel were transferred to a Kjeldahl flask and analysed for its nitrogen content, the results being as follows :—

TABLE V.

Flask No.	*Culture Solution.*	*With or without Sand.*	*Nitrogen Content.*	*Gain in Nitrogen.*	*Gain in Nitrogen on 1 grm.*	*Average Gain in Nitrogen.*
1.	Bottomley's solution—sterile . . .	Without	0·41 mg.			
2.	,, with *Azotobacter*	,,	1·38 mg.	0·97 mg.	1·94 mg.	2·08 mg.
3.	,, ,,	,,	1·52 mg.	1·11 mg.	2·22 mg.	
4.	,, sterile . . .	With	0·41 mg.			
5.	,, with *Azotobacter*	,,	5·67 mg.	5·26 mg.	10·52 mg.	10·80 mg.
6.	,, ,,	,,	5·95 mg.	5·54 mg.	11·08 mg.	
7.	Ashby's solution—sterile	Without	0·41 mg.			
8.	,, with *Azotobacter* . .	,,	0·69 mg.	0·28 mg.	0·56 mg.	0·56 mg.
9.	,, ,, .	,,	0·69 mg.	0·28 mg.	0·56 mg.	
10.	,, sterile	With	0·55 mg.			
11.	,, with *Azotobacter* . .	,,	4·39 mg.	3·84 mg.	7·68 mg.	7·41 mg.
12.	,, ,, .	,,	4·12 mg.	3·57 mg.	7·14 mg.	

The effect of the increased aeration due to the sand slope is made very clear by these figures, and the difference between the simple liquid culture, and that on a sand slope, in rapidity of growth was equally apparent to the eye. On the sand slopes, the gelatinous growth appeared earlier, grew more rapidly, and darkened much more quickly than did that on the liquid. On the sand-slope cultures in Bottomley's solution, the film of *Azotobacter* appeared over the whole surface on the third day ; its formation was deferred until the fifth day in the sand-slope cultures in Ashby's medium and in the ordinary cultures without sand in Bottomley's solution, while in Ashby's liquid cultures no perceptible growth occurred, and the fixation of nitrogen, as shown in the above table, was very slight. The effect on the rapidity of growth of the increased aeration due to the sand was very strikingly shown at the same time by some cultures grown on ethyl alcohol. When an ordinary liquid medium was inoculated, growth took place very slowly, the film appearing over the surface only after the

lapse of about ten days, while at the end of the same period a prolific growth was apparent in the vessels containing the sand slopes, the particles of the white quartz sand which had been used having become almost black, due to the darkening of the film of *Azotobacter* which thus evidently covered each grain. The better aerobic condition maintained by the presence of the sand was also effective in assisting to the purity of the cultures, any anaerobic *Clostridium* with which they had become contaminated developing here with much greater difficulty than under the less aerated conditions found in the ordinary liquid cultures. *Clostridium*, where present, was also seen to develop decidedly better in 100 c.c. cultures than in 50 c.c. ones, the deeper layers of the larger volume being obviously more suited to its requirements than those of the smaller culture. The presence of sodium also seemed to favour the growth of these butyric organisms, and more particularly was this evident in the New Jersey medium, where, if the feeble growth of *Azotobacter* became contaminated with *Clostridium*, the latter developed vigorously, while the further development of the former was inhibited. This is due to the fact that no excess of the neutralizing agent was present in the medium, to correct the acidity of the products of vital activity of *Clostridium*, the accumulation of acid products proving fatal to *Azotobacter*.

Conclusions.

The conclusions which have been arrived at as a result of this investigation appear to be of some importance in connexion with the production of large quantities of vigorous Azotohacters, especially when they are required for soil inoculation, in which case the strongest possible cultures are desirable. The main points are as follows:

In the first place, the presence in the medium of an excess of calcium, or magnesium carbonate, or basic slag, as a neutralizing agent, is more advantageous than that of sodium hydrate; and the former substances not only assist the rapidity of the growth, but also help to maintain its purity.

The figures obtained show clearly that the presence of sodium salts is not only unnecessary, but exerts a depressing influence on the activity of *Azotobacter* as regards the fixation of nitrogen. The beneficial effect of the sodium-chloride solution in inoculating agar plates is due to the fact that this liquid is isotonic with the cell-contents, a solution of similar concentration of many other salts answering the purpose equally well.

Basic slag is an excellent substitute for precipitated chalk as a neutralizing agent in the nutrient medium, and it evidently also contains ingredients which exert a tonic effect upon the organism, increasing its activity in connexion with nitrogen-fixation by as much as 23 per cent. Exactly which of the constituents of the slag have this influence is not known, but probably the iron and manganese present are the most important

in this respect. Appended is an analysis of the slag which, together with samples of the material, was furnished by the courtesy of the Manager of the Chemical Works—late H. and E. Albert.

Silica	6·95	per cent.
Alumina	5·42	„
Ferrous oxide of iron	4·83	„
Ferric „ „	8·49	„
Lime	47·02	„
Phosphoric acid	18·88	„
(Citric acid soluble phosphoric acid =	15·74	„)
Manganese	2·30	„
Magnesium oxide	5·15	„
Sulphur	0·96	„
Total iron =	6·89	„

The proportion of slag required to induce a maximum fixation of nitrogen is 0·4 per cent.

This solution, with basic slag as the neutralizing agent, contains all the ingredients which Mme Krzemieniewski states are essential to the development of *Azotobacter*, besides the iron which Omeliansky and Ssewerowa, and more particularly Remy and Rösing, find to be so important, and the aluminium and silicon which the experiments of Kaserer prove to be so beneficial. Mme Krzemieniewski states that the minimum amounts of the elements requisite for the consumption of 1 grm. of dextrose are potassium 0·38 mg., calcium 0·36 mg., magnesium 0·35 mg., phosphorus 2·46 mg., and sulphur more than 0·49 mg. These requirements are fulfilled by the medium the use of which is here advocated, and the results obtained appear to be in accordance with those of the investigators mentioned.

Although nitrogen-fixation can, and does, under favourable conditions, proceed until the food material is completely exhausted, yet the most active fixation of nitrogen in the small 50 c.c. cultures used in the laboratory is obtained during the course of the first week, possibly owing to some depressing influence which the accumulation of the products of their vital activity may exert upon the organisms.

The yield of combined nitrogen per unit of carbohydrate consumed is apparently not influenced by the depth of the layer of liquid to such an extent as to warrant the use in all cases of the minimum quantity, and, for the purposes of experiment in the laboratory, a volume of 50 c.c. of solution in a 300 c.c. Erlenmeyer flask gives a layer sufficiently well aerated to ensure a good fixation. The best results are evidently not obtained by the supply of only very small amounts of carbohydrate food material, but, once a good growth is obtained, the yield of nitrogen appears to be practically proportional to the amount of food supplied. The use of fairly thin layers of

liquid is, however, to be advocated as assisting in the aeration of the culture, and thus inhibiting, to some extent, the growth of any contaminating *Clostridium*.

The use of sand slopes, as a means of increasing the surface, and therefore the supply of oxygen and nitrogen to the organisms, is to be strongly recommended, the adoption of this method resulting in a more rapid, vigorous, and healthy growth, giving a much greater increase in nitrogen in a given space of time, and being much more free from contamination by anaerobic organisms than is the method of ordinary liquid cultures.

The use of the sand-slope method of culture, with Bottomley's medium in volumes of 50 c.c., results in the fixation of 14 mg. of nitrogen per gramme of mannite consumed, when the most vigorous organisms are used for purposes of inoculation.

Another important result of the present investigation is the confirmation which it gives to the statement of Gerlach and Vogel, that the nitrogen-fixing activity of Azotobacters decreases as their age increases, since in the latter part of the work, when the organisms had presumably become weakened by successive subculture under artificial conditions, they were able to fix only between 8 and 9 mg. of nitrogen, their yield in the earlier part of the work having been 14 mg. per gramme of carbohydrate consumed.

In conclusion, my grateful thanks are due to Professor W. B. Bottomley for the many helpful suggestions given and the kindly interest maintained during the progress of the work.

Addendum.

As a result of the unexpected fixation of nitrogen obtained on 1 grm. of mannite in a medium containing 1 per cent. of slag, nine solutions were made up in order to test the effect of increasing proportions of this neutralizing agent. These solutions each contained 1 per cent. mannite, 0·2 per cent. di-potassium phosphate, 0·02 per cent. magnesium sulphate, with the addition of 1·2, 1·4, 2·0, 2·8, 4·0, 5·0, 6·0, 8·0, and 10·0 per cent. of basic slag respectively. 50 c.c. of the solution containing 1·2 per cent. were put into each of three flasks, and 50 grm. of sand were added to form a slope. These flasks were numbered from 16 to 18, and, similarly, three flasks were used for each of the other solutions, the total number being thus twenty-seven. Nos. 16, 19, 22, 25, 28, 31, 34, 37, and 40 were autoclaved to serve as controls, and the whole series was incubated for seven days at a temperature of 28° C. They were then analysed for their nitrogen content, and the total results obtained with the varying proportions of slag are as follows:

Flask No.	*Proportion of Slag.*	*Nitrogen Content.*	*Fixation on ½ grm. Mannite.*	*Fixation on 1 grm. Mannite.*	*Average Fixation.*
1.	0·2 %	0·55 mg.			
2.	0·2 %	6·54 mg.	5·99 mg.	11·98 mg.	12·30 mg.
3.	0·2 %	6·86 mg.	6·31 mg.	12·62 mg.	
4.	0·4 %	0·70 mg.			
5.	0·4 %	7·56 mg.	6·86 mg.	13·72 mg.	14·28 mg.
6.	0·4 %	8·12 mg.	7·42 mg.	14·84 mg.	
7.	0·6 %	0·77 mg.			
8.	0·6 %	6·58 mg.	5·81 mg.	11·62 mg.	11·34 mg.
9.	0·6 %	6·30 mg.	5·53 mg.	11·06 mg.	
10.	0·8 %	0·84 mg.			
11.	0·8 %	6·02 mg.	5·18 mg.	10·36 mg.	10·78 mg.
12.	0·8 %	6·44 mg.	5·60 mg.	11·20 mg.	
13.	1·0 %	0·98 mg.			
14.	1·0 %	7·56 mg.	6·58 mg.	13·16 mg.	13·02 mg.
15.	1·0 %	7·42 mg.	6·44 mg.	12·88 mg.	
16.	1·2 %	0·28 mg.			
17.	1·2 %	5·87 mg.	5·59 mg.	11·18 mg.	11·18 mg.
18.	1·2 %	5·87 mg.	5·59 mg.	11·18 mg.	
19.	1·4 %	0·28 mg.			
20.	1·4 %	5·64 mg.	5·36 mg.	10·72 mg.	10·58 mg.
21.	1·4 %	5·50 mg.	5·22 mg.	10·44 mg.	
22.	2·0 %	0·71 mg.			
23.	2·0 %	5·53 mg.	4·82 mg.	9·64 mg.	10·01 mg.
24.	2·0 %	5·90 mg.	5·19 mg.	10·38 mg.	
25.	2·8 %	0·71 mg.			
26.	2·8 %	5·28 mg.	4·57 mg.	9·14 mg.	9·00 mg.
27.	2·8 %	5·14 mg.	4·43 mg.	8·86 mg.	
28.	4·0 %	0·64 mg.			
29.	4·0 %	5·43 mg.	4·79 mg.	9·58 mg.	9·29 mg.
30.	4·0 %	5·14 mg.	4·50 mg.	9·00 mg.	
31.	5·0 %	0·74 mg.			
32.	5·0 %	5·14 mg.	4·40 mg.	8·80 mg.	8·94 mg.
33.	5·0 %	5·28 mg.	4·54 mg.	9·08 mg.	
34.	6·0 %	0·28 mg.			
35.	6·0 %	2·86 mg.	2·58 mg.	5·16 mg.	4·87 mg.
36.	6·0 %	2·57 mg.	2·29 mg.	4·58 mg.	
37.	8·0 %	0·43 mg.			
38.	8·0 %	1·86 mg.	1·43 mg.	2·86 mg.	2·57 mg.
39.	8·0 %	1·57 mg.	1·14 mg.	2·28 mg.	
40.	10·0 %	0·71 mg.			
41.	10·0 %	0·64 mg.	—	—	—
42.	10·0 %	0·71 mg.	—	—	

It appears from these results that a proportion of 0·4 per cent. of basic slag has the most beneficial effect upon the activity of the organisms. Their nitrogen-fixing power declines when more than 0·4 per cent. is used, but receives a further stimulation in a medium containing 1 per cent. A steady decrease in nitrogen-fixation follows upon increase in the proportion of slag above 1 per cent., until 5 per cent. is reached, when it begins to fall rapidly and is rendered impossible in a medium containing 10 per cent.

These observations appear to lend support to the hypothesis put forward in a previous paragraph, that the effect of basic slag is not limited to a simple neutralization of acidity, but that there are at least two constituents, probably iron and manganese, present in the slag in different proportions, which exert a tonic influence upon the organisms; the maximum effect of the one being obtained in a proportion of 0·4 per cent., the other being most efficacious in a medium containing 1·0 per cent.

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A Physiological Study of the Germination of Helianthus annuus. II. The Oily Reserve.

BY

EDWIN C. MILLER.

IN a previous paper [1] I reported a study of the general chemical changes which occur in the reserves of the seed of the sunflower during different stages of its germination, and concluded that the carbohydrates which appear in the seedling during germination have their origin in the oily reserve. Only a slight study of the oil itself was made at that time, however, so that it was thought advisable to make a further study of it at different stages in the development of the seedling in order to determine what changes it might undergo during germination.

In the previous paper a review was given of the work carried on by different investigators on the changes which take place in oily seeds during their germination as far as references to these works could be found. One paper, however, which escaped my observation, might well be reviewed here. Deleano,[2] in a study of the seed and seedling of *Ricinus communis*, could not detect the smallest trace of free fatty acid or glycerine at any stage of the seedling. He observed that the quantity of reducing sugar at the time when the disappearance of the oil was most rapid, was small compared to the amount of oil which disappeared, but that the amount of substances soluble in water at that time was large. He found also that the quantity of catalase diminished as the oil disappeared. From these observations he reached the conclusion that the oil contained in the seed as a reserve is not saponified in the cells at all, but that it is transformed into soluble compounds which are easily translocated to the growing parts. These compounds, then, give rise to the carbohydrates which are found in the seedling. He also believes that the catalase plays some part in the transformation of the oil. In the present paper reference will be made only to

[1] Miller, Edwin C. : A Physiological Study of the Germination of *Helianthus annuus*. Ann. of Bot., vol. xxiv, 1910, pp. 693–726.

[2] Deleano, N. T.: Recherche chimique sur la germination. Arch. Sci. Biol. (St. Petersb.), xv, 1910, No. 1, pp. 1–24. Centralbl. f. Bakt., 2. Abt., vol. xxiv, 1909, Nos. 5–7, pp. 130–146.

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those investigations which are deemed necessary for a clear discussion of the results obtained.

Stages of the Seedling examined.

The oil was examined in the resting seed and in five different stages of the seedling. The stages examined were the same as those studied in the previous investigation with the exception of Stage I. Previously the first examination of the seedling was made when the hypocotyls and roots had attained a length of 2·5 to 3·5 cm. Experience showed, however, that it was difficult to obtain a sufficient quantity of hypocotyls and roots to yield the amount of oil necessary for the determinations that were to be made. The seedlings, therefore, were first examined at a later stage of development, when the roots and hypocotyls had a length of 3·5 to 5 cm. The seedling reached this stage in about three and a half to four days.

A brief description of the last four stages of the seedling examined is given here for convenience. If a more detailed explanation is desired, the description and figures of the different stages may be consulted in the former paper.

Stage II. The hypocotyls and roots had a length of from 7·5 to 11 cm., and the arch of the hypocotyl was just breaking through the surface of the ground. The cotyledons had not yet emerged from the seed-coat. The seedlings reached this stage about five days after the planting of the seed.

Stage III. At this stage the hypocotyls had reached a length of from 5 to 6 cm. above the surface of the ground, and the main roots were about the same length with numerous side roots about 2·5 to 3·5 cm. in length. The cotyledons were a bright green, and were spread out perpendicular to the hypocotyl. The plumule had not yet developed. The time required for the seedling to reach this stage was about seven days.

Stage IV. The hypocotyls had now a length of from 8 to 10 cm. from the surface of the ground to the cotyledons. The main roots had reached a length of 6 to 7 cm., and had developed an abundant growth of lateral roots. The cotyledons had broadened and lengthened, and the plumule was slightly developed. The young plant reached this stage of development in about ten days after the planting of the seed.

Stage V. The seedling at this stage differed little from that of Stage IV in general appearance, except that the plumule was more fully developed, and the cotyledons had become more leaf-like. The seedling reached this stage in about fourteen days after the seed was planted.

Preparation of Material for Analysis.

I. *Preparation of Dry Material.* The seedlings for this experiment were grown in ordinary quartz sand in the greenhouse. As soon as the

seedlings had reached the desired stage of development, they were taken up and washed free from the sand. The hypocotyls were then separated from the cotyledons, and the two parts then ground up separately in an ordinary meat chopper. The ground material was then covered with 95 per cent. alcohol and left until it could be treated further.

Previous experience had shown that the ether extract of the sunflower seed and seedling is very easily oxidized. In order to prevent oxidation, or to reduce it to a minimum in the preparation of the dry material of the seed and seedling, the following method was used:

The alcoholic material was placed in two litre glass jars, the lids of which were provided with 'Bunsen valves'. These jars were placed on the steam bath, and the alcohol and water driven off as nearly as possible. The material was then transferred from the jars to large desiccators and dried *in vacuo* over sulphuric acid. When the material was dry it was thoroughly pulverized and placed again *in vacuo* over sulphuric acid until ready for use.

II. *Preparation of the Ether Extract.* The dry powdered material was placed in a large Soxhlet extraction apparatus and extracted for sixteen hours with absolute anhydrous ether. The ethereal solution was then placed on the water bath, and a portion of the ethereal solution distilled off. The concentrated ethereal solution was then placed in a desiccator, and the ether evaporated *in vacuo* over sulphuric acid until no trace of it remained in the oil. The ether extract was then transferred to small tightly corked bottles until ready for use.

III. *Preparation of the Neutral Oil.* It was thought advisable to examine the neutral oil at the various stages of development of the seedling, in order to ascertain what changes, if any, it might undergo as compared to those taking place in the entire ether extract. The method used for obtaining the neutral oil was as follows:

95 per cent. alcohol was added to the ether extract in the proportion of 10 grammes of ether extract to 50 c.c. of alcohol. The mixture was placed on the steam bath and heated to boiling, and then titrated with $\frac{N}{10}$ sodium hydroxide, using phenolphthalein as an indicator. To the cooled mixture was then added an equal volume of petroleum ether, and the mixture shaken gently in a separatory funnel. In this manner is obtained a separation of a solution of soap in 50 per cent. alcohol, and a solution of neutral oil in petroleum ether. The solution of soap was separated from the petroleum ether solution, and the latter again washed with 50 per cent. alcohol to remove all the soap. The petroleum ether solution was then placed on the water bath and most of the petroleum ether driven off. The concentrated solution was then placed in a closed vessel which was connected with the filter pump, and the vessel heated on the water bath until all trace of the

petroleum ether was removed. The neutral oil thus obtained was placed in small tightly corked bottles until ready for analysis.

Methods of Analysis.

Upon the ether extract of the seed and of the cotyledons and hypocotyls at the different stages, the following constants were determined in duplicate: free fatty acid, saponification value, total insoluble acids, total soluble acids, iodine number, and acetyl value. The saponification value, total insoluble acids, total soluble acids, and iodine number were determined for the neutral oil of the seed and cotyledons at the different stages of the seedling. The amount of ether extract in percentage of the dry material and in grammes per 100 cotyledons and hypocotyls was determined for each stage of the seedling examined.

All of the determinations, with the exception of those noted below, were made according to the official methods of the American Association of Official Agricultural Chemists.

The iodine number was estimated by the method of von Hübl.

For the estimation of the acetyl value the following course was followed:

A quantity of oil was heated for two hours with twice its volume of acetic anhydride in a round-bottomed flask with a reflux condenser. The acetylated oil was then transferred to a large beaker and boiling water added. After boiling for half an hour the water was siphoned off. This process was repeated a second and a third time, after which the acetylated oil was shaken up with petroleum ether in a separatory funnel in order to remove all the water from the oil. The petroleum ether solution of the acetylated oil was then placed on the water bath and part of the petroleum ether distilled off. The concentrated solution was then transferred to a closed vessel connected with the filter pump, and the vessel heated on the water bath until all the petroleum ether was driven off. A weighed portion of the oil thus acetylated was then saponified in the usual manner and the acetyl saponification value determined. The excess of sodium hydroxide was titrated with $\frac{N}{2}$ HCl, the alcohol driven off on the water bath and the acids precipitated by $\frac{N}{2}$ sulphuric acid. The flask containing the precipitated acid was then filled to the neck with boiling water and heated on the steam bath, until the insoluble acids collected in a layer at the top of the flask. The insoluble acids were then solidified by the immersion of the flask in ice water, after which the liquid content of the flask was filtered and the filtrate collected. Hot water was again added to the flasks, and the same process repeated a second and third time. The collective filtrates were then

titrated with $\frac{N}{10}$ sodium hydroxide, using phenolphthalein as an indicator. From the amount of sodium hydroxide necessary to titrate the filtrate was deducted the amount accounted for by the quantity of free acid present in the acetylated oil used in the determination. The number of centimetres of $\frac{N}{10}$ sodium hydroxide after this correction was multiplied by 5·61 and the product divided by the weight in grammes of the oil used to determine the acetyl value. A further correction was made for the amount of soluble glycerides present in the ether extract of the cotyledons in Stage V of the seedling.

Discussion of Analytical Results.

The results obtained in this investigation are shown in the following tables. Table I gives the values of the constants obtained for the ether extract and neutral oil of the seed and the cotyledons at the various stages examined. Table II gives the results of the work upon the ether extract of the hypocotyls and roots at the five different stages of the seedling. The values obtained at the different stages of the seedling for any given constant of the oily material are discussed under separate headings. For the convenience of the botanical reader a brief discussion is given of the physiological significance of each of the various constants that were determined.

Ether Extract. The oily material of the seed of the sunflower composes 54·1 per cent. of its dry weight. At Stage I, when the hypocotyls have reached a length of 3·5 to 5 cm., over one-third of this reserve has disappeared from the cotyledons. In the germination of the sunflower seed, then, it appears that the oily reserve begins to disappear at a very early stage. As shown in the previous paper, three days after the planting of the seed, when the hypocotyls and roots had a length of only 2·5 to 3·5 cm., over one-fifth of the original oil had disappeared. Seven days after planting the seed, when the cotyledons have reached the surface of the ground and spread out perpendicular to the hypocotyl, two-thirds of the original oil has disappeared from the cotyledons. An examination of the seedling fourteen days after planting of the seed shows that 8·7 per cent. of the dry weight of the cotyledons consists of ether extract, and that 5·3 per cent. of the original amount of oil is present in them.

In the roots and hypocotyls the percentage of ether extract decreases from 9·9 per cent. of the dry weight in Stage I to 1·8 per cent. in Stage V, but the quantity of oil per 100 hypocotyls and roots remains practically constant. The fact that the actual amount of ether extract present in the roots and hypocotyls remains almost constant during different stages of the seedling was shown by the results in the former paper, and has been noted

by Peters[1] for the seedlings of *Cucurbita Pepo*, and by Schmidt[2] for the sunflower and other seedlings.

TABLE I.

Chemical Nature of the Oily Reserve.

Cotyledons.

	Seeds	*Stage I*	*Stage II*	*Stage III*	*Stage IV*	*Stage V*
Dry Weight of the Cotyledons of 100 Seedlings	4·884	3·153	2·785	2·319	2·021	1·621
Ether Extract (Percentage of dry material)	54·1	52·6	49·2	36·0	16·8	8·7
Grammes of Ether Extract per 100 Cotyledons	2·642	1·658	1·370	0·834	0·339	0·141
Percentage of the Original Oil present	——	62·7	51·8	31·8	12·8	5·3
Acid value of the Ether Extract	1·6	1·7	1·5	4·6	27·5	66·8
Percentage of Free Fatty Acid in Ether Extract *	0·008	0·009	0·009	2·3	13·8	33·5
Saponification Value of Ether Extract	189·3	190·9	192·1	184·3	186·1	204·2
Saponification Value of the Neutral Oil	188·4	189·1	190·6	188·2	184·4	226·1
Total Insoluble Acids of Ether Extract	95·4	96·0	95·7	93·6	87·6	84·8
Total Insoluble Acids of the Neutral Oil	94·6	95·2	94·7	95·9	88·9	——
Total Soluble Acids of Ether Extract †	0·005	0·005	0·007	0·005	0·008	4·5
Total Soluble Acids of the Neutral Oil †	0·005	0·003	0·006	0·003	0·002	3·0
Iodine Number of the Ether Extract	125·5	126·4	124·5	125·8	120·1	111·7
Iodine Number of the Neutral Oil	124·9	126·0	124·1	126·0	119·9	111·9
Acetyl Saponification Value of Ether Extract	222·7	225·0	225·1	215·0	236·3	263·6
Acetyl Value of Ether Extract	40·1	39·0	40·6	38·2	36·5	37·5

TABLE II.

Chemical Nature of Ether Extract.

Hypocotyls and Roots.

	Stage I	*Stage II*	*Stage III*	*Stage IV*	*Stage V*
Dry Weight of the Hypocotyls of 100 Seedlings	0·605	0·885	1·21	1·52	2·17
Ether Extract (Percentage of dry material)	9·9	7·4	3·8	3·1	1·8
Grammes of Ether Extract per 100 Hypocotyls	0·060	0·065	0·046	0·047	0·040
Acid Value of Ether Extract	18·2	24·0	27·1	58·3	97·8
Percentage of Free Fatty Acid in Ether Extract *	9·1	12·0	13·4	26·4	49·1
Saponification Value of Ether Extract	190·3	189·2	198·4	236·9	238·3
Total Insoluble Acids in Ether Extract	89·6	85·6	86·5	67·1	57·2
Total Soluble Acids in Ether Extract †	1·0	1·6	1·8	16·1	18·5
Iodine Number of Ether Extract	117·7	118·8	96·1	72·6	48·3
Acetyl Saponification Value of Ether Extract	251·4	300·0	274·8	——	——
Acetyl Value of Ether Extract	71·2	114·0	95·1	——	——

* Estimated as oleic acid. † Estimated as butyric acid.

Acid Value. By the acid value of an oil is meant the number of milligrams of caustic potash necessary to neutralize one gramme of the oil. It is

[1] Peters, Ed.: Zur Keimungsgeschichte des Kürbissamens. Versuchs-Stationen, iii, 1861, pp. 1–9.

[2] Schmidt, R. H.: Ueber Aufnahme und Verarbeitung von fetten Oelen durch Pflanzen. Flora, lxxiv, 1891, p. 300.

thus a measure of the amount of free fatty acid present in the oil, and indicates the degree of hydrolysis which it has undergone. The acid value of the ether extract of the seed is only 1·6, and this value remains constant for the oily matter of the cotyledons for the first two stages of the seedlings, until one-half of the ether extract has disappeared. In Stage III the value has risen to only 4·6. This value increases to 12·8 in Stage IV and to 66·8 in Stage V, when only 5·3 per cent. of the original ether extract remains in the cotyledons. The acid value of the ether extract of the hypocotyls and roots is 18·2 in Stage I, and gradually increases until it has a value of 97·8 in the last stage examined.

The acid value, however, indicates nothing as to the nature of the free acids present in the ether extract. They may represent organic acids other than the fatty acids, since many of the former are soluble in ether and would thus be present in the ether extract. In this investigation no attempt was made to identify the free acids present in the ether extract, and they were arbitrarily calculated as oleic acid. If fatty acids of lower molecular weight, however, are present, the value obtained would indicate a greater percentage of free fatty acid than really occurs.

The amount of free fatty acid estimated as oleic is less than 1 per cent. of the ether extract of the seed, and that value remains constant for the oily extract of the cotyledons until Stage III of the seedling, when it amounts to 2·3 per cent. of the ether extract present in the cotyledons. The amount gradually increases until it constitutes one-third of the oily material present in the cotyledons at the last stage examined. Some of the free acids present in the ether extract of the cotyledons at the later stages of the seedling are those of low molecular weight and soluble in water, as the estimation of the total soluble acids shows. Not all the free acids, however, are those of the lower groups, for the determination of the soluble acids present in the oil shows a quantity too small to account for all the free acid present in the ether extract of the cotyledons. For example, take the ether extract of the cotyledons at Stage IV. The free fatty acid calculated as oleic that is present in the oily material amounts to 13·8 per cent. If the acid were calculated as butyric it would amount to 4·3 per cent. of the ether extract. But the total quantity of soluble free and combined fatty acids, estimated as butyric, at that stage amounts to less than 1 per cent. of the ether extract. It seems probable, then, that most of the free acids present in the ether extract of the cotyledons represent those of higher molecular weight than those that are soluble in water.

In the hypocotyls and roots the amount of free fatty acid, estimated as oleic, increases from 9·1 per cent. of the ether extract at Stage 1 to 49·1 per cent. at Stage V. During the first three stages examined there is evidence that a considerable part of the free fatty acid present in the ether extract of the hypocotyls and roots represents acids of higher molecular weight. Thus

the amount of free acid in the ether extract estimated as butyric gives a value two to three times greater than that found in the estimation of the total soluble acids. In the last two stages, however, the amount of free acid estimated as butyric would be less than that of the total soluble acids shown to be present. This fact is probably due to the presence of glycerides of lower molecular weight in the ether extract at those stages.

From the above it seems evident that in the ether extract of both the cotyledons and hypocotyls of the sunflower free fatty acids of high molecular weight are present. This can be determined definitely, however, only by a qualitative study of the acids present.

Saponification Value. The saponification equivalent or value of an oil is the amount of caustic potash in milligrams that is neutralized during the saponification of one gramme of the oil by the combined and free fatty acids which it contains. It is in reality an indication of the mean molecular weight of the fatty acids which enter into the composition of the oil. Thus oils which are glycerides of the higher fatty acids give a lower saponification value, while those that are glycerides of the lower fatty acids give a higher saponification value.

The saponification value of the ether extract and neutral oil in the cotyledons of the seed is the same. There is no change in the saponification number of either the ether extract or the neutral oil until the last stage of the seedling examined, when only 5·3 per cent. of the original ether extract remains in the cotyledons. The marked increase of the saponification value of both the neutral oil and ether extract at the last stage of the seedling is significant in the fact that it is the first indication given of the presence of glycerides or free fatty acids of lower molecular value than those originally contained in the cotyledons of the seed.

In the hypocotyls and roots the saponification value of the ether extract begins to increase only in the later stages of development of the seedling. It is a very striking fact that the saponification value of the ether extract in the hypocotyls and roots is identical with that of the oil contained in the cotyledons of the seed and seedling up to a period when the hypocotyls and roots have attained a length of 7·5 to 11 cm. and one-half of the oil originally present in the seed has disappeared. Even after the cotyledons are above ground and spread out perpendicular to the hypocotyl, and after over two-thirds of the original oil has disappeared from the seedling, the saponification value of the ether extract of the hypocotyls and roots has increased but slightly over that of the oil of the cotyledons. Up to the time when two-thirds of the original oil has disappeared from the cotyledons we can say, then, that the saponification values of the oil in the cotyledons and hypocotyls and roots are practically identical with the saponification value of the original oil of the seed.

Total Insoluble Acids. By the total insoluble acids of an oil is meant

the percentage of fatty acids insoluble in water that is yielded on the saponification of the oil. The determination, then, of the insoluble fatty acids of an oil gives some information as to its composition. Thus, if the oil contained in the seed shows during the course of germination a decrease in the percentage of insoluble fatty acids, it indicates that the higher fatty acids are being replaced by those of the lower groups which are soluble in water.

The percentages of total insoluble fatty acids present in the ether extract and in the neutral oil of the seed are, respectively, 95·4 and 94·6. The values thus are practically identical. These values remain constant for both the neutral oil and ether extract until Stage IV of the seedling, when seven-eighths of the original oil of the seed have disappeared. At this period there is a considerable decrease in the amount of insoluble fatty acids in both the neutral oil and the ether extract. The decrease, however, is almost identical in both, so that the amount of insoluble fatty acids remaining in each is practically the same. The amount in the ether extract is 87·6 per cent. and in the neutral oil 88·9 per cent. In the last stage of germination the amount of insoluble fatty acids in the ether extract falls to 84·8 per cent.

The percentage of insoluble fatty acids in the ether extract of the hypocotyls and roots in Stage I when they had reached a length of 3·5 to 5 cm. was 89·6, a value considerably lower than that of the original seed. This value remains the same during the two stages of the seedling, and then rapidly decreases until the value has fallen to 57·2 in the last stage of the seedling examined.

The determination of the insoluble fatty acids indicates that there is no change in the solubility of either the combined or free fatty acids of the oil of the cotyledons until a late stage of development, when nearly all the oil has disappeared. At least, up to that time there is, as will be explained later, no accumulation of soluble acids that can be detected. In the hypocotyls and roots, however, the decrease in the amount of insoluble fatty acids of the ether extract at the first stage of the seedling indicates that in these organs from the beginning of germination the insoluble fatty acids are being replaced to a small extent by the fatty acids which are soluble in water. This replacement becomes very marked in the last two stages of the seedling.

Total Soluble Acids. The total soluble acids of an oil represent both the free and combined acids of the oil which are soluble in water, and represent the fatty acids of lower molecular weight. The amount of acids soluble in water in the ether extract and the neutral oil of the seed is less than 1 per cent. This value remains almost constant for both until the last stage of the seedling examined, when it suddenly rises to 4·5 per cent. of the ether extract and 3 per cent. of the neutral oil. It seems evident from the results that no fatty acids of the lower series are produced from the oil in the cotyledons until only a small part of the original oil remains.

The large amount of soluble acids in the neutral oil of the cotyledons in Stage VI indicates that there are present considerable quantities of the glycerides of the lower fatty acids. The quantity of soluble acids in the ether extract of the roots and hypocotyls, however, amounts to 1 per cent. at the first stage of the seedling and to 1·6 per cent. and 1·8 per cent. respectively in the next two stages. The amount of soluble acid rises rapidly in the last two stages and composes 16·1 per cent. and 18·5 per cent. of the ether extract in Stages V and VI respectively.

The determination of the soluble acids supplements remarkably well the results obtained in the estimation of the total insoluble fatty acids. Wherever there is a decrease in the percentage of insoluble fatty acids there is an increase in the amount of the soluble acids.

The Iodine Number. By the iodine number of an oil is meant the amount of iodine that the combined and free unsaturated fatty acids of an oil will take up expressed in percentage of the weight of the oil. It is really a measure of the quantity of unsaturated fatty acids in the oil. Thus, if the iodine number of the oil decreases during germination, it is indicative of the fact that the unsaturated fatty acids of the oil are becoming saturated, probably by the addition of oxygen.

The iodine numbers of the ether extract and neutral oil of the seed are practically identical, being 125·5 and 124·9 respectively. This value holds constant for both the ether extract and neutral oil of the cotyledons until Stage IV, when there is a like decrease in the value of the iodine number of each. The iodine number for each at this stage is 120. At this period of development seven-eighths of the original oil have disappeared and 13·8 per cent. of the ether extract remaining is composed of free fatty acid. In the last stage of germination, when only 5·3 per cent. of the original oil remains in the cotyledons, and one-third of the oily material present is composed of free fatty acid, the value of the iodine number for both the ether extract and neutral oil is the same and amounts to 111·8.

These results are significant in that they indicate that neither the combined nor the free fatty acids of the ether extract of the cotyledons show any signs of becoming saturated until a late stage of the seedling, and even then the degree of saturation is comparatively slight. It further indicates that the combined and free fatty acids of the ether extract are equally saturated, and that this is the case even when one-third of the ether extract is composed of free acid.

In my previous work I found that the iodine number of the ether extract in Stages IV and V fell to 77·5 and 67·4 respectively. I attribute this low result, however, to the oxidation of the oil during the preparation of the material. In the preparation of the material for this investigation, however, methods were employed, as explained elsewhere, which reduced the oxidation of the oil to a minimum.

In the ether extract of the hypocotyls and roots, however, entirely different results are obtained from that of the cotyledons. At Stage I the iodine number has fallen to 117·7. It stays constant during the next stage and then falls rapidly, amounting to only 43·3 in the last stage of the seedling. This indicates that the free and combined fatty acids of the ether extract of the hypocotyls and roots become saturated to a considerable extent at the beginning of germination, and that this saturation becomes more and more complete as germination progresses.

The Acetyl Value. The acetyl value of an oil gives the number of milligrams of caustic potash required to neutralize the acetic acid that is set free when one gramme of the acetylated oil is saponified. The acetyl value is really a measure of the amount of the hydroxyl groups which an oil contains.

The acetyl value of the ether extract of the seed is 40·1. The value remains practically constant for the oily material of the cotyledons during all stages of the seedling. The slight decrease in the acetyl value at the last stages comes within the limit of error of the process. It seems, then, that there is no increase in the amount of the hydroxyl groups of the ether extract of the cotyledons during any stage of germination.

The acetyl value of the ether extract of the roots and hypocotyls was determined for the first three stages of the seedling. The value amounts to 71·2, 114·0, and 95·1 for each of the respective stages. The results are thus much higher than those obtained for the ether extract of the cotyledons. The amount of soluble glycerides present is far too small to account for this high value. It seems, then, that the ether extract of the roots and hypocotyls has a much larger amount of hydroxyl groups than that of the cotyledons.

General Considerations.

The Oily Material of the Cotyledons. The saponification value, total insoluble acids, total soluble acids, and iodine number of both the ether extract and neutral oil of the cotyledons of the seedling remain constant up to the time when seven-eighths of the original oily reserve present in the seed has disappeared. A marked change in these values takes place only in the last stage examined, when only 5·3 per cent. of the original oil remains in the cotyledons. The change in values for the above constants indicates that the oily material at that stage consists to a small extent of glycerides and free fatty acids of lower molecular weight than those composing the original oily reserve of the seed. The change in the iodine number indicates that both the free fatty acids and glycerides are becoming partially saturated, probably by the addition of oxygen.

The acetyl value of the ether extract remains constant for all stages of the seedling, and indications are thus given that the amount of hydroxyl

groups of the oily material remains the same until all the reserve has disappeared from the cotyledons.

The only marked change in the nature of the oily reserve in the cotyledons is the increase in the amount of free acid present. The acid value of the oily reserve remains constant until over two-thirds of the ether extract have disappeared from the cotyledons, when it rises to 4·6.

The most marked change in the ether extract at the last stage of seedling examined is the amount of free acid present. The acid value of the ether extract at that stage is 66·8. Calculated as oleic acid the amount of free acid present would amount to one-third of the oily material found in the cotyledons at that stage. Some of the free acids present, however, are of low molecular weight and soluble in water; but the greater part of the free acid is composed of fatty acids of high molecular weight which are insoluble in water.

The results obtained in this investigation indicate that, with the exception of an increase in the amount of free acid, no change takes place in the oily reserve of the cotyledons until nineteen-twentieths of the oil originally present has disappeared from them.

The Oily Material of the Hypocotyls and Roots. The saponification value of the ether extract of the hypocotyls is practically the same for the first three stages of the seedling examined as that of the ether extract of the cotyledons at those stages. After the third stage of the seedling the saponification value increases rapidly.

The amount of insoluble fatty acids remains constant during the first three stages, but is about 10 per cent. less than the amount of insoluble acid present in the ether extract of cotyledons at the same stages. After that the amount of insoluble fatty acids falls rapidly and amounts to only 57·2 per cent. of the oily material present at the last stage of the seedling. The amount of soluble fatty acids during the first three stages increases but little, but the amount present is greater than that found in the ether extract of the cotyledons during those respective stages. During the last two stages of the seedling the amount of soluble fatty acids increases rapidly, and amounts to 18·5 per cent. of the oily matter at the last stage of the seedling. The acid value increases gradually from 18·2 at the first stage of the seedling to 97·8 at the last stage examined. During the first three stages there is evidence that a considerable portion of the free acid is composed of acids of higher molecular weight which are insoluble in water. During the last two stages, however, the free acids present seem to be composed entirely of those of low molecular weight which are soluble in water.

The iodine number is constant for the first two stages, but is considerably less than that of the ether extract of the cotyledons at that time; after the second stage of the seedling the iodine value falls rapidly and amounts to only 48·3 at the last stage examined.

The acetyl value for the first three stages averages about twice as great as that of the ether extract of the cotyledons at the respective stages.

It will be noticed that the constants which were determined for the ether extract of the hypocotyls and roots for the first three stages have a striking resemblance to those of the ether extract of the cotyledons during those stages. The results indicate, however, that the changes in the oily material during the first two stages consist in a gradual but evident breaking down of the higher free fatty acids and glycerides into those of lower molecular weight, the saturation of the fatty acids, and an increase in the amount of the hydroxyl groups present. During the last two stages, however, these changes are rapid and very marked, as indicated by the value of the different constants determined.

Summary.

1. The acid value of the ether extract of the seed is low, amounting to only 1·6, and this value remains the same for the oily material of the cotyledons until two-thirds of the oily reserve has disappeared from them. After that the acid value increases rapidly. There is evidence that the greater part of the free acid present in the ether extract at all the stages is composed of free fatty acids of high molecular weight which are insoluble in water.

2. The results obtained indicate that, with the exception of the increase in the amount of free acid, no change takes place in the oily reserve remaining in the cotyledons until the last stage, when the seedling has become an independent plant. At that stage only 5·3 per cent. of the original oily reserve remains in the cotyledons.

3. The results indicate that the change taking place in the oily material of the cotyledons when only 5·3 per cent. of the oily reserve remains, consist in a breaking down of the higher fatty acids and glycerides into those of lower molecular weight, a partial saturation of the free and combined fatty acids, and a marked increase in the amount of free acid.

4. The amount of oily material contained in the hypocotyls and roots remains practically constant for all stages of the seedling examined.

5. Some of the constants of the oily material of the hypocotyls and roots during the first three stages of the seedling have a striking resemblance to those of the oily reserve of the cotyledons at those stages. The results in general, however, show that during these stages there has occurred a gradual but well defined breaking down of the oily material into free fatty acids and glycerides of low molecular weight, a marked saturation of the fatty acids, and an increase in the amount of the hydroxyl group of the oily matter. During the last two stages of the seedling these changes are very rapid and very marked.

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The Development of the Grain of Barley.

BY

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With twenty-two Figures in the Text.

A. Processes of Nutrition.

THREE plots, of as diverse treatment as possible, were selected to provide material for experiment. One plot, A, cropped under a four-course rotation, has received a dressing of cake-fed dung one year in every four, the last application falling in the year of the experiment, so that the plot, while plentifully supplied with all the essential plant foods, is specially rich in nitrogen. A second plot, B, comes under the same rotation but receives no manure, and is therefore deficient in plant nutrients, especially nitrogen. The third plot, C, is in the permanent barley field, which has carried barley since 1852, and it has received no phosphoric acid since that date, though an adequate supply of the other essential elements is provided in the form of alkali salts and ammonium salts. Thus the samples collected should show the results obtained from a nitrogenous general manuring, from a rotation without any addition of manure, and also the effect of phosphoric acid starvation in the presence of a sufficiency of nitrogen, potash, and other alkalis.

The same methods of sampling were adopted as in the case of the previous wheat experiments.[1] Ears were marked with ties of red wool on the day on which they first came into flower, thus ensuring uniformity in age. The plots A and B flowered within three days of one another, but the plants on the P_2O_5-starved plot C were ten days later in protruding the first anthers. As might have been expected, the plants with nitrogenous manure were long and heavy in straw, and were badly laid in places within five days from flowering.

Cuttings from these marked shoots were made at three-day intervals between 5 and 7 a.m. After transference to the laboratory the grain was stripped from the ear and the awns cut off close to the grain as rapidly

[1] Brenchley, W. E., and Hall, A. D.: The Development of the Grain of Wheat. Journ. Agr. Sc., vol. iii, part II, 1909, p. 197.

[Annals of Botany, Vol. XXVI. No. CIII. July, 1912.]

as possible. As with the wheat, and for the same reasons, the material contained in 1,000 grains was selected as the unit—a unit which will represent with the minimum of variation from period to period the material grown upon a unit area.[1] The several lots of 1,000 grains were counted out, weighed, dried in a steam oven for three days at 100° C., then weighed again. Various determinations—ash, nitrogen, phosphoric acid—were made, and after being expressed as percentages of the dry weight of the grain the results were recalculated to give the actual amounts associated with 1,000 grains in each case. The results obtained have been expressed graphically as curves, one for each property. In order to make a fair comparison between these curves the zero point was always reckoned as the day on which the first cutting was made, seven or eight days after flowering, so that

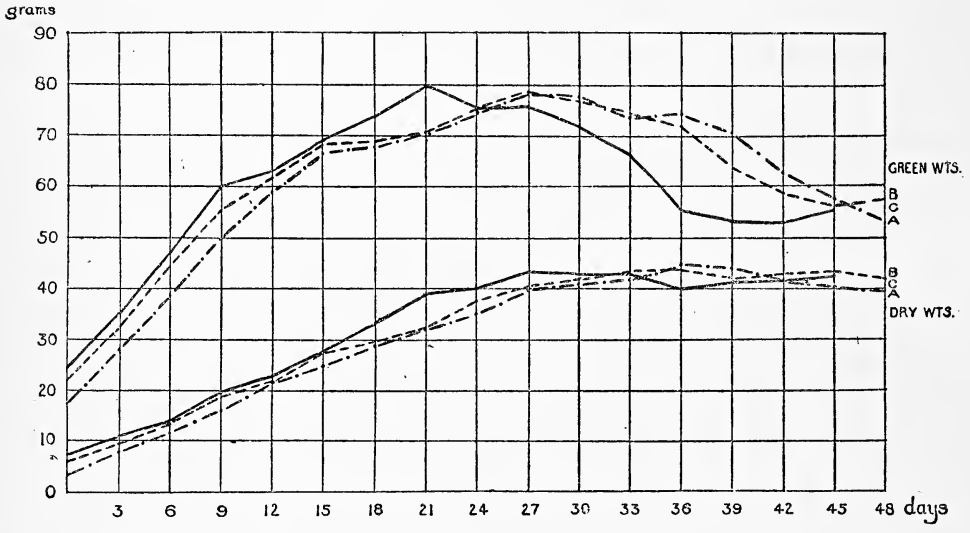


FIG. 1. Green and dry weights of 1,000 grains. Upper set of curves represent green weight, lower set dry weight.

the grains at each period were of the same age, and had approximately reached the same stage of development. As barley is always allowed to become dead ripe in the field the fruiting period of the plant is an extended one, about fifty-six days elapsing between flowering and harvesting the ripe corn.

Weight of Grain, Water Content, &c. Fig. 1 shows the actual green and dry weights of each sample of 1,000 grains. In the two earlier plots (A and B) the green weight rises for twenty-seven days and then falls steadily. The later P_2O_5-starved plot (C) reaches its maximum green weight six days earlier, and then shows a similar but rather accentuated fall. The dry weight rises steadily for twenty-seven to thirty-six days, then

[1] Brenchley and Hall, loc. cit., pp. 197–8.

the P_2O_5-starved (C) and the unmanured (B) plots show a practically flat curve, while the nitrogenous plot (A) indicates a slight but steady fall for the last twelve days before cutting. In each case the maximum green weight of the grain was attained from six to nine days before the maximum dry weight.

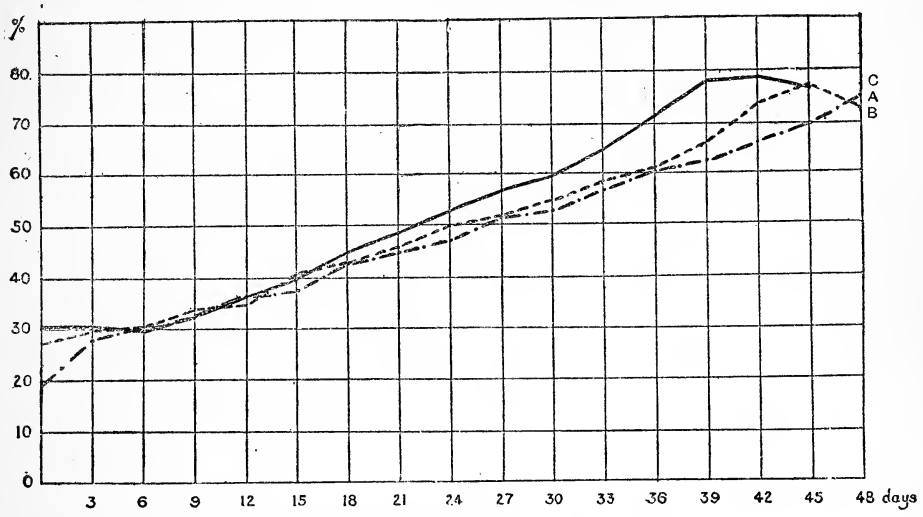


FIG. 2. Percentage of dry weight to green weight.

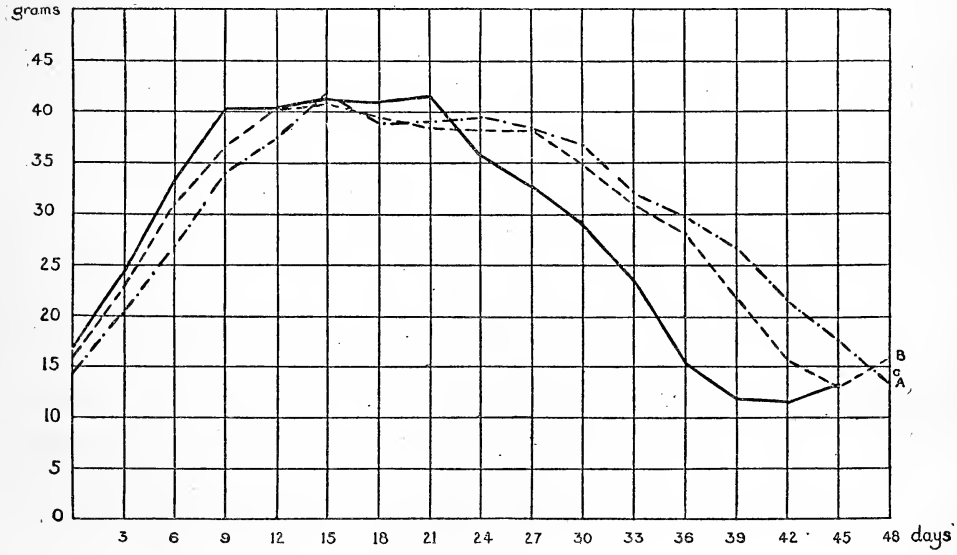


FIG. 3. Actual water contained in 1,000 grains.

In comparing the dry weight with the green weight (Fig. 2), it is seen that the percentage of dry in green rises almost to the end. In all three plots the relationship runs a very equivalent course for about eighteen days,

but then, while A and B keep on rising steadily, C shows a more rapid rise until about six days before harvesting, when the ratio remains steady.

Fig. 3 shows the actual amount of water contained in 1,000 grains. There is a steady and very rapid rise for nine to fifteen days, then a fairly constant level is maintained for about four periods, followed by a gradual fall up to the time of harvesting. Two of the plots show a somewhat inexplicable rise in water content during the last period.

Nitrogen. The percentage of nitrogen in the dry matter of the grain (Fig. 4) falls steadily for four to six periods, after which a rise sets in, this increase being most marked in A, with nitrogenous manure. The rise is much more gradual in both B and C.

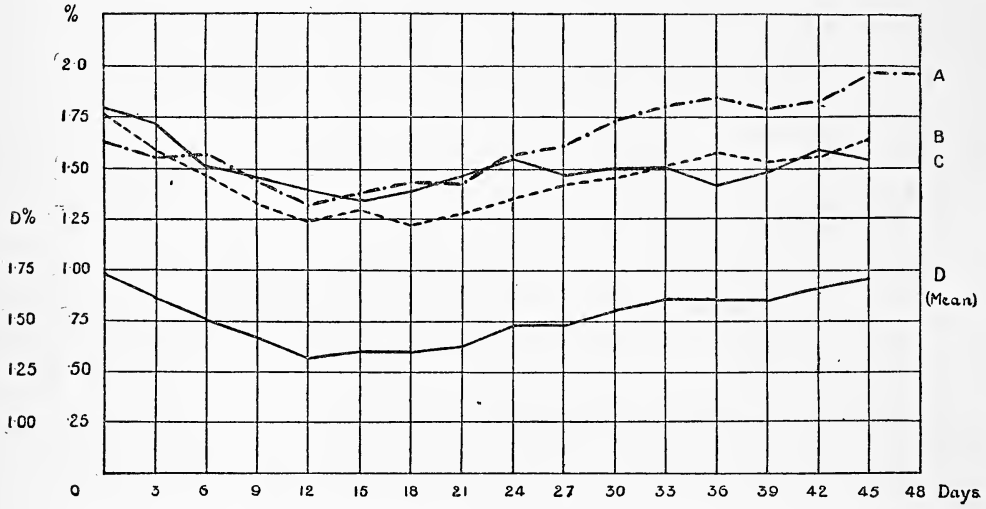


Fig. 4. Percentage of nitrogen in dry matter of grain. Mean curve is placed three squares too low for the sake of clearness.

The actual nitrogen in 1,000 grains (Fig. 5) rises steadily at first in A and B. A reaches its maximum content about twelve days before cutting, and then probably remains constant, while B rises steadily almost to the end. C, on the other hand, is very different in behaviour. After running parallel with the other plots until about the seventh period the curve flattens off considerably, and after rising very slowly for a few days more, the amount of nitrogen present remains practically constant until harvest.

The rise in the percentage of nitrogen in the dry matter is somewhat unexpected, though some indications of it had been noticed in the earlier experiments on wheat. The composite curve D, showing the mean proportion of nitrogen in the dry matter of the grain for the three plots, indicates that this rise is real. There is, of course, the possibility that such an increase is merely a seasonal effect, and further investigations will be necessary to settle the point definitely. Further corroboration of this result

is obtained by a recalculation of results so as to indicate the proportion of nitrogen in the dry matter that entered the grain between successive dates. Owing to the accumulation of small experimental errors, the results are somewhat erratic, but are sufficient to point out that dry matter stored in the later periods is richer in nitrogen than in the earlier periods, a fact that

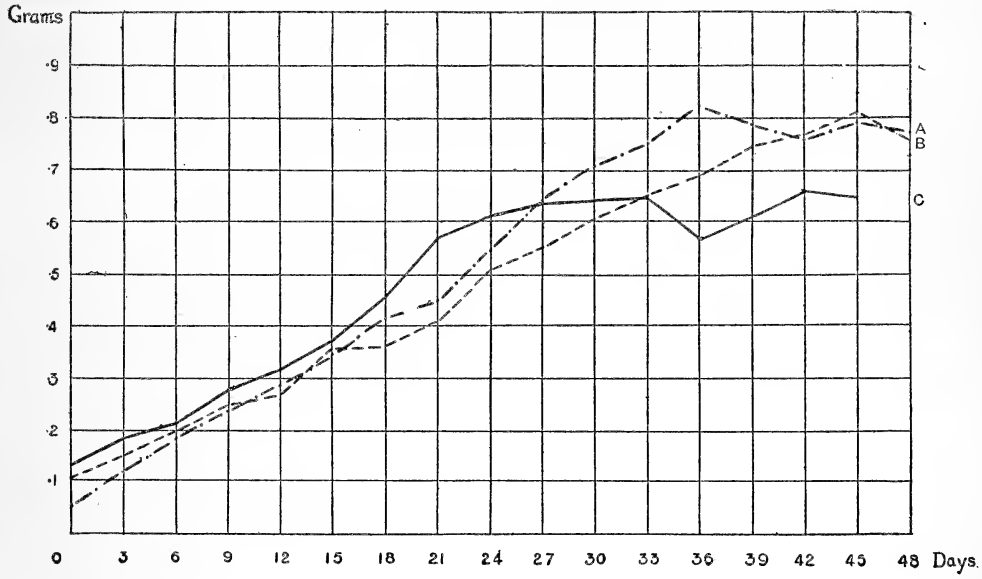


FIG. 5. Actual nitrogen contained in 1,000 grains.

is much accentuated towards the end. The trend of this increase can best be shown by calculations made at nine-day intervals.

TABLE I.

	Per cent. of nitrogen in dry matter entering the grain.	*Actual increase in dry matter.*
	Nine-day intervals.	
	Plot A.	*Plot A.*
July 13–22	1·424	12·660
July 22–31	1·371	12·984
July 31–Aug. 9	2·088	10·877
Aug. 9–18	3·828	4·702
	Plot B.	*Plot B.*
July 16–25	1·119	12·639
July 25–Aug. 3	1·038	10·828
Aug. 3–12	1·750	10·824
Aug. 12–21	3·946	3·540
	Plot C.	*Plot C.*
July 22–31	1·222	11·797
July 31–Aug. 9	1·298	13·808
Aug. 9–18	1·756	10·015
Aug. 18–27	?	—
Aug. 27–Sept. 5	3·318	2·339

This rise is much more evident than with wheat, because of the longer maturation period, during which the migration into the grain has practically ceased while respiration is still continuing, though probably slowing off gradually. The loss due to respiration falls altogether on the non-nitrogenous (carbohydrate) constituents of the grain, hence the relative increase in the proportion of nitrogen present. While the figures at the earlier periods indicate a considerable rise in the proportion of nitrogen in the grain before any great decrease has occurred in the amount of material entered, still there is not sufficient evidence to prove any change in the proportion of nitrogen actually taken in by the plant, since as development continues the

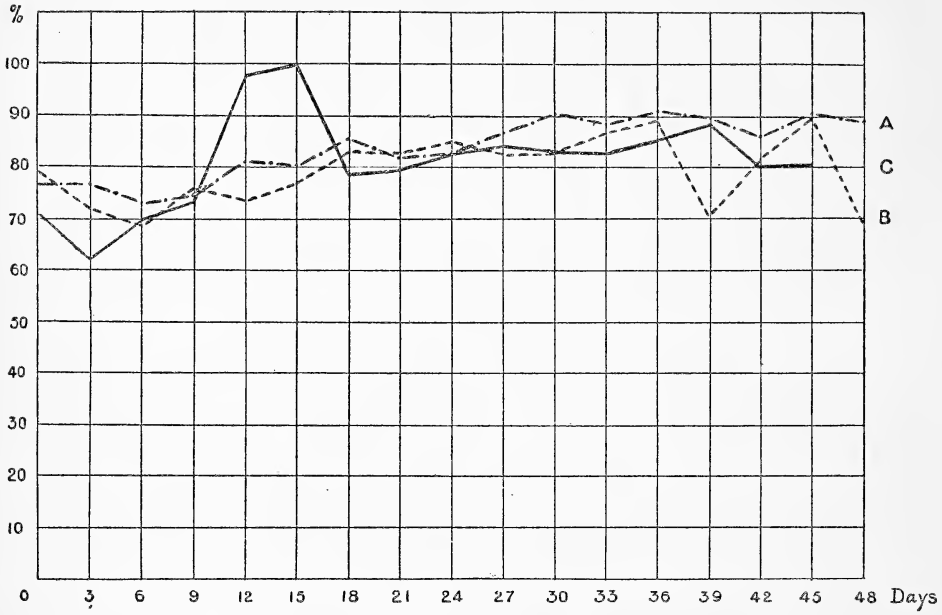


FIG. 6. Percentage of protein nitrogen in total nitrogen.

quantity of respiring substance becomes much greater, causing a corresponding increase in the amount of non-nitrogenous material broken down, so entailing a consequent rise in the percentage of nitrogenous material present.

Fig. 6 shows the results of estimating the proportion of protein nitrogen in total nitrogen. The curves, when smoothed, seem to indicate a slow but steady rise up to about a fortnight before cutting, followed by a period in which the protein nitrogen is either constant or slightly falling off. The actual non-protein nitrogen in 1,000 grains (Fig. 7), though very erratic, tends to increase gradually all along the line.

Ash and phosphoric acid. The proportion of ash to dry matter (Fig. 8) is considerably higher in the two normal plots A and B than in the P_2O_5-starved plot C. In the first two the proportion falls steadily for six periods

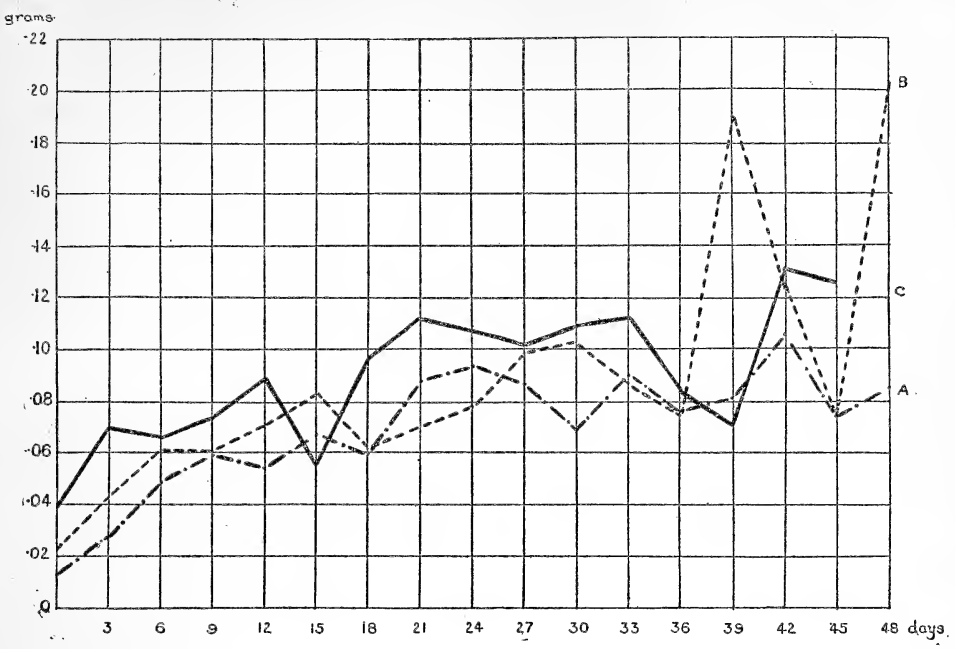


FIG. 7. Actual non-protein nitrogen in 1,000 grains.

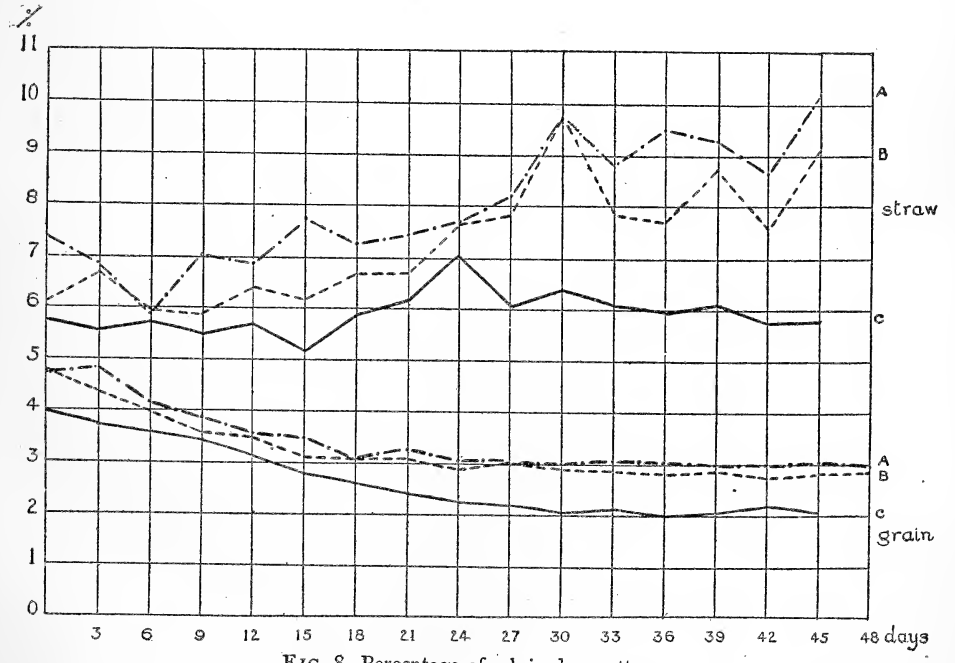


FIG. 8. Percentage of ash in dry matter.

and then remains constant right up to harvest. In C, on the contrary, the fall continues for a much longer time, for thirty days, before a constant level is reached.

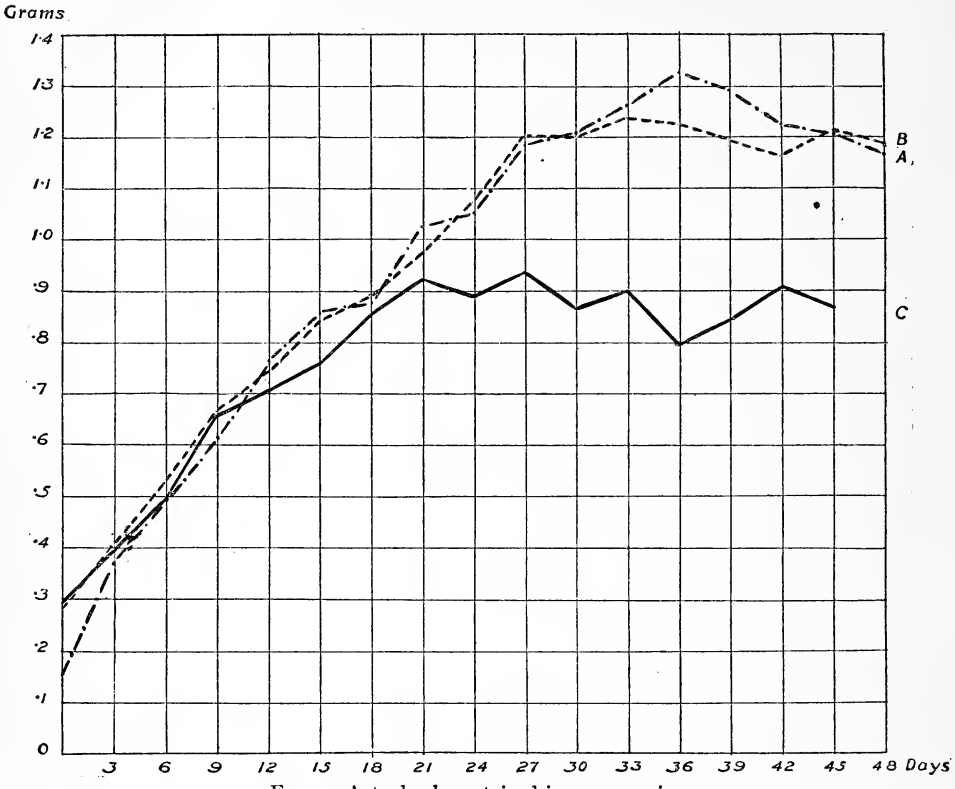


FIG. 9. Actual ash contained in 1,000 grains.

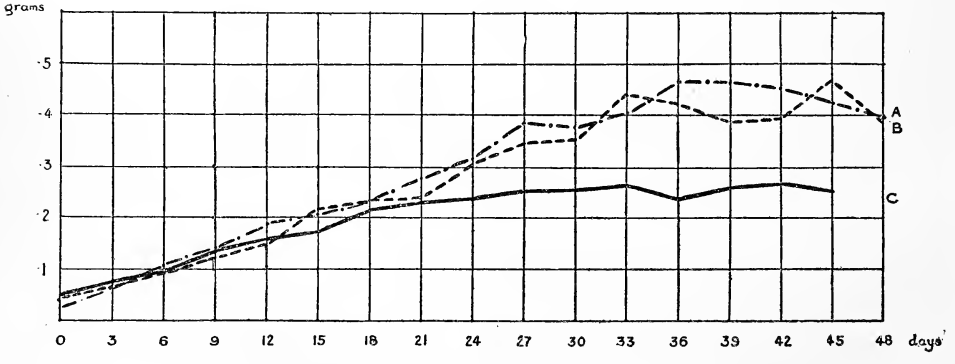


FIG. 10. Actual phosphoric acid contained in 1,000 grains.

The actual amounts of ash and phosphoric acid in 1,000 grains (Figs. 9 and 10) correspond with those of the amount of nitrogen present in that the

representative curves, while differing in proportion, change their direction at corresponding periods in all three cases, indicating some correlation in the entry of these constituents into the grain.

A calculation of the ratio between the nitrogen and phosphoric acid (expressed in Table II and Fig. 11) yields very interesting results. The two normal plots show very similar ratios, while that of C is distinctly higher,

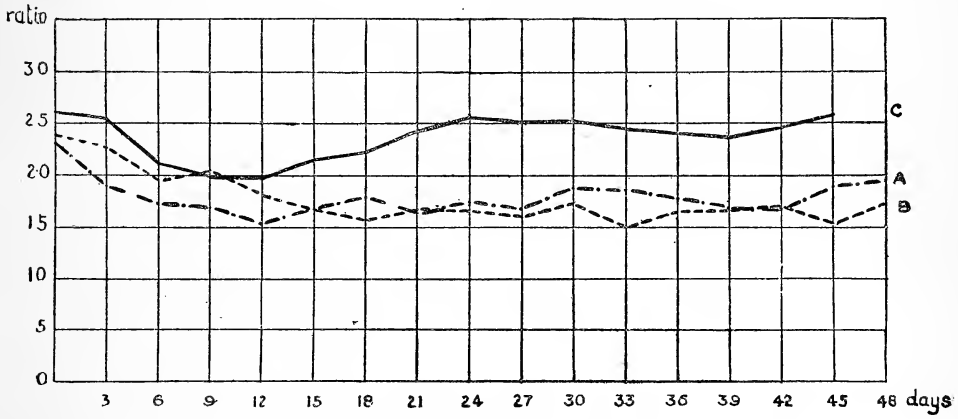


FIG. 11. Ratio between the nitrogen and phosphoric acid in the grain.

TABLE II.

	$\frac{\text{Nitrogen}}{\text{Phosphoric acid}}$		Ratio.
Period 1	2·324	2.399	2·603
,, 2	1·900	2·254	2·544
,, 3	1·724	1·950	2·122
,, 4	1·674	2·043	1·996
,, 5	1·512	1·804	1·987
,, 6	1·666	1·658	2·148
,, 7	1·777	1·558	2·233
,, 8	1·625	1·670	2·428
,, 9	1·734	1·655	2·565
,, 10	1·668	1·597	2·501
,, 11	1·853	1·704	2·503
,, 12	1·846	1·480	2·443
,, 13	1·754	1·631	2·384
,, 14	1·675	1·646	2·354
,, 15	1·670	1·692	2·464
,, 16	1·861	1·512	2·571
,, 17	1·943	1·707	—

showing that in the presence of plenty of available nitrogen in the soil, but with an insufficiency of phosphoric acid, the plant is able to take in a larger proportion of nitrogen than when there is no lack of P_2O_5. The $\frac{N}{P_2O_5}$ ratio falls during the first four or five periods, but eventually rises. In plot C a constant level is maintained after the eighth period.

The theory has been put forward that the P_2O_5 acts in some way as

a vehicle for the conveyance of nitrogen, but while the result from plot C would seem to corroborate this hypothesis, those from A and B do not bear it out, in that on a plot (A) with plenty of available nitrogen and of phosphoric acid the proportion of nitrogen to phosphoric acid is practically the same as on a plot (B) with a deficiency of both substances.

Other Determinations. Fig. 12 shows the percentage of sugar (dextrose) present in the dry matter of the grain. A slight fall occurs at the begin-

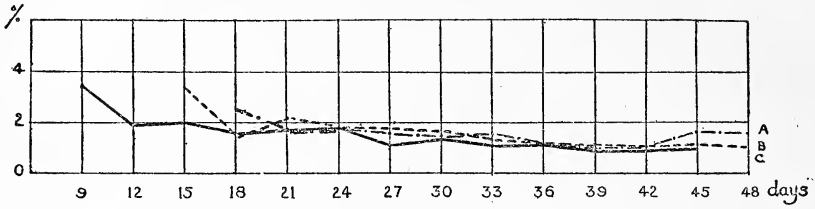


FIG. 12. Percentage of dextrose in dry matter of the grain.

ning, after which the percentage of dextrose is very constant, showing hardly the slightest decline to the very end. The actual dextrose present in 1,000 grains (Fig. 13), after an initial fall, rises slightly for three days in A and B, remains constant for about nine to twelve days, then falls somewhat.

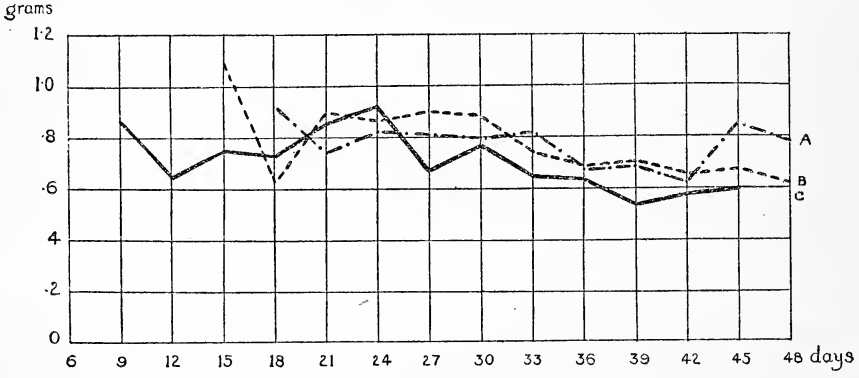


FIG. 13. Actual dextrose contained in 1,000 grains.

In C, however, the rise is prolonged over four three-day periods, and the fall sets in immediately, without any intervening period of constant level.

The *diastatic power* of the grain was determined by macerating the grain and adding it to starch paste ; the quantity of maltose thus produced by 100 parts of dry matter is shown in Fig. 14: the experimental error is very large, but it is evident that the diastatic power of the grain rises steadily for about twenty-seven to thirty-six days, and falls rapidly for a few days more, finally remaining approximately constant.

Relation of Migration Processes to the Nutrition of the Whole Plant.

The straw belonging to each of the marked ears collected was cut off close to the ground, and a series of analyses made parallel to those of the grain.

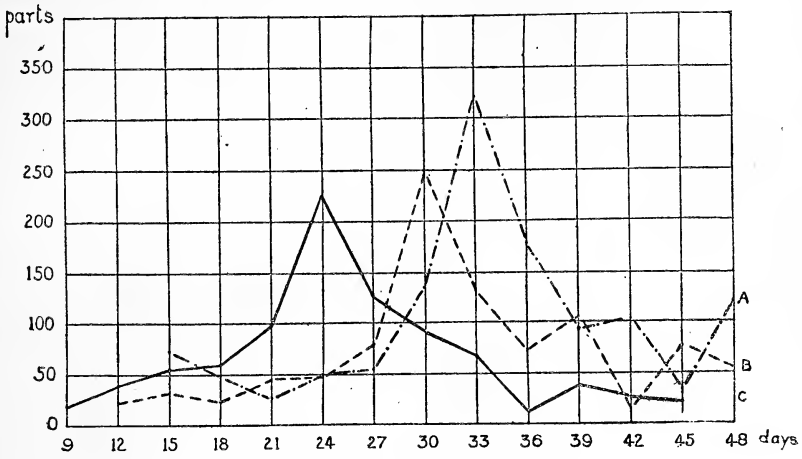


FIG. 14. Maltose produced per 100 parts of dry matter.

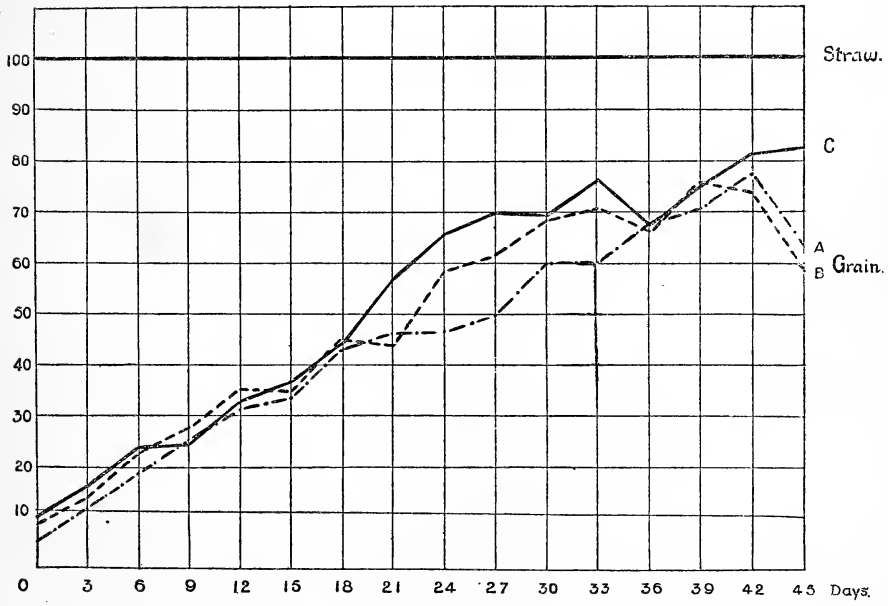


FIG. 15. Curve showing the progressive ratios between the dry weights of the straw and grain. (Weight of straw at each period is taken as a constant, 100.)

Calculations were made to determine the dry weight of the straw associated with 1,000 grains at each period. The progressive ratios between the straw and grain are shown in Fig. 15, in which a constant figure, 100, is adopted for

the dry weight of the straw. This shows that the dry weight of the grain in relation to the straw rises steadily for ten or eleven periods, then remains practically constant for about nine days longer, showing some inclination to fall for a few days before cutting, except in the plot C, which is later than the other two, and which may be somewhat slower in ripening.

Fig. 16 shows the *dry weight* of the whole plant and the grain. The dry weight of the grain increases right up to the time that desiccation begins. As the curve remains flat from that time until harvest, it is indicated that the loss of matter due to respiration in the grain is just

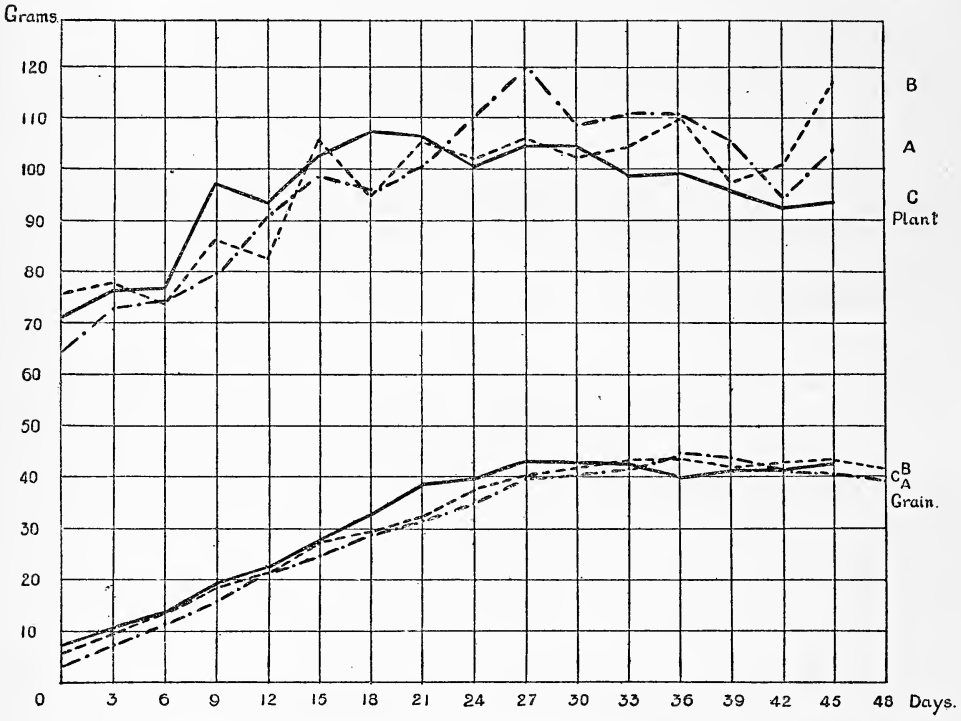


FIG. 16. Dry weights of whole plant and grain. (Whole plant = weight of 1,000 grains + weight of straw calculated as being associated with 1,000 grains.)

counterbalanced by immigration of material from the straw. In the whole plant, however, some fall in dry weight occurs after the beginning of desiccation, showing that from that time onward the intake of food material from the soil, if it still continues, is somewhat overshadowed by the destructive metabolic functions of the plant. In plot C the maximum dry weight of the whole plant is reached at an earlier date than in the other two plots, i. e. at the seventh or eighth period, which is such a critical point in the history of this plot.

The *nitrogen* in the whole plant (Fig. 17) in the two normal plots would seem to reach its maximum at about the time at which desiccation sets in, remaining fairly constant afterwards. In C, on the other hand, the whole of

the nitrogen is in the plant by a much earlier date, by the sixth period (rather before the critical date for the plot), after which a gradual decline in quantity is noticed. The depletion of the straw in favour of the grain is strongly marked during the long ripening period in barley, for the migration of nitrogen into the grain continues for at least a week after the plant has obtained its maximum supply. This is still more evident in plot C, in which the grain continues to gain in nitrogen even when the total nitrogen in the plant is on the downgrade.

Wilfarth, Römer, and Wimmer[1] have demonstrated a great loss in

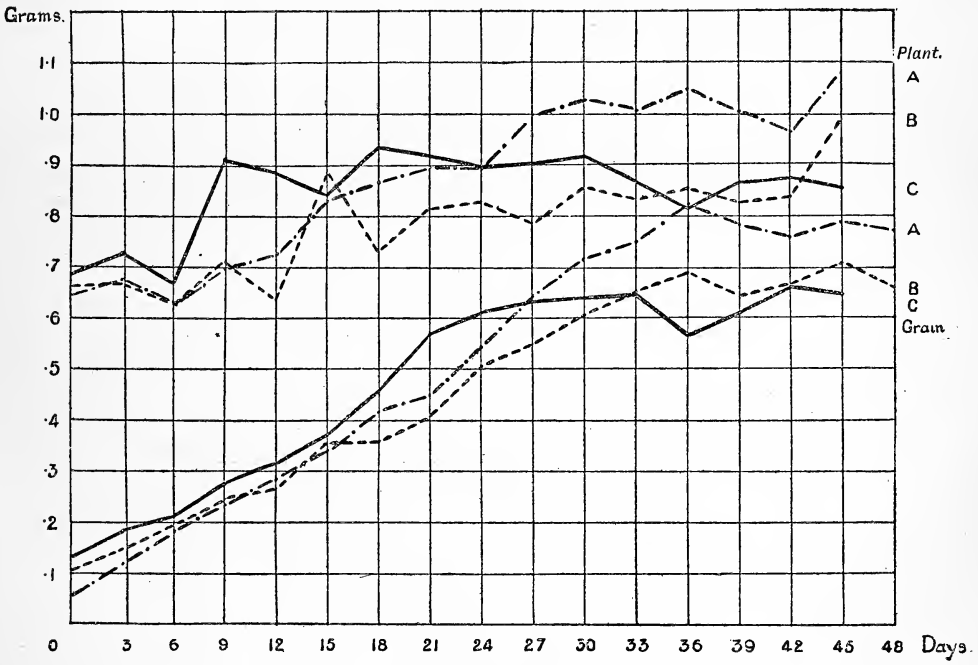


FIG. 17. Actual nitrogen contained in whole plant and in 1,000 grains.

the amount of nitrogen present in barley during the period of ripening, and these authors assume that this loss is due to part of the plant food being returned to the soil by a downward flow of the sap. Le Clerc and Brezeale[2] confirm this loss of nitrogen to some extent, but interpret it as the result of leaching by rain and dew. In the Rothamsted experiments the barley from plot C is the only one in which this loss of nitrogen is evident, and even there it is very slight, being much less than in the investigations of Wilfarth, Römer, and Wimmer, but approximating more closely to the results of Le Clerc and Brezeale.

[1] Landw. Vers. Stat., lxiii, 1905, p. 1.

[2] Plant Food removed from Growing Plants by Rain and Dew. Year-book Depart. Agric. Washington, 1908.

The curves of the intake of ash into the whole plant and the grain (Fig. 18) show that the points of maximum ash content coincide with those of the maximum nitrogen content. When once the maximum is reached,

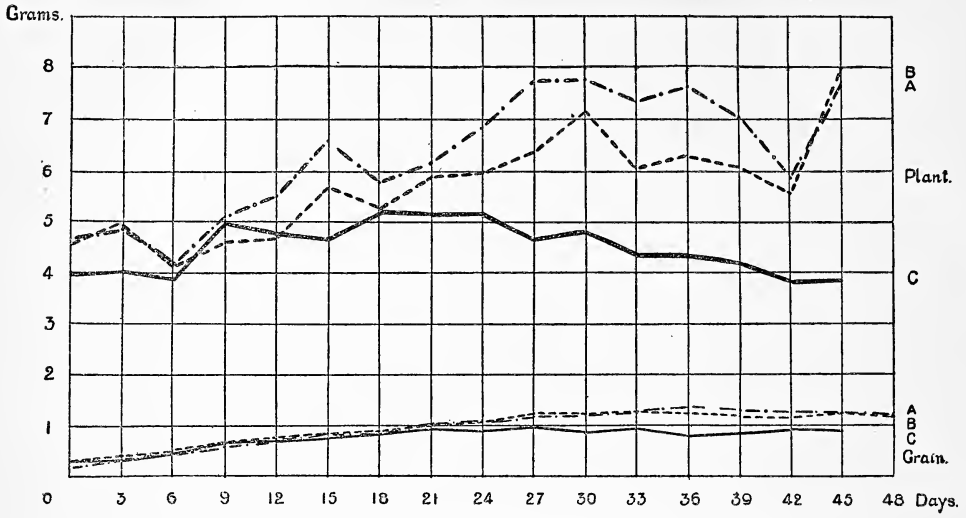


FIG. 18. Actual ash contained in whole plant and in 1,000 grains.

however, the ash in the whole plant begins to fall steadily, though the migration into the grain continues for some little time. In C the total ash in the plant shows a steady fall for four weeks before cutting, corroborating the American experiments.

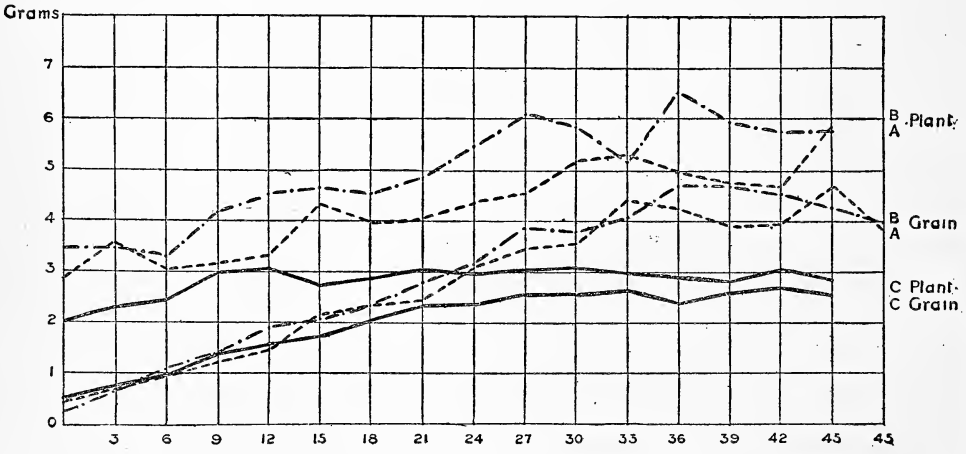


FIG. 19. Actual phosphoric acid contained in whole plant and in 1,000 grains.

The *phosphoric acid* (Fig. 19) remains fairly constant in all the plots when once the maximum has been reached, which coincides with the maximum for the ash. The fall in the latter curve is therefore due to

other ash constituents than the phosphoric acid. This result does not agree with that obtained by Le Clerc and Brezeale, who indicate a possible loss of 36 per cent. of the phosphoric acid in the plant by leaching.

Figs. 20 and 21 illustrate how steadily the proportions of nitrogen and phosphoric acid in the straw decline during the formation of the grain,

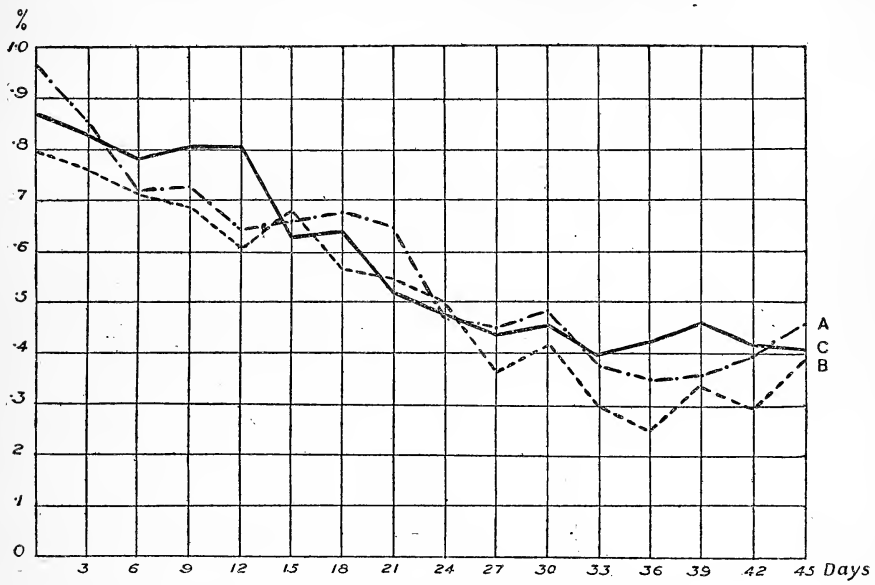


FIG. 20. Percentage of nitrogen in dry matter of straw.

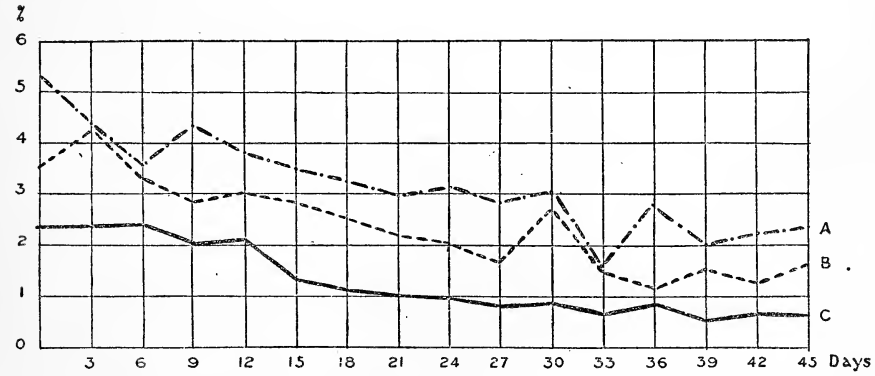


FIG. 21. Percentage of phosphoric acid in dry matter of straw.

owing to the rate of migration into the grain exceeding that of the intake into the straw.

In order to get a bird's-eye view of the relations existing between the various properties examined in barley, certain sets of figures have been averaged for the three plots and the results expressed, with appropriate

scales, in the composite curve (Fig. 22). This shows that the critical point at which maturation or ripening of the grain really begins occurs at the tenth or eleventh period, about fifteen or eighteen days before harvesting. Up to this time the diastatic power of the grain has been steadily increasing. Henceforward it as steadily and rapidly decreases. The dry weight of the grain, the phosphoric acid, nitrogen, and dry weight of the whole plant all

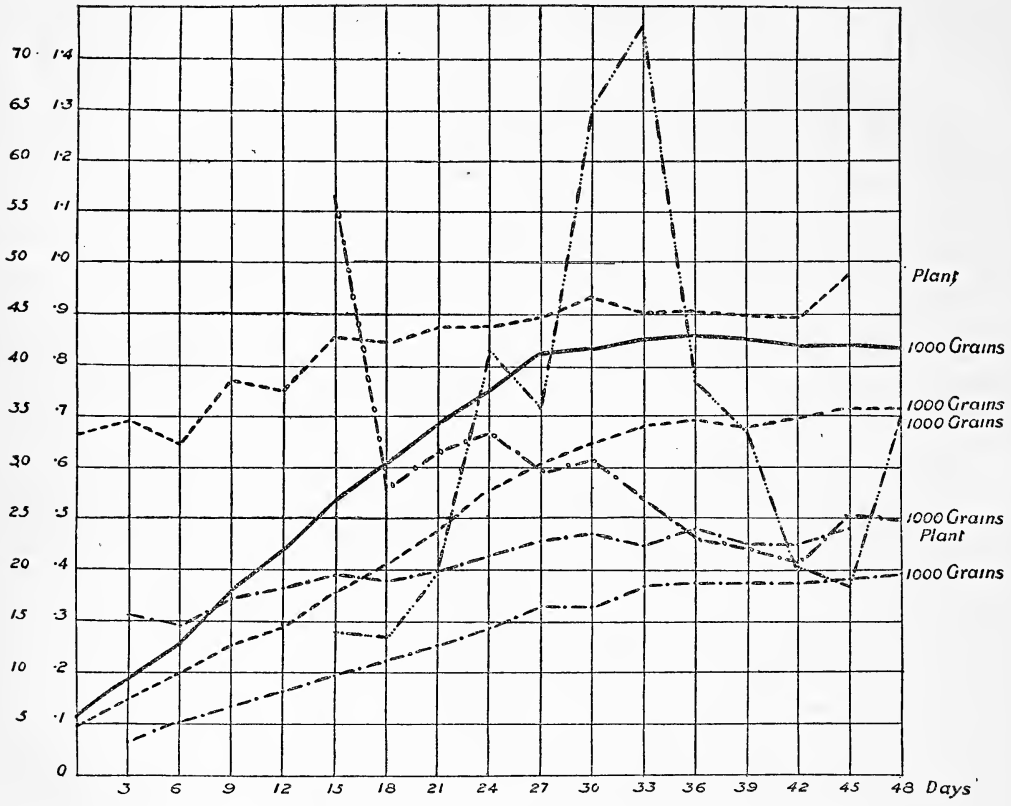


FIG. 22. Composite Curve. Plots A, B, C averaged for corresponding periods.

Dry weight ——————— 1 division = 5 grammes
Maltose produced —·····—·····— ,, ,,
Nitrogen - - - - - - - - - - - - - - - - - 1 division = 0·1 gramme
P_2O_5 —·—·—·—·—·—·—·—·— ,, ,,
Dextrose —o—o—o—o—o—o—o—o—o—o—o— ,, ,,

reach their maximum at approximately the same period. The fact that the P_2O_5 and nitrogen in the grain continue to rise is an indication of the fact that migration of food material from the stem to the grain continues steadily throughout the ripening period. One thing is noticeable—that the amount of dextrose present in the grain apparently does not bear any relation to the diastatic capacity at any given time; the dextrose curve continues to fall for some time after the diastatic power has passed its

optimum, a slight rise occurring towards the end of the maturation period, which may or may not be real.

Comparison between the Composition of the Grain and the Whole Plant of Barley and Wheat at Progressive Stages of Growth. On account of the different morphological structure of the barley and wheat grains no comparison between the two can be strictly concordant. In wheat the grain is simply the true fruit of the plant, consisting of the pericarp and the seed, while in barley the grain has incorporated with itself in addition the palea and the flowering glume. Also, the growing period of barley is considerably the longer, wheat growing for about 39 days from the time of the first sampling, while barley runs on for about 48 days from the same starting-point.

Weight of grain, water content, &c. The *green* and *dry weight* of both barley and wheat reach their maximum in approximately the same time, the decided drop in the case of barley occurring during the long ripening period, which does not exist with wheat owing to the earlier harvesting. The range of weight is much the same in both the cereals. The *percentage of dry weight* is considerably higher in barley than in wheat in the early stages, probably owing to the presence of the dry chaffy glumes.

[At the first cutting the glumes and the grains were separated, and the green and dry weights of each determined. The percentage results show that the glumes contain considerably more dry matter than the succulent grains at such an early stage in development.

Percentage of Dry Weight to Green Weight in Shelled Grain and Glumes.

	Shelled grain.	*Glumes.*
A. July 13	18·47	30·97
B. July 16	19·15	40·96
C. July 22	22·35	48·32]

This difference diminishes as time goes on, so that eventually the percentages correspond fairly closely. The final result in barley is 10 per cent. to 15 per cent. higher than in wheat as desiccation continues during the prolonged ripening period. The actual amounts of water present in 1,000 grains correspond very closely in both cases, though in barley the desiccation is continued until finally the dry grain contains only as much water as the newly formed grain at the first cutting.

Nitrogen. The actual nitrogen in 1,000 grains runs a very parallel course, though the percentage of nitrogen in the dry matter of the grain is at first somewhat higher in wheat than in barley, while later on in development the two grains are very similar in this respect. In barley the percentage of nitrogen steadily increases after it has dropped to its lowest value, whereas in wheat it remains practically constant from that time. Through-

out the *percentage of protein nitrogen* is rather lower in barley than in wheat.

Ash and phosphoric acid. In barley the percentage of ash in dry matter is distinctly higher than in wheat, except in the case of plot C, which is below the normal. The actual quantity of phosphoric acid per 1,000 grains, however, is approximately the same in both the grains.

The $\frac{N}{P_2O_5}$ ratio in wheat exceeds that of the normal barley plots, but falls considerably below that of C, the P_2O_5-starved plot.

Dextrose and diastatic power. The percentage of dextrose in the dry matter of wheat falls far more slowly than in barley, reaching its lowest limit after about 24 days in the former, but in 6–15 days in the latter, after which a constant of about 2 per cent. is maintained in both cases. Consequent on this slower fall there is considerably more dextrose actually present in the wheat grain in the early stages than in barley.

The changes in the *diastatic power* of the grain, as shown by the amount of maltose produced, follow very different courses. Even at its maximum the diastatic power of barley is less than half that of wheat, and the greatest activity is reached at a considerably later date in barley, while the rise and fall are far more sudden.

The *maltose* formed by 1,000 grains, again, shows totally different curves in the two grains, curves between which no correspondence can be made out. In wheat the maltose rises steadily at first, finally remaining more or less constant, while in barley a considerable rise is followed by a corresponding fall.

From the above comparison it is clear that the progressive changes in wheat and barley run a very parallel course though varying in detail. The differences are seen to be chiefly due to the prolonged ripening period of barley, during which maturation changes occur which are not evident in the wheat, owing to the earlier stage at which the latter is harvested.

One difference between wheat and barley is very noticeable in these two sets of experiments. With wheat the manuring of the plots had very little effect upon the analyses of the grain and straw; the course of events ran very parallel, the P_2O_5-starved plot giving results similar to those from more normal plots. With barley, on the contrary, the effect of P_2O_5 starvation is very marked indeed. For a certain time the analyses from plot C run fairly parallel to the others, A and B, but after the seventh or eighth three-day period a radical change sets in. Some limiting factor comes into play, presumably the limitation of available phosphoric acid, and the plant suffers accordingly. The dry weight, nitrogen, and ash contained in the plant show a steady falling off from this period, the phosphoric acid alone remaining constant. In the grain, however, these four properties remain constant or even increase slightly, showing that after immigration from the soil has

ceased, material is still transferred to the grain at the expense of the straw. It seems that in the case of barley the limiting of one factor of the food supply limits the availability of other food substances, i. e. that in the absence of a sufficiency of one essential food the plant is unable to utilize others even in the midst of plenty.

The effect of P_2O_5-starvation is the more intensified with the barley from plot C, since the plant is supplied with an excess of potash and other alkalis in addition to nitrogen, the only shortage being in the phosphoric acid, whereas in the compared wheat plot a general deficiency existed in everything except nitrogen (of which an excess was present), so that the particular effect of lack of phosphoric acid was not so marked.

The difference in the effect of P_2O_5 starvation on barley and wheat is also partly due to the diversity in the root systems. Barley, being shallow rooted, rapidly exhausts the supply of a plant food of which a storage exists in the surface soil. Wheat, being deep rooted, is able to seek its food from a much greater area of soil in the underlying strata, and consequently in a soil continuously cropped and P_2O_5-starved it is probable that such a sufficiency of phosphoric acid is available that the limiting factor does not come into play, and so far as the wheat is concerned phosphoric acid starvation does not exist in the plot considered.

B. Points of Biological Interest.

Preparation of material. While the mature barley grain is of a composite nature, consisting of the fruit and the palea and glume welded inseparably together, the young developing grain can be easily separated into its constituent parts, as the union is a later feature in development. For about fifteen days from the time of flowering, fruit and glumes are distinct individuals, and they can be detached without injury to either member; the actual junction appears to occur fairly rapidly, within a very few days.

The existence of the semi-permeable membrane [1] is a source of difficulty with regard to the penetration of reagents. At least three days before the glumes begin to unite with the grain, this layer is evidently in full activity, as experiments indicated. Grains were taken fresh from the plant, placed for one day in 3 per cent. $AgNO_3$, then one day in 5 per cent. NaCl. Examination showed that the reagents penetrated the glumes freely, but failed to gain access to the endosperm, which remained quite colourless. The outer coats of the grain were a little darkened just under the glumes, showing that the $AgNO_3$ had probably succeeded in penetrating a short distance into the seed coats. In view of this difficulty in penetration, after the first ten days from flowering most of the grains selected for pickling were slightly pricked, so as to secure efficient killing and fixing of the material. The reagents used were acetic alcohol, with an immersion of

[1] Brown, A. J.: Ann. Bot., lxxxi, 1907, pp. 79–87.

a few hours to a day, according to the age of the grain, and Mann's fluid [1] applied for a few hours to two days.

Entry of Starch. The development of the barley grain has been well described by Johannsen,[2] while the mature structure is portrayed by Brown and Morris.[3] These authors, however, do not give any detailed account of the entry of starch into the grain in its progressive stages.

The entry of starch into the grain was investigated by means of material preserved in acetic alcohol. Hand sections were cut, stained with iodine in potassium iodide, washed in water, and mounted in glycerine.

Four days after flowering very little endosperm could be made out, and there were no indications of starch. The pericarp, however, was very thick and fleshy, containing a good deal of starch in all its cells, especially in the layers near the seed. By the next day the endosperm appeared to be still deficient towards the embryo end, though it was fully formed at the other end; but no sign of starch had appeared. Plenty of starch existed in the pericarp, though it was only found thickly in the region of the vascular bundle. By the *sixth* day from flowering starch-formation had begun at the chalazal end of the grain, but the grains were so small that it required an 18 Zeiss ocular and either an AA or DD objective to trace them. Most of the grains had been laid down in cells in the middle of the flanks, none appearing as yet across the bridge. In some cells the association between the starch grain and the nucleus could be made out. A short distance from the chalazal end of the grain no trace of starch had yet appeared.

By the *seventh* day distinct progress had been made, as starch could be discerned for about two-thirds the length of the grain from the chalazal end, occurring as scattered grains in the flanks. The cells in which deposition first began contained considerably more starch than on the previous day, though as yet there was no considerable mass of it, as all the grains remained easily discernible and relatively few in each cell. By the day following that on which the sections were cut all the colour had disappeared from the endosperm starch, though it was retained by the pericarp starch. The pericarp at this time was still broad and uninjured throughout the length of the grain, with a good deal of starch scattered in the cells, though all the grains were easily seen individually. Just round the vascular bundle there was a dense mass of starch, especially on the side towards the endosperm.

By the *tenth* day from flowering starch infilling was in full swing. The embryo was in its early stages of development, and towards the lower

[1] Mann's fluid :—

Water	100 c.c.
Mercuric Chloride	2·5 grm.
Picric acid	1 grm.
40 % Formalin solution	10–15%

(*Vide* Mann : Physiological Histology. Oxford, 1902.)

[2] Johannsen, W. : Résumé du Compte rendu du Laboratoire de Carlsberg, ii, 1884, p. 3.

[3] Brown, H. T., and Morris, G. H. : Journ. Chem. Soc., vol. lvii, Trans. 1890, pp. 460–6.

end a few starch grains occurred in a band of cells stretching across the flanks and the side of the bridge next the furrow. The outer mass of cells adjacent to the embryo remained quite free, while immediately below the level of the embryo the deposition of starch in the flanks was much more dense. The starch grains themselves stained an intense dark blue and retained the iodine coloration for two days after cutting, indicating a more mature stage in development. Proceeding down the grain the dorsal cells continued empty of starch for some little distance, the empty cells forming a kind of wedge in section, showing perfect nuclei. Gradually the wedge of infilled cells became smaller until towards the chalaza starch was evident in all cells right up to the aleurone layer on the dorsal side. One feature was noticeable in those parts of the grain in which a fair quantity of starch was deposited. While the starch grains themselves stained such a deep blue colour, it appeared that the matrix in which they were embedded also tended to stain, but with a violet colour, at least in the cells in the flanks. The violet tint was not noticed in the bridge at this time. Care has to be taken to ascertain that this violet colour is not an optical illusion due to the thickness of the section—i. e. that the colour is not due to superposed starch grains out of focus. Careful examination indicates that this coloured matrix is an actual fact. As a possible explanation the idea presents itself that the soluble sugars come in from the vascular bundle, passing first through the bridge in which the coloured matrix does not seem to occur. Passing outwards into the flanks, chemical changes begin to occur, and the sugars assimilate more to the nature of starch.[1] While still in this transition state it is possible that stages are reached, before the starch grain is actually laid down, which are stainable with iodine in KI to some extent, giving the violet or purplish matrix. As the perfect starch grains come into being they exhibit the characteristic deep blue or black colour with the stain. At this stage in the development of the grain the pericarp was beginning to suffer from the growth of the enclosed seed. At the top of the grain it was still very thick, containing a fair proportion of starch in the deeper seated layers and round the vascular bundle. Towards the middle of the grain the pericarp was becoming badly crushed on the dorsal side by the pressure of the enlarging seed.

By the *eleventh* day from flowering the starch was very thickly deposited in the flanks right through the grain, though it was still noticeable that the grains tended to segregate round the nuclei in the cells. Some little way below the level of the embryo the starch did not extend thickly right across to the dorsal surface, though all cells showed grains in association with the nuclei. A little nearer the embryo the outer cells were quite free from starch grains. High magnification seemed to indicate that each starch grain consists of a central core showing evident striation, surrounded

[1] Cf. Maquenne, M. L. : Comptes rendus Acad. des Sciences. Tome cxlvi, 1908, pp. 542–5.

by a clear hyaline space. Outside all is a kind of bounding layer or 'skin', which also stains with iodine.

Two days later, thirteen days after flowering, little advance had apparently been made in the infilling of starch, as the sector without starch at the embryo level was still very large and extended some distance down the grain. The starch itself, however, was far more retentive of stain, as the iodine coloration was still evident on the fourth day after staining, though the remnants of the pericarp starch had entirely lost their colour during the interval. In the thick body of the grain the matrix in the cells appeared granular and stained yellow with iodine, indicating the protein nature of part of these cell contents.

By the *nineteenth* day from flowering the main body of the starch was apparently laid down. The starch was thick in the flanks and across the bridge, right up towards the tip of the embryo. There was much protein in the flanks, staining with iodine, but there seemed to be less in the bridge. Along the outer edge of the endosperm, adjacent to the embryo, was a band of crushed cells, containing no starch, which persisted throughout the development of the grain. These were probably endosperm cells which had been depleted of their contents and crushed back by the embryo in the course of its growth.

Though the main skeleton of the starch is laid down in the first three weeks from flowering, the entry of starch into the grain is by no means completed in this time. The analytical figures show that the calculated amount of carbohydrate in the grain continues to increase for another fortnight or three weeks, practically doubling in quantity, after which time it remains fairly constant until the barley is cut. The variation in the time of infilling is probably due to weather effects, as bright sunny weather tends to hasten the process, bringing the grain to its full and mature development more rapidly. The change in colour from green to brown is also apparently more closely connected with the weather conditions than with the stage of infilling of the starch, as all the plots examined began to turn at about the same date, on the accession of sunny weather, though the dates of flowering, and in consequence the age of the grains, varied as much as ten days.

Disorganization of Nuclei. While the starch skeleton is being laid down, the nuclei are in full activity and are perfect in constitution, showing their nucleoli clearly. Just about the time that all the endosperm cells have received some quota of their starch, changes begin to occur in the nuclei, at first in those of the cells in the middle of the flanks at the end of the grain away from the embryo. The first indication of change is the disappearance of the nucleoli, the nuclei becoming much more solid and dense in appearance, staining very darkly with haematoxylin. Very soon they become irregular in shape and eventually develop

a coarse network structure. As the days go on these phenomena show a progression upwards, so that by the time the nuclei at the base of the grain have reached the coarse network stage, those a little higher up are getting deformed while those higher still no longer have nucleoli in evidence. The nuclei in the cells of the sub-aleuronic layer, and also those in the immediate neighbourhood of the furrow, do not begin to undergo any change for some time, but remain perfect. In ten or twelve days from the setting in of this 'nuclear senescence'[1] the flank nuclei are involved practically all through the grain, those at the base exhibiting networks of a much finer structure. The nuclei under the aleurone layer have lost their nucleoli, but are still in a solid condition, except in rare cases in which slight deformation into coarse networks has begun. The nuclei round the top of the furrow are still all solid or even perfect, showing no deformation. Three days later great strides have been made, as at the base of the grain many cells of the sub-aleuronic layer show nuclei in the fine network stage, and all the way up the grain to the embryo such cells have either network or badly deformed nuclei. At the base, too, some of the cells round the ventral furrow have now been involved, showing senescence of nuclei to some degree, while the flank nuclei have continued to become finer in a regular progression upwards. In about three weeks from the time that the starch skeleton was first laid down practically all the nuclei of the endosperm cells up as far as the base of the embryo are involved in the senescence, though a few solid or deformed nuclei seem to hang on indefinitely, not developing into networks, especially round the furrow.

While this progression upwards has been occurring in the lower part of the grain, similar changes have been taking place in the endosperm cells in the immediate neighbourhood of the embryo. The first nuclei involved are those in cells adjacent to the embryo, those further away from the embryo and those in the sub-aleuronic layer remaining perfect at first, except just at the point where the embryo abuts on the aleurone layer. The deformation appears to begin earliest at the tip of the embryo, proceeding downwards. The senescence spreads rapidly outwards through the flanks and bridge, until all cells are involved save those of the sub-aleuronic layer and those round the furrow. By the tenth or twelfth day from the beginning of the senescence most of the nuclei at the level of the embryo are deformed or are dense networks (except just under the outer aleurone layer), especially in the deeper seated flank cells and across the bridge. Towards the base of the embryo at this date a few cells in the sub-aleuronic layer show dense networks, though the nuclei round the top of the furrow are still perfect. Three days later, when most of the flank nuclei in the lower part of the

[1] See Brown, H. T., and Escombe, F.: Proc. Roy. Soc., vol. lxiii, No. 389, 1898, p. 18; also Trans. Guiness Research Lab., pp. 123–7.

grain show finer networks, similar nuclei are apparent in cells adjacent to the embryo while those in the middle of the flanks are coarser, some still being only deformed. At the base of the embryo at this date some of the cells of the sub-aleuronic layer are beginning to show deformed nuclei, and at this level too the flank nuclei are finer. The nuclei under the aleurone layer rapidly become more and more involved, and some of those round the furrow also show deformation.

In three or four weeks after the beginning of the nuclear changes most of the inner endosperm cells near the embryo are in the network stage, though some solid ones remain in evidence just under the aleurone layer and in some of the cells round the furrow.

By the time of harvesting, the majority of the nuclei in the grain are in the state of very fine networks, though those in the sub-aleuronic layer and round the furrow have not in all cases made so much progress in disorganization. But up to the last, the relics of the nuclei are still in evidence, and are stainable with Delafield's haematoxylin, indicating that they do not absolutely pass out of existence during the maturation changes. Brown and Escombe state that the cells are quite 'disintegrated' by the time the cells are completely matured. If by the term 'disintegrated' the total disappearance of the nuclei is meant, this statement is hardly borne out by the later observations, 'disorganization' expressing the state of affairs more accurately.

A comparison of the senescence of the nuclei in wheat and barley shows that in most respects the course of events is very parallel. The phenomena of senescence are exactly similar, and in both cases it is the nuclei of the cells in the middle of the flanks which first show signs of disorganization, while those round the furrow are not affected until comparatively late in maturation. One difference is to be noticed—in barley the processes of disorganization appear to advance from both ends of the grain simultaneously, the latest region of the grain to be involved being just below the level of the embryo; while in wheat the disorganization seems to proceed gradually from the tip of the embryo downwards, in exactly the opposite direction to that in which the starch is deposited. In barley, as in wheat, the changes are evidently caused by pressure, due to the deposition of starch, which crushes into the relatively soft nuclei, forcing them out of shape. It is quite possible that the difference in the order of progression in the two grains may be due to differences in the distribution and amount of pressure exerted, not only by the starch grains, but also by the developing embryo.

Summary.

1. The weight of the whole plant increases steadily, until desiccation sets in about three weeks before harvest, after which a fall is evident.

2. The nitrogen, ash, and phosphoric acid increase until a maximum is

reached at about the time at which desiccation sets in. Then, while the nitrogen and phosphoric acid remain fairly constant, the ash decreases somewhat in quantity. The phosphoric-acid-starved plot gives somewhat abnormal results, differing in some respects from those of the other plots.

3. The long growing period of barley gives a prolonged period of desiccation, during which certain maturation changes are evident. These are hardly seen in wheat, as the latter crop is cut just when they are beginning.

4. With wheat the manuring had very little effect on the analyses of the grain or straw, whereas with barley the effect of phosphoric acid starvation is reflected in the results obtained.

5. The infiltration of starch follows a progressive course from the chalazal end of the grain up towards the embryo, the cells in the flanks of the grain being the first to show signs of the carbohydrate.

6. As the barley grain develops nuclear changes set in, due probably to the pressure of the increasing bulk of starch grains. The nuclei first lose their nucleoli and then gradually get deformed and squeezed out into networks of varying degrees of coarseness. The deformation seems to progress from both ends of the grain simultaneously towards the middle, the last cells to be involved being those of the sub-aleuronic layer of the endosperm.

APPENDIX.

Date.	*Green weight of 1,000 grains.*	*Dry weight of 1,000 grains.*	*Per cent. nitrogen in dry matter.*	*Per cent. ash in dry matter.*	*Per cent. P_2O_5 in ash.*	*Per cent. dextrose in dry matter.*	*Maltose produced per 100 parts of dry matter.*
			Plot A.				
July 13	17·52	3·268	1·63	4·71	14·87	—	—
,, 16	27·80	7·710	1·53	4·81	16·76	—	—
,, 19	38·50	11·76	1·54	4·18	21·30	—	—
,, 22	49·69	15·93	1·47	3·87	22·66	—	—
,, 25	58·87	21·51	1·31	3·54	24·50	—	—
,, 28	66·41	24·85	1·37	3·44	23·95	7·75	72·4
,, 31	67·78	28·91	1·42	3·04	26·39	2·49	48·22
Aug. 3	70·65	31·70	1·42	3·24	26·97	1·69	25·41
,, 6	74·33	34·94	1·55	3·01	29·79	1·76	49·00
,, 9	78·01	39·79	1·61	2·97	32·42	1·53	53·10
,, 12	77·29	40·65	1·73	2·97	31·42	1·45	134·2
,, 15	73·65	41·59	1·80	3·04	32·10	1·49	321·5
,, 18	74·05	44·50	1·84	3·01	34·94	1·06	177·9
,, 21	70·42	43·86	1·79	2·94	36·23	1·10	93·89
,, 24	62·83	41·31	1·83	2·97	36·98	1·02	103·8
,, 27	57·68	40·17	1·96	3·01	35·06	1·60	33·26
,, 30	52·65	39·27	1·96	2·97	34·03	1·46	118·7
			Plot B.				
July 16	21·80	5·94	1·76	4·78	15·40	—	—
,, 19	31·96	9·42	1·59	4·35	16·18	—	—
,, 22	44·18	13·32	1·46	3·97	18·88	—	—
,, 25	55·08	18·58	1·33	3·58	18·14	—	—
,, 28	61·72	21·39	1·24	3·49	19·64	7·14	22·05
,, 31	69·04	27·26	1·29	3·10	25·08	3·38	31·97
Aug. 3	68·80	29·41	1·22	3·02	25·94	1·46	22·47
,, 6	70·49	32·01	1·26	3·05	24·78	2·16	44·95
,, 9	75·60	37·48	1·35	2·86	28·53	1·78	47·09
,, 12	78·26	40·24	1·36	2·99	28·54	1·73	78·63
,, 15	76·45	41·64	1·45	2·88	29·45	1·63	244·2
,, 18	74·20	43·20	1·50	2·87	35·43	1·26	131·2
,, 21	71·77	43·77	1·57	2·80	34·42	1·10	72·98
,, 24	63·67	41·95	1·53	2·85	32·62	1·19	105·0
,, 27	58·55	42·85	1·55	2·71	33·80	1·06	14·87
,, 30	55·91	43·09	1·63	2·81	38·43	1·10	76·08
Sept. 2	57·55	41·81	1·57	2·84	32·47	1·00	54·99
			Plot C.				
July 22	24·26	7·39	1·79	3·95	17·44	—	—
,, 25	34·77	10·61	1·72	3·72	18·15	—	—
,, 28	47·07	13·89	1·51	3·56	20·04	6·92	—
,, 31	59·22	19·18	1·44	3·41	21·19	3·45	19·57
Aug. 3	62·92	22·75	1·38	3·11	22·38	1·96	39·08
,, 6	69·01	27·50	1·35	2·76	22·72	1·99	55·38
,, 9	73·76	32·99	1·38	2·59	23·94	1·59	59·75
,, 12	79·98	38·65	1·46	2·39	25·26	1·69	97·02
,, 15	75·50	39·98	1·52	2·22	26·70	1·80	223·6
,, 18	75·59	43·01	1·47	2·18	26·89	1·08	125·7
,, 21	71·55	42·68	1·50	2·03	29·51	1·33	91·78
,, 24	66·13	42·72	1·50	2·11	29·22	1·04	67·21
,, 27	55·48	40·00	1·42	1·99	29·86	1·08	11·99
,, 30	53·04	41·18	1·48	2·05	30·66	0·82	36·79
Sept. 2	53·02	41·53	1·59	2·19	29·50	0·89	25·16
,, 5	55·44	42·34	1·52	2·05	28·87	0·94	21·00

Notes on the Anatomy and Morphology of Pachypodium namaquanum, Welw.[1]

BY

D. G. LEE, B.A.

With Plate LXXXIV and eight Figures in the Text.

PACHYPODIUM NAMAQUANUM is a member of the family Apocynaceae; the genus is placed in the tribe Echitoideae by both Bentham and Hooker and Engler and Prantl. Its nearest relative is *Adenium*, from which it differs by being covered with spines. There are about twelve species, four of which occur in South Africa, the others in tropical Africa and Madagascar. One of the South African species is found in Zululand, two in the Coast and Central Regions. The fourth, *P. namaquanum*, which has the smallest range, has been found in two places: in Great Namaqualand by the Lions River,[2] and in Little Namaqualand at Dabainorup.[3] In addition it was found in Namaqualand by Wyley,[4] who gives no definite locality.

It was first recorded in Lieutenant Paterson's Travels in Africa, Journey 4, Oct. 1779, where it is figured over the class-name Pentandria monogynia, and is represented as having only two spines on each protuberance. He writes as follows[2]: 'At noon we passed the Lions River, the banks of which are in general inhabited by those animals. The country is extremely barren, and covered with small sharp stones, which proved very injurious to our horses' hoofs. In the evening we arrived at a small brackish fountain where we staid all night, and next day our way lay through a narrow path between two mountains. I found here the most beautiful plant I ever saw of the Pentandria monogynia class. It grows to six feet high, and is full of long spines from the ground to the top, and forms a large crown of crisped leaves and reddish tubelar (*sic*) flowers, tinged with yellow and green.' Paterson's description is quoted in Harvey's 'Thesaurus Capensis', where, in addition, the leaf and flower are figured and described under the

[1] Percy Sladen Memorial Expedition in South-west Africa, 1908–9, Report No. 16. This investigation was assisted by a grant from the Union Government.

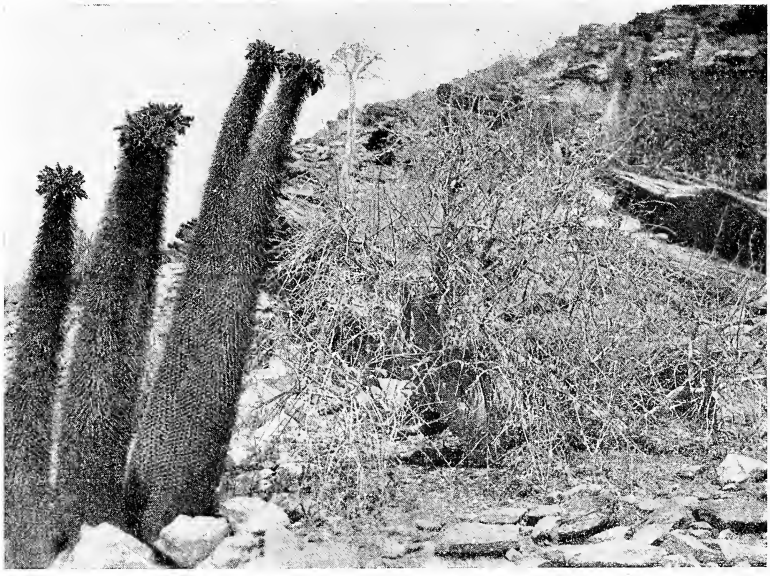
[2] Paterson (2), p. 124 (with plate). The exact position of this locality is uncertain.

[3] Pearson (3). [4] Harvey (1).

[Annals of Botany, Vol. XXVI. No. CIII. July, 1912.]

name *Adenium namaquanum*[1] from material supplied by Wyley. It is here stated that the plant was popularly known as Elephant's Trunk; the aptness of this name can be seen from Text-fig. 1, which is a photograph of the whole plant in its natural habitat. Welwitsch transferred this species from the genus *Adenium* to *Pachypodium*.[2] The account in the 'Flora Capensis' is taken from that in Harvey, and hence, as in Paterson's figure, the spines are again erroneously said to occur in pairs.[3]

The material for this investigation, consisting of the upper parts of two stems preserved in spirit, was obtained by Dr. Pearson on the Percy Sladen Memorial Expedition, 1908–9. In the 'Gardeners' Chronicle' of Dec. 4, 1909, there appeared a photograph of the growing plant, together with an



TEXT-FIG. 1. Photograph of *Pachypodium namaquanum*.

article by Dr. Pearson,[4] from which the following is quoted: 'It [*Pachypodium*] occurs on the very barren schistose ridges at Dabainorup, a few miles south of the Orange River, where it is associated with *Aloe dichotoma*, a species of *Commiphora*, and a few Acanthaceous bushes. The stout fleshy stem emerges from rocks which daily become so heated in the sun that a thick-soled boot is quite inadequate as a protection, and the nails therein become so enlarged that as soon as they cool they fall out. The inner tissues store an enormous quantity of water,[5] and the development of hard-walled cells is so slight that the whole mass can be cut through with the

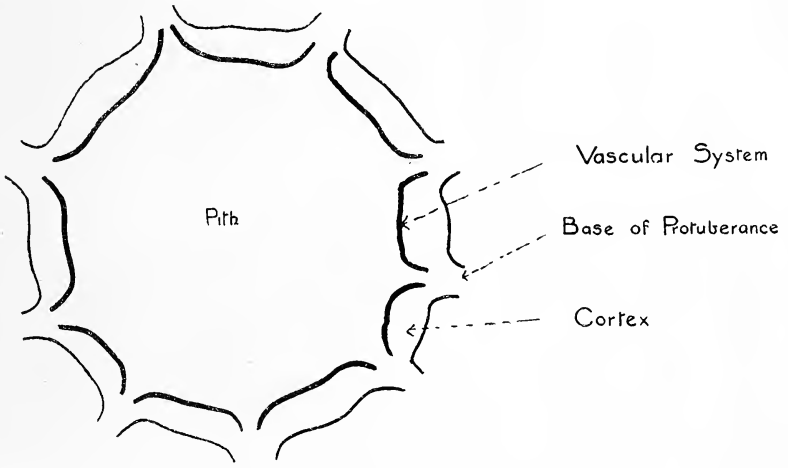
[1] Harvey (1). [2] Welwitsch (7). [3] Stapf (6). [4] Pearson (3).

[5] This water is stored in both the cortex and the pith, and drips from the stem when the latter is broken.

greatest ease by a pocket-knife. The large yellow flowers occur in June among the lower of the leaves which crown the stem; in January the seeds are already scattered and only vestiges of the dried-up corollas remain' (Text-fig. 1).

1. The Stem.

(*a*) *External Appearance.* A number of protuberances are arranged in a close spiral all over the stem (Pl. LXXXIV, Fig. 1, A). At the apex of the stem each protuberance is seen to be situated in the axil of a leaf; the leaf soon falls off, and its scar is carried up the protuberance by subsequent growth at the base of the latter. The protuberances, when young, project from the stem at approximately right angles to it; later they are recurved. On the lower surface of each is the leaf-scar (*l. s.*, Fig. 1, B), and on the upper

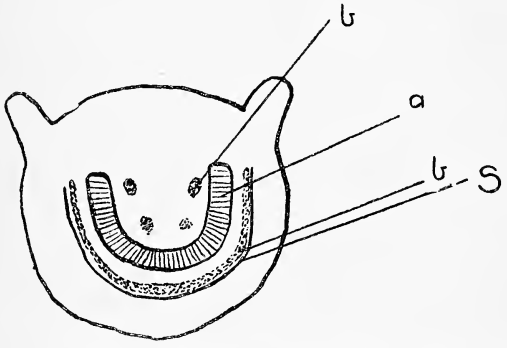


Text-fig. 2. Diagrammatic transverse section across the stem, showing the proportions of pith and cortex.

surface a scar which is no doubt left by the inflorescence (*i. s.*, Fig. 1, C). Each protuberance is fleshy and ends in three hard sharp thorns which bend downwards. The two lateral spines are longer than the median spine; they arise nearer the back of the protuberance, and bend down more sharply, than the median spine. Occasionally four spines are found in a group, in which case the two inner are shorter than the outer.

(*b*) *Anatomy.* The thickness of the stem is mainly due to the bulky pith (Text-fig. 2), the growth of which is almost entirely responsible for the increase in diameter of the stem. This growth is not caused by a cambium, but by the irregular division of the cells of the pith; this is clearly seen in section near the stem apex. The pith is very complex, consisting of large-celled water-storing parenchyma, in which medullary vascular bundles and laticiferous elements run in all directions (Fig. 2). With the limited amount

of material available it has been impossible to ascertain whether the laticiferous elements are vessels or cells; from their appearance in section near the apex of the stem they appear to be the former. Some of the large parenchymatous cells of the pith contain much starch in the form of grains split in the centre (Fig. 3). These are aggregated in very large numbers in the cells around the medullary bundles and laticiferous elements, being comparatively scarce in the other cells of the pith. Sclerosis and the formation of islands of soft bast, both of which are characteristic of Apocynaceae,[1] are absent from the pith in *Pachypodium*. The medullary bundles (Fig. 2) are composed of scalariform xylem elements and phloem, with a little parenchyma lying between the two. They are always accompanied by laticiferous elements (D, Fig. 2), which usually lie next to the xylem. Unlike those of some Apocynaceous plants, they have no definite orientation. They are given off by the vascular ring into the pith; owing to the lack of young material it has been impossible to ascertain whether they owe their origin entirely to the ring bundles or not. They pursue a most irregular course in the pith, both branching and anastomosing so as to penetrate every part of it. When a portion of the pith is macerated the medullary bundles alone are left, forming a complex network. The laticiferous cells branch and run in all directions through the pith, either with or separate from the bundles. In a transverse section of the stem they are seen to enter the pith from the cortex through the medullary rays (Fig. 4), as in many other members of the order.[2]



TEXT-FIG. 3. Diagrammatic transverse section of petiole, showing the arrangement of vascular tissue. *a*. xylem; *b*. phloem; *S*. starch sheath.

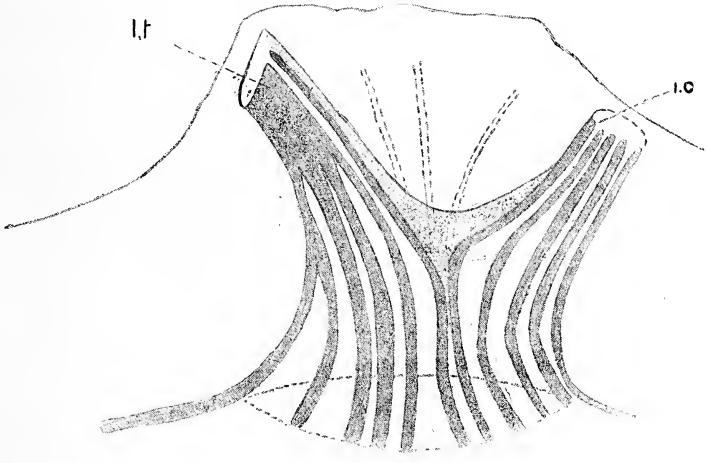
The vascular bundles of the ring are collateral; very little wood is present, and the medullary rays are broad (Fig. 4). The xylem is composed of scalariform elements and parenchyma; cambium is present (C, Fig. 4), but the small amount of secondary tissue formed by it remains parenchymatous. The phloem (B, Fig. 4) consists of narrow sieve-tubes with sieve-plates on their terminal walls. The apparent absence of intraxylary phloem is very striking, for in all other investigated genera of Apocynaceae it has been found.[3] Mr. Worsdell suggests that the intraxylary phloem bundles are present, but that they possess xylem, hence the medullary bundles. Evidence in support of this view will be given in the description of the structure of the protuberance and the petiole. No

[1] Solereder (5), p. 531. [2] l. c., p. 530. [3] l. c., p. 531.

laticiferous cells occur in or accompanying the bundles. Outside each bundle is a number of pericycle fibres (F, Fig. 4), whose walls, which are very thick, give the cellulose reaction with iodine and sulphuric acid. The cortex is massive and fairly compact, composed of rounded parenchymatous cells, many of which contain calcium oxalate, a substance characteristic of the order.[1] It is found in highly refractive clustered crystals. Laticiferous cells are very numerous, traversing the cortex in all directions. Both here and in the pith they have thin walls and granular contents in which nuclei can sometimes be seen. These contents include proteid matter, which stains deep yellow with iodine and light yellow with nickel sulphate. The whole surface of the mature stem, protuberances, and spines is covered by a thick layer of tissue derived from a phellogen which arises in the second layer of the cortex. This tissue is suberized on the stem, but stains with haematoxylin on the young protuberances and spines.

2. The Protuberances and Spines.

(*a*) *Structure of Protuberance.* Each protuberance projects from the stem at approximately right angles to it (Fig. 1, A). A number of ring bundles pass out into the protuberance in the form of a narrowing hollow



Text-fig. 4. Diagram showing the course of the vascular bundles through the protuberance. *l.t.* = leaf-trace; *i.c.* = inflorescence supply.

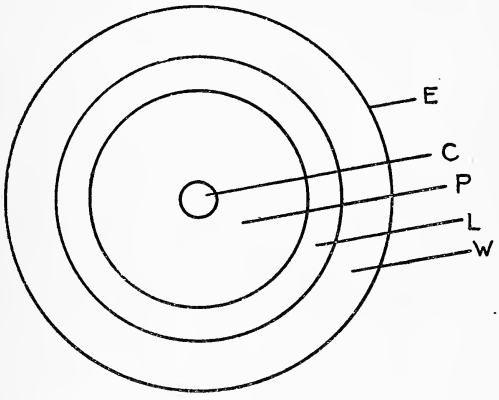
cylinder (Text-fig. 4). Some medullary bundles also pass out into the protuberance, running up within the cylinder. On their way up, the ring bundles are joined by a few of the medullary bundles, which may branch off again. Beneath the level of the scar left by the inflorescence the cylinder of ring bundles can be seen dividing into two unequal portions:

1. A trough of vascular tissue, with its open end upwards, bending

[1] Solereder (5), p. 530.

towards the lower side of the protuberance to supply the leaf (*l. t.*, Text-fig. 4). As the trough continues up the protuberance it becomes more and more compact in structure, as the separate bundles gradually join up. Inside the trough run a few medullary bundles, whose xylem gradually decreases in amount as they continue upwards, until they enter the petiole. Their subsequent appearance and behaviour in the petiole will be described later.

2. A cylinder of vascular tissue bending towards the upper side of the protuberance to supply the inflorescence (*i.c.*, Text-fig. 4). In section this cylinder is seen as a ring bounded by an irregular line staining deeply with haematoxylin, representing crushed laticiferous cells, phloem, or both (Fig. 6). The parenchyma inside the ring is traversed by laticiferous vessels running in all directions, the majority appearing in transverse section.



TEXT-FIG. 5. Diagram of transverse section across young spine. E = epidermis; P = parenchyma; W = water-storing region; C = central tissue; L = lignified tissue.

The vascular tissue consists of a ring of several groups of exceedingly small tracheides just inside the deeply staining line (Fig. 7), surrounding and distributed among which are small thin-walled cells resembling phloem. The inflorescence scar has round its margin a few minute spines; in a longitudinal section of one of these, one tracheide was seen running up the middle, but this appears to be exceptional. From their position around the base of the inflorescence these spines are taken to be morphologically bracts.

The remaining medullary bundles run up between and beyond the ring bundles, and eventually separate into three portions which enter the three spines.

(*b*) *Structure of Spines.* A transverse section of a *young* spine (Text-fig. 5 and Fig. 8) shows a small central cylinder of thin-walled tissue (Fig. 9), in which are five small groups of xylem (A, Fig. 9) arranged roughly in a ring, and similar groups of smaller cells resembling phloem in appearance (B, Fig. 9). There is very little pith. The greater part of the spine is composed of parenchymatous ground tissue, consisting of rounded cells, irregular in shape, with intercellular spaces between them. Towards the periphery the parenchyma of the ground tissue becomes thickened and lignified (L, Fig. 8). Outside this layer the cells are again thin-walled, smaller, and more compactly arranged, passing off into several layers of square water-

storing cells surrounded by a definite thick-walled epidermis. In transverse sections of spines slightly older cork cambium is seen forming in one of the layers of water-storing cells (Fig. 10). It has been seen arising near the periphery in the second layer of the water-storing belt, and has also been seen arising as deep as the seventh layer of this belt. When the cork cambium has arisen the lateral walls of the cells immediately outside it disintegrate, causing the tissue external to it to fall off.

A transverse section of an old spine shows the small central group of cells now embedded in a mass of sclerenchyma. The parenchyma present in this central group of tissue in the young spine has been lignified, so that in transverse section the original xylem tracheides are no longer distinguishable. The phloem becomes crushed and eventually disappears, leaving small spaces in the sclerenchyma (Fig. 11). The belt of thick-walled tissue, which gives to the spine its tough character, is formed by the lignification of the cell-walls of the parenchymatous ground tissue of the young spine; the cells of which it is composed do not elongate. The surface of the spine is covered by a thick layer of tissue formed by the cork cambium, whose origin has been described above. This tissue resembles cork in origin and appearance, but stains with haematoxylin, and can therefore only be tissue destined to form cork, whose walls have not yet been suberized in the oldest spines examined. The lignification of the parenchyma progresses centripetally, finally obliterating the vascular tissue to the extent described above. Owing to this the mature spine loses the function of water-storing and all the phloem elements possessed by the young spine.

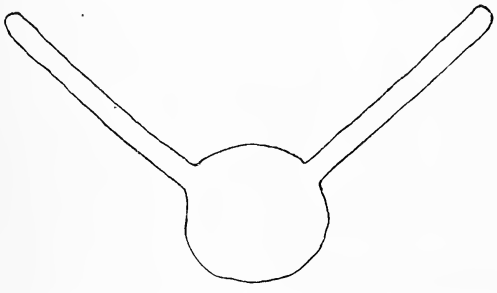
3. The Leaf.

The leaves which crown the stem, borne singly on each protuberance, are $1\frac{1}{2}$–2 in. long and about 1 in. broad, with an obovate-oblong lamina narrowing at the base into a very short hairy petiole. They are densely velvety on both sides, with a wavy margin and obtuse or retuse apex. The blade is bent up on either side of the midrib (involute), forming two grooves in which stomata are situated in small groups (Text-fig. 6). The stomata are also evenly distributed on both surfaces. They are very small, have no subsidiary cells, and are slightly above the level of the epidermis (Text-fig. 7 and Fig. 12); material has not been available for the study of their development. The epidermis is composed of oblong cells (E_1 and E_2, Fig. 13), which have on their outer walls a thin cuticle which becomes very apparent when stained with a saturated solution of chlorophyll. It bears numerous trichomes and emergences whose complex structure is noteworthy when compared with the simple uniseriate or unicellular hairs found in all other members of the order [1] (except in the genus *Oncinotis*, where antler-

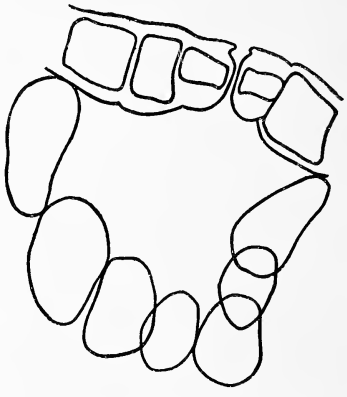
[1] Solereder (5), p. 529.

like trichomes are found [1]). The trichome of *Pachypodium* usually consists of a multicellular base from which radiate a number of straight hairs, which have walls cuticularized like those of the epidermis, and well-marked nuclei. Near the apex unicellular hairs are common. The trichomes tend to pass into emergences which consist of several cells, varying in number from 6 to 10, arranged in a manner similar to that found in the trichomes, but possessing a massive stalk, in the formation of which the subepidermal cells take part (Text-fig. 8). Examples of these structures can be seen forming a regular series ranging from unicellular hairs to stellate trichomes and emergences.

The structure of the leaf is very compact, the mesophyll showing practically no differentiation into palisade and spongy tissue (Fig. 13). It is made up of oblong cells with well-marked nuclei, compactly arranged



TEXT-FIG. 6. Outline of leaf in transverse section, showing the lamina bent up on either side of the midrib.



TEXT-FIG. 7. Longitudinal section of a stoma.

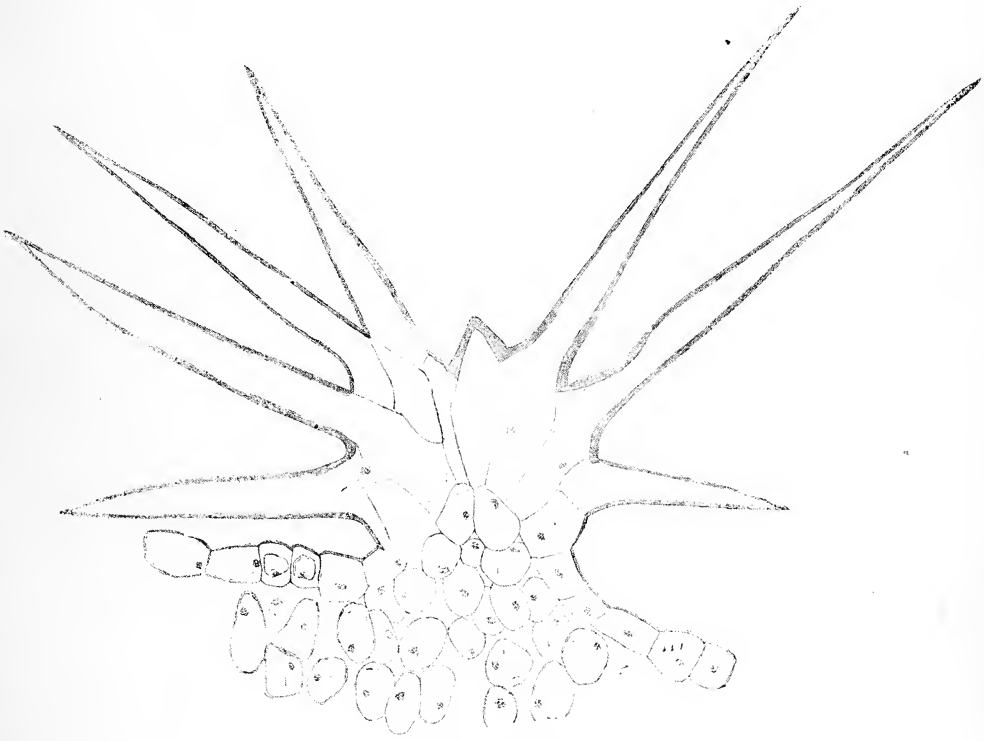
except underneath the stomata, where there are large stomatal chambers. The cells underneath the upper epidermis are usually somewhat longer and more compactly arranged, forming a tissue slightly resembling palisade, in which there are no sclerosed cells such as are sometimes present in members of the Apocynaceae.[2] Calcium oxalate is present in the inner cells in crystals similar to those in the stem cortex. The vascular bundles are collateral, have no cambium, and are smaller than those in the stem (Fig. 13). They are accompanied by one or more laticiferous cells (D, Fig. 13) which are similar to those found in the stem, but here are never found apart from the bundles.

The bundle of the petiole is arc-shaped in transverse section (Text-fig. 3). The xylem (A, Fig. 5) forms a band interrupted by parenchyma; the phloem (B, Fig. 5) forms a band of very small sieve-tubes with sieve-

[1] Solereder (5), p. 530, Fig. 121.

[2] l. c., p. 529.

plates on their terminal walls; between the two is a distinct layer of cambium (C, Fig. 5). On the adaxial side of the protoxylem, embedded in the parenchymatous ground tissue, are several small islands of tissue (B^1, Fig. 5, and Text-Fig. 3) resembling phloem in appearance, but in which the presence of sieve-tubes has not been ascertained. In addition to these phloem groups there are also, on the adaxial side of the protoxylem, a few scattered xylem-elements, some of which are in contact with some of the phloem groups. Some of the latter, however, are entirely without xylem-



TEXT-FIG. 8. Longitudinal section through emergence.

elements. As the petiole bundle characteristic of the order is bicollateral,[1] it was at first thought that the bundle in *Pachypodium* was derived from a bicollateral bundle by the degeneration of the adaxial band of phloem to these small isolated groups. This, however, gives us no explanation of the scattered xylem-elements on the adaxial side of the protoxylem. After carefully following the course of the leaf-trace through the protuberance it was found that these isolated bundles in the petiole were the continuations of the medullary bundles which accompany the leaf-trace, and in which, as has been stated before, the xylem had gradually decreased.

[1] Solereder (5), p. 529.

We have here then a connexion established between the medullary bundles of the stem and the internal phloem groups of the petiole; a connexion which very strongly upholds Mr. Worsdell's opinion that internal phloem groups represent medullary bundles which have lost their xylem.[1]

On the lower side of the arc-shaped bundle is an irregular layer of cells similar in appearance to the cells of the ground tissue, but containing starch in large grains (C, Text-fig. 3). This layer forms a definite sheath on the lower side of the vascular bundle. Much smaller starch grains may be present in the other cells of the ground tissue, but in no definite layer or group of cells. The ground tissue consists of loose spongy parenchyma. There are a few scattered laticiferous cells running up the petiole.

As the arc-shaped bundle of the petiole passes up the midrib it gives off successive branches from its free edges; these branches pass off into the lamina. In transverse section the structure of the midrib is seen to be the same as that of the petiole, except that the vascular bundle is smaller owing to its branching.

4. The Morphology of the Protuberances and Spines.

The morphology of the spines and protuberances is not clear. Little morphological importance can be attached to the arrangement of the vascular tissue of the spine, which might occur in either a stipule or a stem. Thus the stipules of *Acacia* have a structure similar to that seen in these spines. The course of the vascular bundles in the protuberance has been already described, but it is advisable to restate the facts briefly before discussing the morphology. Several vascular bundles leave the ring and run out through the protuberance in the form of a hollow cylinder, which later divides into two portions, the inflorescence supply and the leaf-trace (Text-fig. 4). Running up through the tissue enclosed by the hollow cylinder of ring bundles are a number of medullary bundles. A few of these join on to the cylinder of ring bundles, rarely branching off again, but the majority run straight up and separate into three portions, which supply the three spines. We see, therefore, that while the vascular supply of the inflorescence and leaf is derived from the bundles of the ring, together with some medullary bundles, that of the spines is derived entirely from the medullary bundles. It is known that medullary bundles generally originate from leaf-trace bundles which, after running down one or more internodes as part of the normal ring, turn into the pith.[2] The material available has not been sufficient to determine whether or not the medullary bundles of *Pachypodium* originate in this way.

[1] I wish here to express my thanks to Mr. Worsdell for his valuable suggestions and assistance in this question.

[2] Scott and Brebner (4).

Perhaps the most probable view of the morphology is that we have here two serial buds placed one above the other in the axil of an adnate leaf. The upper of these is an inflorescence whose vascular supply is derived from the vascular ring of the stem. The lower is the apex of the protuberance which receives its bundles from the medullary region only. This view leaves the morphology of the spines themselves an open question. They arise from the lower of the two buds, i. e. the apex of the protuberance, and not being merely emergences they are either stem-spines or modified leaves. That the middle one is a leaf and the two lateral stipules is improbable in view of the facts that stipules are uncommon in the order and when present are intrapetiolar. The three spines are therefore all stem structures, or all modified leaves, or the two lateral are leaves, the terminal and shorter being a stem. Against the first of these is the absence of any sign of reduced leaves subtending the stems; against the second is the apparently terminal position of the median spine; the third then seems the most probable, and is supported by the fact that the median spine has sometimes been seen branching into two.

If the above interpretation of the morphology of the spines and protuberances is correct, we have here two serial axillary buds differing entirely in the origin of their vascular supply. It will be seen that the knowledge of the origin of the medullary bundles, which has been impossible to determine, is necessary before the discussion can be carried any further. The facts that the mature spine loses its water-storing capacity, and that by the time it has matured the inflorescence has fallen off, suggest that the spines store water which is used up in the formation of the inflorescence belonging to the same protuberance. When the flower has fallen off the spines may perhaps be of biological importance in protecting the superficial tissues of the stem from the intense sunlight.

5. Anatomical Relationships.

The most striking anatomical feature which distinguishes *Pachypodium* from all other members of the order is the presence of an anastomosing network of medullary bundles and the absence of intraxylary phloem bundles. With regard to this one of two opinions may be held.

I. That intraxylary phloem bundles, wherever found, are due to the degeneration of medullary bundles. In all other members of the order this degeneration has taken place. This is the opinion of Mr. Worsdell, and is supported by the appearance in the petiole of medullary bundles degenerated into internal phloem groups.

II. That in *Pachypodium* the intraxylary phloem bundles are present and have acquired xylem as an adaptation due to the immense water-storing pith.

Stated briefly, the question resolves itself into whether the medullary bundles are a primary character, or whether they have been acquired later owing to the necessity of some means of conduction in the water-storing pith.

In this connexion it is interesting to note that Scott and Brebner, in a paper on internal phloem, say: 'It is probable also, that the pith-cells themselves may be able to discharge both storing and conducting functions more efficiently when brought into direct relation with the phloem and its proteid contents.'[1] In *Pachypodium* the efficiency of the pith as a water-storing tissue is much increased by the presence of medullary bundles. Correlated with the presence of storage parenchyma and of medullary bundles is the small quantity of wood, in which *Pachypodium* differs from most Apocynaceous plants. Another adaptation to the habitat is seen in the possible water-storing and protective functions of the spines. In other characters, e.g. the occurrence of calcium oxalate and laticiferous elements, and the superficial origin of the cork, *Pachypodium* agrees with the other members of the order.

Regarding the origin of the medullary bundles all that can be said is that they have been seen to branch off from the ring bundles, and have no definite orientation. A study of the seedling structure would be necessary to follow up their development. In other genera of Apocynaceae[2] which possess medullary bundles there is present a cambium outside the inner phloem which gives rise to xylem, so that inversely orientated medullary bundles are formed. No sign of such a cambium has been seen in *Pachypodium*, where the medullary bundles are simply branches of the ring bundles.

In conclusion, I wish to express my thanks to Dr. Pearson for advice and many helpful suggestions in this investigation; and also to Miss E. L. Stephens for her kind assistance in preparing it for the press.

6. Summary.

1. The stem is fleshy and consists to a great extent of the pith.

2. Branching and anastomosing medullary bundles varying in size and orientation traverse the pith in all directions.

3. The bundles of the ring are collateral, separated by wide medullary rays, and have very little secondary tissue.

4. Apparently no intraxylary phloem bundles are present. It is suggested that they are present, but possess xylem, and so form the network of medullary bundles.

5. Laticiferous cells are present in the cortex and pith of the stem, the

[1] Scott and Brebner (4), p. 261.

[2] Solereder (5), p. 531.

petiole, midrib, and lamina of the leaf, and the cortex of the spines. They have thin walls and contain nuclei and granular matter of a proteid nature.

6. It is probable that there are two serial axillary buds placed one above the other in the axil of an adnate leaf. The upper of these is an inflorescence, and the lower is the apex of the protuberance.

7. Of the three spines the median is probably a stem spine, the two lateral being modified leaves.

8. The functions of the spines may be to store water for the formation of the flower, and then to reflect the intense sunlight away from the stem. Near the base of the stem the spines fall off, their function here being probably performed by a thick covering of cork.

Botany Laboratory,
South African College,
March, 1912.

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EXPLANATION OF FIGURES IN PLATE LXXXIV.

Illustrating Miss Lee's paper on *Pachypodium namaquanum*.

A = xylem ; B = phloem ; C = cambium ; D = laticiferous cells.

Fig. 1. A, drawing of external tissue of stem, showing protuberances and spines; B, protuberance from lower side, showing its leaf-scar (= *l.s.*); C, protuberance from upper side, showing the scar left by the inflorescence (= *i.s.*).

Fig. 2. Part of transverse section of pith, showing two medullary bundles. × 220.

Fig. 3. Starch grains as seen in the cells of the pith. × 220.

Fig. 4. Part of transverse section of stem, showing two ring bundles. F = fibres. × 95.

Fig. 5. Part of vascular bundle of petiole in transverse section. × 220.

Fig. 6. Transverse section through protuberance parallel to scar left by inflorescence, showing vascular supply of the latter.

Fig. 7. One of the small groups of vascular tissue as seen in section parallel to the inflorescence scar. × 110.

Fig. 8. Diagram of part of young spine in transverse section. E = epidermis; W = water-storing layer; L = lignified tissue; P = parenchyma; C = central tissue.

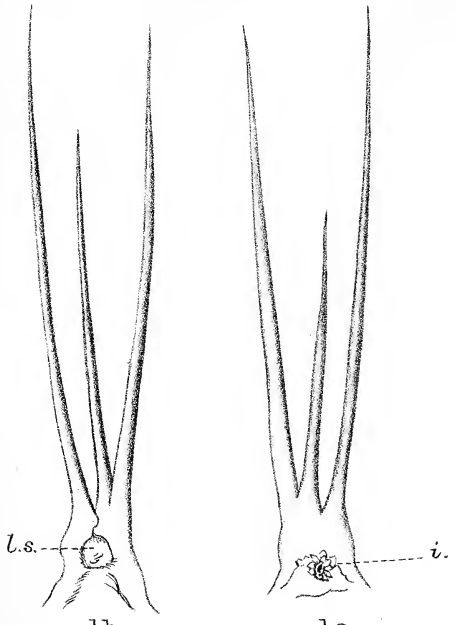
Fig. 9. Central tissue of young spine in transverse section. × 220.

Fig. 10. Transverse section of portion of water-storing tissue of young spine, showing origin of phellogen (*Ph.*). W = water-storing tissue; E = epidermis. × 220.

Fig. 11. Part of central tissue of mature spine, showing space (*sp.*) left by disappearance of phloem.

Fig. 12. Surface view of stoma.

Fig. 13. Lamina of leaf in transverse section. E_1 = upper epidermis; E_2 = lower epidermis.



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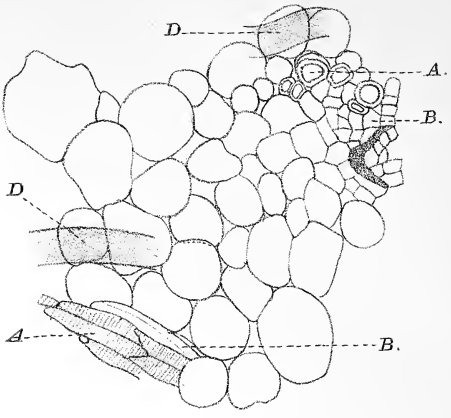

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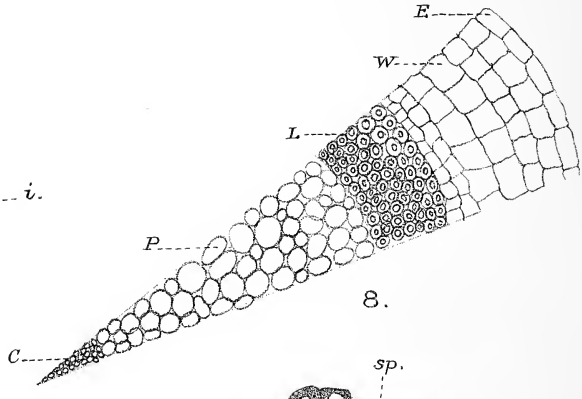

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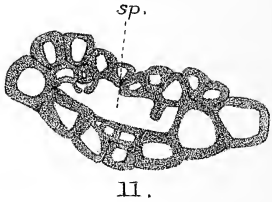

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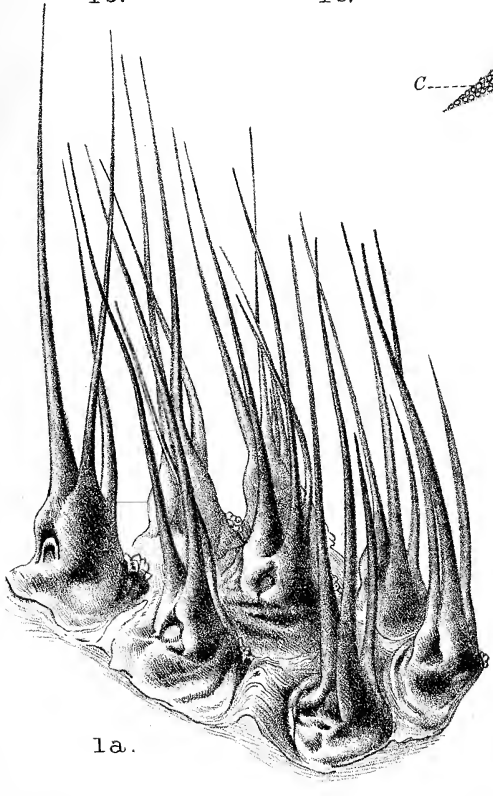

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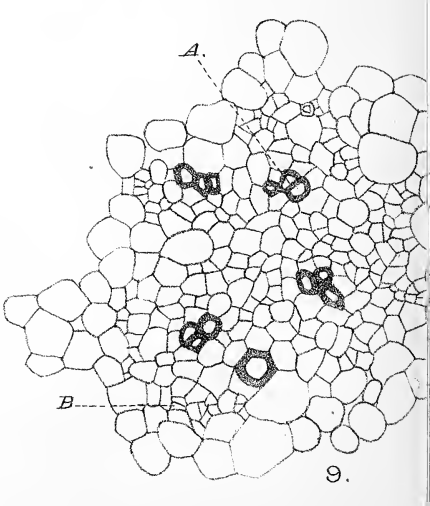
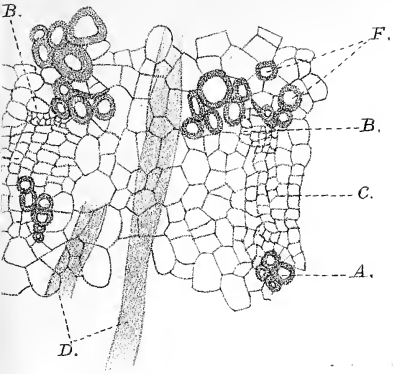


9.

D.Lee, del.

LEE — PACHYPODIUM.



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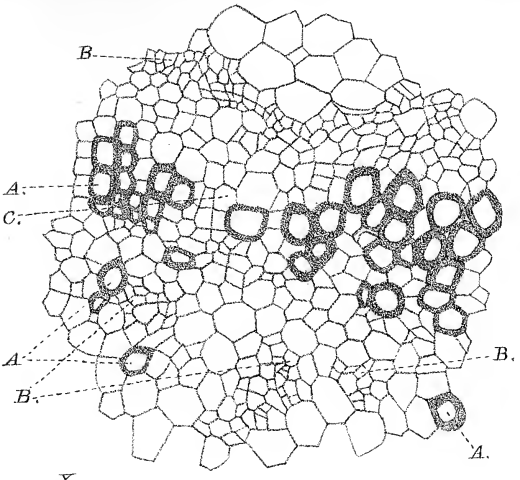

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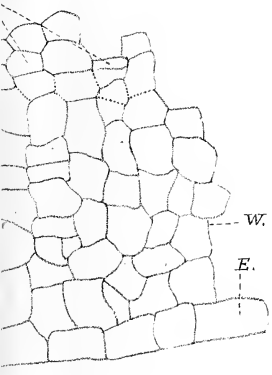

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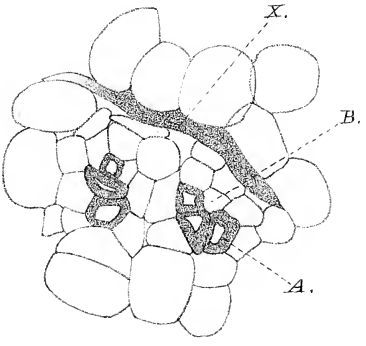

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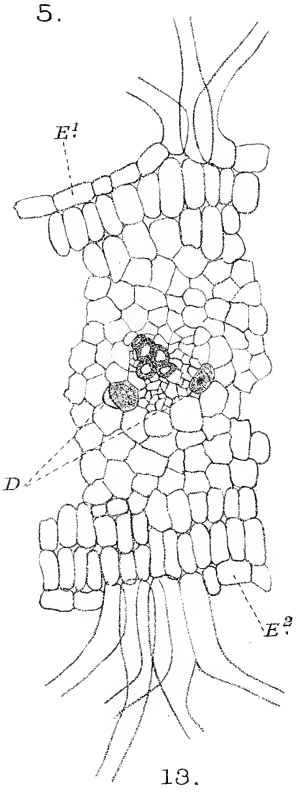

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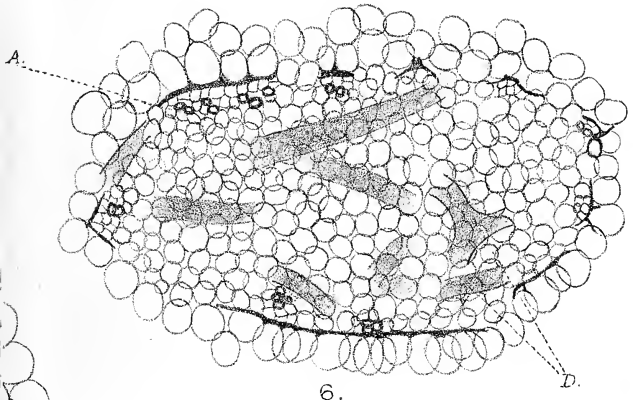

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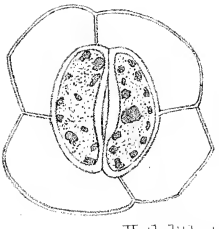

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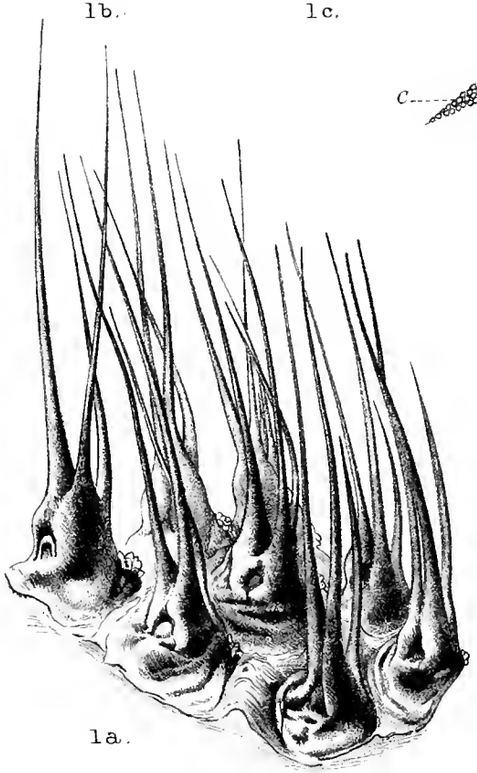
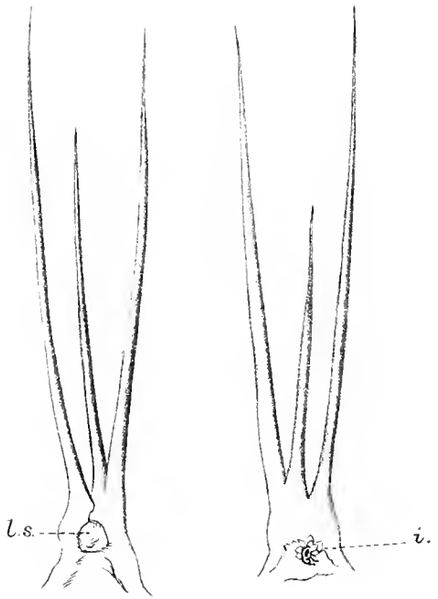
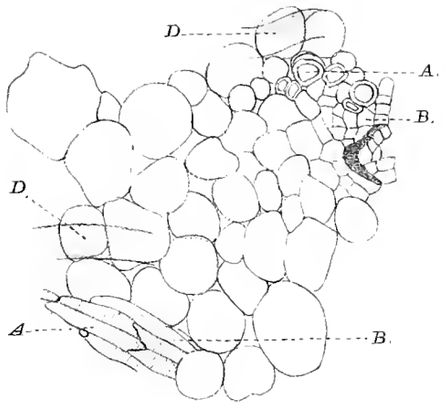
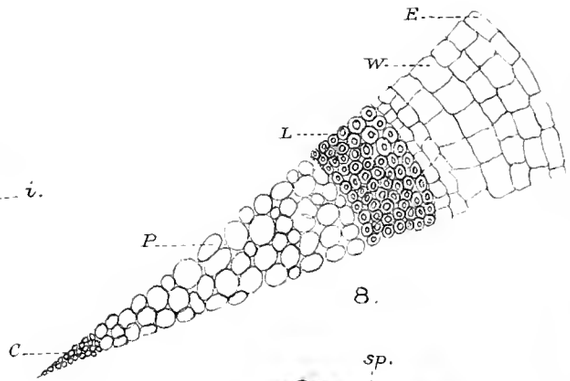
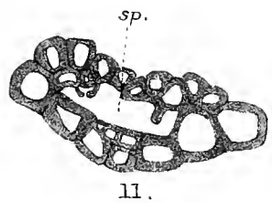
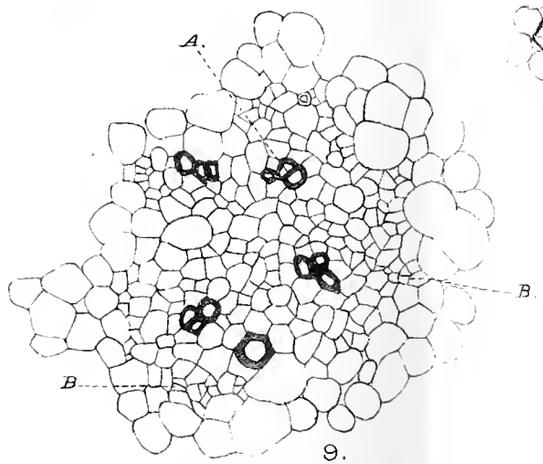


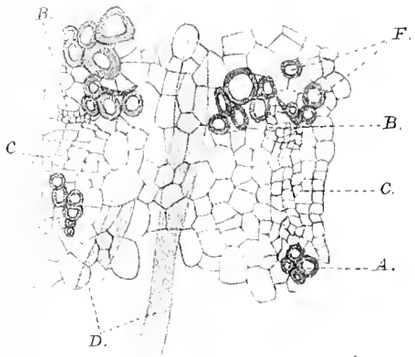


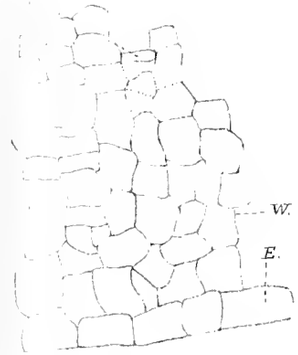


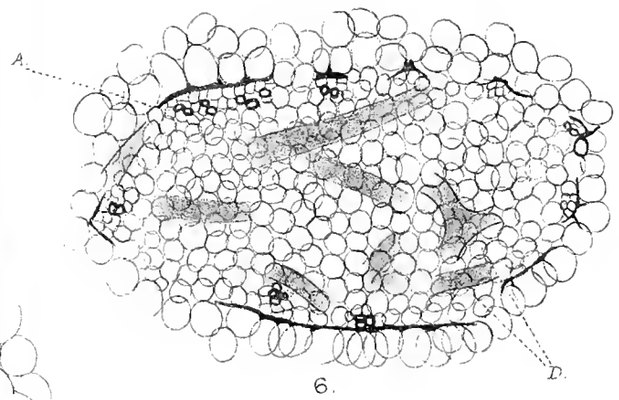


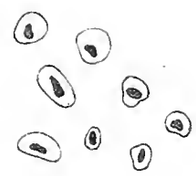


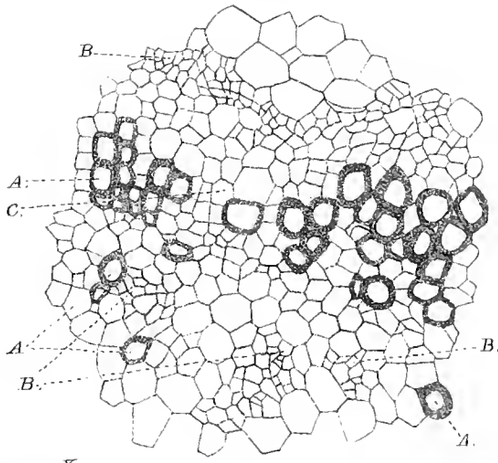


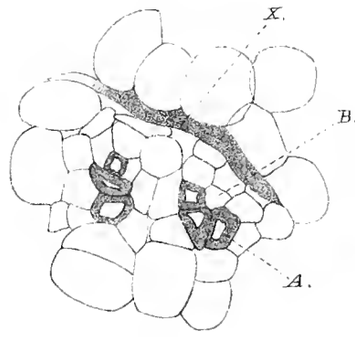


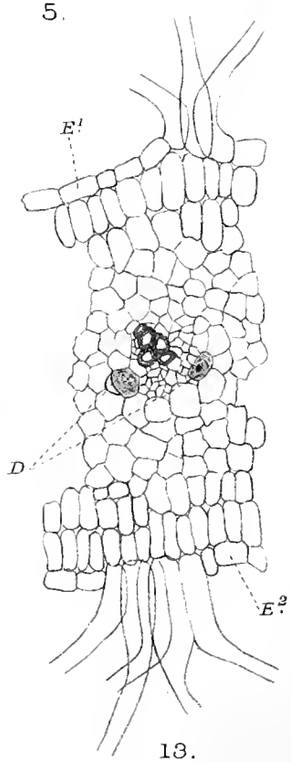


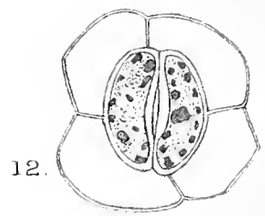












D. Lee, del.

Huth, lith. et imp.

LEE — PACHYPODIUM.

NOTES.

NOTE ON AN ABNORMAL PROTHALLUS OF PINUS MARITIMA, L. —A large number of Pine prothalli were collected during October and November, 1911, with the object of making a comparative study of certain fixing agents, and of various methods of treating fixed material prior to embedding. Only a small part of this material has at present been sectioned, but in clearing it was noticed that one prothallus was quite different in structure to all the rest. This was embedded separately.

Externally, this prothallus was of about the normal size, but instead of showing the usual terminal group of two or three archegonia, there were two lateral groups of two archegonia each. The two groups were only slightly separated, and were almost, but not quite, in the same longitudinal plane.

In sectioning, great care was taken in orienting the material so as to get a median longitudinal section of the prothallus which should pass as nearly as possible through the median plane of each archegonium. For it to do so exactly was impossible, owing to the arrangement of the archegonia as described above, but the accompanying drawing was made almost entirely from one section, the details of the lower archegonia, however, being filled in from adjacent sections.

The prothallus was fixed on Nov. 5, 1911, in the following solution:

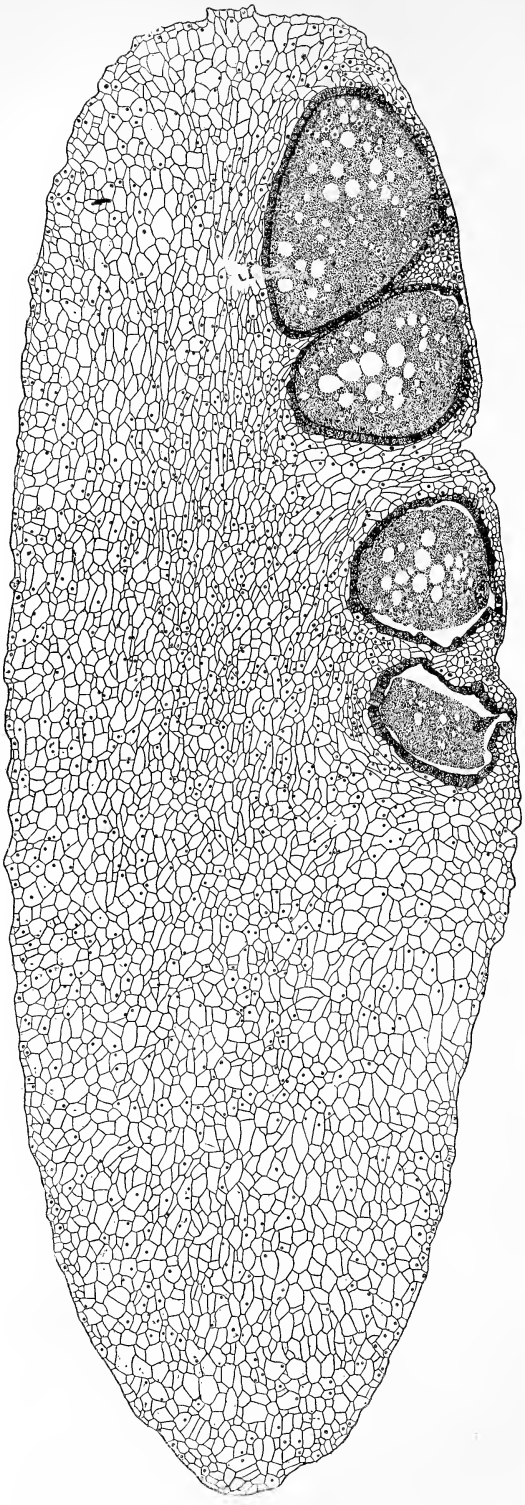
Picric acid, saturated solution in 50 per cent. alcohol, 100 c.c.
Glacial acetic acid . . . . . 5 c.c.
Mercuric chloride . . . . . 5 grammes.

washed in 50 per cent. alcohol, and embedded through cedar-wood oil.

The structure of the prothallus, as seen in section, is sufficiently explained by the drawing.

As far as the writer is aware, no case is recorded of a prothallus of any of the Abietineae which bears only lateral archegonia. The nearest is that figured and described by Miss Ferguson in *Pinus montana uncinata*, which formed archegonia *not only at the top*, but also along the sides of the prothallus, so arranged as to suggest a cock's comb.

The case here described, however, differs fundamentally from Miss Ferguson's in the *entire absence of apical archegonia*. Its chief interest lies in the resemblance to certain other groups—Araucarineae, Sequoiineae, and Callitrineae—in which lateral archegonia are the rule, and terminal archegonia entirely absent, except in Araucarineae, where they may apparently occur as well, especially in *Araucaria*. The resemblance is not a very close one, since in these tribes the archegonia may be, and often are, deep-seated; but this condition is paralleled in other recorded cases in the Abietineae. Here, however, there is no tendency for the archegonia to become embedded, each of



Section of Abnormal Prothallus of *Pinus maritima*, L.

the four being, in fact, less deeply sunk below the surface than in an average terminal archegonium of the same age, the neck being only a few microns from the megaspore membrane. The megaspore membrane seems to be fairly uniform over the whole of the prothallus, instead of being thinner over the apex as is normally the case, and is seen to be stretched over the depressions leading to the necks, instead of following the cell outlines.

The question naturally arises whether this lateral position of archegonia in *Pinus*, perhaps the most stereotyped of all Conifers in the details of its development, is to be regarded as a reversion to an ancient type, a new departure of the nature of a 'freak' or 'mutation', or a pathological condition induced by injury. As regards the last suggestion, it may be dismissed at once. Collections were only made from properly grown and uninjured ovules in healthy-looking cones, and neither before nor after sectioning was any trace seen of injury at or near the apex of the prothallus. The slight crushing seen in and near the two lower archegonia was almost certainly caused in dissecting out the prothallus for fixing. Between the two remaining alternatives it is not easy to decide. Most of the workers on the genera of Conifers with normally lateral archegonia (including the present writer) have regarded this feature as an ancient or 'reversionary' character, but the evidence is perhaps not very conclusive. In the only Cycad with lateral archegonia, the genus in which they occur appears in some other respects the most specialized member of the group.

Of all groups of plants the Conifers are probably the most puzzling in the apparent association of both 'ancient' and relatively 'modern' characters in the same plant, and it must not be hastily assumed, because lateral archegonia are associated in Araucarians with some undoubtedly ancient characters, anatomical and otherwise, that they therefore constitute a primitive feature. The discovery of wholly lateral archegonia in another tribe of Pinaceae may probably be best regarded in the present instance as rather of the nature of a 'mutation' than a 'reversion'; if so, it lends support to the view that the condition is a modern specialized one. This view certainly does not simplify the already rather complicated problem presented by the Araucarians, but if applied to the Callitrineae, it brings this character into better agreement with the reduced cones and male gametophyte, and other 'modern' characters which need not be further specified. In *Sequoia* the other evidence is also conflicting and therefore does not largely affect the problem one way or the other.

The view suggested is put forward tentatively, but in any case it seems clear that the abnormal prothallus, here described, must be taken into consideration as one link in a chain of evidence, not yet completed, which will eventually settle the question of the 'primitive' or 'specialized' nature of lateral archegonia in Conifers. Further investigation of the Araucarineae on the one hand, and of the Callitrineae on the other, is likely to shed considerable light on the problem.

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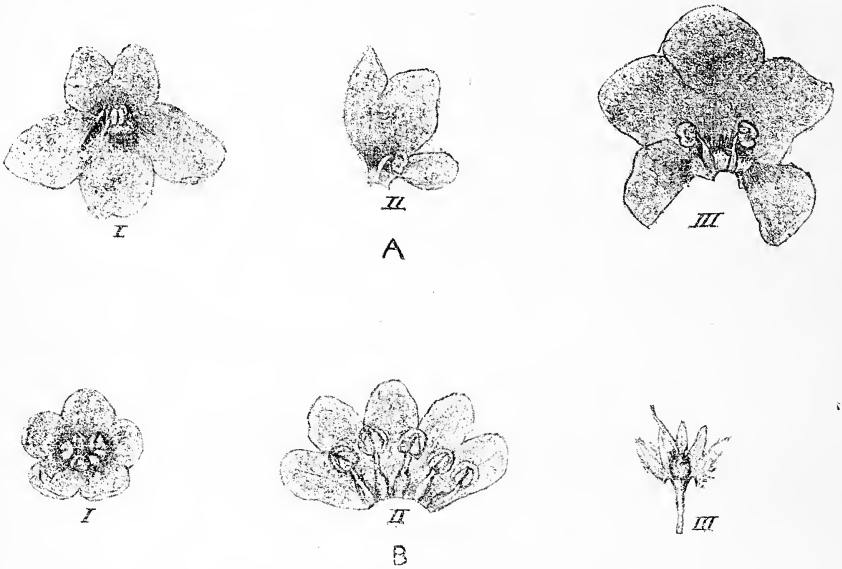
PELORIA IN SAINTPAULIA IONANTHA, WENDLAND.—The detection of the peloric form of *Linaria vulgaris* in 1742, near Upsal, was considered by Linnaeus so remarkable a circumstance, that he not inaptly applied the term *peloria* (signifying prodigy) to describe this phenomenon.

Since his time, however, such cases have not been infrequent, and their occurrence in widely separated groups of plants, especially under cultivation, point to a universality only really thoroughly appreciated by teratologists.

Masters, in his work on Vegetable Teratology, p. 219 (1869), distinguishes two forms of peloria, viz. regular and irregular, the former implying a non-development



FIGS. A and B. *Saintpaulia ionantha*, Wend. A. I. Normal flower, showing projecting style. II. Half profile. III. Inverted and laid open to show insertion of the stamens. B. I. Peloric form. II. Same, laid open. III. Calyx and gynaeceum.

of the irregular portions, and the latter suggesting a formation of irregular parts in increased numbers so as to render the symmetry of the flower perfect. Both forms are sometimes observed on the same plant, as is exemplified in *Linaria vulgaris*, and the same remarks apply to the peloria under consideration. Since its introduction from the Usambara Mountains, Tropical Africa, *Saintpaulia ionantha*, a Gesnerad, which, owing to a superficial resemblance to the Violet, has earned for itself the popular name of African Violet, has responded to cultural treatment by producing a galaxy of colours varying from reds to blues and white, the tints not always being confined to individual plants, but often exhibiting solitary blue and red flowers on the same inflorescence. Indeed, Pynaert[1] describes and figures a plant bearing red, blue, and half red and half blue flowers, a circumstance which is paralleled by certain Azaleas, but which latter anomaly may doubtless be explained by the influence of stock upon scion. The existence of peloric flowers appears, however, not to have been observed, though the allied genera *Gesnera*, *Gloxinia*, *Streptocarpus*, and *Columnea* are recorded as exhibiting this peculiarity. The two forms alluded to

[1] Rev. Hort. Belge, xxii, 1894, p. 109.

occurred in the preceding year on the same inflorescence of a plant flowering in the Begonia House at Kew, but the disposition of the individual flowers, whether terminal or lateral, was at that time not noted, nor has the writer observed a recurrence of the phenonenon. A reference to the accompanying sketches will illustrate the chief points of distinction; it will be noticed that the addition of three stamens in the peloric form has resulted in a consequent diminution of the coralline segments, the corolla having become rotate, and its segments more or less rotundate and slightly incurved, the stamens alternating with these, and except for an increase in number, and a slight dilation at the base of the staminal filaments, identical with those of the normal form. The calyx and gynaeceum are apparently the same (the style, however, not exserted), the ovoid ellipsoid pollen-grains showing no points of distinction microscopically.

Intermediate 3–4 stamened forms connecting these two extreme types were also noticed, but in the majority of cases they were extremely depauperate and could only be designated as malformations.

There appears little doubt that these peloric types represent a retrogression of zygomorphism to a primordially actinomorphic form, or what Morren was pleased to term 'epanody'. As to the cause, much conflicting evidence exists, but a fertile explanation suggested by various authors lies in the excess of food many plants are liable to receive under cultivation. The plants at Kew are generously treated from a cultural standpoint, and the prevalence of peloriae, especially in gardens, suggests that this explanation is not without truth.

Chorisis (?) in Aristea dichotoma, *Ker-Gawl.*

A point of teratological interest worthy of record was noticed by the writer during the preceding year at Kew. A profuse blue-flowering specimen of *Aristea dichotoma*, a dwarf Iridaceous type, exemplified a solitary instance of a four-staminate flower instead of the characteristic three-stamened type (see Fig. C). In its other characters the particular individual conformed to the species, being, with the exception of the androecium, trimerous in its remaining whorls. A close inspection of the flower infers that the supernumerary stamen may be explained by *chorisis* or the division of a normal stamen, as is evinced by the union of the two staminal filaments towards their base. The anthers are in all cases normally dithecous, with the exception of one which exhibits an atrophied anther-cell, the pollen-grains being globular, smooth, and surrounded by a comparatively thick translucent cell-wall. In this particular Order these cases are remarkably rare, but Messrs. C. H. Wright and W. R. Dykes inform me that they have observed it in several Irises.

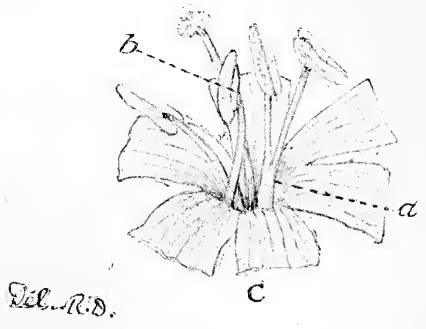
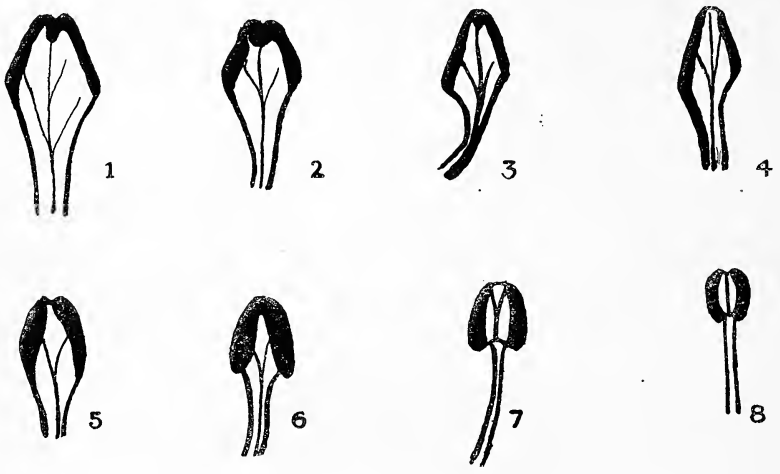


Fig. C. *Aristea dichotoma*, Ker-Gawl. Four-staminate flower: (*a*) point of union of two staminal filaments, suggesting *chorisis*; (*b*) atrophied anther-cell.

R. A. DÜMMER.

Kew.

ABNORMAL FLOWERS OF AMELANCHIER SPICATA.—Double flowers resulting from petalody of the stamens occur so often in the Rosaceae, that it is interesting to meet with a reversal of this state of things where the flowers have staminoid petals. Such an instance is afforded by the flowers of two small plants of *Amelanchier spicata*, Koeh., growing in the Royal Botanic Gardens, Kew. *Amelanchier spicata* is common in the Northern States of America, and is regarded by Sargent as a variety of *Amelanchier canadensis*. Like those of the latter, its petals are white, strap-shaped,



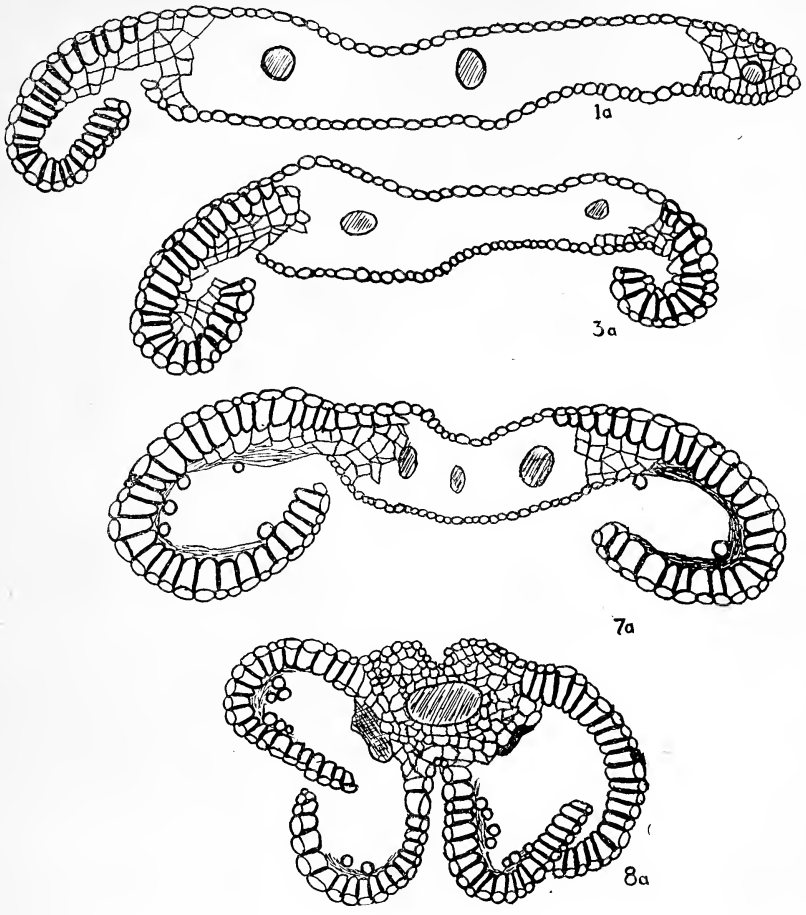
FIGS. 1–8.

or slightly obovate, varying in length from 8 to 12 mm. In the flowers of the plants growing at Kew the petals show a more or less complete infolding of their lateral margins. The more complete this infolding, the less the development of the petal-tip and the more nearly do the resulting structures resemble stamens. (Figs. 1–7 show a series of the abnormal petals.)

The most petal-like of the structures are of a white colour slightly tinged with pink; they are considerably smaller than normal petals. The most staminoid have white filaments bearing at their tips what appear to be anthers of a light brown colour, whilst the normal stamen has white filaments with cream-coloured versatile anthers (Fig. 8).

That the resemblance is not merely superficial is shown by transverse sections of these structures, as well as by the fact that the more completely developed of them undergo dehiscence disclosing yellow pollen-grains. Some sections were kindly prepared by Mr. Boodle, Keeper of the Jodrell Laboratory. Figs. 1 *a*, 3 *a*, 7 *a*, and 8 *a* show sections corresponding respectively to Figs. 1, 3, 7, and 8. In the first of these there is a development of the fibrous layer at one end only; in Figs. 3 *a* and 7 *a* this development

occurs at both ends. In Fig. 7 *a* pollen-sacs have completely developed and dehiscence has taken place, leaving disorganized cells and a few pollen-grains within the fibrous



FIGS. 1 *a*, 3 *a*, 7 *a*, and 8 *a*.

layer. It may be added that the flowers showed a further deviation from the normal in having the top of the ovary and the styles glabrous.

J. J. CLARK.

ROYAL BOTANIC GARDENS,
KEW.

ON WELL-MARKED AEROTROPIC GROWTHS OF BACILLUS MEGATHERIUM.—For nearly three years I have had under my observation aerotropic growths of *Bacillus megatherium* which present a remarkable annual periodicity. These growths were first noticed on the removal of some boards forming the front of a large box in which the palm *Demonorops* was growing. On the projecting dead extremities of the roots and the surrounding earth thus exposed, white fungal masses

were found. The growths on the earth were better marked than those on the roots, and varied from small flat, whitish films to patches 8 mm. square and up to 4 mm. in height. The latter were amorphous masses of Bacteria which cultural and microscopical methods showed to be *B. megatherium* in a practically pure condition. The vitality of the growths was well marked during the late winter months, during which I easily obtained typical and rapidly growing cultures on agar-agar and gelatine. The greatest efflorescence was attained towards the end of spring. Later on the growths looked drier and more scanty, their creamy colour becoming dead white, and they did not flourish so readily on culture media. At this stage more spores than bacilli were observed in both growths than in the period of greatest development. The chalky, desiccated appearance, poor reaction to cultural methods, and preponderance of spores were intensified during the summer.

As a site for Bacteria the roots of the palm would not be unusual, but well-marked and diffuse bacterial growths on the surface of the earth are unique and less easy to account for. Although Bacteria are found plentifully in the upper six feet or so of undisturbed soil (not deeper, unless corpses or other sources of pollution exist below),[1] a well-defined positively aerotropic growth, even of aerobes, is not seen. The only exceptions seem to be the Myxobacteriaceae described by Thaxter,[2] which are found on various animal excrementa, and exhibit Myxomycetes-like fructifications. Their nutritive conditions are, therefore, eminently favourable. Whether this may help to account for their specialized fructification is problematical, but there is no doubt that the question of nutrition exerts an important effect on the exuberance to which a bacterial development will extend, and I was inclined to attribute the peculiar efflorescence of *Bacillus megatherium* on the earth to some unusual source of nourishment, which with the favourable temperature of the plant house (average 80° F.) would conduce to free development. The interest of the conclusion that the earth-growth existed saprophytically on the persisting remains of products of the decayed wood in the earth was minimized by later observations. On the open, horizontal surfaces of the boxes and tubs in which other tropical plants were placed, I afterwards noticed several growths of the bacillus. Here the areas were mostly circular in outline. The smaller ones were flat, the larger somewhat raised, particularly towards the centre, and measured up to an inch in diameter. In these situations the existence of a marked supply of food material is not so obvious. I hope to note any gradual change from a flat film to raised growths like those on the earth around *Demonorops* which appear to connect the ordinary free living Bacteria with the more highly specialized fructifications of the Myxobacteriaceae.

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[1] Young: Proc. Roy. Soc. of Edin., xxxvii, Part IV, p. 759.

[2] Thaxter: Bot. Gaz., xiv, 1892, p. 389; xxiii, 1897, p. 395; xxxvii, 1904, p. 405.

THE EXTENT OF THE ROOT-SYSTEM OF CUCUMIS SATIVUS.—In Sachs's 'Lectures on the Physiology of Plants' (Eng. ed., p. 13) the following sentence occurs: 'S. Clark has taken the trouble to measure the length of all the roots of a large gourd plant and found that it amounted to 25 kilom.' This statement is repeatedly quoted in other treatises on Physiology, e.g. 'Pfeffer's Physiology,' Eng. ed., vol. i, p. 153, Jost's 'Lectures on Plant Physiology', Eng. ed., p. 28, &c. The reference is not given in any of the more recent works nor by Sachs himself. It may be of interest to quote the original statement, which occurs in a paper by W. S. Clark, President of the State Agricultural College, Amherst, Mass., and published in the 22nd Annual Report to the Massachusetts State Board of Agriculture. The paper in question deals with a wide range of subjects, and is more in the nature of a popular discourse than a scientific research. Some of the statements made by the writer are sufficiently startling. For instance, he states that roots of Clover one year old penetrated the soil perpendicularly to a depth of 8 feet, Lucerne roots to a depth of over 20 feet, and that, on the authority of an unnamed Indian officer, those of *Prosopis spicigera* reached a depth of over 69 feet! The last statement, at least, must be accepted with reservations, more especially when resting on an anonymous statement by one presumably without special botanical knowledge. Pfeffer (Phys., vol. i, p. 258) quotes Clark as his authority for the record that the Birch gives off 6·8 kilos. of sap per day in cases of active bleeding. Clark's statement is, however, that a Paper Birch 15 inches in diameter gave off, in less than two months, over 1,486 lb. of sap, a maximum of 63 lb. 4 oz. being reached on May 5th. This would give on an average 24·4 lb., or approximately 11·1 kilos. per day over 60 days (nearly twice the amount quoted by Pfeffer), while the maximum would represent about 28·5 kilos.

In 'Nature' of June 3, 1875, Clark's paper is reviewed at some length, and the reviewer, although he says he has 'no reason to doubt the accuracy of the statements contained in Mr. Clark's paper', expresses a desire 'to see the observations repeated'—a scepticism justifiable in view of the remarkable observations recorded by Clark. One of these deals with the length of the entire root-system of a 'squash-vine' (*Cucurbita maxima*), and since Clark's measurements have been quoted again and again without comment, and as the original paper is not very accessible, it may be worth while to give the quotation in full:

'But our squash-vine affords the most astonishing demonstration of all that has been said about root-development. Growing under the most favourable circumstances, the roots attained a number and an aggregate length almost incredible. The primary root from the seed, after penetrating the earth about four inches, terminated abruptly and threw out adventitious branches in all directions. In order to obtain an accurate knowledge of their development, the entire bed occupied by them was saturated with water, and, after fifteen hours, numerous holes were bored through the plank-bottom, and the earth thus washed away. After many hours of most patient labour, the entire system of roots was cleaned and spread out upon the floor of a large room, where they were carefully measured. The main branches extended from twelve to fifteen feet, and their total length, including branches, was more than two thousand feet. At every node, or joint, of the vine was also produced a root. One of these nodal roots was washed out and found to be four feet long, and to have four hundred and eighty branches, averaging, with their branchlets, a length of thirty inches, making

a total of more than twelve hundred feet. As there were seventy nodal roots, there must have been more than fifteen miles in length on the entire vine. There were certainly more than eighty thousand feet; and of these, fifty thousand feet must have been produced at the rate of one thousand feet or more per day.'

I cannot find any record that Clark's observations have been repeated or confirmed. Further, Clark does not state how the measurements were made, nor is there any evidence in his paper that he took these measurements himself, or checked them if carried out by others. Thinking that it might be worth while to estimate as carefully as possible the length of the root-system of another member of the same family, I asked the British Botanical Association, Holgate, York, to cultivate for me, under the *most favourable conditions*, a plant of *Cucumis sativus*, and requested the Scientific Director, Dr. Burt, to take special precautions as to the separation of the plant from the soil and preservation of all roots of whatever calibre. This, Dr. Burt assures me, was done with extreme care, under his own supervision.

The plant was grown in a frame 10 feet by 6 feet, and was a full-sized plant bearing fourteen fruits, none of which were removed until quite mature, and until the plant was ready for lifting. The soil was washed away very carefully, beginning at one end of the frame and working through to the other. No roots were lost, even a few fibres, disconnected unavoidably in the process of removal, being preserved for subsequent measurement. The shoot region, including all the smaller branches, measured approximately 32 feet with about 140 leaves, counting in those that had been functional but had withered away at some date previous to lifting the plant. All the roots were then cut off close to the stem, both the primary one as well as the adventitious roots springing from the nodes. After the statements made by Nobbe, that the root-system of cereals may reach a length of 500 to 600 metres, and by Clark, as quoted above, I was much surprised to note the comparatively limited root extent of *Cucumis sativus*. After trying various methods I came to the conclusion that the only satisfactory way of obtaining reliable data was to stretch the root taut, and by aid of compass and centimetre scale to measure the length of the roots individually. The total length obtained was only 85·75 m., or, taking a metre as = 3·28 feet, in English measurements and in round numbers, 281¼ feet. The actual scale measurements were checked by a calculation of (*a*) volume, (*b*) dry weight.

In the former case, the entire root-system was vacuum dried and pressed down in a graduated measure; in the latter the vacuum-dried material was carefully weighed, the volume and weight of a definite length of roots of various thicknesses having been previously estimated. On the basis of the data so obtained, the volumetric estimate gave a length of 285⅓ feet, and the estimate by weight 279½ feet. It may be taken, therefore, that the total length of the root-system of this particular plant of *Cucumis* was about 280 feet.

Wheat cultures were also made, but, owing to the excessive dryness of the summer of 1911, the cultures were to a large extent spoilt, and it was thought better to discard them, and trust to obtaining more normal material in the present year. These measurements, for obvious reasons, have not as yet been made.

R. J. HARVEY GIBSON.

HARTLEY BOTANICAL LABORATORIES,
THE UNIVERSITY, LIVERPOOL.
June 6, 1912.

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.
LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

The general plan of the work may be gathered from Part I, which was published in 1908 as a royal 4to volume of 211 pages. In it are described the following twelve types of floral structure, selected from familiar garden flowers that bloom in the early part of the year (January–April):—

Helleborus niger . .	Christmas Rose.	*Viola odorata*	Sweet Violet.
Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

The description of each type is illustrated by a full-page coloured plate, giving an accurate longitudinal section of the flower, and by a black-and-white plate giving the inflorescence, the floral diagram, and other structural details. As each subsidiary species has also a coloured plate allotted to it, the volume contains no less than forty coloured and fourteen uncoloured plates, in addition to a large number of figures, chiefly developmental, included in the text. It can be obtained at the original price of £1 1*s.* net by subscribers to Part II.

It was hoped that the reception of so striking a volume as Part I would have been such as to justify the Delegates of the Press in proceeding forthwith to publish Part II, the material for which is in readiness. Inasmuch as this anticipation has unfortunately not been realized so far, the Delegates are not disposed to undertake the publication of Part II without some assurance that the necessarily large expenditure involved will meet with the general support of those who, in one way or another, are interested in flowers. But the University Press has received such warm commendations of the work from Botanists who desire to push on the study of Botany in the English-speaking countries that they desire, if possible, to continue publication. They propose, therefore, to ask for subscriptions for copies of Part II at One Guinea each, on the understanding that Part II will, like Part I, consist of descriptions of twelve types of flowers, with allied forms, and be similarly illustrated, though it may be found necessary to reduce somewhat the number of coloured plates. Any copies not subscribed for will not be sold at less than thirty shillings each.

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The Anatomy and Morphology of the Inflorescences and Flowers of Ephedra.

BY

MARY G. THODAY (SYKES),

Girton College, Fellow of Newnham College, Cambridge, and Honorary Research Fellow in the University of Manchester,

AND

EMILY M. BERRIDGE, B.Sc., F.L.S.

With Plate LXXXV and twenty-one Figures in the Text.

WE undertook this investigation because it seemed to us that the genus *Ephedra* required re-examination for the sake of comparison with recent work on the other genera of the Gnetales.

The material on which our work is based is largely supplied from collections, embedded material, and slides previously made by one of us for other work on the embryology of the genus,[1] and includes four species, *Ephedra altissima*, *E. distachya*, *E. fragilis*, and *E. nebrodensis*. Additional inflorescences of *E. altissima* in various stages were obtained from the Manchester University Botanical Laboratory, and herbarium material of various other species, *E. alata*, *E. Torreyana*, *E. trifurca*, and others, was supplied from the Manchester University Herbarium.

I. General Morphology of the Inflorescences.[2]

The male inflorescence in *Ephedra* consists of an axis arising in the axil of one of the ordinary leaves, and often dichasially branched, bearing generally one terminal and two lateral strobili (Fig. 1, Pl. LXXXV). The bracts at the point of branching have acute apices, like those in a similar position in *Welwitschia*.

In *E. distachya*, *fragilis*, *nebrodensis*, and *antisyphilitica*,[3] &c., the female inflorescence axis also springs directly from the axil of a leaf on the

[1] Berridge and Sanday: Oogenesis and Embryogeny in *Ephedra distachya*. New Phyt., vi. 1907.

[2] Strasburger, 1872, pp. 76 ff. and 132 ff.; Land, 1904.

[3] Coulter and Chamberlain, 1901, p. 372. In this and several other species the peduncle is so short that the strobilus appears to be sessile in the axil of the bract.

[Annals of Botany, Vol. XXVI. No. CIV. October, 1912.]

vegetative stem; it generally bears one strobilus only; in *E. distachya*, however, it is sometimes branched. In some cases, which are specially common in *E. fragilis*, two or three such peduncles appear in one axil; here the lateral ones are in reality branches of the median peduncle, the first internode of the latter having failed to elongate. This suppression of the first internode seems to be characteristic of the shrubby Ephedras; in *E. fragilis* as many as fourteen branches are sometimes found crowded together at a single node. In *E. altissima* the arrangement, though different in appearance, is the same in essentials; the tendency to suppression of internodes at the base of the branches is not so marked in the vegetative shoots and the peduncles branch freely, the strobili, some of which are abortive, drooping in loose clusters from the climbing stems (Fig. 2, Pl. LXXXV).

The frequent dichasial branching of the female inflorescences of this species is a feature of marked resemblance to the inflorescences of *Welwitschia*. It is interesting to find dichasial branching a recurrent feature in the Gnetales, as a comparison has already been made between the inflorescences of *Welwitschia*[1] and the dichasially branched inflorescences of *Wielandiella*,[2] the flower of which also approaches that of the Gnetales.[3]

In all the above species each female strobilus is made up of three pairs of decussate fused bracts which form three cupules, the lowest very small, the next larger, and the uppermost forming a large protective cup within which the ovules (or ovule) are enclosed (Figs. 2 and 3, Pl. LXXXV).

In the tribe Alatae, which includes some of the other species examined, the bracts of the female strobilus are more numerous and are not fused, but become during the ripening of the fruit chaffy and membranous instead of succulent[4] (Fig. 3 *c*, Pl. LXXXV, *E. Torreyana*; there were here ten pairs of bracts). In *E. Torreyana* there was one case with as many as five ovules in the strobilus, and examples with three were fairly frequent. In the strobili bearing three ovules the alternating whorls of bracts are sometimes trimerous throughout. Usually, however, even in these species, where the cones have numerous membranous bracts, there are only two ovules in each cone.

In the cones of *E. distachya* and *E. fragilis*[5] there are also two ovules, occurring one in the axil of each of the topmost pair of bracts, the main axis terminating in between them. In microtome series of *E. distachya* the true apex of the stem is visible between the ovules as a small projection of a few cells.

In *E. altissima*[6] there is commonly only a single terminal ovule.

[1] Sykes, 1910.

[2] Nathorst, 1888 (*Williamsonia angustfolia*) and Nathorst, 1902, 1910, 1911.

[3] p. 975.

[4] See Stapf's monograph on the Ephedras, p. 23 and Pl. I–IV.

[5] Var. *campylopoda*, Strasburger, 1871; also *E. antisyphilitica*, Coulter and Chamberlain, 1901, *E. helvetica*, Jaccard, 1894, and many other species, Stapf, 1889.

[6] As in *E. trifurca*, Land, 1904; *E. Alte*, *E. campylopoda*, &c., Stapf.

Occasionally, however, a form with two ovules is met with in which the arrangement of the ovules is the same as that found in the other species; but in this case no sign of the stem apex could be detected, the two outer integuments being fused together at the base.

The ovule has two coverings similar in position and character to those immediately surrounding the ovules in the other Gnetales, and here regarded as the outer and inner integuments.

The male strobilus is similar to the young female, but above the single basal pair of sterile bracts there are several pairs of fertile bracts, in the axil of each of which is a male 'flower'. The male flower consists of two perianth segments and a stalk, frequently more or less bifid, bearing two groups of bilocular synangia (Figs. 4–6, Pl. LXXXV). The number of the synangia varies from eight in *E. distachya* and *nebrodensis* to two in *E. altissima*.

In *E. fragilis*, var. *campylopoda*, the strobilus is bisexual, with male flowers in the axils of the lower pairs of fertile bracts and ovules in the axils of the uppermost pair. The latter, however, never reach full development.

The strobili of *Ephedra* are obviously far more closely comparable with those of *Welwitschia*[1] than with those of *Gnetum*.[2] The branched male inflorescence with its compact strobili, and both male and female strobili with their basal sterile bracts and their upper fertile bracts with axillary sporangiophores, are strikingly similar in both genera, but in *Ephedra* the strobilus has a much more limited growth than in *Welwitschia*.

II. The Anatomy of the Inflorescences and Flowers.

(*a*) The Bracts.

The bracts of both male and female strobili are similar in character,[3] though those of the female are tougher in the early stages than those of the male, and also undergo later various changes connected with the ripening of the fruit, becoming, in the different species, succulent or chaffy, &c. The members of each pair are fused together in the female strobilus, but only slightly connate at the base in the male.

Each bract receives two vascular bundles, which run unbranched nearly to its apex. As in the vegetative leaves, the bundle is accompanied by a small number of reticulate transfusion tracheides, occurring laterally in two groups (Fig. 7, Pl. LXXXV). These increase in number and size towards the apex of the bract, where the bundles approach one another. Finally, the endings of the bundles are lost in one common group of transfusion tissue

[1] Pearson, 1906, 1909; Sykes, 1910. [2] Thoday (Sykes), 1911; Pearson, 1912.

[3] Stapf, 1889, pp. 25 ff.; Bertrand, Fig. 12, Pl. III, figure showing similar structure of vegetative leaf.

which extends for a short distance further into the apex (Text-fig. II. 5, 6, 7, 8).

Fibrous cells with lignified walls are scattered through the tissues of all the bracts and are especially numerous just under the inner epidermis, where in the cupules of the female strobilus they form a definite layer, one or two cells deep in the outer cupules, two, three or more cells deep in the inner cupules (Fig. 7, Pl. LXXXV).

The outer epidermis is very thick and strongly cuticularized. The outer wall consists of three layers, an inner cellulose, a middle containing crystals, and an outer cuticularized layer. The stomata are mainly in the outer epidermis, they are very small and deeply sunk, and the inner surface of the guard cells is strongly cuticularized (Fig. 8, Pl. LXXXV). The epidermis and stomata of the vegetative leaves are very similar in structure.

The structure of the bracts in *Ephedra* is closely comparable with that in *Welwitschia*,[1] the main differences being due to the unbranched nature of the vascular bundles in the former genus. The distribution of the fibrous cells, the curious character of the outer epidermis,[2] the structure of the stomata, and the transfusion tissue are all points of similarity.

(*b*) The Peduncle.

(i) *General.* The peduncle or naked axis of the inflorescence closely resembles the young vegetative stem. It has a strongly thickened and cuticularized epidermis with stomata similar to those of the bracts; the cortex is mainly composed of thin-walled assimilating cells, but contains also strands of hypodermal sclerenchyma which, however, do not project and form marked ridges as in the stem. The phloem of each bundle is accompanied by a strand of fibrous cells. In *E. altissima* the hypodermal strands of thickened cells are absent, but the pith is strongly lignified, the cells showing simple pits, and well-marked lignified strands accompany the vascular bundles; in *E. distachya*, on the other hand, the pith is thin-walled and the strands bordering on the phloem are reduced to a few fibrous cells, but the hypodermal groups are constant and regular, although smaller than in the vegetative stem. *E. fragilis* shows an intermediate condition; the hypodermal strands are irregular and scattered, while the pith and bundle strands are thick-walled but unlignified.

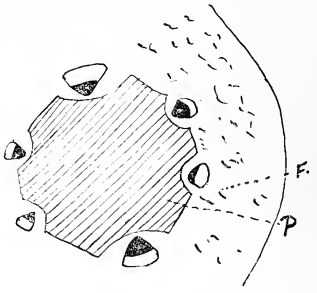
In the vegetative stem of *E. altissima* the lignification of the pith is confined to the region near the node, while in *E. distachya*, *E. fragilis*, and *E. nebrodensis* signs of lignification appear only at the margin of the pith in the neighbourhood of the bundles.

[1] Sykes, 1910, pp. 184–6, Figs. 5, 9 *a* and *b*, Pl. XVII.

[2] It compares still more closely with the epidermis of the vegetative leaves in *Welwitschia*. Ibid., Fig. 2, Pl. XVII.

The varied distribution of the lignified tissue in the different species of this genus is paralleled in *Welwitschia*, where in the male inflorescence the entire pith is lignified, while in the female inflorescence this happens only in the extreme base; elsewhere lignification is confined to strands of tissue on the periphery of the pith accompanying the vascular bundles.[1]

(ii) *Vascular Anatomy of Naked Axis.* In *E. distachya* the peduncle is traversed by eight collateral bundles, occurring in a regular ring and remaining unbranched except where, in the male inflorescence, branching of the axis occurs. At this level and at the branchings of the axis of the female inflorescence in *E. altissima*, the behaviour of the bundle system is similar to that described later in connexion with the vegetative buds in the axils of the ordinary vegetative leaves. In the upper branches of the female peduncle of *E. altissima* the structure is somewhat different; there are here four small bundles and two large ones, which latter are seen by their behaviour in the strobilus each to represent two of the bundles in the lower parts of the peduncle.



TEXT-FIG. 1. Transverse section of peduncle of *E. altissima* near the base of a strobilus, showing six bundles, lignified pith (P), and fibres scattered in the cortex (F).

(*c*) Anatomy of the Female Strobilus.

The axis of the strobilus itself is similar in general structure, distribution of fibres, &c., to the naked axis bearing the strobilus. In describing the course of the vascular bundles in the axis of the female strobilus it will be necessary to treat the two species specially examined separately.

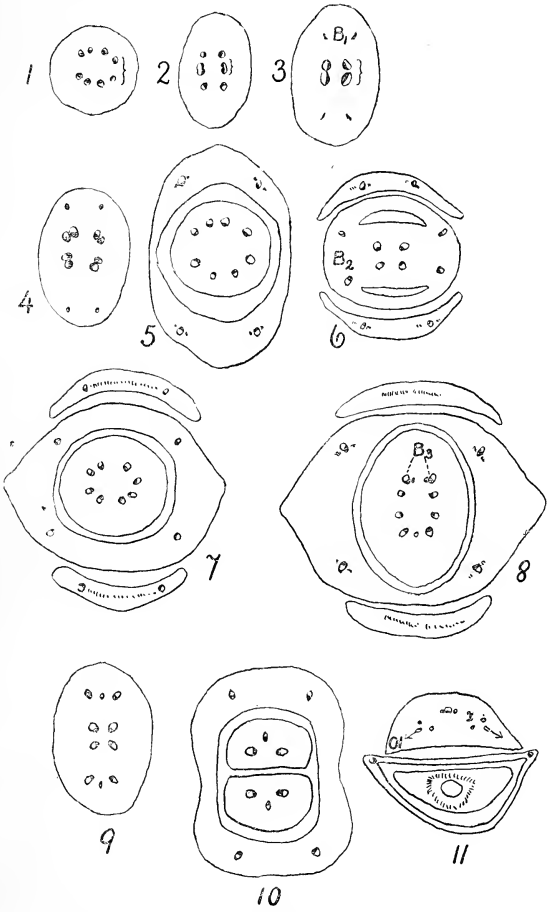
(i) *E. distachya.* The eight bundles which enter the base of the strobilus become arranged in two groups separated from one another by gaps in the ring (Text-fig. II. 1). Then the bundles on either side of each gap (Text-fig. II. 2 and 3, B) pass out to the first pair of bracts.

Four bundles are left in the axis; these now divide to form again the original number (Text-fig. II. 4). Next the eight bundles are rearranged into two groups at right angles to the previous arrangement, and two pairs of bundles pass out to the second pair of bracts (Text-fig. II. 5 and 6, B_2).

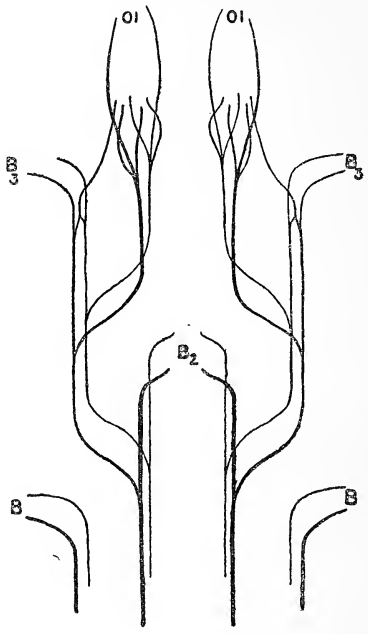
Four bundles are again left in the axis and once more divide to form eight, arranged in two groups corresponding in position to those below the first pair of bracts. Before, however, the lateral members of each group pass out to supply the third pair of bracts, a small bundle originates from each of them and fuses with the opposite one in the gap between the

[1] Sykes, 1910, pp. 191 and 201–2.

groups (Text-fig. II. 8 and 9). The two pairs of foliar bundles now pass out in the ordinary way towards the bract, and the little median bundles pass out a short distance with them (Text-fig. II. 9). After the bundles have entered the base of the bract the median bundle curves backwards a little towards the centre of the axis and with two of the axial bundles



TEXT-FIG. II. 1–11. Series of transverse sections through the female strobilus of *E. distachya*, described in text. B_1, B_2, and B_3 = bundles supplying lowest, middle, and upper pairs of bracts respectively; OI = bundles supplying outer integument. The middle cupule has been omitted in 9, and the upper cupule in 11.

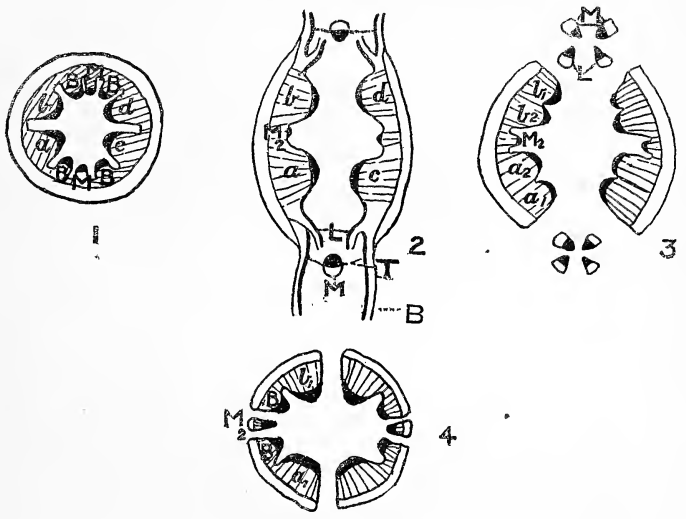


TEXT-FIG. III. Plan of course of vascular bundles in the female strobilus of *E. distachya*; letters as in Text-fig. II.

enters the ovule. With the formation of the two ovules the growth of the axis ceases, and all the vascular elements are therefore used up in the ovular supply (Text-fig. II. 10).

The behaviour of the bundle system at the level of origin of the ovule is similar in essentials to its behaviour at the origin of an ordinary *vegetative*

axillary bud. The vegetative axillary bud receives a median trace,[1] derived from the two foliar bundles, which passes out first to the abaxial side of the bud and there divides into two again; also two lateral bundles which supply the adaxial side of the bud (Text-fig. IV. 1–5). The lateral traces are derived in some small part from the foliar bundles as they pass out to the subtending leaf, but chiefly from branches of the adjoining main bundles. In the case of the ovule none of the elements composing the two lateral traces are derived from the foliar bundles, and the adaxial side of each ovule is entirely supplied by two of the four main bundles left in the axis, which do not branch but themselves form the lateral traces; consequently no vascular elements remain to supply the minute stem apex.



TEXT-FIG. IV. 1–5. Diagrams of series of transverse sections through the node of the vegetative stem (*E. nebrodensis*). M = median axillary bundle; L = lateral axillary bundle; B = bundle supply of subtending bract; a, b, c, d = four main bundles, which fork to form eight, $a_1a_2b_1b_2$, $c_1c_2d_1d_2$, in between each node; M_2 = median axillary bundle for axillary bud at next node; T = bridge of transfusion tracheides connecting the bract bundles with the median axillary bundle.

The course of the *bundle system in the ovule*[2] itself is very simple. The three bundles, one median axillary and two lateral, which enter the base of the ovule usually each branch into three; sometimes the median or abaxial one remains unbranched. In either case the median bundle does not contribute anything towards the supply of the outer integument, but the latter receives the middle branch from the other two groups only.[3] The outer integument thus receives two bundles which traverse the angles

[1] The earlier account by Strasburger states that this is not the case: the vascular supply of the ovule differing in this respect from that of an ordinary axillary bud; 1872, pp. 78 ff.

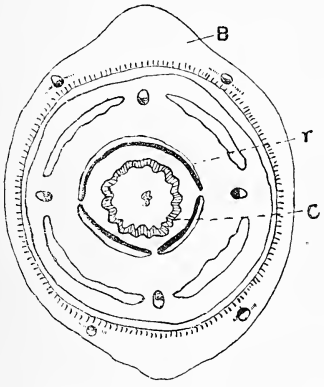
[2] See also Strasburger, 1879, p. 99.

[3] The two bundles supplying the outer integument in *Welwitschia* originate in a very similar manner, each being derived from the middle bundle of a group of three; Sykes, 1910, p. 197, Diagram IX, especially Case 2.

adjoining its flattened side (Text-fig. II. 11) and run unbranched into the apex. In *E. fragilis* the median bundle does commonly contribute a small bundle to the outer integument.

In *E. alata* and *E. Torreyana* there are three large bundles in the outer integument, derived exactly alike from the three bundles entering the base of the ovule. The integument in these species has three large projecting wings, one in the median and two in the lateral planes.

The five or seven bundles left in the flower axis form a ring which dies out low down in the base of the ovule and does not as in *Welwitschia* and *Gnetum* run up into the base of the inner integument. From the position of the ring the constituent bundles here also are clearly integumental.[1]



TEXT-FIG. V. Transverse section through base of ovule described as Case 2. The section is taken just at the level at which the outer integument with its four bundles is in the act of becoming free. B = upper cupule; *r* = ring of vascular tissue entering base of inner integument; C = suberized layer at the base of nucellus.

The simple nature of the vascular system of the ovule in *Ephedra* as compared with that of the other Gnetales is very striking, and, like the unbranched pair of bundles supplying the leaves and bracts, would appear to point to reduction in this genus.

(ii) *E. altissima.* The main differences between this species and *E. distachya* in the course of the bundles in the axis of the strobilus and flowers depend on the fact that in *E. altissima* there is usually only one ovule. There is, however, a considerable range of variation in this species, and it becomes clear from an examination of the different cases that the uniovulate is a modification from the biovulate condition.

Case 1. Biovulate cones with both ovules fertile are occasionally found. In these cases the course of the vascular bundles is practically identical with that described in the cone of *E. distachya*, except that in the cases examined the integument always received three vascular bundles, and not two as is the rule in *E. distachya*, the median axillary bundle branching into three and contributing as well as the other two [2] to the integument.

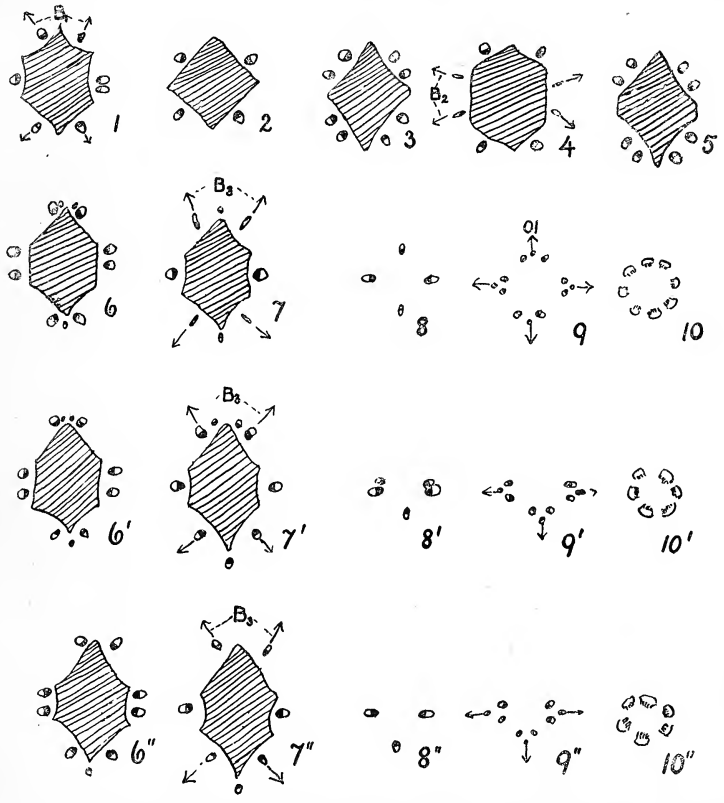
Case 2. Among the material in the laboratory in Manchester, cases frequently occurred in which the single nut of the uniovulate cone had four angles instead of the normal three, and four bundles supplied the outer integument [3] (Text-fig. V), running up these four angles. The course of the

[1] See pp. 966, 967. The tissues of nucellus and integument in *Ephedra* are differentiated from one another down to the base of the ovule.

[2] In *E. trifurca*, in which species also there is commonly only one ovule in the strobilus, biovulate cones also occasionally occur, in which the course of the bundles is the same as in Case 1 of *E. altissima*.

[3] This is the normal case in *E. trifurca* (see also Land, 1904).

vascular bundles in this case is seen in Text-fig. VI. 1–10. Text-fig. VI. 8 represents the level above the origin of the uppermost bracts (B_3), and it is seen that four bundles run into the base of the single ovule. Of these the two smaller represent the median traces derived from the bract bundles and the two larger each represent two of the main axial bundles fused together. In all the uniovulate cones of *E. altissima* examined, the course of the bundles in the cone axis below this level only differed from that in *E. distachya* in that six bundles instead of eight occurred in the internodes (six



TEXT-FIG. VI. Illustrates three transverse series through the female strobilus of *E. altissima*, described in text. Case 2 = 1–10, Case 3 = 6′–10′, Case 4 = 6″–10″; letters as before.

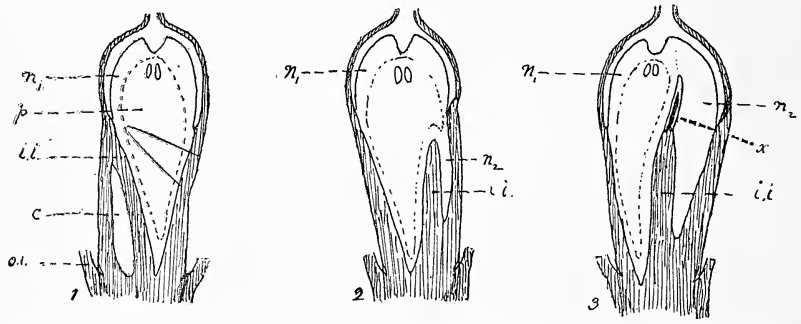
is also the number in the peduncle, p. 957). This appears to be the result of the tendency of the four main axial bundles, which ultimately supply the two ovules in the biovulate cone, but which in the uniovulate cone fuse into two in the base of the ovule, to fuse into pairs lower down also.

Cases 3 and 4. In other uniovulate cones the integument is three-angled and receives three vascular bundles. The two median traces may still both be formed from the bract bundles as in Cases 1 and 2, but in Case 3 one of them divides into two in the base of the ovule, and the halves fuse with the two main bundles, so that three bundles run up into the ovule

instead of four (Text-fig. VI. 6′–10′). This case is intermediate between the first two cases and Case 4, in which one of the median axillary bundles is not formed at all, and only three bundles enter the base of the ovule (Text-fig. VI. 6″–10″). This is the case described by Strasburger[1] as normal, but it here seemed to be exceptional, for it only occurred in one ovule out of seven or eight. Case 3 was the most common.

(*d*) Relation of E. altissima to E. distachya.

The series of cases just described seems to indicate that in fairly recent times changes must have taken place in *E. altissima* which have resulted in the modification of the biovulate to produce the uniovulate condition. There is other evidence which indicates that this change has been brought about by the fusion of the two axillary ovules to form one apparently terminal one. In some of the biovulate cones each ovule has



TEXT-FIG. VII. 1–3. Diagrams of three of the numerous uniovulate cones of *E. altissima* which show evidence of derivation from a biovulate condition. In Diagram 1 the abortive ovule is merely a mass of undifferentiated tissue with a cavity; in Diagram 2 the abortive nucellus is fused at the apex with the fertile nucellus; in Diagram 3 the abortive nucellus is much better developed and the wall of common integumental tissue which separates it from the fertile nucellus is continued upwards above the region of fusion of nucellus and integument, a small free portion being present. *p.*, prothallus; n_1, fertile nucellus; n_2, abortive nucellus; *i.i.*, common portion of inner integument free from the nucellus higher up (*x*); *o.i.*, outer integument; *c.*, cavity representing abortive nucellus.

both outer and inner integuments, but in many cases a common outer integument is present. In one example in which there was a common outer integument both ovules were fertile, but usually one is more or less abortive. This abortive ovule is often fused to the lower part of the inner integument of its fully developed companion, distorting and pressing aside its base; frequently it is merely a mass of undifferentiated tissue with a cavity in the middle (Text-fig. VII. 1), having an independent cup of suberized tissue at its base like that always found at the base of the fertile ovule. Sometimes, however, nucellar tissue[2] occurs within the cavity of the abortive ovule. This is the case in the example represented in Fig. 10,

[1] Strasburger, 1872, Fig. 55 *a* and *b*, Taf. XVI.

[2] It is remarkably easy in *Ephedra* to distinguish the nucellar tissue from the surrounding layers of the inner integument, even in the common basal region; pp. 966, 967, Text-fig. XI. 2.

Pl. LXXXV, and in Text-fig. VII. 2. Here the common wall of integumental tissue between the two nucelli is incomplete, and the small nucellar mass of the abortive ovule is fused at its apex with the fertile nucellus, so that in this section it appears as a long lobe of the latter extending downwards into the integumental tissue.

In a somewhat similar case (Text-fig. VII. 3) fusion of the two nucelli has only taken place at their extreme apices, and the common integumental wall runs up between them and ends in a small free lamina in the chink below the point of fusion. Here again only one nucellus contains a prothallium with archegonia.

Fully one-third of thirty-four strobili of *E. altissima* examined showed traces of the presence of a second abortive ovule within the outer integument.

(*e*) The Anatomy of the Ovule.[1]

E. distachya. The ovules in *E. distachya* and other biovulate species are roughly triangular, compressed and flattened on their adjacent sides, but rounded abaxially.

Two vascular bundles traverse the *outer integument,* running in the two sharp angles of the flattened side. The outer epidermis of the integument is composed of large columnar cells and the inner epidermis of similar smaller cells except where it clasps the micropylar tube; there each cell is drawn out into a papilla. In the old ovule these become lignified and are firmly fused on to the micropylar tube (Text-figs. VIII and IX and especially XI. 3, and Fig. 11, Pl. LXXXV).

At the base of the integument it consists mainly of a layer of brown cells underneath the outer epidermis, which is continuous right round the bundles (Text-fig. VIII. 5), and internal to this is a band of tissue which even in the oldest ovule examined was still parenchymatous.

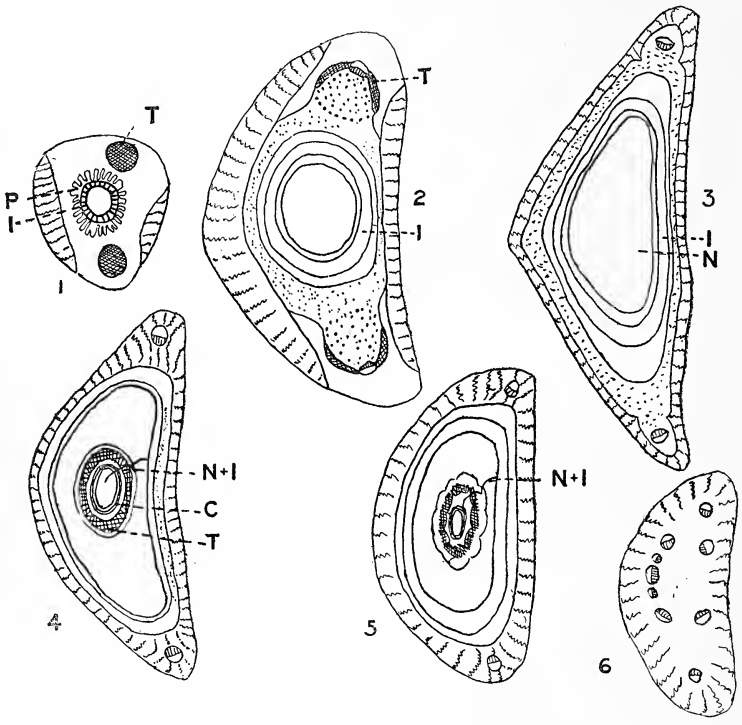
A little higher up (Text-fig. VIII. 4), fibrous cells with thick walls become differentiated in this parenchymatous band, and very soon there is a thick layer of fibrous cells on the inner side of the brown layer (Text-fig. VIII. 3). In the upper part of the outer integument there is, as well as the fibrous layer, a more conspicuous strand [2] of larger fibrous cells accompanying each vascular bundle on its inner side (Text-fig. VIII. 2); but at the tip, where the integument surrounds the micropylar tube, both the fibrous layer and the separate strands die out (Text-fig. VIII. 1). The brown layer gradually diminishes in prominence till in this region it is represented only by two small bands of tissue alternating with the two vascular bundles.

[1] Strasburger, 1872, pp. 86 ff., Pl. XVI, &c.

[2] In *E. nebrodensis,* the outer integument of which is very like that of *E. distachya,* these fibrous strands accompany the vascular bundles right to the base of the integument. The fibrous layer is also well marked and strongly lignified.

The vascular bundles throughout the upper third of the ovule are accompanied by large wings of transfusion tissue, which extends some distance round the fibrous strands (Text-fig. VIII. 2), and at their termination the bundles are lost in two large groups of transfusion tracheides (Text-fig. VIII. 1, T).

The *inner integument*, which becomes free about two-thirds of the way up the nucellus, projects in the early stages considerably beyond the outer covering, but in the mature seed this projecting portion is generally broken

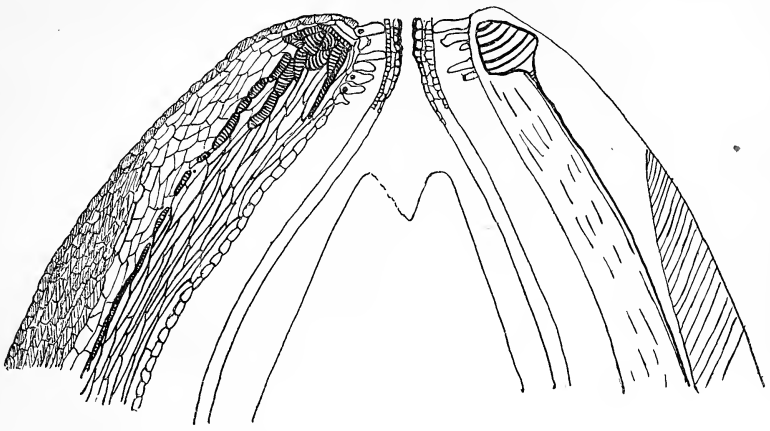


TEXT-FIG. VIII. 1–6. Diagram of series of transverse sections through the ovule of *E. distachya*. P = papillae; T = transfusion tissue; I = inner integument; N = nucellus; C = layer of suberized cells at base of nucellus; cross hatching = xylem; dots = fibres; jagged lines = brown tissue.

off. The whole integument is very thin and its base is made up of thin-walled parenchymatous cells only; in the mycropylar tube there are three layers of cells, the inner epidermis, composed of large cells which are cuticularized to a most remarkable extent (Fig. 11, Pl. LXXXV), and two outer layers of much smaller cells whose walls are thickened in a minor degree, but not cuticularized. A little mucilage appears in the tube shortly before fertilization; later this becomes hardened into a solid mass[1] which closes

[1] A similar secretion ot mucilage is recorded by Pearson ('06) at the fertilization stage in *Welwitschia*; but it does not appear to be known whether this persists and becomes hardened afterwards.

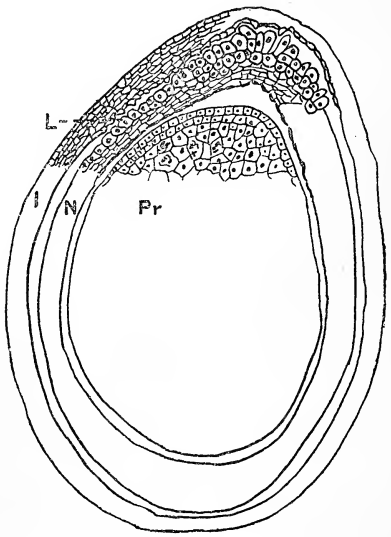
the tip of the tube and which is continuous downwards as a thick mucilaginous lining to the tube. The chink between the micropylar tube and the integument is also closed by the papillae of the outer integument, and



TEXT-FIG. IX. Longitudinal section of ovule of *E. distachya*, older than the one drawn in Text-fig. VIII. The fibres internal to the vascular bundles are seen to be strongly lignified.

in this manner the developing embryo would appear to be as effectively protected as in *Gnetum*[1] with its special apparatus.

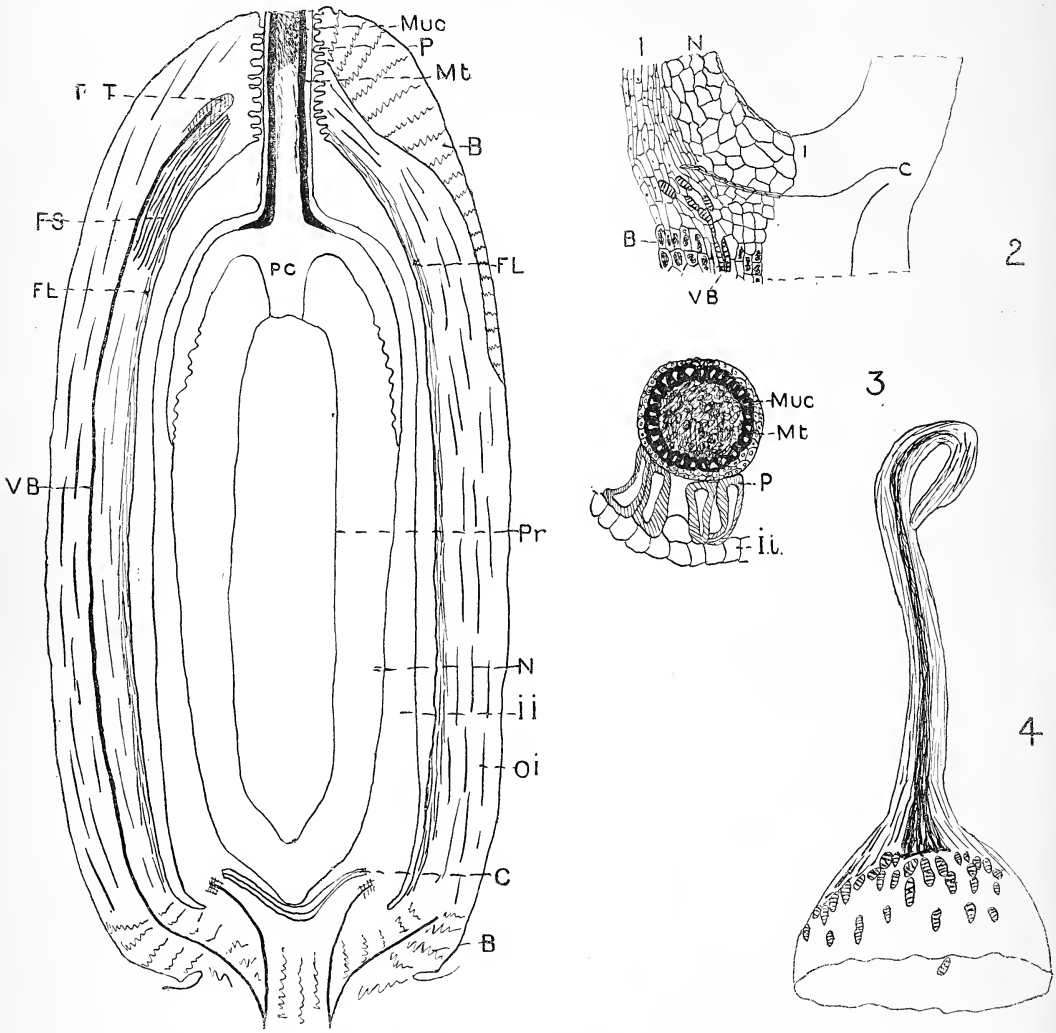
The ring of vascular bundles which enters the base of the ovule does not run up as far as the free region of the inner integument, but terminates quite low down in a mass of transfusion tracheides. One of the most characteristic features of the *Ephedra* ovule is the sharp demarcation of the large empty cells of the nucellar tissue from the smaller celled tissue which forms the free part of the inner integument and is also prolonged downwards right round the base of the nucellus (Text-fig. XI. 2). The ring of vascular tissue is situated in the integumental region. This differentiation is not of course present in the earliest stages, its cause being the proximity and growth of the prothallus, in consequence of which the cells of the nucellus become flattened and empty. Still the differentiation is of some interest, nothing of the kind having been seen in *Gnetum* or *Welwitschia*.



TEXT-FIG. X. Transverse section of nucellus and inner integument just above the level at which the integument becomes free. I = inner integument; N = nucellus; *Pr* = prothallus; L = layer of papillate cells on periphery of nucellus.

[1] Berridge, 1911; Thoday (Sykes), 1911.

The *nucellus* becomes very thin and papery in the seed, except at the apex, where it is still possible to distinguish the remains of the remarkably deep pollen-chamber, which has already been described in detail by other

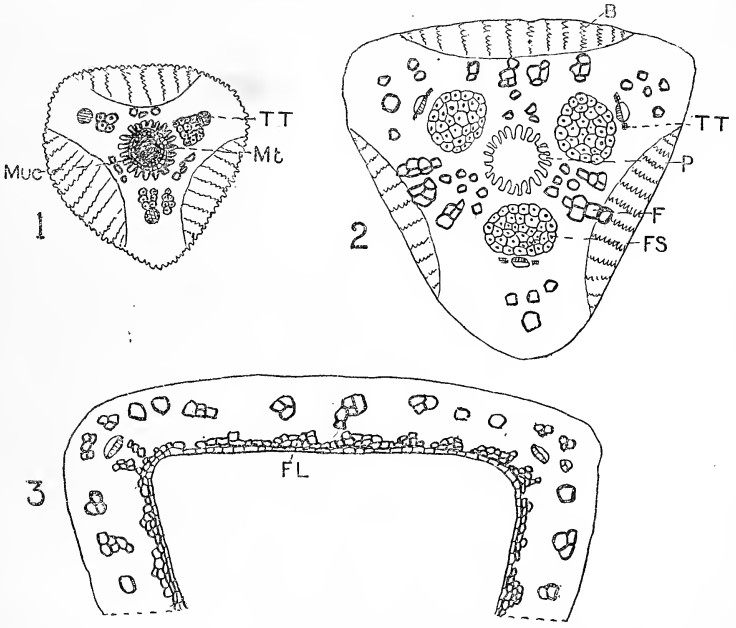


TEXT-FIG. XI. 1–4. *E. altissima* ovule. 1. Longitudinal section showing general structure. N = nucellus; *Pr* = prothallus; PC = pollen-chamber; *ii* = inner and *oi* = outer integument; *Muc.* = mucilage; FS = fibrous strand accompanying vascular bundle; FL = fibrous layer on inner edge of outer integument; P = papillae; *Mt* = micropylar tube; B = hypodermal tissue; C = cup; VB = vascular bundle; TT = transfusion tissue. 2. Base of fused nucellus and inner integument, showing sharp line of demarcation. 3. Transverse section through apex of micropylar tube, closed by hardened mucilage, and part of edge of inner integument showing lignified papillae attached to micropylar tube. 4. Free region of inner integument, showing coiled micropylar tube and reticulately thickened strengthening cells.

authors[1] (Figs. 9 and 12, Pl. LXXXV). In the seed of some species the walls of the cells surrounding the pollen-chamber become thickened and lignified,

[1] Strasburger, 1872, Fig. 54, Taf. XVI; Jaccard, 1894; Land, 1904; Berridge and Sanday, 1907.

forming a little cap as in *Gnetum*.[1] For some little distance above the level at which the nucellus becomes free from the inner integument, the cells composing its epidermal layer are drawn out into papilla-like outgrowths (Text-fig. X).[2] At its base the nucellus is separated from the small-celled tissue belonging to the region of the inner integument by a thin layer of crushed cells, empty of contents, with suberized walls, which forms a cup, internal to the larger cup formed by the vascular bundles and their transfusion tracheides, extending upwards to about the same level, and separated from it by two or three layers of parenchyma (Text-figs. V, C, and XI. 2, C). It is very difficult to imagine what may be the function of such a cup of suberized cells.



TEXT-FIG. XII. 1–3. *E. altissima.* Diagrams of transverse sections through ovule; 1 = through apex of micropylar tube; 2 = through the outer integument at the level of the lower part of micropylar tube; and 3 = at a level about half-way up the ovule. Letters as before.

E. altissima. The single ovule of *E. altissima* differs to some extent from the ovule of the bisporangiate *E. distachya.* The great difference in size may be seen by a comparison of Figs. 2 and 3 *a*, Pl. LXXXV, which are magnified to the same scale. It is of course no longer laterally compressed by the presence of another ovule, though it is still angled, its angles being

[1] Thoday (Sykes), 1911, pp. 1113–14.

[2] Pearson has suggested that during the disorganization of the nucellar apex in *Welwitschia* a good deal of the mucilage afterwards found in the micropylar tube is formed, and he also produces evidence to show that some of this mucilage is secreted by the outer layers of the nucellar cone (1909, p. 343). It appears probable that in *Ephedra* also, while some of the mucilage originates by disorganization to form the pollen-chamber, some of it is similarly secreted by the papillate cells of the nucellar epidermis.

three or four in number, with a corresponding number of vascular bundles in the outer integument. The angles are not very prominent, but it is quite easy to tell their number by examination with the naked eye.

In the *outer integument* there is an outer soft and an inner fibrous layer as in *E. distachya*. In the outer layer the hypodermal brown tissue is only differentiated in the extreme base of the outer integument, where it forms a ring, and in the tip, where it is distributed in bands alternating with the vascular bundles. Text-fig. XI. 1 shows one of these bands cut longitudinally on the right side of the ovule, while on the left the section has passed through the region in between the bands and has cut one of the alternating vascular bundles. The inner fibrous layer is relatively less thick than in *E. distachya*; probably this is correlated with the abundant development of sclerenchyma throughout the outer parenchymatous layer (Text-figs. XI. 1, and X). The strands of fibres described in *E. distachya* accompanying the vascular bundles in the apical region of the ovule are differentiated here also, but are strongly lignified at a much earlier stage (FS, Text-figs. XI. 1, and XII). The vascular bundles have less transfusion tissue than in *E. distachya*.

The *inner integument* differs only in the fact that in the fertilized seed the tip of the micropylar tube is sometimes coiled over, and that reticulately thickened strengthening cells[1] are scattered through the base of the thin free portion below the micropylar tube (Text-fig. XI. 4).

The mass of hardened mucilage closing the apex of the micropylar tube was particularly well seen in this species (Text-fig. XI. 3).

(*f*) The Development of the Ovule.

The development of the ovule has been already described by other authors, and it is not proposed to give any details here, but merely to draw attention to the relations of the growth of the various parts of the ovule at each stage of development.

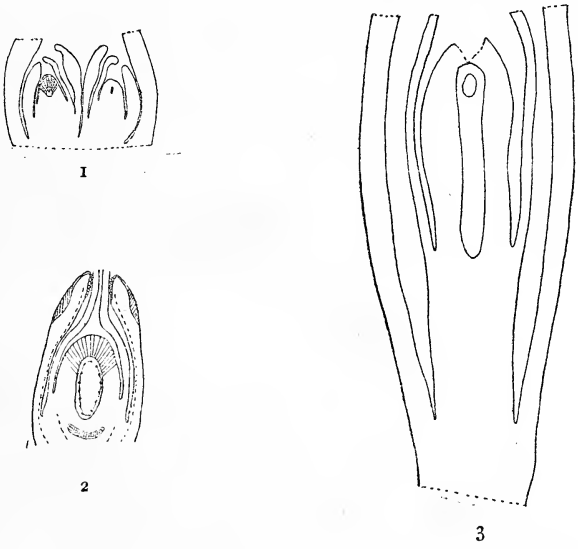
In the very young ovule the two integuments arise close together at the base of the ovule;[2] at the stage shown in Text-fig. XIII. 1 the bases of the two integuments are still almost on a level. From this stage onwards for some time the growth is mainly confined to the free apex of the nucellus, while very little development takes place in the region between the levels of origin of the two integuments. It will be seen that in Text-fig. XIII. 2, which is clearly a much older ovule than Text-fig. XIII. 1, this region has not begun to develop. Growth here begins about the time of cell-wall formation within the megaspore and continues with the enlargement of the prothallus; in the ovule figured in Text-fig. XIII. 3, which is not yet fertilized, considerable growth of this region has taken place. From this stage onwards the nucellar apex enlarges less in proportion to the rest

[1] It is probable that these were the cells once mistaken for tracheides. See reference in Thoday, 1911, p. 1117, note 3.

[2] See Fig. 2, Pl. XXII, in Berridge and Sanday, 1907.

of the ovule, and consequently the free part of the inner integument gets carried further up; in Text-fig. XIII. 3 its level of freedom is about half-way up the ovule, but in the ripe seed it is about two-thirds of the way up (Text-fig. XI. 1).[1] Even in the mature seed, however, the free apex of the nucellus is conspicuous and fairly massive.

It is interesting to compare the relative development of the different regions of the ovule in the three genera of the Gnetales. In *Gnetum* the method of development is similar to that in *Ephedra* in that the apical region of the ovule develops first, but in the later stages this region appears practically to cease to grow, and it is almost entirely to the great growth of



TEXT-FIG. XIII. 1–3. Three stages in the growth of the ovule in *Ephedra*. 1. Early stage with two integuments arising together at the base of the ovule. 2. Young ovule in which the apex has grown considerably, but the two integuments still originate close together. 3. Fertilization stage in which the region between the integuments has begun to enlarge.

the intermediate region, as in the Cycads, that the enlargement of the ovule is due. The apex of the nucellus after fertilization becomes hardened and withered, as it does also later and to a much smaller extent in *Ephedra*.

In *Welwitschia* we unfortunately know little of the earlier stages. In Text-fig. VIII, p. 196 of an earlier paper,[2] a stage is drawn in which the integuments are fairly close together. Here also the early growth in the apical region is followed by the enlargement of the intermediate region, but it also appears that here further great expansion of the apical region takes place, producing a massive nucellar apex far larger in size than that in *Ephedra*, though more like *Ephedra* than *Gnetum*. It has already been suggested [3] that the Angiosperms illustrate the further development of this tendency.

[1] Or even three-quarters, Strasburger, 1872, Fig. 50, Pl. XVI.
[2] Sykes, 1910.
[3] Sykes, 1910, pp. 218–19.

(*g*) Anatomy of Male Strobilus.

The anatomy of the male strobilus is similar in all essentials to that of the female. The pair of lower sterile bracts have usually no buds in their axils, and each is supplied with two bundles in the same way as the pairs of sterile bracts of the female strobilus. At the other nodes, at each of which arise two bracts and two male flowers, the course of the vascular bundles is closely comparable with that at the origin of a vegetative bud.[1] The male flower receives, similarly, three bundles: the median trace, derived itself from the fusion of two bundles and originating early from the two bundles which supply the subtending bract, and two lateral traces which originate from the bract bundles as they pass out. Unlike the lateral traces of the vegetative bud, these lateral traces receive only a very small contribution from the bundles of the main stem, and are derived mainly from the foliar bundles (Text-fig. XVIII. 1–4).

(*h*) Anatomy of Male Flower.[2]

I. *General.* The male flower consists of a short axis which generally arises free in the axil of the bract, but in *E. fragilis* is fused for a short distance with the bract. On this short axis are inserted the two membraneous appendages. Above their insertion the antherophore bearing the synangia extends upwards, its long axis being a continuation of the long axis of the flower. In *E. fragilis* it is folded back on itself in the bud (Text-fig. XIV) showing circinate vernation, but this curious configuration has not been found in any of the other species, in the buds of which the axis is very short and straight (Fig. 5 *c*, Pl. LXXXV).

TEXT-FIG. XIV. Sporangiophore of *E. fragilis* in bud, showing the manner in which it is folded over. P = base of perianth. × 23.

The antherophore varies considerably in the different species. It is generally cylindrical in the earliest stages, and it may remain so when mature, or may broaden out into a fairly wide lamina. In *E. Torreyana* (Text-fig. XVII) and *E. aspera*[3] this lamina is well developed, and the synangia are borne on long stalks. In *E. nebrodensis* also the sporangiophore is flattened, as it is in several of the species figured by Stapf. In *E. distachya* and *E. fragilis* it is much more cylindrical, and in *E. altissima*, where it is probably most reduced, it shows little if any sign of broadening out into a lamina.

In some species, e.g. *E. distachya*, *E. nebrodensis*, the antherophore is clearly bifid. Each half bears four synangia, situated on the side of the

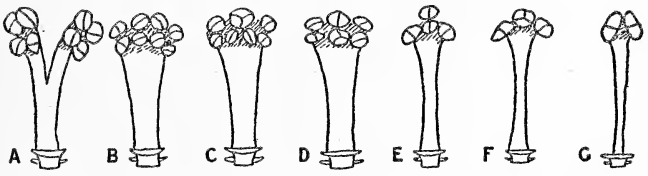
[1] p. 959 and Text-fig. IV.

[2] See also Strasburger, 1872, p. 132.

[3] Both of these species have female strobili with numerous membranous bracts, and are presumably (pp. 954 and 976) among the more primitive of the species of *Ephedra*.

antherophore away from the main axis of the strobilus, but borne somewhat laterally and thus facing away from the axis of the antherophore. Apparently this arrangement represents the original form of the antherophore from which the other more reduced forms are derived by fusion of the synangia. The fused synangia are often bilocular, quadrilocular, or irregular in form. In *E. distachya* the division of the antherophore into two extends occasionally almost to its base,[1] but in other cases it may be quite absent, the two uppermost synangia being thus brought almost into contact with one another.

From this arrangement it is but a short step to the first stages of fusion and reduction in number of the synangia; this has taken place in *E. Torreyana* (Text-fig. XVII) and *E. aspera*, in which the two uppermost synangia, one belonging to each half of the antherophore, are fused, giving rise to a single apical, commonly trilocular synangium (Text-fig. XV, C). There is often considerable variation within the limits of a single species, various stages of fusion occurring in the different antherophores even of the same



TEXT-FIG. XV. Diagram of antherophores of the various species, illustrating the reduction in number of the synangia by fusion. A = *E. distachya*; B = *E. distachya*, *nebrodensis*, or *fragilis*; C = *E. aspera* or *E. Torreyana*; D = *E. aspera* or *E. Torreyana*; E = *E. Alte*; F = *E. aspera* or *E. altissima*; G = *E. altissima*.

strobilus. In *E. Torreyana*, for instance, cases occur in which the two inner synangia, like the uppermost pair, also fuse with one another (Text-fig. XV, D), so that there are two single median and two pairs of lateral synangia.

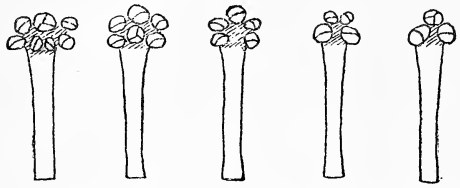
In *E. campylopoda*[2], a further fusion has occurred, the outer synangia of each half of the antherophore having fused with one another (Text-fig. XV, E), so that there are only four synangia, two median and two lateral. Next there are species in which the original eight synangia are represented only by three, one terminal and two lateral (*E. aspera*, *E. altissima*, *E. Alte* (Stapf), Text-fig. XV, F), and finally in *E. altissima* the terminal synangium, which when present is generally trilocular, is commonly abortive, and the antherophore bears only two lateral synangia (Text-fig. XV, G; Figs. 6 *a* and 6 *b*, Pl. LXXXV).

It is not intended to assert that these fusions here described are the only possible fusions among the synangia, all kinds of irregular fusions occur, distributed among the various species; even in *E. distachya* and

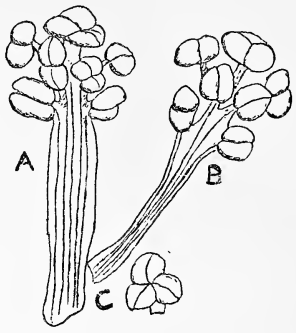
[1] Also recorded by Stapf, 1889, p. 21.

[2] Also in *E. Alte*, Stapf.

E. nebrodensis, cases were met with in which two otherwise independent synangia were fused by their adjacent ends. One of the most irregular species is *E. aspera*, in which occurred all the forms indicated in Text-



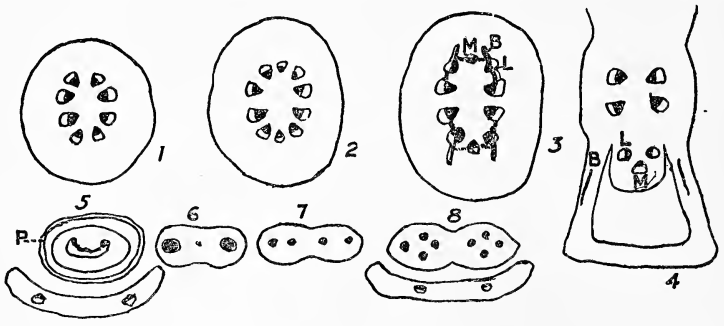
TEXT-FIG. XVI. Diagram of antherophores of *E. aspera*, showing the great variation in this species.



TEXT-FIG. XVII. Two antherophores of *E. Torreyana*, each showing a single terminal synangium, quadrilocular in A, trilocular in B. C is the trilocular synangium of B drawn from another point of view.

fig. XV. It is only meant to suggest that the forms shown in the diagram in Text-fig. XV indicate the main lines along which reduction has taken place.

II. *Vascular*. The three bundles which enter [1] the axis of the male flower fuse more or less completely into a single crescentic bundle (Text-fig.

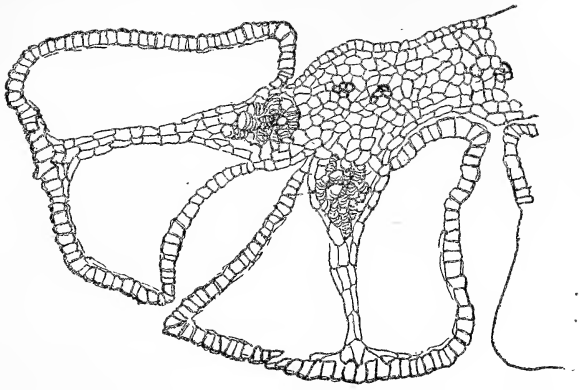


TEXT-FIG. XVIII. 1–8. Series of transverse sections through axis of male strobilus and antherophore of *E. nebrodensis*. 1–4 = axis of male strobilus, showing contribution of one median and two lateral traces to the antherophore, all being derived from the foliar traces. 5–8 = antherophore in axil of bract; 5 shows crescentic bundle, in 6 it has broken into two with a minute bundle between which dies out, in 7 the two have divided to form four, and in 8 to form eight. P = 'perianth'; other letters as before.

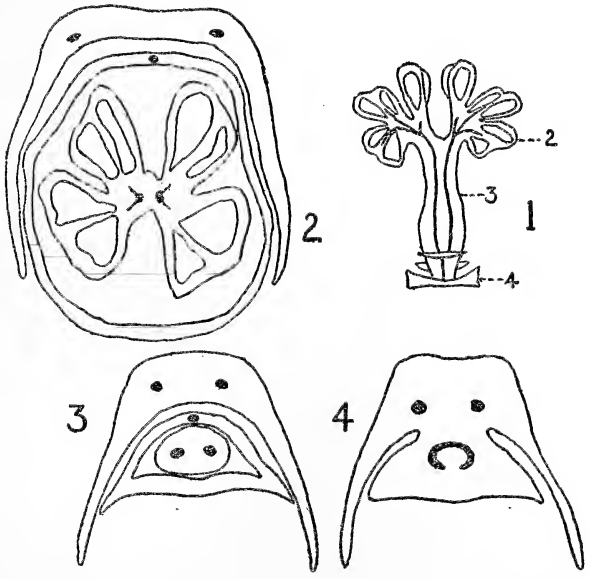
XVIII. 4, 5). The fusion lasts only for a very short time, and takes place just below the level of insertion of the two leaf-like appendages. The crescentic mass thus formed almost immediately separates again into two large bundles, and between these a minute third one is commonly found on the abaxial

[1] p. 970.

side. In *E. fragilis* this minute bundle passes out into the adaxial 'perianth' member (Text-fig. XX. 3, 4), and rarely this may even receive two bundles, but the other member appears to be always without a vascular



TEXT-FIG. XIX. Longitudinal section through two synangia of *E. nebrodensis*, showing transfusion tissue in septa.



TEXT-FIG. XX.[1] Diagram of male flower of *E. fragilis*. 1 = Longitudinal section; 2, 3, 4 = transverse sections from levels 2, 3, 4 in Diagram 1. The two bundles are seen to pass up unbranched to the level of origin of the synangia. The third bundle supplying the perianth is seen in 3, and the fusion of the base of the flower-stalk with the bract in 4. Higher up than 2 the perianth segments become separate from one another. 2, 3, 4 × 23.

supply.[2] In all the other species the leaf-like appendages receive no bundles, and the minute third bundle dies out, while the two larger portions

[1] This figure is adapted from drawings kindly lent by Dr. Benson of Royal Holloway College.

[2] As in *Welwitschia*, but in *Gnetum* these structures have a vascular supply.

of the crescentic mass supply the two halves of the bifid antherophore. In each half in *E. distachya* and *E. nebrodensis*, the bundle branches into two and then again into four, the eight bundles thus formed supplying the two groups of four synangia; each bundle ends in a mass of transfusion tracheides [1] in the base of the septum separating the two loculi of each synangium (Text-fig. XIX).

In most of the other species also the two main bundles branch quite early into a number of bundles corresponding with the number of synangia borne on the antherophore, but in *E. fragilis* they remain unbranched almost up to the level of insertion of the synangia, before they each divide up to form four traces supplying the four synangia.

III. Morphological Considerations.

A. *The Male Flower.* In *Ephedra* the male flower axis is axillary, and it receives its vascular supply like an axillary bud; it bears two leafy appendages and a bifid antherophore. It therefore has the characteristics of an axillary shoot. The appendages are not however in the same plane as the first pair of leaves of a normal axillary shoot, but at right angles to it, that is in the position of the second pair of leaves; and where a vascular supply is present it corroborates this view: while the two halves of the antherophore are in the plane of the first or third pairs of leaves. Arber and Parkin [2] have adopted the view that the parts corresponding to the first pair of leaves are missing, and the second and third only are represented. The antherophore, they think, consists of two members originally standing laterally, but now by reduction of the flower and by fusion brought into a median position.

The male flower then becomes a very reduced strobilus, consisting of an axis bearing two pairs of appendages, the antherophore itself being a disc consisting of two fused sporophylls. Whether or no this view be the correct one, it does at any rate appear fairly clear, that whatever be the nature of the axis on which it stands, the antherophore is of foliar nature [3]: its broadened lamina in the species in which it is best developed, and especially its circinnate vernation in *E. fragilis* (Text-fig. 12), both combine to emphasize its leaf-like character: further, it is found that the two halves of the antherophore receive their bundles in the same manner as do the first and third pair of leaves of an axillary shoot, each half receiving at its base a contribution both from one of the lateral traces and from the median trace.

In view of the rapidly accumulating evidence [4] suggesting that the

[1] Cf. *Welwitschia* antherophore; Sykes, 1910.

[2] Arber and Parkin, 1908, p. 499.

[3] Coulter and Chamberlain assume it to be axial; 1910, p. 471.

[4] Arber and Parkin, 1908; Sykes, 1910; Berridge, 1911; Thoday (Sykes), 1911.

Gnetales and Bennettitales are derived from the same stock, the leaf-like character of the disc of male organs in *Ephedra* and *Welwitschia* is of interest as tending to support this relationship. We now know, among the Bennettitales themselves, many examples of discs of male sporophylls much reduced from their original leafy and pinnate form. In *W. rajmahlensis*,[1] for example, the segments of the disc are few in number and no longer flattened, but consist of a branched axis-like structure, bearing each a row of bilocular synangia, seemingly very suggestive of the male organ in *Ephedra*. Wieland[2] also has lately drawn attention to the resemblance between the small male discs of *Wielandiella*, the reduced structure of which has recently been redescribed by Nathorst,[3] and the disc of six fused male sporophylls in *Welwitschia*. Such simple discs as those of *Williamsonia whitbiensis*[4] are perhaps even more suggestive of the staminate disc of *Welwitschia*. In this species there is a disc of fifteen simple leaf-like segments, fused at the base, projecting freely above; the free portion of each segment bears a row of paired bilocular synangia, while below the free portion this row is continued downwards by synangia which have become abortive. So that here reduction in the size of the segments and abortion of the lower synangia has already begun. Granted only the further continuation of reduction and abortion, the separate flattened segments of such a disc may easily be compared with one of the two flattened segments, fused below, free above, of the sporangiophore of such a species as *Ephedra distachya*, in which a row of only two pairs of bilocular synangia is borne by each half of the bipartite disc. *Welwitschia* differs in that the six members of its disc, similarly fused below, are continued upwards, not as flattened segments bearing paired bilocular synangia, but as cylindrical axes each terminating in a single trilocular synangium. In Wieland's staminate disc of El Consuelo,[5] in some respects less reduced than *W. whitbiensis*, the free portions of the disc are not flattened but cylindrical, and bear paired lateral synangia on stalks, and we have already seen in *Ephedra* itself[6] how easily a stage with paired lateral synangia can undergo reduction by fusion and abortion of the synangia, in the process of fusion trilocular synangia being produced. All that is required to derive the male disc of *Welwitschia* from Williamsonian discs such as these is the abortion of the lower pairs of synangia, already begun in *W. whitbiensis*, and the fusion of the topmost pair to form a trilocular synangium, such as is produced by fusion in *Ephedra*.

B. *The Female Strobilus* (cone). The restricted number of ovules in the female strobilus (cone) of *Ephedra* stands in marked contrast to the numerous ovules found in the strobili of the other Gnetales. In the groups of species in which the bracts are succulent the whole strobilus is very

[1] Wieland, 1911, p. 461. [2] l. c., p. 438. [3] Nathorst, 1910.
[4] Nathorst, 1911, especially Fig. 3, p. 13. [5] Wieland, 1909, p. 433. [6] p. 971.

reduced in size, only three or four pairs of bracts being generally developed. The Alatae, however, the group in which numerous membranous bracts occur below the single fertile pair, is suggestive of an originally greater development than is found at present, which would be more comparable with the strobilus of *Welwitschia* with its numerous membranous bracts and axillary ovules. *E. alata*, *Torreyana*, &c., which have numerous bracts more or less loosely arranged and ovules which stand fairly free in the centre of the strobilus, are, we think, nearer the primitive form than the more reduced species. It is probable that more than a single whorl of bracts was originally fertile; indeed in one cone of *E. altissima* small masses of abortive tissue suggestive of undeveloped sporangiophores were found in the axils of the bracts next below the fertile ones; perhaps at the base of the strobilus there were male flowers as now in *E. fragilis*, var. *campylopoda*.

The increase in thickness and succulence of the bracts of groups other than the Alatae is accompanied by their decrease in number and by greater pressure on the ovules. These no longer stand free in the centre of the strobilus, but are tightly enclosed by the subtending bracts, and the three well-marked wings, each with its vascular bundle characteristic of the ovules of the Alatae, become modified by pressure. The ovules become laterally flattened, the two lateral wings being retained while the median one is lost; in *E. fragilis* and *E. nebrodensis* a small median wing with its vascular bundle is still present, but in *E. distachya* there is little trace of a median angle to the seed, and the median vascular bundle is hardly ever formed.

In *E. altissima* the very thick and succulent fertile bracts are fused together and tightly enclose in a cup the single ovule which has finally resulted from fusion of the two ovules originally present. The seed is no longer winged, but is round and almost smooth except at the apex where three or four slightly projecting ribs or angles can be distinguished.

C. *The Female Flower*. The ovule in *Ephedra*, like the male flower, is axillary in position, and perhaps as a consequence of this position it receives its bundles in the same manner as a vegetative axillary bud. Whether these facts can be regarded as proof that it is therefore the equivalent of a vegetative axillary bud is not easy to decide.

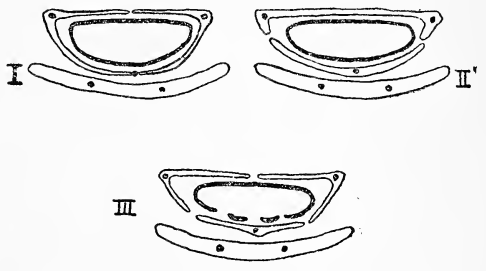
From a study of *Welwitschia* one of us[1] was inclined to conclude that it was wisest to term the axes of both male and female flowers 'sporangiophores', but as has been said in the case of the male flower there is little to support this terminology, and the direct evidence as to the foliar nature of the flattened and bifid male sporangiophore in *Ephedra*, which is emphasized by the fern-like vernation of the organ in *E. fragilis* when in bud, is on the

[1] Sykes, 1910.

whole against it, and supports the view that the male flower represents an axillary strobilus.

Attempts have been made by various authors [1] to show that the coverings of the ovules represent fused bracts, and that the female flower is consequently an axillary bud bearing one or two whorls of bracts and terminating in an ovule. Some have also tried to prove that the ovule is not the termination of the axis of this bud, but is borne on one of the surrounding bracts, which represents a carpel.

Strasburger, in 1872, put forward the view that the outer covering in *Ephedra* and *Welwitschia* is the equivalent of a pair of leaves arranged, like the first leaves of an axillary shoot, transversely to the subtending bract. Jaccard also held the same view. According to it (Plan I, Text-fig. XXI) each half of the nut represents a leaf with a single median bundle instead of the pair of bundles formed in the normal vegetative leaf. But this suggestion is rendered unlikely by the fact that there is often a third bundle, situated in a position which would correspond to the fused margins of the leaves. Strasburger changed his opinion in 1879 and came to regard both coverings of the ovule as integuments.



TEXT-FIG. XXI. Plans of the ovule of *E. distachya*. I. According to the view of Strasburger (first pair of leaves). II. According to the view of Van Tieghem (second pair of leaves). III. According to the view of Lignier (whorl of three leaves).

Van Tieghem regarded the outer covering as composed of two leaves, corresponding in position to the second pair of leaves of an axillary bud, in the same plane as the subtending bract; the flattened surface of the nut with its two angles representing one leaf, traversed by two vascular bundles, the rounded, sometimes angled, outer portion representing the more or less abortive second leaf, sometimes supplied with a single vascular bundle. He regarded the ovule as borne on the inner leaf of the pair, a suggestion for which there is no evidence.

Lignier attempts to overcome the difficulties involved in demonstrating that the outer covering of the ovule represents a pair of leaves by the suggestion that it represents three leaves, the three vascular bundles belonging each to one of the three leaves; the female flower would thus become trimerous, the outer of the three leaves being more or less abortive. The inner covering he also regards as representing a whorl of three bracts, forming a tricarpellary ovary, the inner bract being the most developed, the two outer more or less abortive. There is no evidence to support the latter suggestion, and trimery is very infrequent in *Ephedra*. His attempts to

[1] Strasburger, 1872; Van Tieghem, 1869; Jaccard, 1894; Lignier, 1901; Arber and Parkin, 1908.

use the four-angled ovules of *Ephedra altissima* to support his view are not successful in the light of the anatomical investigations described in the current paper; for so far from it being possible that the outer covering in these cases represents only two of the leaves composing the outer covering in one of the ovules of the biovulate cones, it is found that it represents the fused outer coverings of both ovules, and its four vascular bundles represent the six bundles of the two ovules.

All theories which attempt to explain the outer coverings as composed of leaves do not meet the difficulty of the different method of vascular supply. Each of the bundles supplying the integument originates as the centre one of a group of three bundles formed from one of the bundles in the ovular base, the two lateral bundles remaining in the axis (Text-figs. II. 11, and III); the integumental bundles of *Welwitschia* are commonly formed in the same manner, but leaf-trace bundles do not arise in this way.

It appears to us that all attempts to compare the outer covering to leaves requires some distortion of the evidence, and that it is more correct to regard it as an integument in the ordinary sense of the word—a covering of the ovule of problematical origin. This is still more true of the inner covering, which with its micropylar tube is obviously comparable with the integuments of other Gymnospermous ovules. The complex structure of the outer covering is also unlike that of the ordinary leaves and is much more comparable, not only with that of the outer covering in *Welwitschia* and the middle one of *Gnetum*,[1] but with that of the outer covering of other ovules, more especially those of the Bennettitales. The resemblance[2] between the outer covering of *Gnetum* and the integument of *Bennettites* to which attention has already been drawn is still further emphasized in *Ephedra*; for here the three or four fibrous strands representing the star-like rays of the fibrous layer in *Gnetum* and *Bennettites* each correspond to an external angling of the seed, such as is present in *Bennettites*.[3] The remarkably detailed resemblance in the structure of their outer integuments between the three members of the Gnetalean alliance, their leaves being so different, is alone a striking fact, and suggests that the coverings of the ovule are independent structures, less plastic than the leaves.

The ovule with its two integuments thus remains an isolated structure in the axil of the bract. There is no evidence to suggest that the ovule was ever borne on the subtending bract, and there is no direct evidence, except the analogy between its vascular supply and that of a vegetative bud, that

[1] It is clear from the structure that it is the middle covering of *Gnetum* which is homologous with the outer covering of the other two forms; Thoday (Sykes), 1911.

[2] Berridge, 1911; Thoday (Sykes), 1911.

[3] Wieland's recent figures of the ovule in *Cycadeoidea turrita* (1911, p. 458, Fig. 15 *c*, and 1912, p. 90) still further strengthen the comparisons which have been made between the ovules of *Gnetum* and *Cycadeoidea*, though his comparison with *Gnetum*, 1911, p. 458, Fig. 15, *a* and *b*, is evidently based on some misapprehension.

the little axis on which it is borne represents an axillary bud, for it bears no leaves. But the male flower has already been compared with a little strobilus, and it seems probable in view of the evidence of reduction in the family that the female flower also represents a very reduced strobilus. If like the male flower it is to be compared with the strobilus of the Bennettitales, then the single ovule must represent the whole ovulate cone in that family; the comparisons which have been made by other authors between the male flower of *Welwitschia* and the Bennettitean strobilus necessitate this presumption, and direct evidence of such a fusion of ovules and their coverings has here been recorded in *E. altissima*, where the single terminal ovule represents the two fused axillary ovules of other species.

It seems that while there is strong evidence that the male sporangia are foliar, we are driven to the view that the ovule terminates an axillary shoot. In the Bennettitales also the male sporangia are undoubtedly foliar, but we are still not certain as to the seed, though the view that the seed pedicel is the equivalent of an interseminal scale and therefore foliar appears to be gaining ground. If the single ovule in *Ephedra* and *Welwitschia* represent the mass of foliar ovules and scales in *Bennettites*, being derived by reduction and fusion from a strobiloid condition probably more simple than that actually found in that family, this difference between male and female sporangia would be accounted for.

The single ovule now differentiated direct from the plastic apex of the axillary bud would thus be the equivalent of more than one ovule, each originally borne on a foliar organ, but now fused together at the apex of an axis, much as the apical pair of synangia in *E. altissima* and perhaps the single trilocular synangium of *Welwitschia* are the equivalent of several pairs of synangia originally borne on foliar appendages, but now fused together at the top of what falsely appears to be a cylindrical axis since it shows so little trace of its primitively bifid and leaf-like condition. It may be that there is as yet insufficient evidence for this analogy between micro- and megasporangia, but at any rate the great amount of reduction which the microsporophyll has undergone, even among existing forms, as evidenced by its variation from a leaf-like organ bearing eight synangia to a small cylindrical axis carrying only two, renders it quite probable that in the female flower also we have the final stage of a long series of reductions, the last trace of which is still to be seen to-day in the fusion of ovules in *E. altissima.*

IV. Comparison of the Inflorescences of the Three Members of the Gnetales.

Of the three genera of the Gnetales it appears from their anatomy that *Ephedra* and *Welwitschia* are most closely related to one another. The dichasial branching of the male inflorescences of all species of *Ephedra*

and the more or less regular dichasial branching of the female inflorescences of *E. altissima* is a point of similarity with both the male and female inflorescences of *Welwitschia.* In *Welwitschia* the individual strobilus is elongated and produces numerous fertile bracts and flowers, fifty or thereabouts in the female and still more in the male [1] with a few sterile bracts at the base.[2] In *Ephedra* the male strobilus bears only ten to fifteen whorls of fertile bracts, and the female strobilus is of still more limited growth. It has already been remarked that the strobili of the Alatae with their numerous membranous bracts are suggestive of an originally greater development than is found at present, and even the bracts themselves in these species bear a close resemblance to those of *Welwitschia.*

In *Gnetum* also the male inflorescence is dichasially branched and both male and female inflorescences would seem from their general arrangement to be comparable with those of *Ephedra* and *Welwitschia.* But when we come to examine the individual strobili we find that the cupule at each node is the equivalent of the pair of bracts at the node in the other genera, subtending not two but a cushion bearing six or more ovules in the female inflorescence and numerous antherophores and abortive ovules in the male. There are many more points widening the gap between *Gnetum* and the other two genera: the ovule has an extra covering and both this and the outer integument are radially symmetrical and supplied by numerous vascular bundles; the micropylar tube has a complex mechanism for closing it; the membranous appendages of the male flower have a vascular supply. The complicated method of bundle supply [3] of the ovule and antherophores in *Gnetum* is also peculiarly characteristic, while the method of supplying these organs in the other two genera is very simple and quite comparable, there being, however, no median axillary bundle in *Welwitschia.*

In comparing the individual flowers of the three genera, it is again found that *Ephedra* and *Welwitschia* show most resemblance to one another, though here the signs of relationship are sufficiently clear to justify the placing of all three forms in the Gnetales.

In *Welwitschia* the male flower consists of an abortive ovule, surrounded by a disc of six male sporangiophores fused at their base, and four membranous appendages. In *Ephedra* the male flower bears two membranous appendages, and evidence has been brought forward to show that the antherophore probably consists of a disc of two fused sporophylls surrounding an abortive apex.[4] With respect to the form of its microsporophyll (as also in its gametophyte) *Ephedra* appears the more primitive of the two; each

[1] Pearson, 1906.

[2] The lowest pair of sterile bracts is distinguished from the others by their acute apices; the same characteristic also occurs in the lowest pair of sterile bracts in the male strobilus of *Ephedra* (p. 953).

[3] Thoday (Sykes), 1911, and Pearson, 1912.

[4] Thibout, 1896, Part III; Arber and Parkin, Part I, p. 502.

half of the antherophore with its paired bilocular synangia resembling far more closely one element of the disc of a *Williamsonia* than the microsporophyll of *Welwitschia* with its trilocular stalked synangium.

The ovules of *Ephedra* and *Welwitschia* being in similar compressed positions differ in some respects from the freely projecting ovule of *Gnetum*, but in other ways they show remarkable similarity. They all have an inner thin membranous covering, fused with the nucellus below, free above, and prolonged into a narrow tube, lined in *Ephedra*, *Welwitschia*, and some species of *Gnetum* by a thick cuticle. The inner ring of vascular bundles, which in *Gnetum* is prolonged into the free part of this inner integument, in *Welwitschia* terminates at the level at which it becomes free from the nucellus, and in *Ephedra* at a still lower level.

They all also have a thick outer covering. In *Ephedra* it is angled, and in *Welwitschia* it has two well developed wings; in *Gnetum* it is smooth. The stony layer of this outer covering is always strongly developed at points corresponding to the external angles, and even in the smooth seed of *Gnetum* it is angled.

Ephedra and *Gnetum* both have pollen-chambers, one of the signs of their greater primitiveness than *Welwitschia*, but even in *Welwitschia* there is some disorganization at the apex of the nucellus.[1]

The above evidence as to the reduced nature of *Ephedra* and its closer relationship to *Welwitschia* cannot be taken as indicating that it is reduced from *Welwitschia*. Its gymnospermous gametophyte and its well-developed pollen-chamber prohibit such a conclusion. But *Ephedra* and *Welwitschia* together appear to have retained in common numerous points which separate them off from *Gnetum* with its many singular characteristics.[2] Both genera show many signs of reduction, and *Welwitschia* has also, while retaining a less reduced strobilus than *Ephedra* and more primitive male flowers, undergone many remarkable vegetative modifications and a special elaboration of its ovules in the great growth of the nucellar apex correlated with its peculiar methods of fertilization.

V. Summary.

1. The vascular system of the inflorescences and flowers of various species of *Ephedra* is described. It is found that the method of supply of the axillary flower buds is similar in essentials to that of the vegetative buds in the axils of the ordinary leaves. Each vegetative bud and each flower receives three bundles: a median abaxial bundle (afterwards branching into two in the vegetative bud), intimately connected with the bundles of the subtending leaf, and two adaxial bundles which originate partly or entirely from the adjoining bundles of the main stem.

[1] Pearson, 1906, Fig. 28, Pl. XIX, pp. 289–90.

[2] See also Pearson, 1912, p. 614; the male gametophyte, on the contrary, is most closely comparable in *Gnetum* and *Welwitschia*; l. c. p. 618.

2. The three bundles entering the axillary ovule branch each into three. In some species (*E. Torreyana*, *E. alata*, *E. fragilis*) the median bundle of each trio passes out into the outer covering; in other species (*E. distachya*) the abaxial trio does not provide any contribution to the outer covering, which thus receives only two bundles. A special case is afforded by the uniovulate species, *E. altissima* and *E. trifurca*, in which the two fertile bracts enclose only a single ovule. This receives either four or three bundles, which clearly represent the fused vascular systems of the two ovules of the biovulate species.

3. That the single terminal ovule of these species is actually the product of the fusion of the two axillary ovules of the biovulate species is clearly demonstrated by a long series of intermediate forms in *E. altissima*; among which occur biovulate cones, each ovule having two coverings, biovulate cones with a common outer covering, and uniovulate cones in which an abortive nucellus is more or less fused at the apex with a fertile one.

4. The structure of the outer covering and its method of vascular supply do not support the view that it represents the first whorl of leaves of an axillary shoot. It is here regarded as an integument. It is more or less angled in the various species and is composed, roughly, of an outer brown-celled layer and an inner fibrous layer. The vascular bundles traverse the angles and are accompanied towards the apex of the ovule by strands of fibres; they terminate in transfusion tissue. The structure of the outer integument is thus very similar to that of *Gnetum* (middle covering), except that there is here no palisade layer and that the angling of the inner fibrous layer corresponds to the outer angling of the seed which does not occur in *Gnetum*, but is present in *Welwitschia*. The comparison formerly drawn between *Gnetum* and *Bennettites* is thus further emphasized by the study of *Ephedra*.

5. The inner covering has no vascular supply, the ring of bundles entering the ovule terminating near its base. It is free from the nucellus for the upper third of the ovule and is prolonged upwards as a micropylar tube with a very strongly cuticularized lining. The opening of the micropylar tube is closed in the fertilized ovule by a hardened plug of mucilage, and the chink between the two integuments is closed by papillae which grow out from the epidermis of the outer covering and firmly clasp the inner. The fertilized ovule is thus as efficiently protected as in *Gnetum* with its complex mechanism. Both arrangements are, physiologically, abortive attempts at Angiospermy.

6. The different species of *Ephedra* exhibit much variation in the number of male sporangia. In *E. distachya*, *E. fragilis*, *E. nebrodensis*, &c., the sporangiophore is clearly bifid, and each half bears four bilocular synangia. In some species such as *E. aspera*, *E. Torreyana*, &c., there is no clear separation into two halves, and the upper pair of synangia are fused

together. Other species exhibit further stages in this reduction process, more and more synangia fusing together, often forming in the process trilocular or even quadrilocular synangia, until *E. altissima* is arrived at with only two bilocular synangia. The formation of trilocular synangia as a result of fusion affords a link with *Welwitschia*, where terminal trilocular synangia are normally produced. In most species the synangia are nearly sessile, but in *E. Torreyana* and a few others they are borne on a stalk of some length, reminiscent again of the stalk freely projecting above the fused portion of the staminal whorl in *Welwitschia*.

7. From the evidence available it is concluded that the structures in the axils of the fertile bracts in the male cone are to be regarded as flowers, or little strobili, each consisting of one axis bearing four leaves. The first pair of leaves, which, except in *E. fragilis*, receive no vascular supply, are orientated like the second pair borne by a vegetative axillary bud, and the two flattened halves of the fertile organ thus appear to represent the third pair of leaves fused with one another back to back. In *E. fragilis* they exhibit circinnate vernation in the bud.

8. This bipartite sporangiophore with its paired bilocular synangia is compared with the six-partite disc of sporophylls in *Welwitschia* and with the multipartite disc in the Bennettitales. It is thought that we can trace the steps of a reduction series from the disc of *Cycadeoidea* with its bipinnate sporophylls, through such stages as *Williamsonia whitbiensis*, in which the segments are small and simple, and each bears a row of paired bilocular synangia, the lower of which are abortive ; and the disc of El Consuelo in which the freely projecting portions of the disc are no longer flattened but bear stalked synangia ; to *Ephedra*, where the disc is reduced to two segments, each bearing two pairs of bilocular synangia, and *Welwitschia*, where it is composed of six segments, each with a stalked terminal trilocular synangium. From the reduction series in *Ephedra* itself it is seen how from the fusion of bilocular sporangia a single trilocular stalked synangia can be produced.

9. Whether the female flower also in *Ephedra* and *Welwitschia* is, like the male, morphologically a little strobilus and the equivalent of an axillary bud, it is not easy to decide, since it consists of an isolated ovule ; the more complicated relations occurring in *Gnetum* make the matter still more difficult. The male sporangiophores having been related with some show of probability to the disc of sporophylls in the Bennettitales, it seems justifiable, considering the many signs of reduction in the Gnetales, to suggest that the single ovule now developed at the apex of the axillary structure in the male and female flowers of *Welwitschia* and in the female flower of *Ephedra* represents the many ovules and interseminal scales of such a flower as *Cycadeoidea* fused together. This is rendered the more possible by the discovery that fusion of ovules actually occurs in *Ephedra*, resulting in the production of a uniovulate from a biovulate cone.

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DESCRIPTION OF PLATE LXXXV.

Illustrating the paper by Mrs. Thoday and Miss Berridge on the Inflorescences and Flowers of *Ephedra*.

Fig. 1. Young male inflorescence of *E. distachya*, consisting of peduncle bearing three strobili. *St.* = sterile bracts; B = fertile bracts, in the axils of which are the male flowers. The young synangia, enclosed in the two leaf-like appendages (P) are seen projecting a little way out between the bracts. × 16.

Fig. 2. Basal portion of female inflorescence of *E. altissima*, showing loose cluster of female strobili borne on a branched axis which hangs downwards from the climbing stem. × 2.

Fig. 3 *a*. Single female strobilus of *E. distachya*, detached from its position in the axil of a leaf on the ordinary vegetative stem. × 2.

Fig. 3 *b*. Single female strobilus of *E. fragilis* in situ. The micropylar tubes of the two ovules can be seen projecting from the enclosing bracts. × 16.

Fig. 3 *c*. Single female strobilus of *E. alata*, showing the numerous membranous bracts with prominent midribs, and two projecting ovules. × 2.

Fig. 3 *d*. Single ovule of *E. alata*, showing one of the three wings. × 2.

Fig. 4. Male sporangiophore of *E. distachya*, front view, bearing eight synangia. × 16.

Fig. 5 *a*. Male sporangiophore of *E. nebrodensis*, front view. × 16.

Fig. 5 *b*. Male sporangiophore of *E. nebrodensis*, back view. × 16.

Fig. 5 *c*. Young male sporangiophore of *E. nebrodensis*, seen in bud, enclosed by two leaf-like appendages. × 16.

Fig. 6 *a*. Male sporangiophore of *E. altissima*, young, bearing two synangia. × 16.

Fig. 6 *b*. Ditto, older. The long transparent stalk is seen to be traversed by two vascular bundles (*vb*). × 16.

Fig. 7. Transverse section through portion of bract of outer cupule. *Tr* = transfusion tissue. × 78.

Fig. 8. Longitudinal section through stoma of bract, showing cuticularized ridges. × 116.

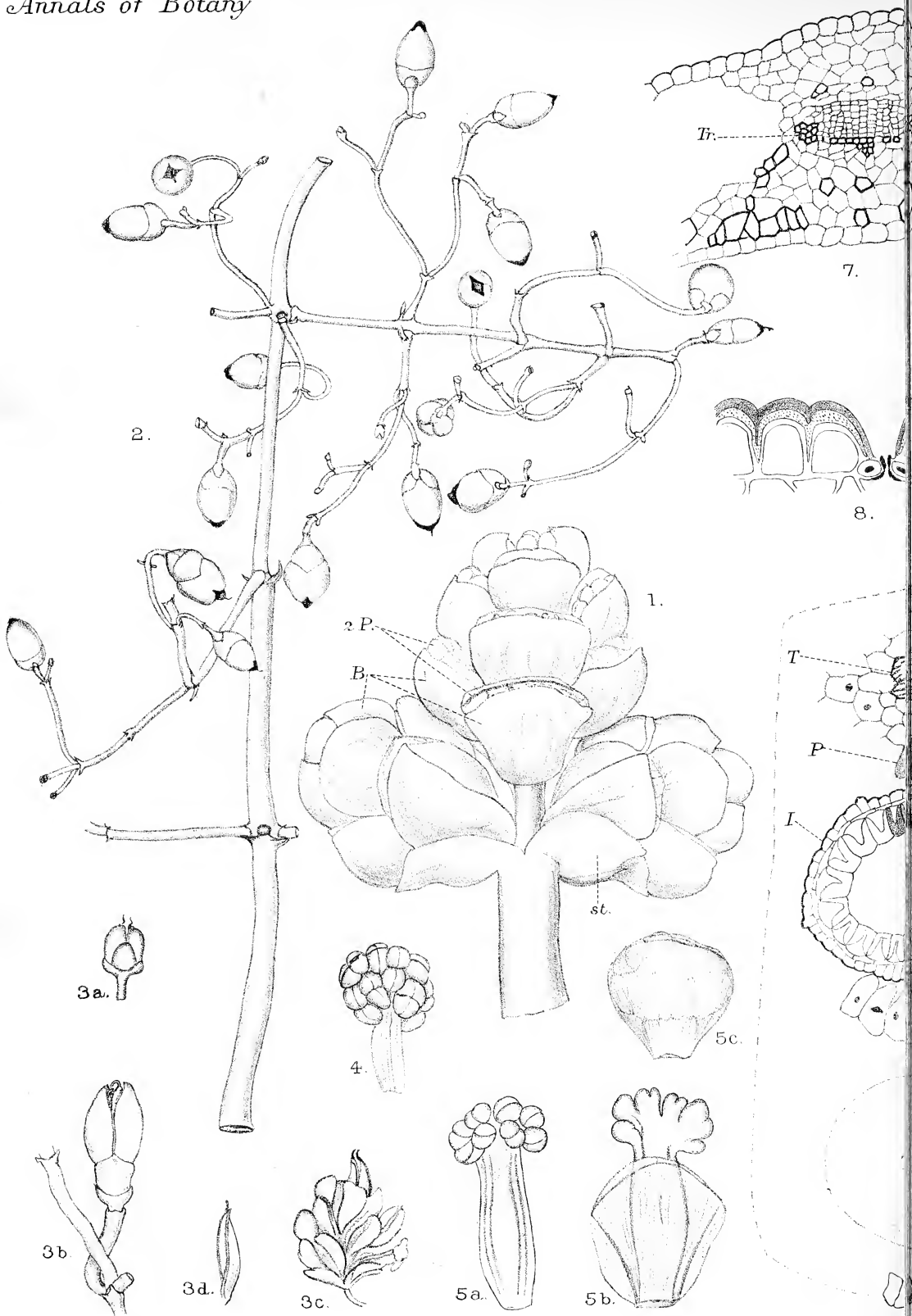
Fig. 9. Apex of cone of *E. fragilis*, showing the two ovules with their integuments. *c* = cup of suberized cells. × 25.

Fig. 10. Ovule of *E. altissima* with double nucellus, only the left-hand half of which is fertile; the two inner integuments, free at the base, are fused at the apex into one. *d. n.* = double nucellus, the left-hand half of which contains a prothallus (*pro.*); *i.int.* = inner integument.

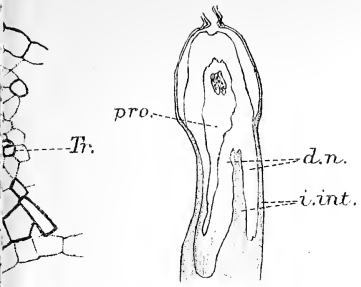
Fig. 11 *a*. Transverse section of apex of young unfertilized ovule of *E. distachya*. *b* = brown tissue; I = still open micropylar tube with cuticularized lining; *p* = papillae; *Tr* = transfusion tissue. × 116.

Fig. 11 *b*. External view of nut of *E. distachya*, showing small angles. × 16.

Fig. 12. Longitudinal section of apex of nucellus of *E. fragilis*, showing deep pollen-chamber. × 90.



THODAY & BERRIDGE.——EPHEDRA.



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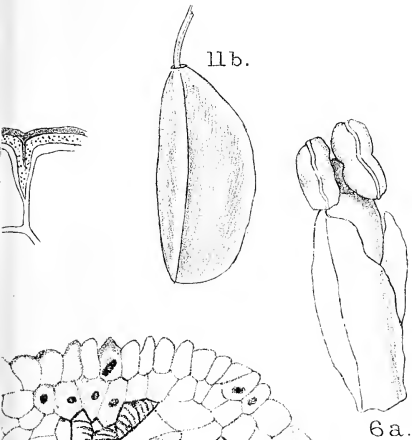

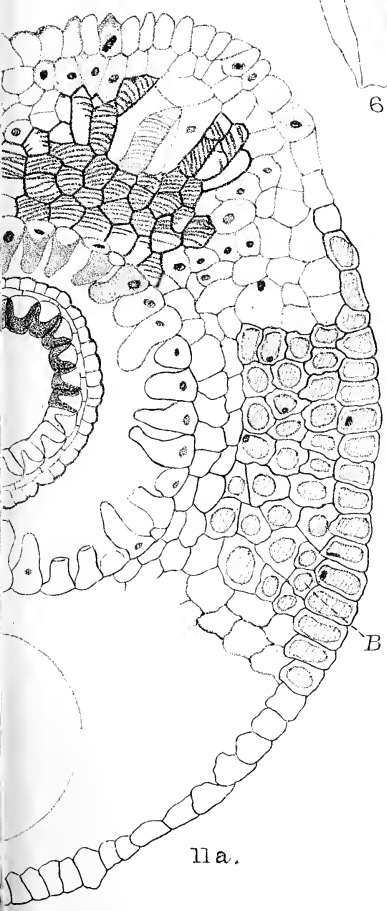

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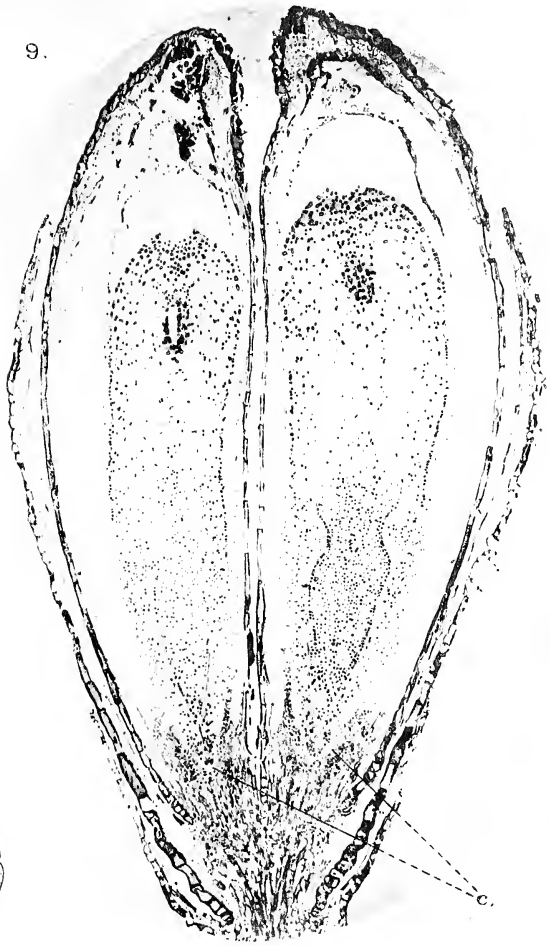

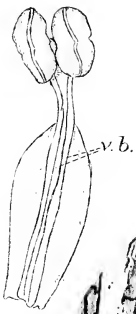

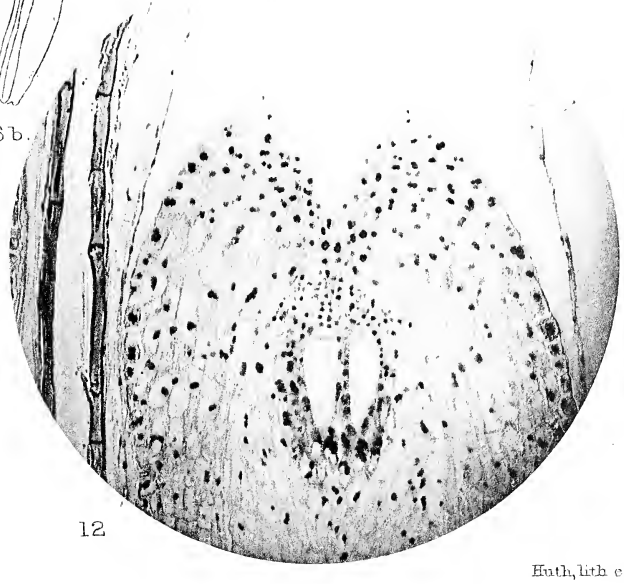

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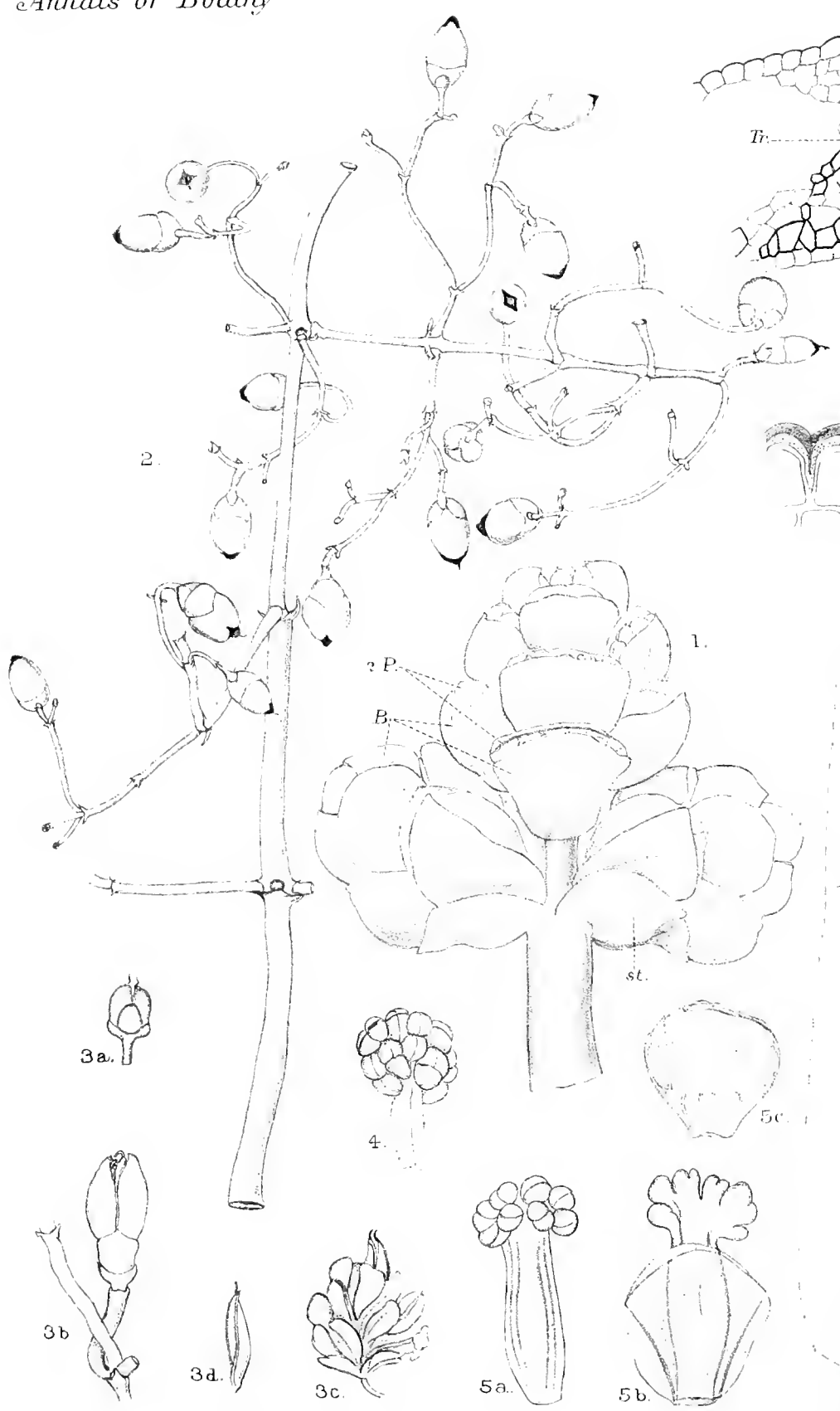

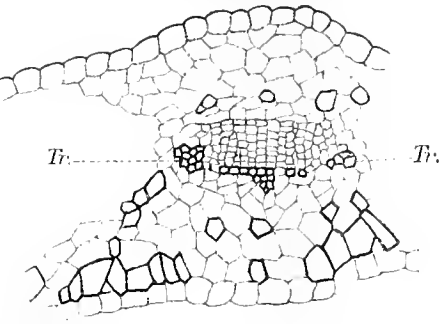

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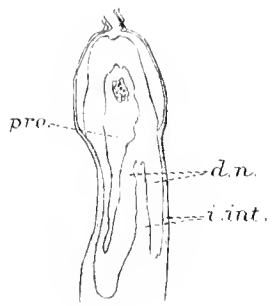

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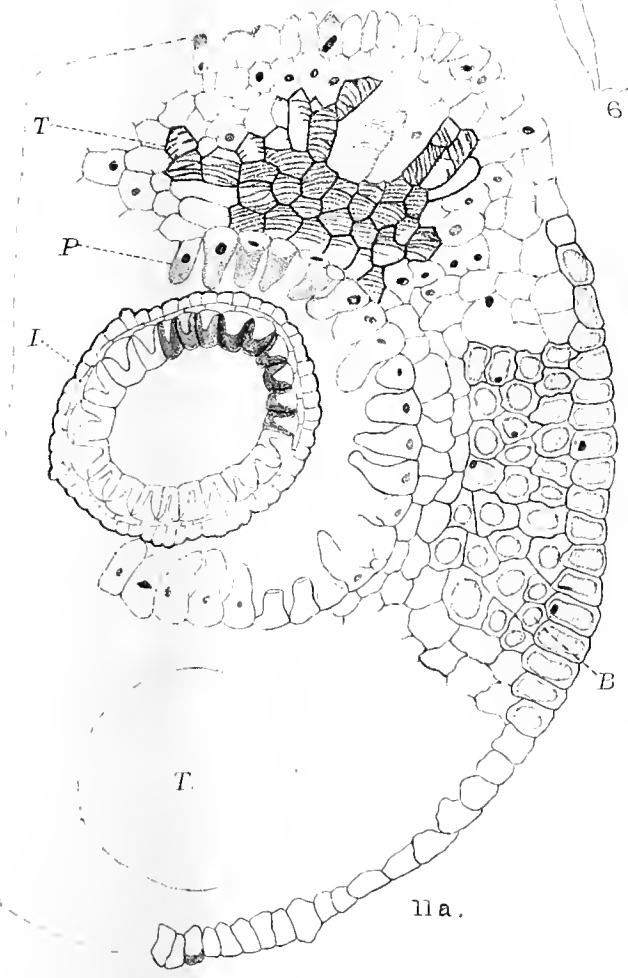

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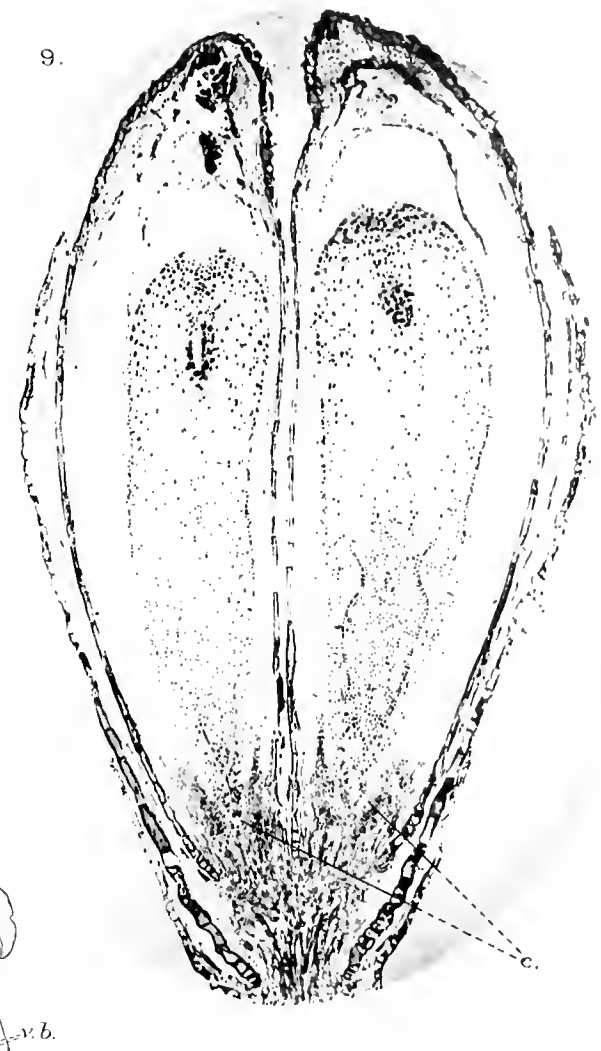

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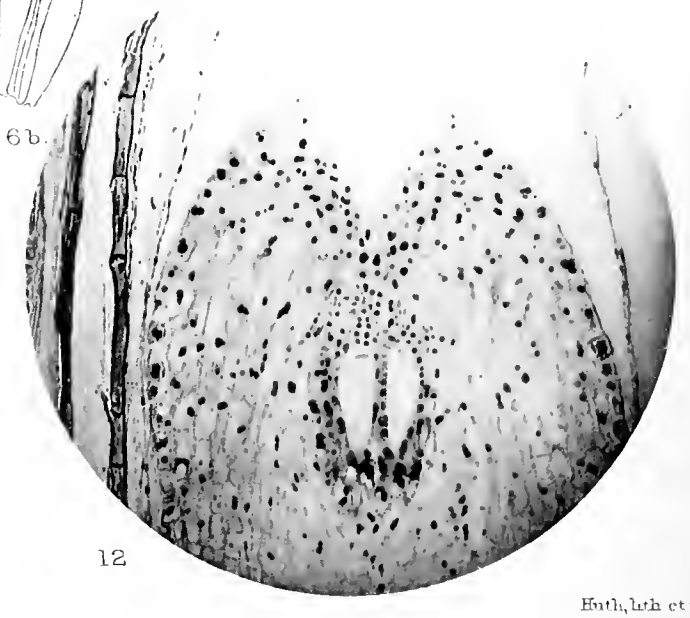

12

THODAY & BERRIDGE.——EPHEDRA.

Huth, lith et imp

The Structure of the Female Strobilus in Gnetum Gnemon.

BY

EMILY M. BERRIDGE, B.Sc., F.L.S.

With four Figures in the Text.

VERY full and detailed accounts of the vascular supply to the flowers of various species of *Gnetum* have recently been published,[1] but in no case apparently do the ovules investigated show a certain series of complexes which constitute a striking feature in the vascular system of the female 'flower' of *Gnetum Gnemon*.

In this species, as in other Indo-Malayan forms,[2] the 12–14 bundles which traverse the base of the female flower all spring from the bundles which supply the cupule, and only a very few late-formed branches originate directly from the main vascular system of the inflorescence. Each of these bundles, on reaching the level of insertion of the outermost of the three coats of the ovule, becomes very broad and gives off two traces to this outer coat or 'perianth'; then turning inwards and upwards each passes on to supply the two inner coverings, which are probably best regarded as integuments.

Just above the point of departure of the two traces to the outermost coat (Fig. 1, p_1, p_2) each main bundle gives rise to a curious complex of vascular strands.

Fig. 2 shows a tangential section of one of these, while Figs. 3 and 4, representing two sections through a single complex, show more clearly the relative positions of the main bundle, *m.*, the traces p_1, p_2 passing to the outermost covering of the ovule, the complex, *c.*, and the main bundle, *int.*, passing on towards the integuments.

Although, as in this case, the complexes often take the form of loops, they also frequently consist of three or four vascular strands, which end

Thoday, M. G. ('11) : The Female Inflorescence and Ovules of *Gnetum Africanum*, with notes on *Gnetum scandens*. Ann. of Bot., xxv, p. 1101. Pearson, H. H. W.: The Microsporangium and Microspore of *Gnetum*. Ann. of Bot., xxvi, p. 614.

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[Annals of Botany, Vol. XXVI. No. CIV. October, 1912.]

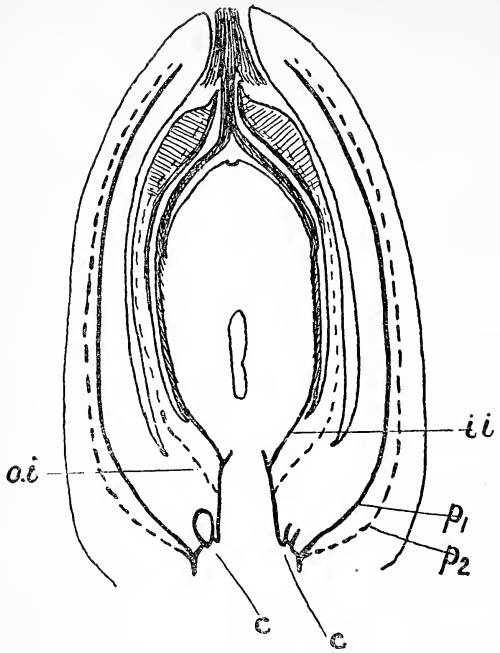

FIG. 1. Longitudinal section through young seed of *Gnetum Gnemon*. p_1, p_2, bundles supplying outermost coat; *o.i.*, bundle supplying outer integument; *i.i.*, bundle supplying inner integument; *c.*, complex of vascular strands. The bundles indicated by dotted lines do not lie in the plane of the section.

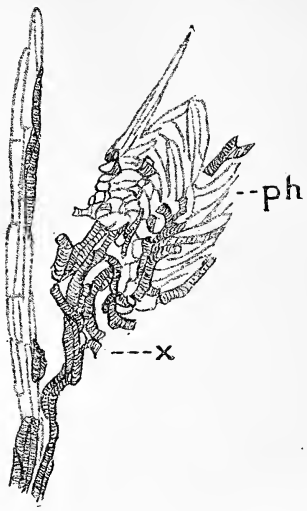

FIG. 2. Tangential section of a single vascular complex. *x*, xylem; *ph.*, phloem.

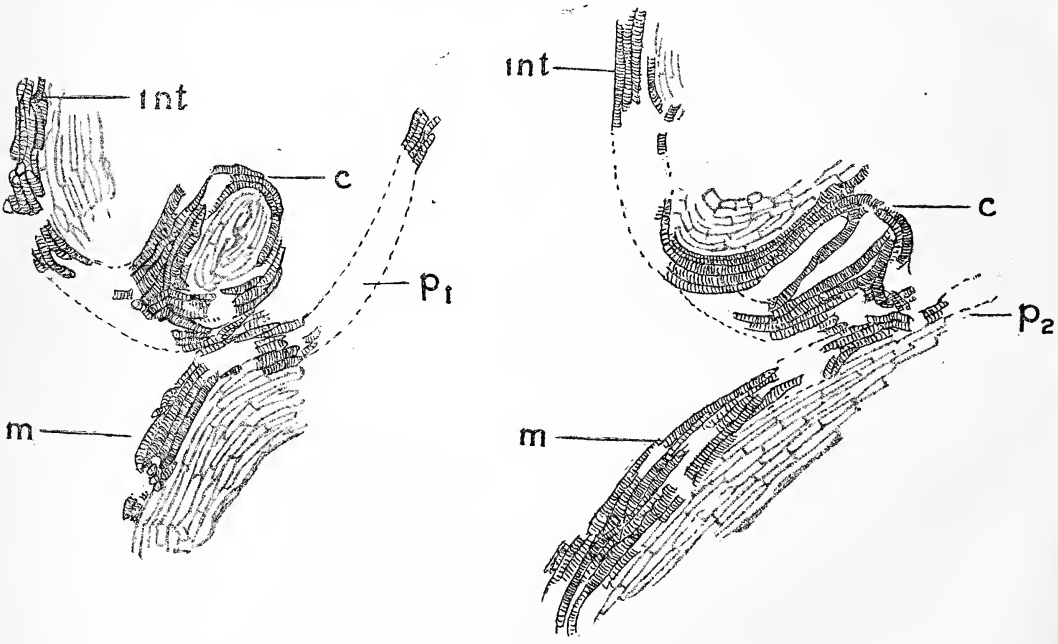

FIG. 3. Longitudinal sections through a single vascular complex. *m.*, main bundle; p_1, p_2, bundles to outermost coat; *c.*, complex; *int.*, bundle to integuments.

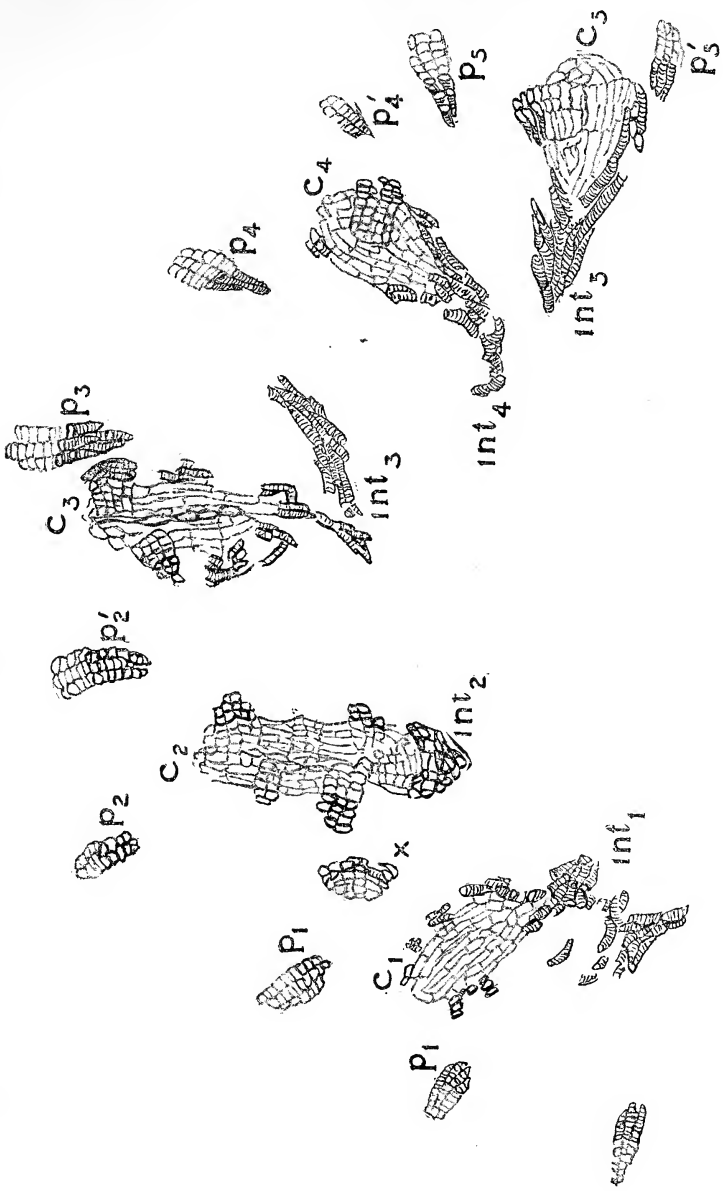

FIG. 4. Transverse section through half the base of a flower. p_1, p_1, p_2, p_2, &c., bundles to the outermost coat; int_1, int_2, &c., bundles to the integuments; c_1, c_2, c_3, &c., vascular complexes.

blindly in the tissue below the groove between the second and third coats of the ovule.

In Fig. 4 the vascular complexes are shown as they appear in a cross-section through the base of the flower. They always contain a considerable mass of phloem, but the xylem tends to break up into separate strands, and in some cases, as at *x*, independent bundles are formed.

This whorl of twelve or more complex groups of vascular tissue, constantly appearing in the same position below the ovule, can hardly be dismissed as a mere irregularity of the bundle system. It seems not improbable that we have here the vestiges of the vascular supply of some whorl of organs which was situated between the outermost covering and the outer integument of the ovule.

It seems justifiable to assume that these complexes did at one time serve a whorl of organs surrounding the ovule, for although in the process of reduction the vascular bundles seem usually to dwindle and disappear before the organ itself is lost, as in the case of the perianth of the male flower of *Ephedra* or the abortive ovule of that of *Welwitschia*, yet these complex vascular structures may have survived, as has been suggested by Dr. Scott, because they were adapted to the storage of water. Also a very clear and somewhat similar case of the presence of vestigial bundles has been met with in the oak. In some cross-sections of a young flower the stamens were found to be represented by small outgrowths alternating with the stigmatic lobes. Just below these minute outgrowths, small branches from the vascular bundles supplying the perianth end in little irregular masses of reticulately thickened cells. In another flower of about the same age, the outgrowths are absent, but the small branch bundles persist.

The position of these vascular complexes in the base of the female flower of *Gnetum Gnemon* recalls that of 'the small complexes of four or more bundles' which Prof. Pearson describes as supplying the male flowers in other species of *Gnetum*.[1] In both cases they spring from bundles which pass on to form the vascular supply of an ovule, and they are situated just above the junction of these ovular traces with the traces of the protective bracts which in one case form the so-called perianth, in the other the cupule of the male inflorescence. If the presence of these vascular structures in *Gnetum Gnemon* is evidence for the existence, originally, of a whorl of male flowers surrounding the base of the ovule, the primitive form of the strobilus would have been an axis terminated by a female flower and bearing a single ring of male flowers, the whole protected by a cupule—the so-called perianth. This closely resembles the type of inflorescence suggested by Prof. Pearson as possibly the ancestral form; in his study of the structure of the male inflorescence he draws the following conclusion: 'Since the inflorescence may consist of as few as two flowering nodes and a terminal segment (which in some cases is an ovule with appendages) it is conceivable that the ancestral type of inflorescence was an axis bearing a single lateral ring of male flowers and a terminal female flower.'

The vascular knots may possibly have supplied a whorl of microsporophylls rather than complete male flowers; the female flower of *Gnetum* would then be comparable to the male flower of *Welwitschia*, and the presence

[1] Ann. of Bot., vol. xxvi, p. 614.

of these vascular structures might be taken as evidence for the hypothesis that the whole family originally possessed bisexual flowers. There are indications that the organs in the whorl were grouped in four sets of approximately three members, for the main bundles just above the insertion of the complexes are linked together by anastomosing strands in this manner. Hence the whorl may have consisted of four members of threefold nature similar to the microsporophylls of the *Welwitschia* flower. The structure of the male inflorescence in *Gnetum*, however, points to an association of male flowers rather than of microsporophylls with the ovule; hence it seems best to regard the female flower as representing at the present day a partial inflorescence and not a proanthostrobilus.

It follows from such a view that the apparently simple spike now constituting the whole female inflorescence of *Gnetum* is really of compound nature, consisting primitively of a central axis with a series of cupules from the axils of which sprang secondary axes bearing a ring of male flowers and a single terminal female flower. From such a compound inflorescence, moreover, could be derived an inflorescence closely resembling, both morphologically and anatomically, the male spikes of *Gnetum Gnemon*, *G. scandens*, and other Indo-Malayan species. Suppression of the first internode of the secondary axis, a modification which constantly occurs in many species of *Ephedra*, would bring the terminal female flower and the ring of male flowers into the axil of the cupule, and the outermost protective covering of the partial inflorescence, being now unnecessary, would disappear. The shortening of the first internode of the vegetative branches is accompanied in *Ephedra altissima* and frequently in *E. nebrodensis* by a shifting of the axillary buds of the first node from their normal lateral position to the abaxial side of the branch. The suppression of the internodes in the branches of the primitive inflorescence of *Gnetum* may have been similarly accompanied by a shifting of the male flowers so that they have become crowded together in the axil of the cupule below the abortive ovule, and reduced in number.

In the vegetative branches of *Gnetum Gnemon* suppression of the first internode is not clearly evident, but the presence of small buds in the axil between the branch and its subtending leaf, basipetally developed and deriving their vascular supply from the bundles of the branch alone, may indicate that the same thing occurs here also.

It seems probable therefore that *Gnetum* originally bore compound bisexual inflorescences from which both male and female spikes have been derived. The former have preserved in certain Indo-Malayan species their bisexual character, but the latter have as a rule lost all trace of it, for the strobilus of *Gnetum Gnemon* alone, as far as is known, retains in its series of vascular complexes surrounding the base of the ovule some vestige of its original complicated structure.

SUMMARY.

The presence of a ring of complex groups of vascular strands arising from the bundles in the base of the female 'flower' of *Gnetum Gnemon* may indicate that the ovule was primitively surrounded by a whorl of male flowers. If this were the case the female inflorescence of *Gnetum* would have been originally compound and bisexual, and from such a form the existing male inflorescence can easily be derived.

Somatic Mitoses in Oenothera.

BY

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With Plate LXXXVI.

THE purpose of the present paper is to record certain facts regarding the somatic mitoses in the Oenotheras. For several years I have made incidental observations on the somatic divisions, particularly in flower tissues. But recently, in connexion with a study of the formation of the megaspores in *O. lata*, certain peculiar phenomena were observed in the rapidly dividing cells of the nucellus. In order to understand these peculiar appearances, which stood in various possible relations to the phenomena of reduction, it was found to be necessary to make a special study of normal somatic mitoses.

A brief account of normal nuclear division in the cells of the nucellus will therefore be given, followed by a description of the few rare cases in which the shape of the chromosomes differs from the normal, and certain instances in which an actual reduction division is apparently taking place in somatic tissue.

The slides from which these observations were made were all prepared from material collected in July, 1909, from a single plant of *O. lata* for a study of öogenesis. The fixing fluid used was a chrom-acetic solution which gave excellent fixation of the nuclei, and the stains employed were Heidenhain's iron-alum-haematoxylin and the triple stain. My actual acquaintance with the somatic mitoses is much wider, including several years' observations on a large number of forms.

CHROMOSOME NUMBERS.

It may first be stated that the sporophyte number of chromosomes in this *O. lata* plant was 15. Several counts were made in various mother-cells, which yielded incontestably 15 in every case. A large number of counts made in the nucellar tissue showed that 15 was the characteristic number here also, though certain variations from that number were found to

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occur. Thus, in over fifty cells of the nucellus the number was determined to be certainly 15, in three cases it was impossible to determine whether the number was 14 or 15 owing to the relative position occupied by certain chromosomes, in one cell only 12 chromosomes could be found though the cell was undisturbed by the knife, in two cells the number was certainly 16, and in one cell 20 or 21 chromosomes were found.

These counts were all made with great care, nothing short of certainty with regard to the number being considered satisfactory. The counts were made chiefly in two stages of mitosis, (1) the late prophase just before the spindle begins to appear, and (2) the metaphase in polar view. In the former case only nuclei were considered which could be shown with certainty to be uncut. This stage is a peculiarly favourable one for counting the chromosomes, because the latter are never in contact. They apparently repel each other, as they are invariably found at this time distributed just within the periphery of the nucleus (cf. Pl. LXXXVI, Fig. 11). In the equatorial plate stage nuclei only were chosen for counting when the chromosome group was in a plane parallel to the plane of section and undisturbed (cf. Fig. 17).

I mention these circumstances to show the care with which the counts were made. They show that the sporophyte number of chromosomes in this plant of *O. lata* was undoubtedly 15, although in nucellar tissue there are occasional variations to a lower or a much higher number. The significance of such variations is not at present clear. The smaller variations may be due to the temporary fusion or the fragmentation of certain chromosomes, though it was impossible to obtain evidence for this from the size of the chromosomes themselves. Or they may be due, as E. B. Wilson has suggested, to the occasional failure of a chromosome to condense from the resting nucleus. In the single case in which about 21 chromosomes were to be seen in the equatorial plate (Fig. 21), the chromosome group appeared certainly larger than in normal nuclear plates, the cell also being larger than its neighbours. It seems probable that in this case the increase in the number of chromosomes occurred in some other way. Careful examination showed that all the chromosomes occupied approximately one plane, so that the increase in number could not have been due to precocious fission of certain chromosomes, unless, possibly, it had occurred in the prophase before the nuclear membrane disappeared and the equatorial plate was formed. We can, therefore, conclude that, whatever their significance or manner of origin, variations in the chromosome number in the nucellus do occasionally occur.

The fundamental number of chromosomes, both in the somatic cells and the germ cells of this individual, is evidently 15—one more than is found in *O. Lamarckiana*. Miss Lutz ('09) has also reported 15 chromosomes in the root tips of certain plants of *O. lata* as well as in several individuals of certain other mutants. As I pointed out (Gates, '08, p. 28), these deviations of one chromosome from the number (14) in *O. Lamarckiana*

very probably arise during meiosis, for one of the striking features of diakinesis in *O. Lamarckiana* and its mutants is that the chromosomes are but loosely paired (cf. Fig. 38), and there is, therefore, exceptional opportunity for the occurrence of irregularities in the distribution of the meiotic chromosomes. I further showed that during meiosis in the pollen mother-cells such distributional irregularities actually do occur.

From all the counts thus far made of the chromosomes in *O. lata* it appears that the number 15 occurs at least in most individuals, though the counts are perhaps not yet numerous enough to show that 15 is the number for all individuals, and that the *O. lata* characters are therefore constantly associated with the extra chromosome. The inconstancy of this mutant when self-pollinated (in a fertile race) or crossed may perhaps be accounted for by the presence of the extra chromosome, for it is probable that part of the megaspores produced will have 8 chromosomes, and part 7.

Somatic Mitosis.

Prophase.

Though the somatic cells of *Oenothera* are relatively small, yet the nuclear structures are very clear, and in any meristematic tissue it is easy to get a complete series showing every stage of mitotic division within a small area. It is not to be expected, however, that the finest details of the chromatin in the earliest prophase stages can be depicted as minutely as in larger nuclei, so that such questions as the earliest stages of the transformation of the nuclear reticulum into the prophase chromosomes will not be critically considered here. The later prophase stages are, however, remarkably clear, and admit of no doubt in interpretation. The following description and figures refer only to the cells of the nucellus, though in other tissues the processes are essentially the same.

When in the complete resting condition (Pl. LXXXVI, Fig. 1) the nucleus contains a delicate reticulum whose meshes are equally distributed through its whole cavity. Several nucleoli of varying sizes usually are present, and a small area more or less free from threads is usually found around the nucleoli, especially the larger ones, in fixed material. The reticulum in fixed preparations consists of the usual delicate interwoven threads, having a more or less beaded appearance owing to the presence at intervals of deeper staining and sometimes thicker portions of the threads, the same structures occurring also where threads cross. This moniliform appearance of the threads becomes more conspicuous at the beginning of the preparations for prophase (Fig. 2). The threads also begin to lose their uniform character, some threads becoming visibly thicker and more conspicuous, while others retain the delicate character of the completely resting nucleus. Very soon this differentiation becomes more marked (Fig. 3), a few of the threads retaining their original delicate character, but most of them becoming

progressively thicker and more conspicuous. This is accompanied in some threads by an intensification of the moniliform character, while other portions of the threadwork become thicker and quite homogeneous in appearance (Fig. 3), having a rather loose texture and presumably formed by the fusion of several threads. This is, frequently at least, accompanied by a movement of material towards the periphery of the nucleus, for the thicker threads are usually more conspicuous in this region. They are also more strongly moniliform, thus giving the periphery of the nucleus a more densely chromatic appearance.

At a slightly later stage than that of the last figure the chromosomes begin to be sufficiently definite so that they can be counted approximately. There is some variation at this time in the manner of their first appearance as definite chromosome bodies. Sometimes they first appear as very long and narrow, deep-staining, twisted threads in the nucleus (Fig. 4). In this figure only a portion of the chromosomes are drawn, in order to show the complete outlines of certain ones. In other cases the bodies which are destined to become chromosomes are shorter and thicker, and much less twisted when they can first be observed (Figs. 3, 5, and 6).

The formation of the body of the chromosome seems, therefore, to be the result of progressive parallel fusion of numbers of strands in the original reticulum. The threadwork is thus swept up into a few thick strands which from the first show a marked tendency to occupy the periphery of the nucleus. The finer threads visible in Figs. 5 and 6 finally all disappear, presumably by absorption into the denser masses, and the chromosomes meanwhile continue to shorten and thicken progressively. In Fig. 7 this process has proceeded farther than in Figs. 5 and 6, and the chromosome number can now be definitely counted—15.

During these processes the nucleoli remain unchanged, floating freely in the nuclear cavity until the nuclear membrane disappears, when they are suddenly dissolved and vanish.

It will be seen from the preceding figures that nothing even remotely resembling prochromosomes is to be found in the resting nucleus. I have sought for these bodies, using a variety of depths of stains, and am convinced there are no such bodies present in the resting nucleus. The nucleoli and the bead-like thickenings of the threads of the nuclear reticulum are the only dense bodies to be observed. The latter are very numerous, and, as can be seen from Figs. 1–3, a number of them occur along any length of thread equal to that of the chromosome. I can only conclude that there is no evidence whatever for the persistence of denser chromosome centres or prochromosomes in the completely resting nuclei of these cells. The passage into a moniliform threadwork or reticulum is complete, no denser aggregations except the easily recognizable nucleoli, which are usually only 3 or 4 in number, remaining in any part of the nucleus.

At the stage represented by Fig. 7 the last threads of the reticulum have been withdrawn, and the nuclear cavity is now occupied only by karyolymph in which the chromosomes and nucleoli float. They are peripherally arranged and undergo progressive condensation as shown in Figs. 7–10. This is the best stage of all for counting.

In a previous paper (Gates, '11 *b*, p. 931) I reached the conclusion, after a preliminary examination of somatic prophases in *O. gigas*, that a continuous spireme is produced, which segments to form the chromosomes. A study of the earlier prophase stages shows that this conception requires modification. The chromosomes are coiled spirally round and round the nuclear cavity in diakinesis in such a way as to give frequently the appearance of an end-to-end arrangement. But they do not originate in an end-to-end position, as shown by Figs. 4–7. They originate, as already described, by a progressive condensation of the threads in certain portions of the reticulum, in a manner similar in some respects to that described by Stomps ('10) in the pollen mother-cells of *Spinacia*. When first differentiated from the reticulum they are neither end to end nor side by side, but are irregularly arranged with regard to each other, a single chromosome in the earliest stage of its appearance sometimes extending across the whole diameter of the nucleus (Fig. 4). They are also at first much coiled and very irregular in shape, often forming a loop, though usually of nearly uniform thickness throughout. They thus correspond to Boveri's ('09) conception of the origin of the chromosomes from the resting reticulum as shown in the blastomeres of *Ascaris*. While we may consider that each portion of the resting reticulum represents in general an alveolated chromosome, yet anastomoses between adjacent parts, as well as perhaps movements between parts of the nucleus, must cause more or less intermixture of portions of the reticulum derived from separate chromosomes. This may perhaps account for the manner in which the chromosomes are intermingled when they first appear, but it of course assumes a more strict individuality of the resting chromosomes than can be visibly demonstrated.

I was unable to obtain any satisfactory evidence that the chromosomes are paired at all in arrangement when they are first differentiated from the reticulum. Later, in Figs. 8–11, indications of a side-by-side pairing begin to appear. In these figures each nucleus is drawn in two parts, representing the chromosomes seen in upper and lower focus. In each case the full number of 15 chromosomes is present. In Figs. 8 *a* and 8 *b* the chromosomes are still considerably looped and twisted. In Figs. 9 and 10 they have condensed to a shorter and thicker, more rod-like shape. Delicate (linin?) connexions are still to be seen attached to some of the chromosomes in Fig. 9.

In a former paper (Gates, '11 *b*) I mentioned that the chromosomes in somatic prophases usually undergo a longitudinal split. A single

chromosome in the nucleus of Fig. 9 shows such a split precociously. Fig. 10 represents the definitive shape of the chromosomes just before this split usually appears, and in Figs. 11 *a* and 11 *b* the fission is clearly visible in nearly every chromosome. At about this time the chromosomes also take up a more uniformly distributed peripheral position, and they may frequently have the appearance of an end-to-end arrangement, though they are not actually in contact, and their original arrangement was quite different. At the stage represented by Fig. 11 the chromosomes have also usually reached entire uniformity as regards size and shape. There are usually one to several nucleoli at this time, but occasionally, as in Fig. 10, no nucleolus is present. All the Figs. 1–11 are drawn from nuclei which were uncut by the knife.

The first definite indications of lateral chromosome pairings appear at about the same time as the longitudinal split. Such a feature cannot be shown satisfactorily when the chromosomes are lying in three planes, but there are indications of such pairs in Fig. 11.

The stage of the prophase in which the chromosomes are longitudinally split appears to last for some time. But it is soon followed by a stage (Fig. 12) in which the nuclear membrane has disappeared, the spindle has made its appearance as a delicate weft of fibrillae in the cytoplasm immediately surrounding the nucleus, and the split in the chromosomes is no longer visible. The chromosomes are still grouped around the periphery of a sphere, as in the last stage, but the cavity of the nucleus has decreased somewhat, so that the chromosomes are more closely grouped, but they still partly retain the shape of curved rods, a shape assumed by the dia-kinetic chromosomes (Fig. 11) in accommodating themselves to the periphery of the nucleus. This stage is of short duration. Fig. 13 shows a later prophase, in which the spindle is beginning to assume a bipolar shape, and the chromosomes are being arranged on the equatorial plate. In this particular cell the chromosomes vary considerably from their usual shape, and the difference is not merely due to foreshortening. In studying large numbers of somatic mitoses, such variations in the shape of the chromosomes are occasionally found. Their significance is not at present understood.

Another case in which the shape of the chromosomes differed markedly from their normal shape in nucellar tissue is shown in Fig. 14. In this metaphase side view the chromosomes are short and thick, dumb-bell-shaped bodies, and they are also peculiarly distributed on the spindle, although the cell was undisturbed in cutting. All the chromosomes are not represented in the figure, and the full number could not be exactly determined. Fig. 15 shows the position of this cell (*b*) in the ovule near the megaspore mother-cell (*a*), in which the heterotype mitosis is just completed, but no cell-wall has been formed. Such cases of variation in chromosome shape in particular

cells occur only occasionally, and no explanation of their occurrence is to be offered in the present paper.

Metaphase.

Fig. 16 represents a normal metaphase in slightly oblique view. The chromosomes are not all represented, but the longitudinal split can be clearly seen. Figs. 17–21 represent equatorial plates of the metaphase in polar view. Figs. 17 and 18 each contain 15 chromosomes, while 16 chromosomes are present in Fig. 19, 12 in Fig. 20, and 20 or 21 in Fig. 21, though for the sake of clearness only 18 are drawn in the figure. As already stated, the regular number in this plant is undoubtedly 15, and the departures shown in Figs. 19, 20, and 21 represent rare cases. Such wide fluctuations as 12 and 20 were particularly surprising to find, but accurate study of other somatic tissues will probably disclose equally striking cases of occasional variations in number in certain stages of mitosis.

Farmer and Shove ('05) described similar variations in the root tips of *Tradescantia virginica*. After a large series of counts they found the chromosome number to vary from 26 to 33 (p. 562). They also (p. 565) found the number inconstant during the heterotype mitosis, varying from 12 to 16. This may be connected with the fact that the plant commonly fails to set seed.

Wilson ('09, p. 185), in his study of the chromosomes in the Hemipteran genus *Metapodius*, also found occasional variations of one in the number of chromosomes in the spermatogonia and ovaries.

It will be seen that in Fig. 20 two of the chromosomes, and in Fig. 21 one chromosome, show a precocious split. As a possible suggestion regarding the origin of the increased number of chromosomes in Figs. 19 and 21, we may assume that the fission which regularly occurs in the prophase chromosomes in diakinesis, instead of closing up, becomes accentuated, resulting in the separation of those daughter chromosomes and their regular orientation in a single plane afterwards, on the equatorial plate of the spindle. Of course it does not follow that such chromosomes will undergo another fission on the spindle, and thus perpetuate the increased number. It might well be, and is indeed perhaps more probable, that those chromosomes which, on our hypothesis, completed their precocious fission and separation in the prophase, would merely travel to opposite poles of the spindle and thus restore the normal number. The determination of this possibility by observations of fixed material is practically an impossibility, for the moment such chromosomes pass towards the poles in anaphase, there remains no evidence that they separated immediately after the precocious split in the prophase. The probability of this explanation is, however, increased by the fact that the aberrant numbers were, all but one, found in metaphase groups.

Critical examination of these figures will show clear evidence of pairing between the chromosomes on the equatorial plate. The paired arrangement first begins to be seen in the late prophase (Fig. 11) at the time of the precocious chromosome split. The pairing naturally becomes much more evident to an observer when the chromosomes arrange themselves in one plane on the equatorial plate. Figs. 17, 18, and 20 show indisputable evidences of it. Nearly all the chromosomes in these figures are clearly arranged in pairs. It is often impossible, however, to determine any definite pairing at all. In Figs. 17 and 18 the chromosome number is odd, from which it follows of necessity that one chromosome is unpaired. In Fig. 18 any observer would scarcely hesitate to decide that all the chromosomes are in pairs except the one marked *a*, while in Fig. 17 there are several equally clear pairs, and the chromosome marked *a* is probably the odd one. The twelve chromosomes in Fig. 20 are all evidently arranged in pairs.

Anaphase and Telophase.

The anaphases of mitosis are represented by Figs. 22–5. Figs. 22 *a* and 22 *b* are polar views of the two chromosome groups of the early anaphase from the same cell, just after the daughter chromosomes have separated. Each group contains 15 chromosomes, and the great apparent variation in size and shape of the chromosomes is a result of the varying positions they occupy in their movement towards the poles. Fig. 23 is a side view of a later stage of anaphase, in which the chromosomes, in the form of rods or loops, are just reaching the poles. The poles of the spindle have lost their sharp-pointed character, and have become broad with the fibres of the spindle nearly parallel throughout.

In Fig. 24 a more compact group of the daughter chromosomes is being formed, the spindle is somewhat shortened and broadened, and the first indication of a cell-wall has appeared in the median plane of the spindle. In Fig. 25 are found the first indications of coalescence between parts of chromosomes which are in contact. The first thickening of the spindle fibres in the median region gives a shadowy appearance to this part of the spindle. In Fig. 26 the process of condensation of the chromosome group has proceeded farther, until a compact and apparently almost solid mass of chromatin is formed at each pole. The thickenings on the spindle fibres in the median plane are more definite and sharply localized than in the previous figure. The nuclear membrane has not yet begun to appear.

In the telophase stages the chromosomes frequently change their shape in an important manner. The origin of the nuclear membrane has not been studied, but it doubtless agrees with the method already known for the nuclei of the pollen mother-cells of *Oenothera* and many earlier-described forms. Fig. 27 represents an early stage in the reconstruction of the two daughter nuclei. One of these nuclei shows clearly 15 chromosomes,

while in the lower nucleus the full number could not be seen owing to the angle from which the lower nucleus was observed. It will be seen that in this figure and the following (Fig. 28) the chromosomes nearly all have a characteristic dumb-bell shape. This was first observed several years ago in other floral tissues (Gates, '07, p. 19). In Fig. 28, also, 15 chromosomes are present and can be counted very easily. These chromosomes are in the form of tiny loops, or in many cases have a median constriction which gives them the appearance of dumb-bells. It will be seen from the figures that the size of the chromosomes varies strongly, not only according to the size of the cell and nucleus in which they are found, but according to the stage of mitosis. The mass of chromatin at the poles in the stage represented by Fig. 26 is frequently greater than that in the early telophase after the nuclear membrane has been formed. These telophase stages also show that the chromosomes retain their individuality as independent bodies during the period represented by Fig. 26, during which they are closely massed together. The slightly later telophase in Fig. 29 also contains 15 chromosomes. A nucleolus has not yet appeared. The nucleus has grown in size (its larger size in this figure also depends partly upon its presence in a larger cell); the chromosomes also are larger, have mostly lost their median constriction, but are still sharply individualized. It is not certain that the median constriction always appears, although it is of frequent occurrence. The increase in size of the chromosomes appears to be due at first to a process of swelling, which is often accompanied (Fig. 30) by the chromosome becoming less deeply staining, and sending out processes which begin to anastomose with those of adjacent chromosomes. In Fig. 30 both daughter nuclei of a nucellus cell are shown in the same stage of alveolization. Figs. 31–3 show other nuclei in the same condition. The chromosomes can still be counted, at least approximately. The chromatin content of the nucleus is here, as in the prophase, frequently coiled, though quite irregularly, round the periphery of the nucleus. Before this stage has been reached the new cell-wall is formed, but these stages can of course be easily distinguished from prophases by the comparative size as well as position of the nuclei and cells.

Fig. 34 shows the two daughter nuclei in a still later stage. The nuclei have markedly increased in size, and their anastomosis and transformation into a threadwork has proceeded farther. The chromosomes can still be counted, however, the whole 15 being shown in Fig. 34 *b*; and the median constriction still remains in some of them. Nucleoli have also appeared. This telophase stage is easily distinguished from the prophase stages represented by Figs. 5–10, not only in the size of the nuclei but in the shape of the chromosomes. If Fig. 34 were mistaken for a prophase the dark bodies might then easily be considered 'prochromosomes'. In Fig. 35, though the nucleus has passed still farther into the resting condition, and

a delicate network of threads fills much of the nucleus, yet the chromosome centres are still visible and can be approximately counted. It is possible that when the mitoses follow each other very rapidly the nucleus begins a new division before it has passed much farther than this stage into the resting condition. But if the growth is not too rapid, a completely resting nucleus is formed (Fig. 36), corresponding exactly with Fig. 1 except in its smaller size. In the subsequent growth of the nucleus up to the size of Fig. 1, its chromatin content remains in the form of a uniformly distributed reticulum. It is impossible to see in these facts any support for a theory of pro-chromosomes. That each section of the resting reticulum is derived, in large part at least, from a given chromosome is probable, but there is no centre or core of the chromosome which retains its condensed form through the completely 'resting' nucleus.

Other Observations.

For comparison, I have shown in Fig. 38 a single megaspore mother-cell in *O. lata* in metaphase of the heterotype mitosis. Not only the cell, but the spindle and chromosomes are enormously larger than in the surrounding nucellar tissue. Fifteen chromosomes can be counted, though they are rather closely grouped. As is well known, allotypic chromosomes generally differ from somatic or typical chromosomes, not only in size but in shape. The heterotypic chromosomes of Fig. 38 are not slender rods or loops, but are short and stout or almost spherical. But for unknown reasons the chromosomes in occasional somatic cells may approach the shape of the heterotypic chromosomes, as in Fig. 13, or may take other forms, such as that of Fig. 14, which resembles the ordinary shape in early telophase of somatic mitosis (Figs. 27, 28). It should be stated that the cells represented by Figs. 13 and 14 were both surrounded by dividing cells in which the shape of the chromosomes was normal, so that the variations in shape cannot be attributed to the fixation or subsequent treatment.

Another observation which is worth recording here, as showing the relative stability of the spindle as a structure in the cyloplasm of the cell, and that it is not merely a reflection of osmotic stresses or electro-magnetic lines of force, is the following. By an accident which occasionally happens to every cytologist, a preparation which had been mounted in Canada balsam some time previously was slightly crushed, so that the cell-walls of a small area of tissue were destroyed by the pressure. On the margin of this area were several cells which were burst open and their contents extruded. One such cell contained a spindle in metaphase, and this was pressed out of the cell through the broken cell-wall. The spindle itself remained perfectly intact, with the chromosomes attached and undisturbed, though the spindle had been moved out of the ruptured cell and turned through an angle of 90°. This spindle remained floating freely in the

Canada balsam, and permanently unchanged as regards the arrangement of its fibres and the attachment of the chromosomes. Even were it not already abundantly evident from other sources, one could not fail to be convinced by this simple observation that the spindle is a structure more stable than the rest of the cytoplasm, and that the chromosomes are definitely attached to the spindle fibres.

In this connexion certain observations of Lillie ('08) on the eggs of the annelid worm, *Chaetopterus*, are worth citing because they lead to exactly the same conception of the relations between spindle and cytoplasm. Eggs of *Chaetopterus* suspend their maturation processes in the metaphase of the first reduction division to await the entrance of a sperm, the spindle being attached by one pole to the periphery of the egg. If eggs in this condition be centrifuged, the spindle with its attached chromosomes is frequently torn loose and displaced by the centrifugal force to one side of the egg. But the chromosomes remain attached, though the spindle may be more or less distorted by too rapid centrifuging or by the impact of the heavy yolk globules thrown against it. It was further found that if such centrifuged eggs are afterwards fertilized and allowed to develop, the spindle migrates back to its original position in order to complete the maturation processes.

Reduction Divisions (?) in Somatic Tissue.

The primary purpose of the preceding account of normal mitosis in nucellar tissue was to afford a basis for comparison with certain cases which appear to represent actual reduction divisions in the same tissue. Two such cases were carefully observed which agreed in all essentials, and one of them is represented in Fig. 37. The cell containing this spindle occurred near the margin of the nucellus of an apparently normal ovule. Adjacent cells were in various stages of normal mitosis. As will be seen from the figure, this spindle was exceptionally large. Comparison with Fig. 38 shows it to be nearly as long, though not so broad, as the heterotypic spindle, though the containing cell was not conspicuously larger than surrounding cells of the nucellus. The spindle is conspicuously larger than in normal nucellus cells such as are represented in Figs. 14, 16, 23–6.

As regards the chromosomes, which are not all represented in Fig. 37, they could not all be determined with certainty, owing to the crowded positions occupied by some. But as far as could be determined, there were certainly two very clear pairs or bivalents in which the individual chromosomes could be seen, one pair in which the two chromosomes appeared to be closely appressed or fused, two pairs less clear owing, apparently, to partial fusion, one other probable pair, one certainly single chromosome, and probably another single chromosome. This makes 14 chromosomes. The presence of the fifteenth could not be determined.

The two very clear pairs are shown in Fig. 37, the members of each pair having the shape of normal somatic chromosomes (i. e. long, somewhat curved rods), but lying clearly in longitudinal pairs and oriented quite differently from their normal position on the equatorial plate. These are separately represented by *a* and *b* in Fig. 37 *a*. The partly fused pair are represented by *c* in Fig. 37 *a*, while *d* represents a chromosome which is clearly single.

Critical study of this and one other similar spindle demonstrates that a portion, at least, of the chromosomes are closely paired with each other. Such cases are evidently rare, and it will require much further study to demonstrate the actual fate of the chromosomes in such mitoses. Even the loosest pairs are much more closely associated than the pairs of an ordinary somatic metaphase (cf. Fig. 37 with Figs. 18, 19, 20), while in several pairs the chromosomes appear to be partly fused. Their peculiar orientation on the spindle also reminds one of the heterotypic mitosis (Fig. 38) in which the chromosomes are always irregularly scattered in the median region of the spindle and not in regular alinement. On the whole, it seems probable that this pairing in the equatorial region of the spindle (Fig. 37) will be followed by the separation of the pairs, and hence by a reduction division. It is impossible not to be struck by the numerous differences between this figure and a normal somatic metaphase. While the inference that this intimate pairing is to be followed by a reduction or segregation division is as yet unproven by actual observation, yet it seems more probable than any other explanation I can offer.

Discussion.

The present paper is meant to be a record of facts rather than a discussion of literature, but a few points may be briefly referred to.

A paired condition of the chromosomes was first found in the somatic tissues of plants by Strasburger in 1905. References to subsequent papers, showing the undoubted occurrence of regular somatic chromosome pairings, were made in my paper on the mode of chromosome reduction (Gates, '11 *a*). It is undoubtedly true that this condition in the metaphase of each somatic division is widespread in the sporophytic tissues of plants. It is obvious from Figs. 4–10 that in the early prophases of sporophytic mitoses in *Oenothera* the chromosomes are at least less evidently paired than in the metaphase. Whether they remain associated throughout the whole intermitotic period, or whether they go through a regular series of evolutions leading to fresh pairing in each mitotic cycle, must therefore remain undecided, though it is certain that chromosomes which are paired on the spindle in metaphase are more likely to be closely associated with each other during the passage into the resting condition than those which occupy

widely separated positions in the equatorial plate. It is, therefore, uncertain whether the pairing of chromosomes in the metaphase is but a part of a constant association between homologous maternal and paternal chromosomes of the sporophyte, or whether in each mitotic cycle there is a re-entering into a paired arrangement of chromosomes which in the resting nucleus are less closely or but little associated with each other. The fact that in heteromorphic chromosome groups, as in *Funkia*, *Galtonia*, *Yucca*, &c., the pairings are always between chromosomes of similar morphology is a strong argument for the belief that the paired arrangement represents an actual association of homologous maternal and paternal elements. However, until it is definitely shown that the chromosomes in gametophytic mitoses, such as in pollen grains or in Fern prothallia, have no paired arrangement, too much stress cannot be laid upon the general significance of the pairing in sporophytic tissue. Meantime, the evidence from the chromosome behaviour in animals, both in spermatogonial divisions and in synapsis, very strongly supports the hypothesis of homologous pairing. If the gametophytic chromosomes of plants should prove to be also paired in metaphase (a view for which I am not aware that any evidence exists at the present time), then the explanation would necessarily be a mechanical one, and the same explanation would then apply to sporophytic pairings. It seems improbable, however, that a purely mechanical explanation of these pairings will ever be necessary.

Another point worth emphasizing is that in the late prophase of somatic mitoses a split occurs, just as frequently occurs in the heterotype prophase, and may afterwards close up, again as in the heterotype prophase. This serves to emphasize a fact which I referred to in a previous paper (Gates, '11 *a*), namely, that the only essential difference between the behaviour of the somatic and the heterotypic chromosomes is that the latter merely segregate in metaphase, while the former undergo a split. In other words, this removes one more of the differences which were formerly supposed to exist between the somatic and the heterotype mitoses.

Again, though the chromosomes usually show a median constriction in the telophase, yet this is only of transient duration, and apparently bears no relation to the appearance of a split in the succeeding prophase. The chromosomes of the telophase pass into the resting condition without any indication of a split in their substance, such as has been described for certain forms. The earliest appearance of any split in the chromosome structure is in the rather late prophase of mitosis.

Variations in the shape of somatic chromosomes have been comparatively little studied. Němec ('10, p. 263) has shown that the chromosomes in root tips of *Allium montanum*, *Vicia faba*, and *Galtonia candicans* become much shorter and thicker after treatment with benzine vapour, and Kemp ('10) in a careful paper has shown that chloral hydrate produces

a marked change in the shape of the chromosomes of the root tip, often producing chromosome shapes closely resembling heterotype tetrads.

The results, particularly of Němec, in producing tetraploid and octoploid nuclei by subjecting growing root tips to chloral hydrate, are too well known to require discussion here. The question whether such tetraploid cells afterwards undergo reduction divisions which restore the normal number of chromosomes, or whether the disappearance of tetraploid cells from the root tips (which gradually takes place after subsequent growth) is wholly due to other causes, such as division into several smaller cells, fragmentation, and passing into permanent tissue, has been much disputed. Kemp found nothing in her studies which could be interpreted as true reduction figures, and she evidently, like Strasburger, doubts the existence of such. Němec ('10, pp. 63–5) describes two types of reduction figures in chloralized roots: (1) indirect reduction figures which differ from the typical (*a*) in that the chromosomes are longitudinally split at the poles of the spindle, and (*b*) in their occurrence in abnormally large (tetraploid) cells; and (2) direct reduction figures, which also occur in large (tetraploid) cells, and in which a segregation of the chromosomes takes place, half going to each pole of the spindle.

If my interpretation of Fig. 37 of this paper is correct, the pairing of chromosomes in metaphase will be followed by a direct reduction division. Němec ('10, p. 245) describes somewhat similar pairings with partial fusion of the chromosomes as occurring frequently in the root tips of *Ricinus zanzibariensis* (cf. his Figs. 92, Pl. III, and 97, Pl. IV). The possibility therefore remains that an actual reduction will not take place, though the large size of the cell and spindle suggests that something exceptional is occurring in the cases I have described.

Summary.

The results recorded in this paper may be briefly summarized as follows:

The individual of *O. lata* from which the figures for this account of the somatic mitoses were drawn contained 15 chromosomes in its megaspore mother-cells and in the cells of the nucellus. In the nucellus, however, there were occasional cells containing a different number. Thus over 50 cells were shown to have 15 chromosomes, one contained only 12, two contained 16, and one 20 to 21. These aberrant numbers were all, with one exception, observed in the metaphase equatorial plate, and it is possible that the higher numbers are to be accounted for by separation of certain of the daughter chromosomes immediately after their formation by the prophase split. If this is the real explanation of such cases, it does not follow that the increased chromosome number will be perpetuated, for the chromo-

somes which separated precociously may merely segregate in metaphase instead of dividing again, and thus restore the normal number.

From the various counts now made it is evident that more individuals of the mutant *O. lata* contain 15 chromosomes than 14, and it may be that the *O. lata* characters are constantly associated with the presence of an extra chromosome.

The account of somatic mitosis is taken entirely from the nucellus, though the phenomena are closely similar in other tissues. The resting nucleus contains a uniform reticulum of somewhat moniliform threads. There is no evidence whatever of prochromosomes or denser chromatic centres in the completely resting nucleus.

The chromosomes first appear in early prophase by a thickening in certain threads of the reticulum. They are at first long and narrow, much twisted, and are not evidently paired in any way, but are scattered irregularly through the nucleus, being connected by a meshwork of finer threads.

The chromosomes then shorten and thicken, the finer threads gradually disappear, and the chromosome bodies can easily be counted.

A conspicuous longitudinal split then, usually at least, appears in the chromosomes in the late prophase shortly before the nuclear membrane disappears. The first evidence of lateral pairing of the chromosomes also appears about this time.

In the latest prophase stage, after the nuclear membrane has disappeared and the spindle has begun to form, the split in the chromosomes may close up entirely.

In metaphase the chromosomes are often in evident pairs on the equatorial plate, it being possible in some cases to determine with a high degree of probability the particular chromosome which is odd and unpaired.

Certain cases are described in which the chromosomes in metaphase differed from the normal in shape, being shorter and thicker and more like the heterotype chromosomes, or dumb-bell shaped like the somatic chromosomes in one stage of the telophase.

In the very early telophase, after the nuclear membrane is formed, the chromosomes, at least in many cases, assume a characteristic dumb-bell shape owing to a median constriction. The chromosomes continue to be countable until a later stage, but finally pass into a completely resting condition in which no remnant of a central denser core of the chromosome remains.

It was discovered accidentally that if the wall of a cell in a preparation be ruptured and the contents squeezed out, the spindle may be isolated and retain its shape and the chromosomes remain attached, showing that it has greater stability of structure than would be produced by osmotic or electromagnetic forces.

Finally, two cases were observed in which the spindles were exceptionally large and the chromosomes were many of them closely joined in pairs to form bivalents, though retaining the shape of the somatic chromosomes. These bivalents were peculiarly oriented on the spindle, and from all analogy it seems very probable that this pairing would be followed by an actual reduction division in somatic tissue.

In conclusion, I am greatly indebted to Professor J. Bretland Farmer, F.R.S., for suggestions and criticisms in connexion with this work.

Note added Aug. 29.—In a paper which has just appeared ('Triploid Mutants in *Oenothera*,' Biolog. Centralbl., 1912) Miss Lutz affirms the incorrectness of certain of her earlier counts of 14 chromosomes for *O. lata*, and finds 15 chromosomes in the root tips of all the *lata* plants she has examined. In a re-examination of the preparations of *O. lata* (now somewhat deteriorated) described in my first paper on the subject ('Pollen Development in Hybrids of *O. lata* and *O. Lamarckiana*, and its Relation to Mutation,' Bot. Gaz., Feb., 1907) I was unable to determine with certainty whether this *lata* plant contained 14 or 15 chromosomes. But from all the evidence now at hand, it is safe to conclude that *O. lata* nearly always contains 15 chromosomes, though it is quite possible there may be exceptions in the case of certain forms of *lata* which produce plenty of pollen. It is probable that the almost complete sterility of the pollen in typical *lata* is concerned with the presence of the extra, unpaired chromosome.

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EXPLANATION OF PLATE LXXXVI.

Illustrating Dr. Gates's paper on Somatic Mitoses in *Oenothera*.

The figures were all drawn with an Abbé camera lucida, using a 2 mm. Zeiss apochromat. objective, ap. 1·40, and an 18-compensating ocular, and reproduced natural size, giving a magnification *circ.* 3,800 diameters; except Fig. 15, which is drawn with a low power. The figures were all taken from the nucellus of *Oenothera lata*, except Fig. 38, which represents a megaspore mother-cell.

Fig. 1. Completely resting nucleus, containing a uniform moniliform reticulum.

Fig. 2. Cell showing the earliest prophase stage of the nucleus. Certain threads of the reticulum are becoming thicker and more markedly moniliform.

Fig. 3. Slightly later than last figure. The distinction between the thicker and more delicate threads is becoming more apparent.

Fig. 4. Prophase stage, showing the first appearance of definite chromosomes. They are long and irregularly coiled. For the sake of clearness only part of them are represented in this figure, the fine threads being omitted.

Figs. 5 and 6. Slightly later stage in which nearly all the chromosomes can be counted. The chromosomes are shorter, thicker, and less coiled than in the previous figure, and portions of the fine threads of the original reticulum still remain.

Fig. 7. Later prophase, in which the remnants of the reticulum have disappeared and only the chromosomes (fifteen) and the nucleoli are left floating in the cell-sap.

Figs. 8–11 each represent two views of a single uncut nucleus, showing all the chromosomes (fifteen) in two planes of focus.

Figs. 8 *a*, 8 *b*. A single (uncut) nucleus in two foci, showing all the chromosomes (fifteen). The latter are still thin rods, somewhat coiled.

Figs. 9 *a*, 9 *b*. Showing all the chromosomes (fifteen) of another nucleus slightly later than the last. The chromosomes are shorter and thicker, one showing a precocious split. Fragments of 'linin' remain attached to some of them.

Figs. 10 *a*, 10 *b*. Same stage as last. Chromosomes peripherally arranged, full number (fifteen) present, no nucleolus.

Figs. 11 *a*, 11 *b*. Later stage of diakinesis than last, in which the chromosomes all show a clear longitudinal split.

Fig. 12. Last stage of prophase. The longitudinal split in the chromosomes has closed up, the nuclear membrane has disappeared and the first indications of the spindle appear as a weft of delicate fibrillae surrounding the nucleus. The full number of fifteen chromosomes is present.

Fig. 13. Slightly before metaphase. The chromosomes are just being drawn into the equatorial plate. In this cell the chromosomes are somewhat abnormal in shape. Only twelve are represented in the figure.

Fig. 14. Another abnormal nucleus in metaphase, side view. The chromosomes are arranged in two series on the spindle, and are in the shape of dumb-bells. Nine are shown in the figure.

Fig. 15. An ovule (integuments not shown); *a* represents the megaspore mother-cell which has just undergone the heterotypic division; *b* is the cell represented in Fig. 14.

Fig. 16. Normal metaphase, side view, slightly oblique. The chromosomes have split for the anaphase. The spindle is sharp-pointed.

Fig. 17. Metaphase, polar view. The chromosomes (fifteen) probably all in pairs except *a*, which is unpaired.

Fig. 18. Same as last. Chromosomes (fifteen) clearly paired, except *a*, which is the odd chromosome.

Fig. 19. Equatorial plate, showing (exceptionally) sixteen chromosomes with some indications of pairing.

Fig. 20. Equatorial plate, showing (exceptionally) only twelve chromosomes. They clearly form six pairs. The two outermost show the beginnings of a precocious split.

Fig. 21. Equatorial plate showing (exceptionally) eighteen chromosomes. The whole number present was twenty or twenty-one. One chromosome shows the beginning of a precocious split. See text.

Fig. 22. Early anaphase, showing the two daughter groups of chromosomes (fifteen each) in equatorial view, from different foci of the same cell.

Fig. 23. Later anaphase, showing the chromosome loops being drawn to the poles. Spindle poles now broad, and spindle fibres nearly parallel.

Fig. 24. Later stage than last. The chromosomes have reached the poles and the thickenings of the fibres in the median region of the spindle have already appeared.

Fig. 25. Same stage as last. First indications of cell-wall.

Fig. 26. Slightly later stage. The chromosomes have formed a compact group at each pole.

Fig. 27. Telophase. The membrane has been formed around the daughter nuclei, and the chromosomes—now very small—have mostly taken on the shape of dumb-bells owing to a median constriction. The full fifteen chromosomes can be counted in the upper nucleus.

Fig. 28. Same stage as last, showing the fifteen chromosomes, nearly all having a clear median constriction.

Fig. 29. Later telophase than last. The nuclear cavity has increased in size, the chromosomes are larger and have mostly lost their constriction. Fifteen chromosomes present.

Fig. 30. Later telophase, showing both daughter nuclei. The drawing-out and anastomosis of the chromosomes has begun.

Figs. 31–33. Same stage as last. Not all the chromosomes represented.

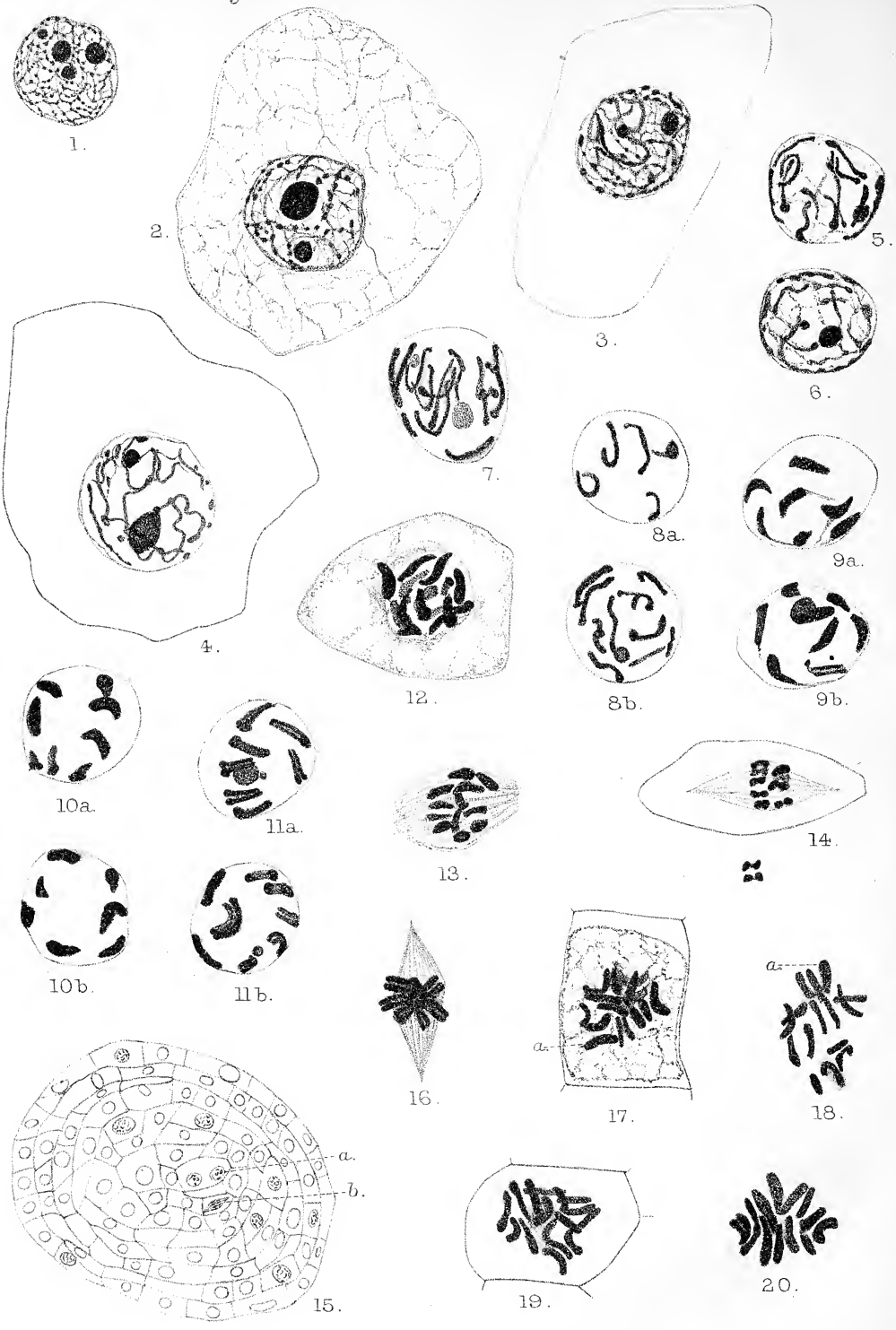
Figs. 34 *a*, 34 *b*. Two telophase nuclei from one cell. The centres of the chromosomes still remain condensed. In 34 *b* the full fifteen chromosomes are shown, several of them still showing the median constriction.

Fig. 35. Later stage than Fig. 34. The reticulum is becoming better developed, but portions of the chromosomes still remain in the condensed form.

Fig. 36. The completely resting condition, like Fig. 1 but smaller. A uniform, slightly moniliform reticulum fills the nuclear cavity.

Fig. 37. An abnormal cell from the nucellus. The spindle is much larger than in an ordinary nucellar cell, being nearly as long—though not so broad—as the heterotype spindle (Fig. 38). The chromosomes are mostly in close pairs, lying side by side but irregularly oriented on the spindle. The members of these pairs are much more closely associated than in normal mitoses (cf. Figs. 17–20), and it seems probable that this will be followed by a reduction of the chromosome pairs. In Fig. 37 *a* several of these chromosomes are drawn separately; *a* and *b* are two chromosome pairs, *c* a partly fused pair, *d* a single chromosome, and the other three represent partly fused pairs.

Fig. 38. Megaspore mother-cell drawn on same scale, showing heterotype mitosis with fifteen chromosomes present. The chromosomes differ sharply in size and shape from those of the somatic cells.



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GATES —— OENOTHERA.


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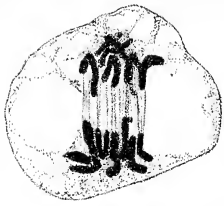
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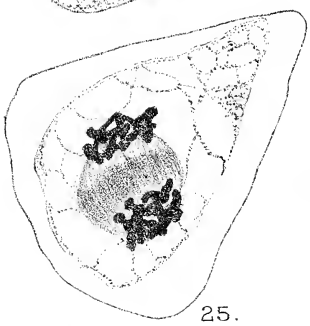
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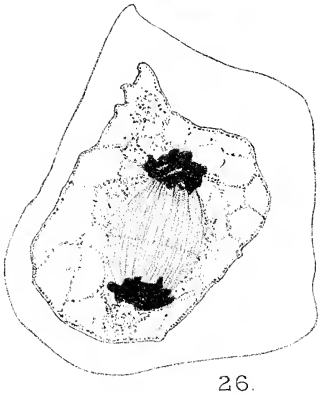
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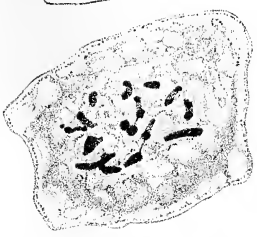
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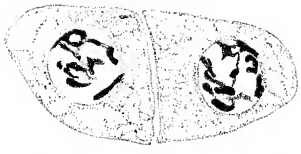
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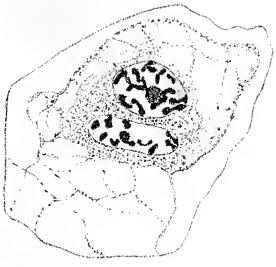
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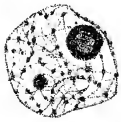
34a.


34b.


31.


32.


33.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37a.


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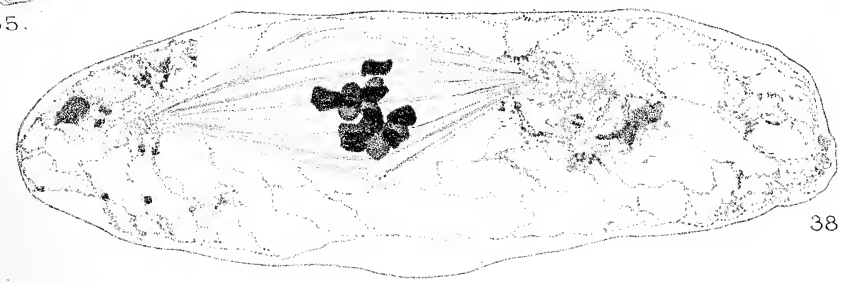
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Huth, lith. et imp.

The Structure of Mesoxylon Lomaxii and M. poroxyloides.

BY

D. H. SCOTT, F.R.S.

With Plates LXXXVII-XC.

THE genus *Mesoxylon* was established by Mr. Maslen and myself in 1910 for certain Palaeozoic stems, intermediate in structure between the genera *Poroxylon* and *Cordaites*. *Mesoxylon Sutcliffii*, one of the five species briefly characterized in our original Note, was very fully described by Mr. Maslen last year (Maslen, '11). His admirable account of this typical form, of which very ample material was available, enables me to deal somewhat more briefly with the two species now to be described, which are not so richly represented. The question of the specific value of the anatomical characters by which we distinguish the fragmentary remains of petrified Palaeozoic plants is a difficult one; we have thought it advisable to give distinct names to well-characterized forms, even though we cannot in all cases be certain that the distinction may not be bridged, as more numerous specimens come under observation.

It will be well to begin by recalling the characters assigned to the genus *Mesoxylon*:

Pith relatively large, discoid. Wood dense, with narrow, usually uniseriate medullary rays, and relatively narrow tracheides. Leaf-traces double where they leave the pith, the two strands uniting at a lower level, but undergoing further subdivision in the pericycle and cortex, before entering the leaf.

Centripetal xylem present in the stem, where it forms part of the leaf-traces at the margin of the pith and throughout their course outwards into the leaves.

Outer cortex strengthened by a system of sclerenchymatous bands of the *Dictyoxylon* or *Sparganum* type. Wood of the kind usual in Cordaitales, the bulk of the secondary tracheides having multiseriate bordered pits on the radial walls. Tracheides of the leaf-traces, spiral or scalariform.[1]

[1] The generic character is very slightly abridged from that given in our Note (Scott and Maslen, '10, p. 237).

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Mr. Maslen has pointed out that '*M. Sutcliffii* (as well as the other species of *Mesoxylon*) exhibits structural characters intermediate between those of *Cordaites* and *Poroxylon*, but on the whole stands much nearer to the former genus' (Maslen, '11, p. 409).

I think this opinion is justified; the chief character in which *Poroxylon* is approached is the very definite one of the presence of centripetal wood in the stem. It was on this ground that I at first put *M. Sutcliffii* provisionally in the genus *Poroxylon* (Scott, '09, p. 511). As regards most, if not all, of the species referred to *Mesoxylon*, the characters common to *Cordaites* are more numerous, if less important. The relation of our genus to certain other genera, recently established, will be briefly considered at the close of the paper.

Mesoxylon Lomaxii, Scott and Maslen.

The specimens of *M. Lomaxii* are larger than most of the others. The three fragments known at present, which may possibly have come originally from a single stem, measure respectively:

5·5 × 2·3 cm. (Slide 2325)
6·0 × 3·5 cm. (Slide 2326)
and 5·0 × 4·0 cm. (Slides 2327 and 2328).

These rough measurements are probably rather under the mark, owing to the difficulty of allowing accurately for the partial destruction of the cortex.[1]

All the specimens are from roof-nodules at Shore, and the preservation is of the usual roof-nodule type: much tissue has been lost altogether, but the remaining portions are often well preserved, though not remarkably so in this case. Fortunately the inner margin of the wood is one of the best preserved regions (Pl. LXXXVII, Figs. 1 and 2).

The pith is large: in the least distorted of the specimens (see Fig. 1) it measures 22 × 17 mm. in diameter. There is a continuous and persistent outer zone of pith, while the central region is in the usual discoid condition (Pl. LXXXIX, Fig. 17).

The other striking features of the species are the dense zone of wood, with numerous prominences, corresponding no doubt to the position of the leaf-traces, on the side towards the pith (Figs. 1 and 2); the evident groups of centripetal xylem in the primary strands of the wood (Pl. LXXXVII,

[1] The longitudinal sections, from the same specimen as the transverse section 2326, are numbered 2375–2383. There are five more sections in the Collection at University College, London, lent me by Prof. F. W. Oliver, F.R.S. Q 6 m is a transverse section cut immediately above my 2328, while Q 6 n, also transverse, belongs to the same fragment as 2326, and was probably cut below that section. Q 6 o–Q 6 q are three longitudinal sections cut from the same specimen as my transverse section 2325. All the University College sections are good, and of quite equal importance to those in my own collection.

Fig. 5; Pl. LXXXIX, Fig. 18): the phloem-zone with its large and conspicuous secretory sacs (Figs. 6 and 7), the broad cortex, mostly of secondary origin, and the rather narrow external, Dictyoxylon zone (Figs. 1, 2, and 6), which, however, is seldom preserved.

The Pith.

The persistent zone of the pith shows little differentiation; the outer portion has somewhat thicker walls than the inner layers, which consist of a delicate tissue; the cells of both portions are short.

The diaphragms constituting the central, discoid part of the pith are made up of thin-walled cells, similar to those of the inner portion of the persistent zone; they have the form usual in Cordaiteae; the diaphragms are thickest at the outer end, where they abut on the persistent zone, and thin out towards the centre of the pith, where they are usually broken and distorted (see the radial section, Pl. LXXXIX, Fig. 17).

The Leaf-traces.

As regards the course of the leaf-trace strands our data are incomplete, for no serial sections are available; the characteristic points, however, are clear. Traced inwards through the secondary wood the twin bundles of a leaf-trace converge (Pl. LXXXVII, Fig. 4), and fuse as soon as they reach the margin of the pith (Fig. 5). This rapid convergence and fusion of the twin strands may be taken, provisionally, as a specific character. As a result, the numerous circum-medullary primary strands are, in the case of *M. Lomaxii*, single bundles (Pl. LXXXIX, Fig. 18), with perhaps some slight indication of recent fusion. The tangential section shown in Fig. 3 passes through a pair of bundles (at *l.t.*), where they have not yet fused. If we now follow the trace outwards, we find that its course approaches the horizontal in passing through the wood, as is necessarily the case, where much secondary growth has taken place. In the phloem the two bundles reappear in approximately transverse section (Fig. 6). In passing through the cortex further subdivision goes on. Thus the four bundles of a single trace are seen in Fig. 7. Later stages of subdivision are not clearly shown, but it is evident that the bundles of the trace increased in number, for at least five are seen in a tangential section through the secondary cortex (slide 2376). Probably the ultimate number was eight, as in the other species.

The outgoing leaf-traces observed are not numerous. Though the loss of tissue, both in the wood and cortex, has no doubt often led to their being missed, it is probable that the leaves to which they ran were really more scattered than in the three species, *M. Sutcliffii*, *M. poroxyloides*, and *M. multirame*. At the most not more than four traces have been observed outside the wood, nor more than three in the wood itself, in

any one transverse section. It must, however, be remembered that the great development of periderm in the cortex must give the bundles a more horizontal course in this region, so that the conditions, even apart from the state of preservation, are not so favourable for observing them in transverse section as in specimens where there is less secondary growth.

On the other hand, the single primary xylem-strands, formed by the fused pairs, are present in large numbers around the pith; in each transverse section there are about twenty which show evident centripetal xylem, while other prominences, which are without this tissue, no doubt represent the lower ends of similar leaf-trace strands or the products of their anastomosis. Thus the whole inner margin of the wood is strongly undulated (Fig. 1), the intermediate secondary xylem only reaching the pith in the bays between the prominences. From the great number of primary xylem-strands it is evident that the phyllotaxis was complex.

In the structure of the leaf-traces, the first point to be noticed is the centripetal xylem. This is well developed, both in the bundles passing through the cortex (Pl. LXXXVII, Figs. 6 and 7), and in those which are approaching or have reached the edge of the pith (Pl. LXXXVII, Fig. 5; Pl. LXXXIX, Fig. 18). It forms, as seen in transverse section, an arc or crescent of considerable thickness; its irregularly grouped elements are sharply distinguished from the radial rows of the centrifugal wood (Fig. 18). The tracheides of the centripetal xylem are spiral or finely scalariform. It appears that the tissue persisted with little diminution for some distance downwards after the bundle reached the pith, for many of the circum-medullary strands have a well-developed centripetal portion. In others, however, it is extremely reduced, while it is altogether absent from many of the prominent xylem-strands. There can thus be no doubt that the centripetal wood eventually died out in a downward direction, as in other species of *Mesoxylon* and in *Poroxylon*, *Calamopitys Beinertiana*, &c.

The position of the protoxylem appears to have been, as is usually the case in this group, in contact with the centripetal wood (Pl. LXXXIX, Fig. 18, *px*). Here, as in other species, it is accompanied by some thin-walled parenchyma, which partially separates the centripetal from the centrifugal xylem.

A characteristic feature of the circum-medullary strands is the presence, in many cases, of a definite bundle-sheath separating the xylem from the pith. The sheath is limited to those strands which are seen in the upper part of their course; it dies out lower down. In a transverse section the sheath is found in about two-thirds of the strands which still have definite centripetal xylem. The simplest form of sheath occurs low down in the course of the strand. Here it consists of an arc, not more than two or three cells in thickness, of more or less radially elongated cells, with walls a little thicker than those of the adjacent pith-cells (Pl. LXXXIX, Fig. 18, *sh*).

The sheath encloses the whole prominent part, both centripetal and centrifugal, of the xylem-strand. Following the strand upwards in its course to the point where it is about to leave the pith, the sheath is here a much more important structure, consisting of numerous layers of radially arranged thick-walled cells, which have evidently increased in number by secondary divisions (Pl. LXXXVII, Fig. 5, *sh*). The double bundle shown in the photograph is in the act of fusion, or rather of separation, for we are tracing it in the upward direction. Still higher up, where the twin bundles are beginning to pass out into the wood, the sheath forms a double arc, corresponding to the two strands (Pl. LXXXVII, Fig. 4). In this region the cells of the sheath have reticulate, tracheoidal markings, which may be seen in Fig. 4 on using a lens. As we follow the trace on its outward course, we find that the sheath becomes merged in the secondary wood which here closes in behind the outgoing strands; in the transitional region the short cells of the sheath are interspersed with the long, sinuous tracheides.

The Wood.

The structure of the wood, apart from the centripetal xylem of the leaf-traces, is of the usual Cordaitean type. In the parts corresponding to the primary strands a considerable amount of the inner centrifugal wood consists of scalariform tracheides, or in some cases elements with crossed spirals; in the case of an entering bundle, fifteen layers of such elements were counted, and in one of the blunter prominences, representing a bundle lower down in its course, there were ten such layers.

In places, however, where the strictly secondary wood abuts on the pith, as in the depressions between the bundles, the pitted tracheides may extend right up to the inner margin. The scalariform elements are, as a rule, sculptured on all sides, while in the pitted tracheides the pits are limited to the radial walls. Transitional elements sometimes occur.

The tracheides of the secondary wood are rather small, ranging from 30 to 60 μ, the usual dimensions being 40 to 50 μ. The medullary rays are very narrow, 12 to 18 μ in width. As the tangential sections show, they may reach a considerable height, up to about twenty-five cells. They range from this down to a height of two cells or even one. Only in quite rare cases do we find a radial division, making the ray locally biseriate; otherwise the rays are uniseriate throughout (Pl. LXXXIX, Fig. 19). In certain places the pits are well preserved. They form two or three series on the radial wall of the tracheide, and have the somewhat angular outline usual in Cordaitales. In a few cases the border of the pit is sufficiently perfect to show the inclined, elliptical slit, but usually the pore appears round (Pl. LXXXIX, Fig. 21). On the walls of medullary ray-cells in contact with tracheides the pits are arranged in two or three horizontal rows on the

ray-cell; they are less regular in form than elsewhere, and seem to be unbordered, though the preservation leaves this uncertain.

In the outer secondary wood the position of a double leaf-trace is marked, in tangential section, by the presence of two knots of irregularly interwoven tracheides, enclosing in their meshes the tracheides of the outgoing strand.

The Phloem and Pericycle.

The phloem-zone is traversed by long, sac-like elements, possibly of a secretory nature. In one of the specimens especially (slide 2325), they are very conspicuous; they are from 100 to 200 μ in diameter; their thick walls appear black and their contents brown (Pl. LXXXVII, Figs. 1 and 6). These elements extend into the pericycle, which may be distinguished from the phloem in longitudinal section by the fact that only short cells are present between the sacs.

In the phloem proper, which is imperfectly preserved, there are long tubes, about 40 μ in diameter, which in tangential section are seen to form a kind of mesh-work, enclosing the phloem-rays (LXXXIX, Fig. 20). Transverse or oblique walls appear to occur at long intervals. These tubular elements may probably be the sieve-tubes.

The Cortex.

The inner cortex is remarkable for the great development of secondary tissue, resembling periderm (Pl. LXXXVII, Figs. 2 and 6). The whole of the thin-walled zone has its cells radially arranged; the radial cell-rows, however, are not continuous throughout; the tangential divisions appear to have gone on in more than one layer, and not to have been limited to a definite phellogen. The zone in question, which reaches a thickness of at least 5 mm., may perhaps be more properly called secondary cortex rather than periderm.

In the hypodermal zone the radial sclerotic bands are usually narrow, and vary much in depth. There is no extensive tangential section of this zone, but some cases of anastomosis between the bands have been observed. Hence we may speak of the mechanical hypoderm as of the Dictyoxylon type. Towards the exterior the sclerotic bands unite in a continuous zone of moderately thick-walled tissue. The outer tissues are very imperfectly preserved, but the contour, as far as shown, appears to prove that the leaf-bases were scattered, and not crowded as in *M. Sutcliffii*.

Diagnosis.

An amended diagnosis of the species may now be given:

Mesoxylon Lomaxii, Scott and Maslen, 1910.

Leaf-bases scattered.

Pith large and discoid, with a persistent outer zone.

Twin-bundles of the leaf-trace converging rapidly in the wood, and fusing immediately on reaching the edge of the pith.

Centripetal xylem well developed, persisting for a long distance below the fusion of the twin-bundles.

Xylem-strands at the margin of the pith with a distinct and wide sheath in the upper part of their course.

Spiral and scalariform tracheides constituting the whole of the leaf-trace xylem and the inner part of the corresponding secondary wood, but almost absent from the intermediate secondary wood.

Bordered pits in two or three rows.

Medullary rays (with rare exceptions) uniseriate, 1–25 cells in height.

Secondary inner cortex or periderm of great thickness.[1]

Dictyoxylon zone relatively narrow.

Roof-nodules; Shore, Littleborough. Lower Coal Measures.

This species is named after Mr. James Lomax, to whom its discovery and that of the other species of *Mesoxylon* is due.

Mesoxylon poroxyloides, Scott and Maslen.

This species, like the last, was first named and shortly described in 1910, in the joint Note by Mr. Maslen and myself. It had, however, already been referred to, together with *M. multirame*, in the second edition of my 'Studies in Fossil Botany' (Scott, '09, p. 526), where I mentioned the occurrence of stems from Shore, in which the centripetal wood of a *Poroxylon* co-existed with the discoid pith and other characters of a *Cordaites*. *Mesoxylon poroxyloides* is a fossil from the ordinary coal-balls or seam-nodules of Shore; in this respect it is like *M. multirame*, and differs from *M. Sutcliffii*, *M. Lomaxii*, and *M. platypodium*, which are all roof-nodule specimens. The preservation of *M. poroxyloides* is consequently more complete than that of the species last mentioned, for it is free from the patchiness which is the peculiar defect of specimens from the roof-nodules. The detailed investigation has been carried out on one specimen, represented in my collection by seven sections (slides 2352–2358), of which four are transverse and three longitudinal. This may be called the type specimen.[2]

[1] This character must, of course, vary with the age of the specimen.

[2] There are six more sections of this specimen in the University College collection, for the loan of which I am indebted to my friend Prof. F. W. Oliver, F.R.S. Two of the sections, Q 6 a and Q 6 b, are transverse, and four, Q 6 c–Q 6 f, longitudinal. I have been able to determine the order of four of the transverse sections, which, from below upwards, run thus:

S. Coll. 2352
,, 2353
Univ. Coll. Coll. Q 6 a
,, Q 6 b

The other two transverse sections are not in series with the above, but 2355 comes below 2354.

Another stem, incompletely shown in slide 2397, may probably belong to this species, and is interesting from its close association with numerous leaves of the so-called 'Cordaitean' type (see p. 1022). A third stem (slide 2609) closely resembles the type, though not so well preserved. These are rather small stems from 2 to 3 cm. in diameter. A much larger specimen (slide 2608), of which the *radius*, measured only to the outer edge of the wood, exceeds 4 cm., has some points in common with *M. poroxyloides*, but in such large and incompletely preserved stems specific identification becomes very uncertain.

In the type specimen (Pl. LXXXVIII, Fig. 8), the pith measures about 12 × 4 mm. (8 mm. mean diameter), that of the whole stem being about 2·5 cm. In slide 2397 (the second specimen) the stem is too incomplete to measure accurately: the diameter may have been about 2 cm.; the stem is young and the pith therefore proportionately larger than in the type. The stem in slide 2609 approaches 3 cm. in diameter, that of the pith being little more than 6 mm. It thus appears that a comparatively small pith may be regarded as characteristic.

The pith, as in other species, has a persistent outer zone and a discoid middle (Pl. LXXXVIII, Fig. 13; Pl. XC, Fig. 22). In the convergence and early fusion of the twin-bundles of the leaf-trace as they reach the pith (Pl. LXXXVIII, Figs. 9 and 10) *M. poroxyloides* comes nearest to *M. Lomaxii*, from which it differs in the absence of a definite sheath round the primary xylem.

The centripetal xylem is well developed and is retained for some distance below the point of fusion of the strands (Pl. XC, Fig. 23). The secondary wood has the small tracheides and narrow medullary rays usual in the genus.

The phloem, chiefly secondary, forms a broad zone, well preserved in the type-specimen. The pericycle contains numerous large elements with dark contents, which may have been secretory sacs.

The Dictyoxylon zone of the cortex is wider than in other species. The leaf-traces subdivide in passing through the cortex, and on entering the leaf-base the full number of eight bundles is attained (Pl. XC, Fig. 24).

No axillary shoots or steles have yet been observed.

The Pith.

The pith is decidedly well preserved in places; the most favourable section for showing it as a whole is slide Q 6 c in the University College collection. The section illustrated in Pl. LXXXVIII, Fig. 13, and Pl. XC, Fig. 22, is not quite radial and the pith is somewhat broken and displaced, but the discoid structure is sufficiently evident. It is of rather a peculiar kind and may be described as compound, for the thick horizontal plates stretching inwards from the persistent zone subdivide towards the

middle into a number of thin discs (Pl. XC, Fig. 22; Pl. LXXXVIII, Fig. 13). Thus the rupture of the pith appears to have taken place in two stages. It is possible that this may have only happened locally.

The external, persistent zone of pith is about 0·8 mm. thick, and consists for the most part of thin-walled, fairly isodiametric cells. In the inner layers, the cells appear empty or have light-brown contents; those next the central cavity are sometimes flattened (Pl. LXXXVIII, Fig. 10). In the outer half of the zone the cells are a trifle smaller, and many of them have almost black contents. In contact with the wood a few narrow, vertically elongated, delicate-looking cells may be distinguished (Pl. LXXXVIII, Figs. 10, 11, and 14).

The diaphragms are composed of larger cells than those of the persistent zone; they appear round or somewhat hexagonal in transverse section, and when seen in longitudinal section are found to be much flattened (Fig. 13). The tissue of the thin central diaphragms has collapsed; connecting shreds of membrane show that the diaphragms have been torn apart by the growth of the stem (Fig. 13). The excellent preservation of the persistent zone is a clear proof that the partial destruction of the central tissue was a natural process occurring during life. It will be seen that the structure of the pith in *M. poroxyloides* agrees very closely with that of *M. Sutcliffii*, described by Mr. Maslen (Maslen, '11, p. 391).

The Leaf-traces.

In the zone of wood, as shown in a complete section, Pl. LXXXVIII, Fig. 8, three pairs of bundles are seen passing in, and seven more leaf-traces can be recognized at the margin of the pith, either in course of fusion or already fused. In all these strands the centripetal xylem is well developed.

As the twin xylem-strands come in through the secondary wood they converge (Pl. LXXXVIII, Fig. 9), and almost immediately on reaching the border of the pith they prepare to fuse. At this level the two centripetal xylem groups open out towards each other (see the right-hand bundle in Fig. 9), the xylem-parenchyma of the two strands becoming continuous across the medullary ray between them. At a slightly lower level the two centripetal arcs of xylem unite, so that the joint strand now has a single primary xylem-mass. The photograph in Pl. LXXXVIII, Fig. 10, shows a double strand in the act of fusion; the two centripetal groups of xylem have just become continuous. The drawing (Pl. XC, Fig. 23) shows a similar strand lower down in its course, where union is complete. That this is the actual course of the changes, as traced from above downwards, is shown on comparing the same strands in successive sections. Other strands around the pith have reduced remains of centripetal xylem, or the smallest elements are quite on the inner edge, showing that the structure has become endarch.

As regards the outward course of the leaf-traces, there is no essential difference from *M. Sutcliffii*. The bundles, however, run very obliquely through the secondary wood, the phloem zone, and the inner cortex; they are seldom seen well in transverse sections of these regions. We know, however, that the original pair divided up into eight, for all the eight bundles of a trace are seen in the section shown in Pl. XC, Fig. 24, where they are entering a leaf-base. Some sections show the strands passing almost horizontally through the wood, and subdividing as they enter the cortex.

The structure of the primary strand is essentially the same as in *M. Sutcliffii* and other species. The centripetal xylem is perhaps rather specially well developed; here also the protoxylem, where it can be clearly seen, is in contact with the centripetal part of the xylem, abutting on the internal parenchyma of the strand (Pl. XC, Fig. 23). Longitudinal sections show that the centripetal wood is composed, apart from the protoxylem, of close-wound spiral elements. The somewhat oblique tangential section from which the photograph (Pl. LXXXVIII, Figs. 11 and 12) is taken shows a pair of bundles approaching the pith. In the right-hand bundle the plane of section passes obliquely through the island of parenchyma and protoxylem (Fig. 12, *px.*).

No actual sheath is developed round the xylem-strands as they reach the pith. There is, however, a very near approach to this structure, for, where the double strand is entering through the wood and getting near the pith, the adjacent cells on the inner side have divided tangentially, and some of them show tracheal markings, just as in *M. Lomaxii* (Fig. 9, *sh*). The differences between the two species as regards the bundle-sheath is thus only one of degree.

The Wood.

The inner part of the centrifugal wood is composed of spiral, reticulate, or scalariform elements. The reticulate markings are often evidently formed by crossed spiral bands, such as are shown at one or two places in Mr. Maslen's figure from a leaf-trace bundle of *M. Sutcliffii* (Maslen, '11, Pl. XXXV, Fig. 14). The reticulate condition often forms the transition to the regular pitted sculpturing of the main mass of secondary tracheides. The zone of spiral and transitional elements is of considerable width, twelve to fourteen layers in the leaf-trace, and about six layers even where the wood appears to be purely secondary. This seems to be a specific difference from *M. Lomaxii*.

The typical, pitted, secondary tracheides have only two rows of pits on the radial wall, and sometimes only a single row (Pl. LXXXVIII, Figs. 14 and 16). Where a medullary ray is crossed, the pits are more numerous, narrower, and markedly oblique. The tracheides are about 20 to 40 μ in

diameter, most of them over 30 μ. The rays are almost invariably uniseriate, ranging in height from one to about ten cells; the diameter of a ray is commonly from 20 to 25 μ, the cells being about 36 μ in height. At the end towards the pith the rays become broader, and radial divisions of their cells are frequent. The wood of *M. poroxyloides* thus differs from that of *M. Lomaxii* in the somewhat smaller size of the tracheides, with a corresponding diminution in the number of rows of pits, and, so far as observed, in the absence of very high medullary rays. There is pretty close agreement with the wood of *M. Sutcliffii*, as described by Mr. Maslen. The greater development of the inner spiral and scalariform zone of tracheides in the secondary wood is characteristic of *M. poroxyloides*.

The Phloem and Pericycle.

The phloem in the type specimen forms a zone 0·8 mm. to 1 mm. in breadth (Pl. LXXXVIII, Fig. 15). Nearly the whole thickness is made up of radially seriated, secondary elements. The phloem is very fairly preserved, and is closely similar in structure to that of *M. Sutcliffii*, of which Mr. Maslen has given a full description (Maslen, '11, p. 400). The elongated elements with dense contents are very conspicuous; in some the contents are black, in others light brown, but the two appear to pass over into one another, and I cannot find any sharp distinction between the two forms. It may be mentioned that cells with dark contents also occur in the phloem rays, which are often dilated towards the exterior. It is probable, as suggested by Mr. Maslen in the case of his species, that the long elements with no obvious contents are the sieve-tubes. I postpone, however, any detailed account of the phloem to a later paper, as I have recently received from Mr. Lomax a series of sections from a remarkably well preserved stem, closely similar to *M. poroxyloides* as regards the phloem, though differing in some other details. Beyond the phloem comes another fairly broad zone, which may best be regarded as the pericycle. This contains a number of large sacs with dark contents (Fig. 15, *s.s.*). In longitudinal section the sacs often appear to be divided up into cells, but this may only be due to a breaking up of the contents.

The Cortex.

On the outer border of the pericycle is a layer of internal periderm, very unequally developed, and in some places reaching a thickness of about a dozen cells. Many of the periderm cells are flattened and rather thick-walled, suggesting a true cork. There is, however, no sign of any general desiccation of the outer tissues, so it is evident that they had not yet been completely cut off by cork from communication with the water supply of the stem. At many places the periderm is double, an outer layer or arc having formed in the adjacent cortical tissue. On general grounds one would

expect the outer periderm to have been formed before the inner ; the latter, however, is sometimes the more developed of the two, and as the whole cortex appears to have been living up to the time when the plant itself met its fate, we cannot be certain as to the order in which the peridermal layers appeared.

The inner cortex, like the pericycle, contains large sacs with dark contents, though they are not so numerous here. Except where a leaf-trace is passing out, the inner cortex is little developed ; we almost immediately reach the Dictyoxylon zone, which attains a considerable thickness—quite 2 mm. in places. The radial bands of fibres do not, however, extend quite to the outside of the stem ; the fibres are little thickened, and the differentiation of the mechanical tissues, as seen in transverse section, not very marked.

The outer surface of the stem is incompletely preserved, and hence the arrangement of the leaf-bases is obscured. At first sight they appear to be scattered, but further examination shows that they must have been fairly close together. Thus, in one section (slide 2353) two leaf-traces are seen in the outer cortex; both consist of a number of bundles—probably eight in each case—though all are not preserved. The adjacent bundles of the two traces are only about 4 mm. apart.[1] The corresponding leaf-bases are not complete, but there can have been no great extent of stem-surface between them. The leaf-bases, however, were probably somewhat less closely set than in *M. Sutcliffii*.

As in other species, where a leaf-trace passes out through the cortex into the leaf-base it is surrounded by a large mass of parenchyma, which encroaches upon the Dictyoxylon zone. This is consequently considerably thinner opposite the outgoing leaf-traces than elsewhere.

It may be mentioned that the 'Cordaitean' leaves associated in slide 2397 with a stem, probably referable to *M. poroxyloides*, are of the type recently described by Prof. Margaret Benson under the name *Cordaites Felicis* (Benson, '12). This is shown by the well-developed sheath, the presence of complete longitudinal fibrous partitions between the bundles and of thickened masses of hypoderm between the bundles and the partitions, the partial 'inner sheath', and the arrangement of the centripetal and centrifugal xylem, which is often identical with that shown in Prof. Benson's text-figure (l. c., p. 204). Some of the leaves associated with the stem in question are, however, thicker (i.e. cut, no doubt, nearer the base) than any of Prof. Benson's specimens; one fragment reaches a thickness of 1·8 mm. In these thickest parts some of the partitions are missing; their absence may be correlated with a recent division of the bundles.

[1] The distance between the centre lines of the two traces would be about 8 mm.

It thus appears very probable that *Cordaites Felicis* was the leaf of a *Mesoxylon* of the *poroxyloides* type. Direct evidence from continuity may perhaps be hoped for. At any rate this probability may serve to call closer attention to the British specimens of 'Cordaitean' leaves, which had been much neglected until the appearance of Prof. Benson's paper. Many excellent specimens are now available.

Diagnosis.

The following is an amended diagnosis of the species :

Mesoxylon poroxyloides, Scott and Maslen, 1910.

Leaf-bases moderately crowded, not quite covering the surface of the stem.

Pith not very large, discoid, with a persistent outer zone.

Diaphragms sometimes splitting into finer plates towards the middle of the pith.

Twin-bundles of the leaf-trace converging and uniting soon after reaching the pith, subdividing in the cortex to form eight bundles.

Centripetal xylem very distinct, persisting below the fusion of the twin strands.

Xylem-strands without a sheath, or with only a rudimentary one, limited to the region where they first reach the pith.

Tracheides of the inner part of the intermediate secondary wood, as well as those of the leaf-traces, spiral, reticulate, or scalariform.

Bordered pits in one or two rows.

Medullary rays (with rare exceptions) uniseriate, 1–10 cells in height.

Dictyoxylon zone of cortex very broad, fibres not much thickened.

Seam-nodules; Shore, Littleborough. Lower Coal Measures.

Comparative Considerations.

Important forms of *Mesoxylon* still remain to be described, but the three species which have now been dealt with by Mr. Maslen and myself are sufficient to make the position of the genus clear. The existing classical accounts of the structure of the stem in *Cordaites* (Renault, '79 and '96) are not so full as we could wish, and a reinvestigation is desirable; so far as the stem is concerned we are now better acquainted, in some respects, with the anatomy of *Mesoxylon* than with that of the type-genus of the family. On the available data, however, it is evident that the affinity between *Mesoxylon* and *Cordaites* is a close one, and I agree with Mr. Maslen that our genus may best be included in the family Cordaiteae (Maslen, '11, p. 411). This is, of course, a departure from the position which I took up in the second edition of my 'Studies in Fossil Botany' (Scott, '09, p. 511), when I provisionally included what is now *Mesoxylon Sutcliffii* in the genus *Poroxylon*, though at the same time I referred to other forms, since added

to *Mesoxylon*, under Cordaiteae (l. c., p. 526). The centripetal wood of the stem, no doubt, died out gradually; in *Mesoxylon* it is still sufficiently well developed to justify generic separation from *Cordaites*, but not, I think, a position in a distinct family. The point, however, is of little importance; what really matters is, that the two families Cordaiteae and Poroxylae are now closely linked together.

The points of resemblance and difference between *Mesoxylon*, *Cordaites*, and *Poroxylon* have been well summarized by Mr. Maslen (l. c., p. 410). They hold good essentially for all the species investigated. It will be noticed that no definite distinction between *Mesoxylon* and *Cordaites* is given, beyond the presence of centripetal xylem in the stele of the former. In other respects there appears to be agreement, but we do not know enough about the exact course of the leaf-traces or the detailed structure of the phloem in *Cordaites*, to be certain how close the agreement really was. At any rate, we have the large, discoid pith, the dense wood with narrow rays,[1] and the histology of the xylem-elements, especially the great development of spiral and scalariform tracheides in the leaf-trace region, as definite characters common to the two genera. The species of *Mesoxylon* would have been left in *Cordaites* if it were not for the presence of centripetal wood in the stele. I feel no doubt that most of the British specimens of 'Cordaitean' leaves really belong to *Mesoxylon*, which is a much commoner type of stem in the Coal-Measure petrifactions than that of *Cordaites* itself. If this belief is confirmed, we shall have the close agreement in foliar characters as another proof of the near affinity of the two genera.

I thus regard *Mesoxylon* as the last term in the series of forms leading up from the Seed-Ferns to the typical *Cordaites* described by Grand'Eury and Renault.

Some additions have lately been made to the Cordaitales and their allies by Dr. Zalessky, who has founded no less than five new genera, partly for new forms, partly for plants already described under other names (Zalessky, '09, '11[1], and '11[2]).

The new forms are: *Callixylon Trifilievi*, Zal. (Zalessky, '09 and '11[1]), and *Caenoxylon Scotti*, Zal. (Zalessky, '11[2]). The other new genera are: *Eristophyton* (Zalessky, 11[1]), based on *Calamopitys Beinertiana* (Göpp.), and *C. fascicularis*, Scott; *Mesopitys* (Zalessky, '11[1]), based on *Dadoxylon Tchihatcheffi* (Göpp.); and *Parapitys* (Zalessky, '11[1]), based on *Dadoxylon Spenceri*, Scott.

Callixylon Trifilievi (Zalessky, '09) from the Upper Devonian of the Donetz basin in Russia is a very interesting fossil, evidently allied to the Lower Carboniferous genus *Pitys* (Scott, '02). Numerous (26) small, mesarch

[1] As a matter of fact somewhat wider rays occur in *Cordaites* than have yet been observed in *Mesoxylon* (Renault, '96, p. 334).

strands of primary xylem surround the pith, most of them in contact with the secondary wood, though some are separated from it by 1 to 3 rows of cells. These strands anastomose freely. At a distance from the pith the rays are usually uniseriate. The pith is horizontally ruptured (imperfectly discoid), and the pith-cells surrounding a primary xylem-strand are elongated radially with respect to it. Tangential pits occur in the secondary wood, as in *Pitys antiqua*. The agreement with *Pitys* seems to be decidedly nearer than with the other genera (*Parapitys* and *Eristophyton*) with which the author compares it. He separates the plant generically on the ground of its narrow medullary rays, the wedge-like segments of secondary wood, and the arrangement of the pits, on the radial walls of the secondary tracheides, in groups (Zalessky, '11[1], p. 28). At any rate this new form, of which a fuller description is promised by the discoverer, finds its place in the family Pityeae, and has no near affinity with *Mesoxylon*.

The fragment named *Caenoxylon Scotti* by Dr. Zalessky is of uncertain origin; it is of Upper Palaeozoic age, and possibly comes from the Permian of the Ural (Zalessky, '11[2]). The pith is 2 cm. in diameter, and the zone of wood, so far as preserved, about 13 mm. thick. The presence of distinct annual rings in the wood is an interesting feature, indicating a relatively late age. The pith is not stated to be discoid, but is described as consisting of distinct outer and inner regions, with a meristematic layer between them.

The primary xylem is divided into a number of bundles of various sizes and shapes, separated by medullary tissue with occasional signs of meristematic division. Some of the strands may be a considerable distance apart from the main mass of primary xylem, of which they are ramifications; in all, the structure is endarch. The leaf-traces are double, and the medullary rays uniseriate. The author regards *Caenoxylon* as undoubtedly allied to *Eristophyton*, *Pitys*, *Parapitys*, and *Mesopitys*, and more remotely to *Cordaites*, *Poroxylon*, and *Mesoxylon*. He suggests, on the ground of the arrangement of the primary xylem and the character of the double leaf-trace, that the fossil may possibly be on the line of descent of *Ginkgo*.

At present we have only a preliminary communication from Dr. Zalessky on this form. Except that I cannot recognize any but the most remote affinity to his *Eristophyton*, I have nothing to add to the author's remarks.

In connexion with *Caenoxylon* we may shortly refer to Dr. Zalessky's new genus *Mesopitys*, founded on the *Araucarites Tchihatcheffi* of Göppert. This, again, is a Permian plant, and was the first stem of that age in which the presence of annual rings in the wood was observed. The pith is small (3 mm. in diameter in one case), the stems (without cortex) ranging from 5 × 3 to 13 × 8 cm. No mesarch xylem-strands were observed; the primary wood, as in *Caenoxylon*, is all centrifugal, forming groups of irregularly

arranged tracheides with the smallest elements always on the inner side. The whole of the primary wood is composed of spiral or rayed tracheides which abut immediately on the pitted elements of the secondary wood. Unlike *Caenoxylon*, *Mesopitys Tchihatcheffi* has a single bundle constituting the leaf-trace, where it passes out through the wood. The medullary rays are uniseriate. The genus *Mesopitys* is established for stems with secondary wood of the *Dadoxylon* type, characterized by the arrangement of the feebly developed primary wood in bundles of endarch or even mesarch structure, and by the single bundles traversing the secondary wood (Zalessky, '11[1], p. 28). The author regards his new genus as forming the final term of the group of stems of the *Dadoxylon* type described by me in 1902, and considers it to be closely allied to his *Eristophyton Beinertianum* (*Calamopitys Beinertiana* (Scott)).

It is interesting to note that Dr. Zalessky is prepared to include stems with mesarch as well as those with purely endarch primary xylem in the same genus. I have no objection to this in principle, for the one structure, no doubt, passed over gradually into the other, nor do I dispute a certain degree of affinity between my *Calamopitys Beinertiana* and *Mesopitys Tchihatcheffi*. At the same time, the presence of very highly developed mesarch strands in the former plant (though they become endarch lower down in their course) appears to me a more important difference than Dr. Zalessky recognizes. I quite agree with the author that his *Mesopitys Tchihatcheffi* requires a new genus.

Another of Dr. Zalessky's new genera is *Parapitys*, founded for the reception of *Dadoxylon Spenceri* (Scott, '02). It was inevitable that sooner or later this form should acquire generic rank. It is characterized by the double leaf-traces, the small mesarch strands of primary xylem, and the relatively small pentagonal pith, the wood being of the ordinary Cordaitean type. Of all the forms discussed by Dr. Zalessky, *Parapitys* seems to me the nearest to *Mesoxylon*, from which it only differs (so far as we know at present) in the characters of the pith. It seems to be widely separated from the species of *Calamopitys* placed by Dr. Zalessky in *Eristophyton* by the double leaf-traces and the great reduction of the primary mesarch strands.

Dr. Zalessky devotes a good deal of space to an argument against my inclusion of *Calamopitys fascicularis* and *C. Beinertiana* in the genus *Calamopitys* of Unger, founding the new genus *Eristophyton* for their reception (Zalessky, '11[1]). I do not propose to enter fully into the question in this paper, because, from my point of view, these plants have very little to do with *Mesoxylon*. It is possible that the various, not very important characters, on which Dr. Zalessky lays stress, may in the aggregate justify generic separation. The author, however, has not quite realized what the characters are which I regard as essential to *Calamopitys*. As I stated (Scott, '02, p. 360): 'The *Calamopitys* group is characterized by the

relatively large dimensions and distinct mesarch structure of those primary xylem-strands which are about to pass out from the pith, while the same strands, lower down in their course, are reduced in size, and in some cases assume endarch structure, owing to the dying out of the centripetal xylem. A single strand passed out from the pith to form the leaf-trace.'

The really characteristic point in *Calamopitys* is the presence of the quite peculiar large, round mesarch xylem-strands, which are unlike those of any other plant I have seen. Their structure in *C. Saturni* and *C. fascicularis* (to take an example from each group) is identical, as I hope to show more in detail on another occasion. The only differential character of any weight between *Calamopitys* and the new *Eristophyton* is the width of the medullary rays, which are generally wide in the former and narrow in the latter. This, however, appears to be inconstant, for, in a section of *C. annularis*, very kindly lent me, among many other preparations, by Count Solms-Laubach, the rays are only one or at most two cells in width, thus differing from the pluriseriate rays usual in *C. Saturni* and even in other specimens of *C. annularis*. There is no reasonable doubt that the species of *Calamopitys* described by Count Solms-Laubach were Pteridosperms, as indicated by their *Kalymma* petioles. The important question at issue between Dr. Zalessky and myself is whether the species *C. fascicularis* and *C. Beinertiana* were likewise Pteridosperms, or belonged to a higher group. This goes much beyond the mere question of generic separation, which is of secondary importance.

At present I must adhere to my view that the agreement in the primary characters of the wood, between the species placed by Dr. Zalessky in *Eristophyton* and the type species of *Calamopitys*, is so close as to prove a very near affinity. The question will not, however, be finally decided until the cortex and leaf-bases of the former species are known.

One argument used by Dr. Zalessky must be shortly dealt with, as it appears to me somewhat misleading (Zalessky, '11[1], p. 27). He says that, if my view is just, we must suppose that the species *fascicularis* and *Beinertiana* had fern-like foliage. He regards his *Callixylon Trifilievi* as certainly allied to these species. He finds reason to believe that the latter plant may have had a stem reaching about a metre in diameter, and thinks it improbable that such trunks could have borne the foliage of a Fern.

Now, to begin with, *Callixylon Trifilievi* shows no close affinity to *Calamopitys fascicularis* and *Beinertiana*. It is practically a *Pitys*, and no one has attributed filicoid foliage to *Pitys*, which was more probably of Cordaitean habit. Secondly, there is no reason to doubt that in Palaeozoic days stems of great girth bore fern-like foliage. A specimen of *Medullosa stellata* discovered by Weber (Weber und Sterzel, '96, p. 25), though decorticated, measured nearly $\frac{1}{2}$ a metre (48 × 45 cm.) in diameter. It is well known that the foliage of *Medullosa* was fern-like (e. g. *Neuropteris*,

Alethopteris). Whether *Calamopitys fascicularis* and *Beinertiana* attained a very large diameter or not we cannot say; if they did so, the fact would certainly be no argument against their Pteridospermous affinities.

Summary.

The characters of *Mesoxylon Lomaxii* and *M. poroxyloides* have already been summarized in their specific diagnoses (pp. 1016 and 1023). The genus comes very near *Cordaites*, as shown by the characters of the pith and wood, and further indicated by those of the associated leaves. The affinity with *Poroxylon* is somewhat more remote, and the genus is best placed in the family Cordaiteae; it is at present definitely distinguished from *Cordaites* only by the presence of centripetal wood in the stele of the stem. *Mesoxylon* thus forms the last link in the chain of fossil types connecting the Pteridosperms with the typical *Cordaites* of the Upper Palaeozoic.

Of the new genera recently established by Dr. Zalessky, *Callixylon* is very near *Pitys* and should be placed in the same family. *Caenoxylon* and *Mesopitys* are advanced forms, and had reached an anatomical level corresponding to that of *Cordaites* itself, but on somewhat different lines.

Parapitys is best regarded as a near ally of *Mesoxylon*. *Eristophyton*, even if generically separable from *Calamopitys*, is closely allied to it, and both may provisionally be regarded as probably belonging to the Pteridosperms rather than to the Cordaitales. It is possible, however, that further discoveries may show that the Calamopityeae were an important transitional group.

The results already attained by Dr. Zalessky and others are very satisfactory, as demonstrating a considerable variety among the stems of Palaeozoic age, referable or allied to the Cordaitales.

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DESCRIPTION OF PLATES LXXXVII–XC.

Illustrating Dr. Scott's paper on *Mesoxylon*.

The photographic figures require to be examined with a lens.

PLATE LXXXVII.

Mesoxylon Lomaxii.

Photographs by Mr. W. Tams.

Fig. 1. General transverse section of the stem, showing the patchy preservation characteristic of roof-nodule specimens. × nearly 2. Slide 2328.

Fig. 2. Half of another transverse section, better preserved. *d*, Dictyoxylon zone; *pd*, periderm. × about 3. Slide 2325.

Fig. 3. Tangential section from inner part of wood, showing the twin-bundles of a leaf-trace (*l.t.*) and their junction below. × 10. Slide 2380.

Fig. 4. Somewhat oblique section, showing the converging twin-bundles of a leaf-trace (*l.t.*). *sh*, bundle-sheath; *p.*, pith. × about 30. Slide 2325.

Fig. 5. Leaf-trace in the act of fusion. *x*, centripetal xylem; *sh*, bundle-sheath; *p*, pith. × about 40. Slide 2328.

Fig. 6. Double leaf-trace in the phloem: bundles slightly displaced. *x*, centripetal xylem of a bundle; x^2, secondary wood of stele; *pd*, periderm; the dark bodies are the 'secretory sacs'. × about 45. Slide 2325.

Fig. 7. Quadruple leaf-trace in the inner cortex. *x*, centripetal xylem of a bundle. × about 45. Slide 2325.

PLATE LXXXVIII.

Mesoxylon poroxyloides.

Photographs by Mr. W. Tams.

Fig. 8. General transverse section. *d.s.*, displaced segment of stem, which has slipped from a different level; *d*, Dictyoxylon cortex. × 3·2. Slide 2352.

Fig. 9. Twin-bundles of a leaf-trace converging on approaching pith. *x*, centripetal xylem of bundles; *sh*, imperfectly developed sheath; *p*, outer zone of pith, here alone preserved. × about 40. Slide 2352.

Fig. 10. Double leaf-trace, just fused, at margin of pith. *x*, centripetal xylem; *p'*, inner, *p''*, outer zone of pith. × about 40. Slide 2352.

Fig. 11. Approximately tangential section through the twin-bundles of a leaf-trace reaching the pith. The plane of section passes gradually inwards towards the bottom. *l.t.*, leaf-trace; *p*, outer zone of pith. × about 30. Slide 2358.

Fig. 12. Lower end of right-hand bundle from Fig. 11 in slightly oblique tangential section. x^2, centrifugal xylem of bundle; *x.p.*, island of xylem-parenchyma; *px*, protoxylem; *x*, centripetal xylem. × 100. Slide 2358.

Fig. 13. Longitudinal section through the middle region of the pith, showing the finer diaphragms. × 32. Slide 2356. Cf. Pl. XC, Fig. 22.

Fig. 14. Radial section through peripheral pith and wood, passing in places through a primary xylem-strand. *p'*, inner, *p''*, outer layer of pith; *x*, primary xylem; between *p''* and *x* a few elongated cells can be detected; x^2, secondary wood with medullary rays. × 38. Slide 2356.

Fig. 15. Transverse section of wood, phloem, and pericycle. x^2, secondary wood; *ph*, broad zone of phloem, mostly secondary; *s.s.*, 'secretory sacs' of pericycle. × 42. Slide 2352.

Fig. 16. Tracheides of secondary wood, in radial section, showing pits. × about 160. Slide 2356.

PLATE LXXXIX.

Mesoxylon Lomaxii.

From Drawings: Fig. 17 by Mr. G. T. Gwilliam, Figs. 18–21 by Miss G. C. Harrison.

Fig. 17. General radial section of fragment of stem. *pd*, periderm or secondary cortex; *x*, wood; *p*, persistent zone of pith; *d.p.*, discoid pith, much broken. × $2\frac{1}{2}$. Slide 2383.

Fig. 18. Transverse section of a xylem-strand, after fusion, at the border of the pith. *sh*, bundle-sheath; *x*, centripetal wood, some of the elements badly preserved; *px*, probable protoxylem; x^2, centrifugal wood; *m.r.*, medullary ray. × about 150. Slide 2325.

Fig. 19. Tangential section of secondary wood. *m.r.*, medullary rays, of very varying length. × about 120. Slide 2377.

Fig. 20. Tangential section of phloem. *s.t.*, probable sieve-tubes; *m.r.*, medullary rays. × about 120. Slide 2377.

Fig. 21. Tracheide from a radial section, showing three rows of bordered pits. × about 300. Slide 2383.

PLATE XC.

Mesoxylon poroxyloides.

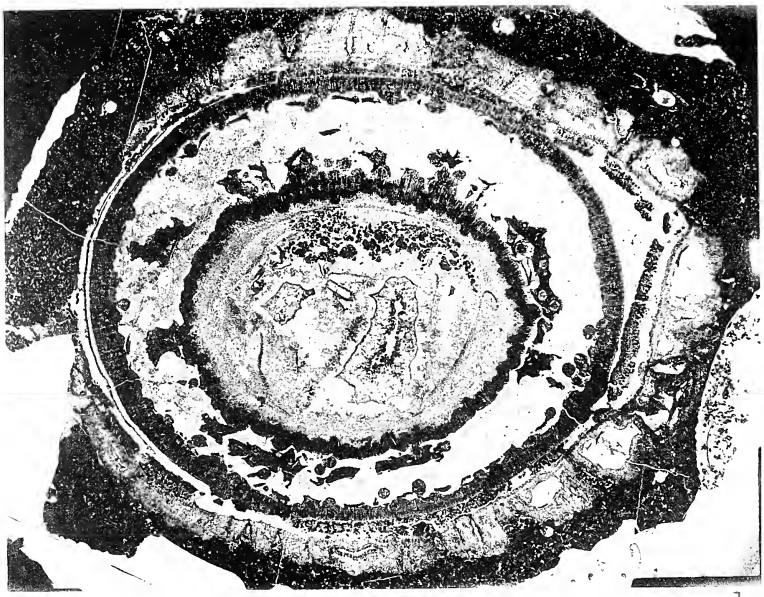
From Drawings: Fig. 22 by Mr. G. T. Gwilliam, Figs. 23 and 24 by Miss G. C. Harrison.

Fig. 22. General approximately radial section. *c*, cortex; *ph*, phloem; *x*, wood; *p*, pith, showing the persistent outer zone and the coarser and finer diaphragms of the middle discoid portion. × about 3. Slide 2356.

Fig. 23. Transverse section of a fused xylem-strand at the border of the pith. *x*, centripetal xylem; *px*, protoxylem; x^2, centrifugal xylem; *m.r.*, medullary rays. × about 200. Slide 2354.

Fig. 24. Row of eight bundles (*v.b.*) constituting a leaf-trace entering the base of a leaf. In the better-preserved bundles centripetal and centrifugal xylem can be distinguished. × 33. Slide 2352.

The slide-numbers all refer to the Scott collection.



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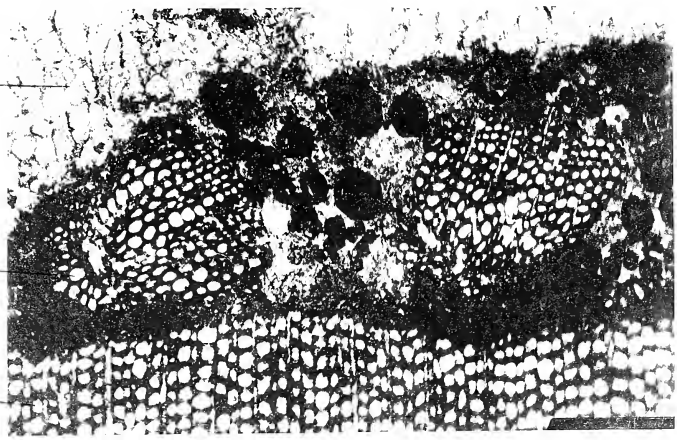

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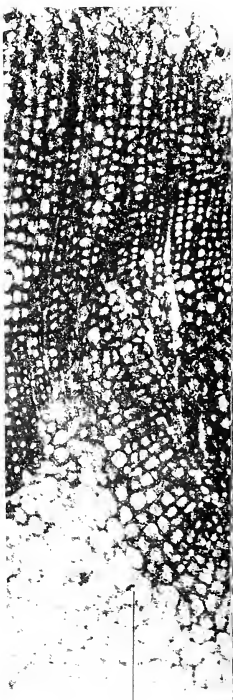

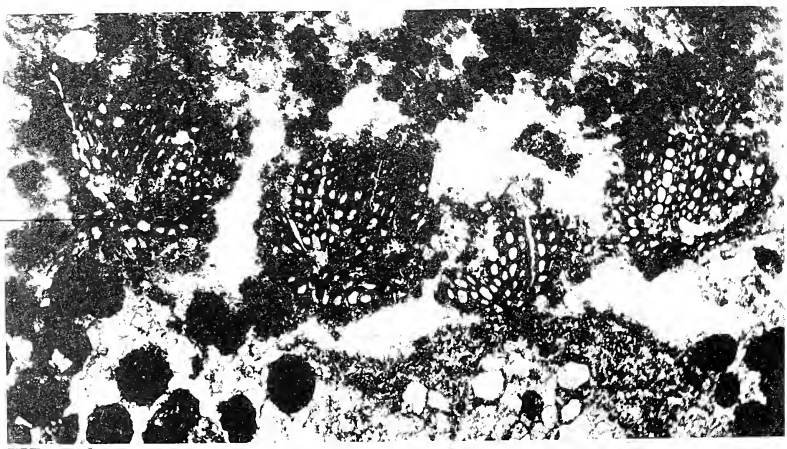

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W. Tams, photo.

SCOTT—MESOXYLON LOMAXII.

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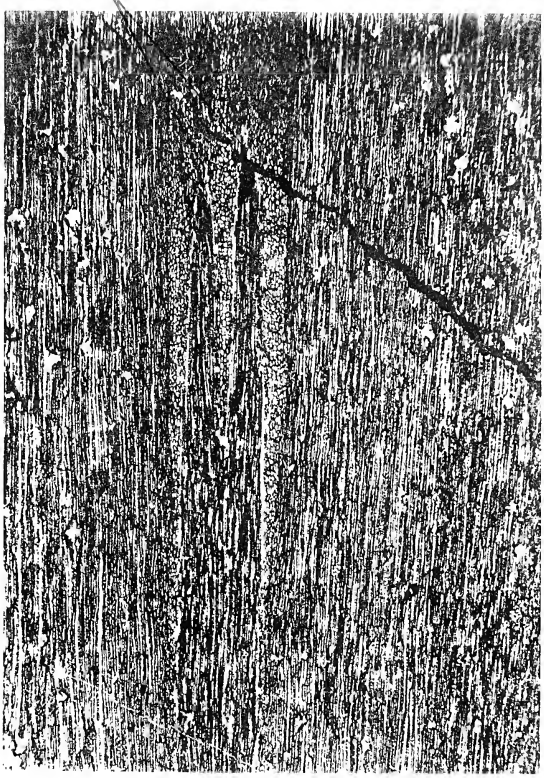

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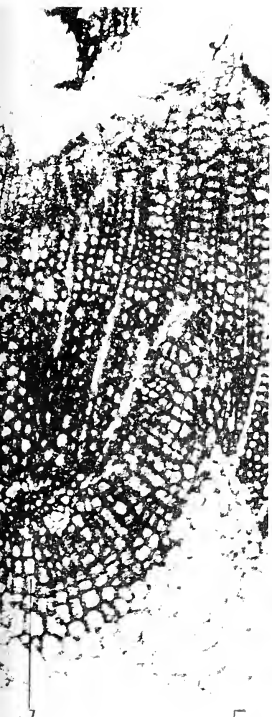

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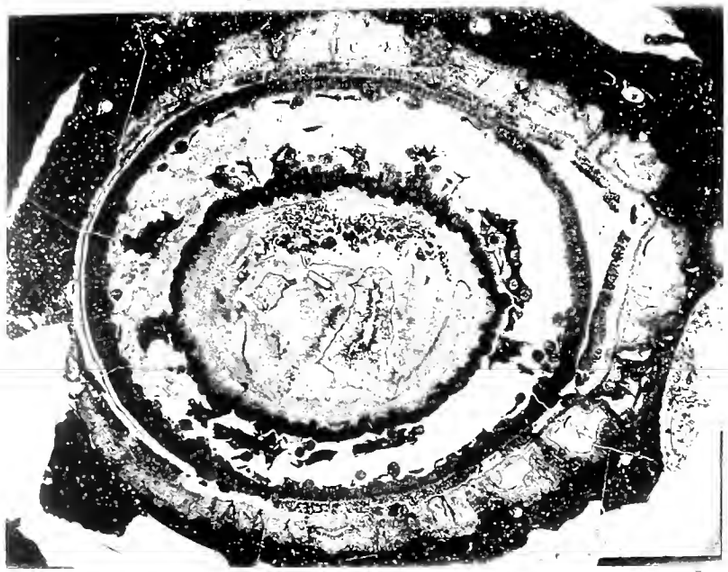

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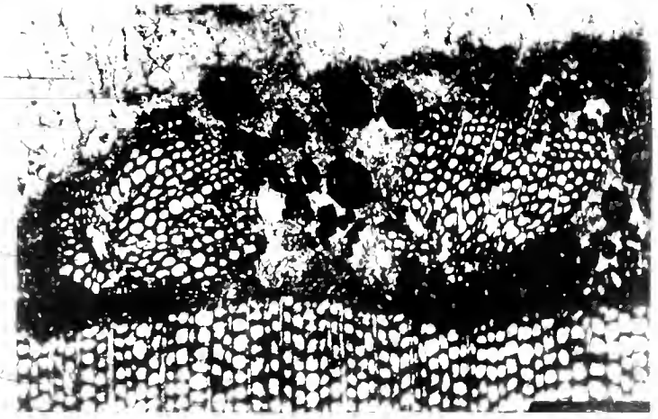

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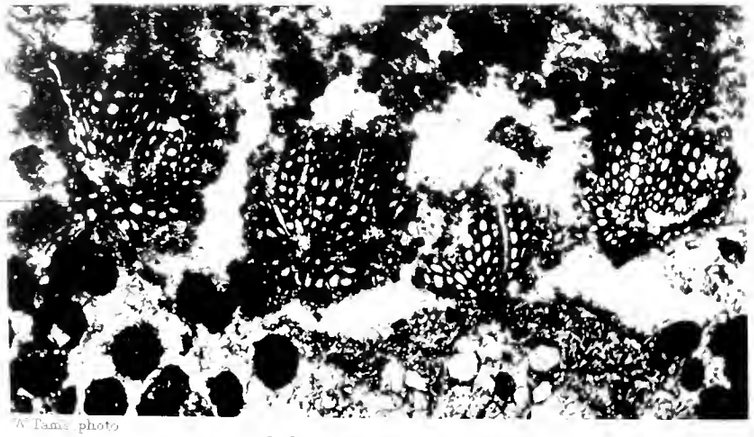

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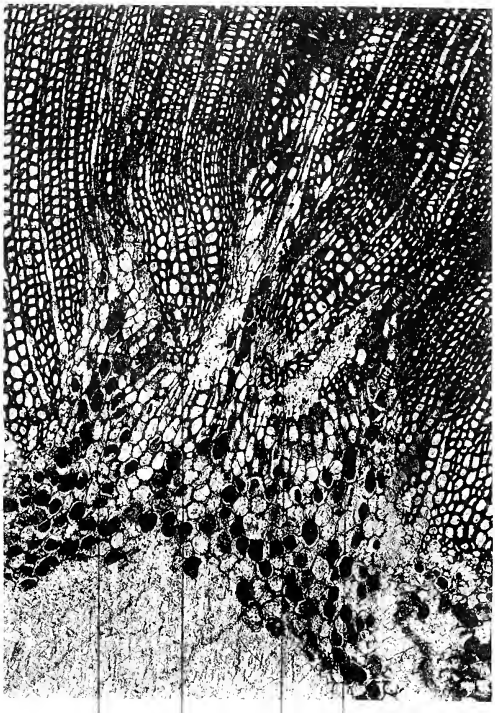

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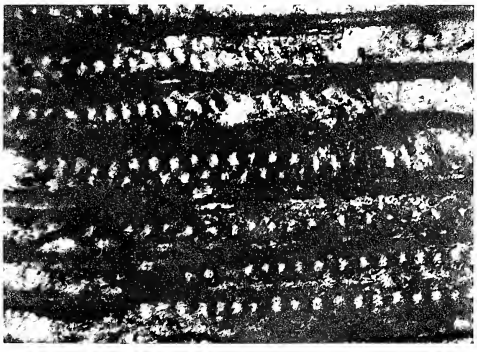

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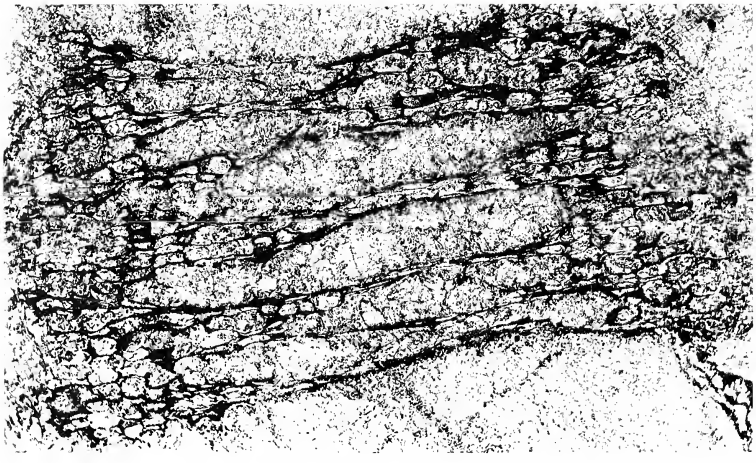

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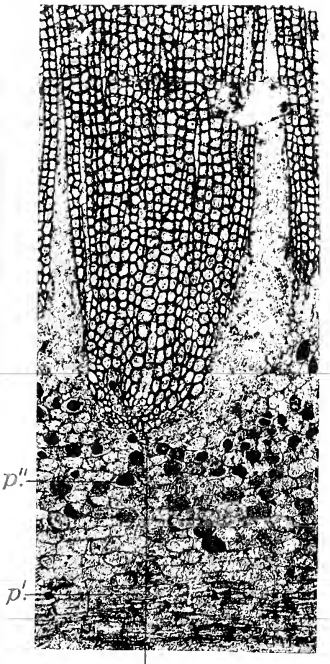
SCOTT——MESOXYLON POROXYLOIDES.



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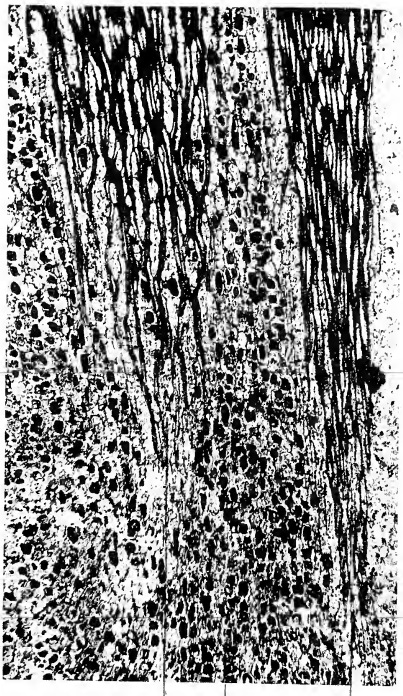

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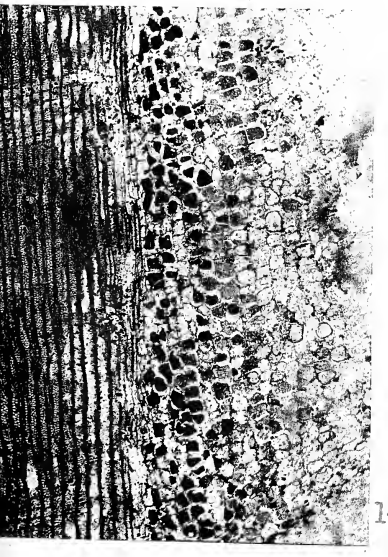

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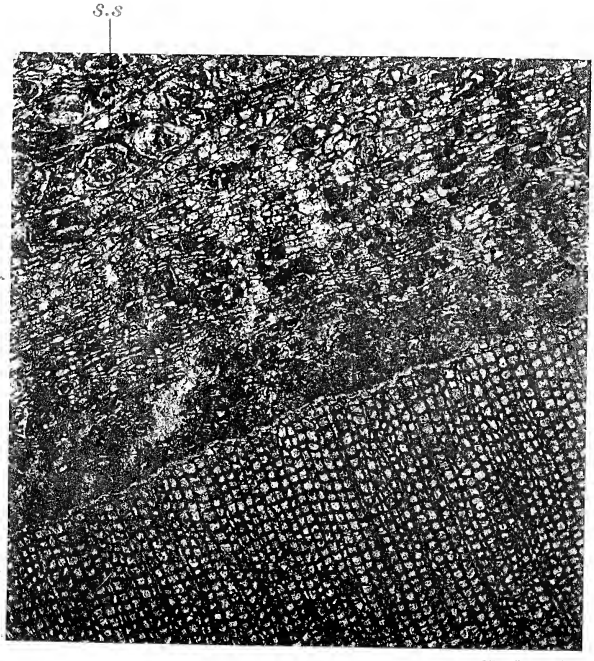

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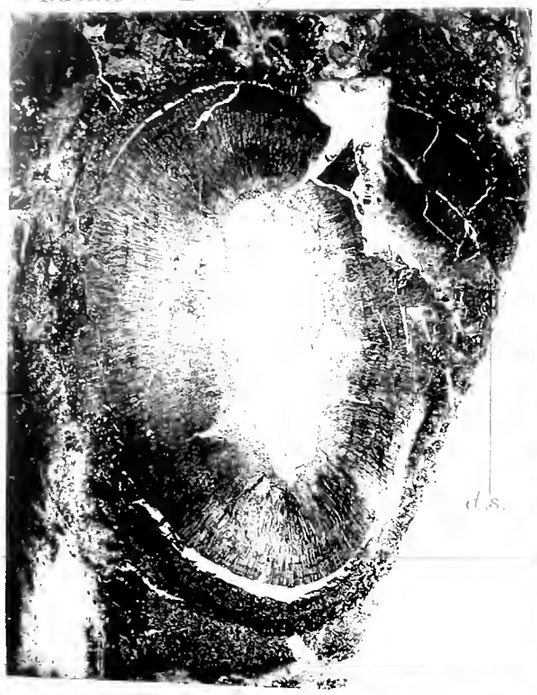

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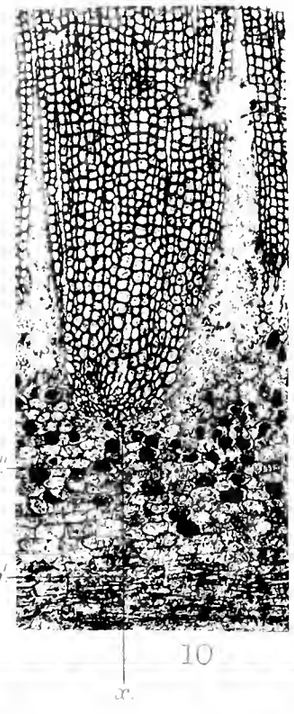

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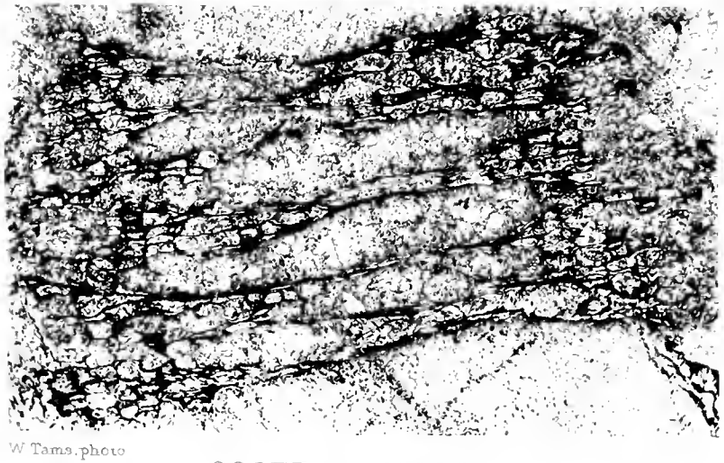

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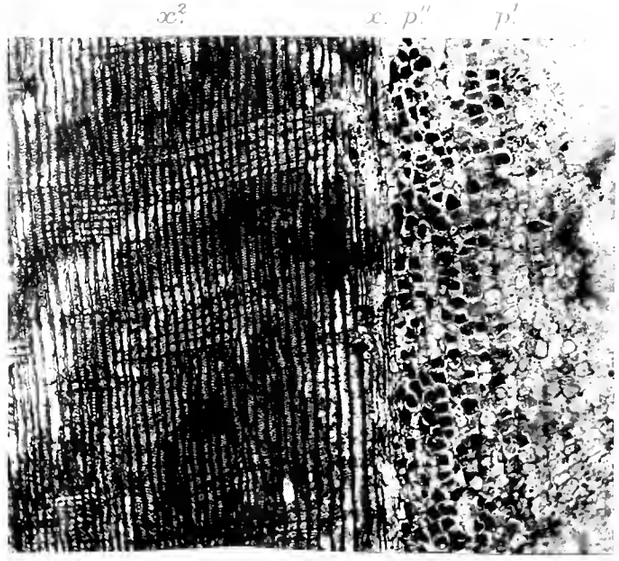

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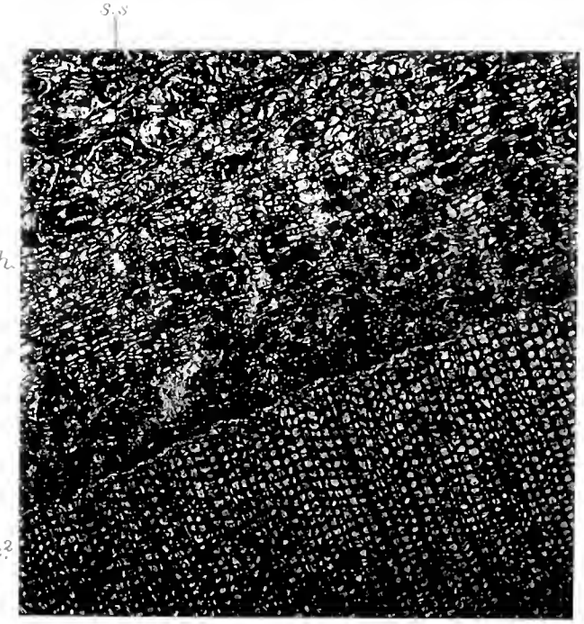

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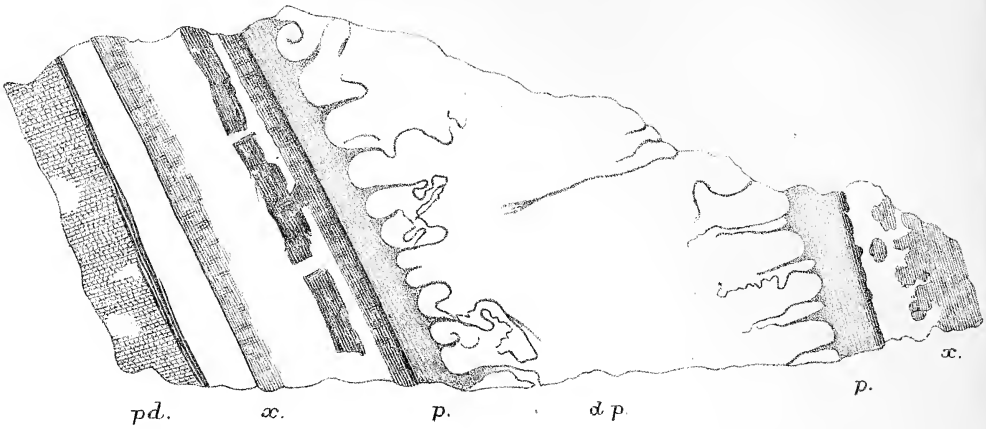

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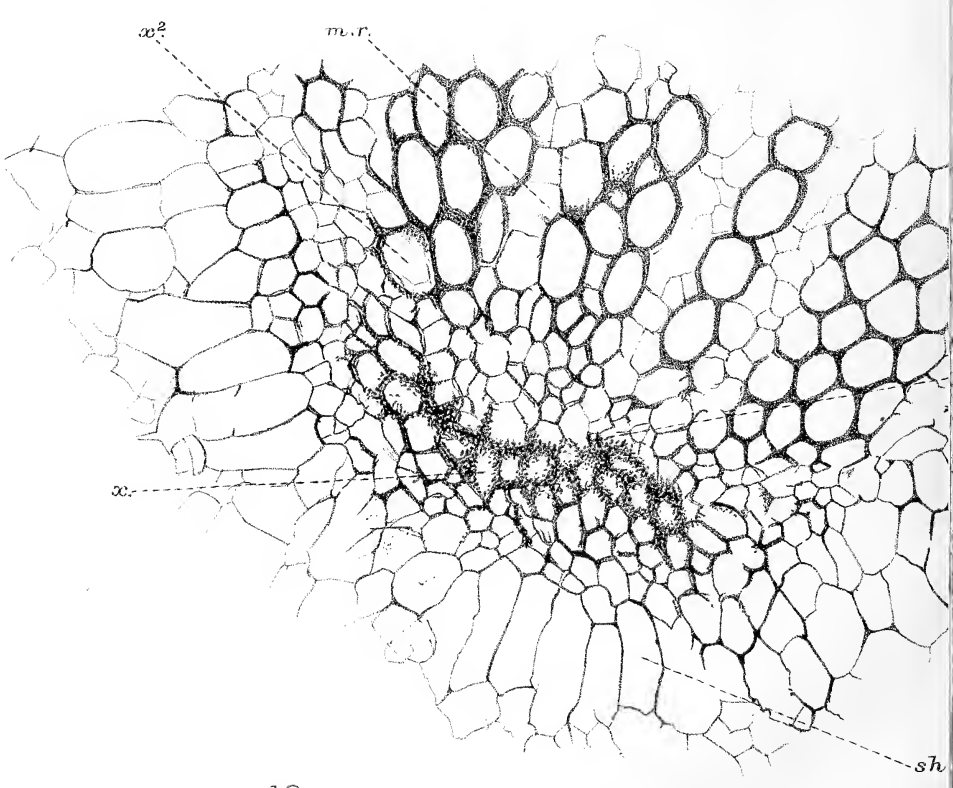

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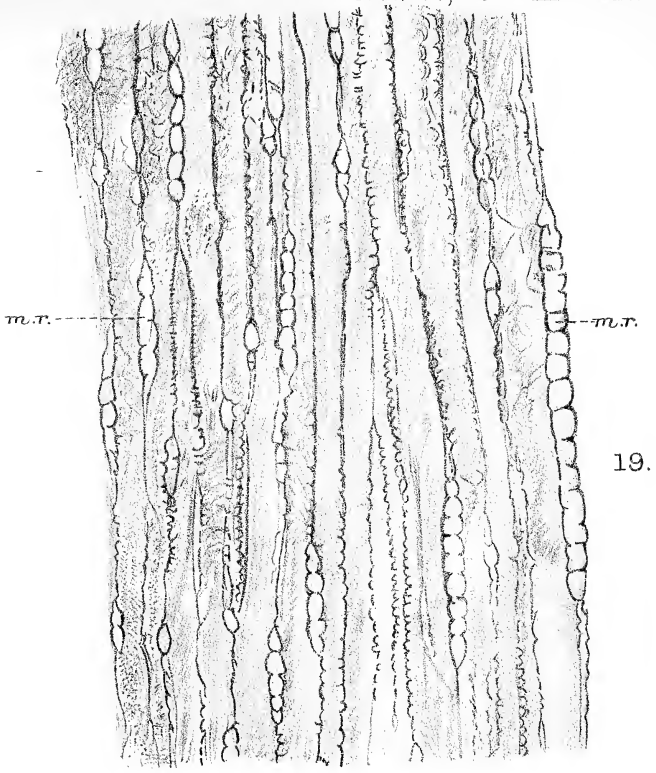

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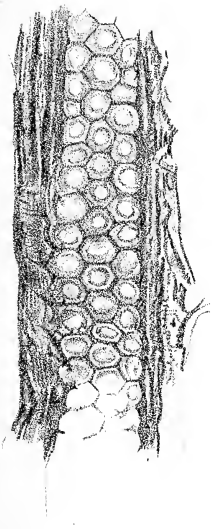

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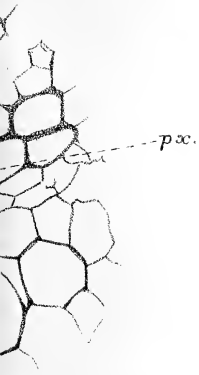

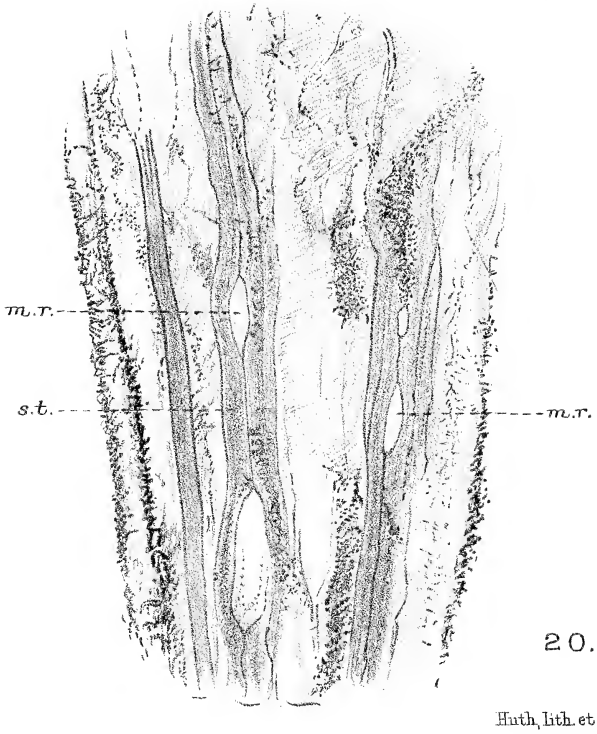

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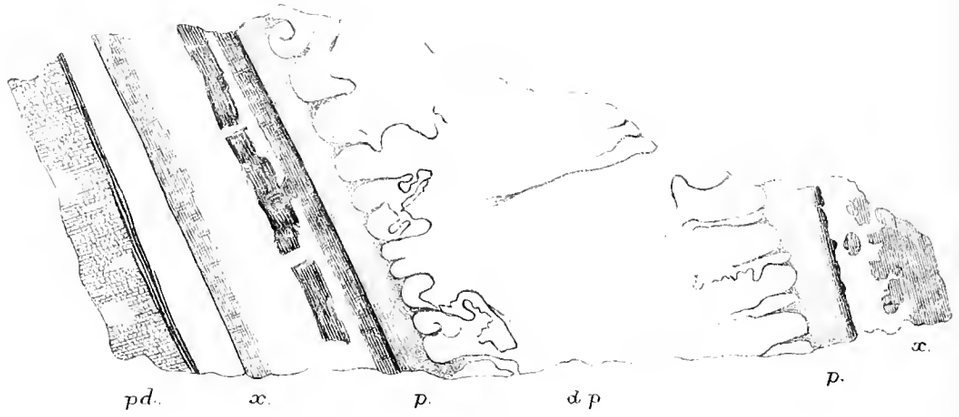

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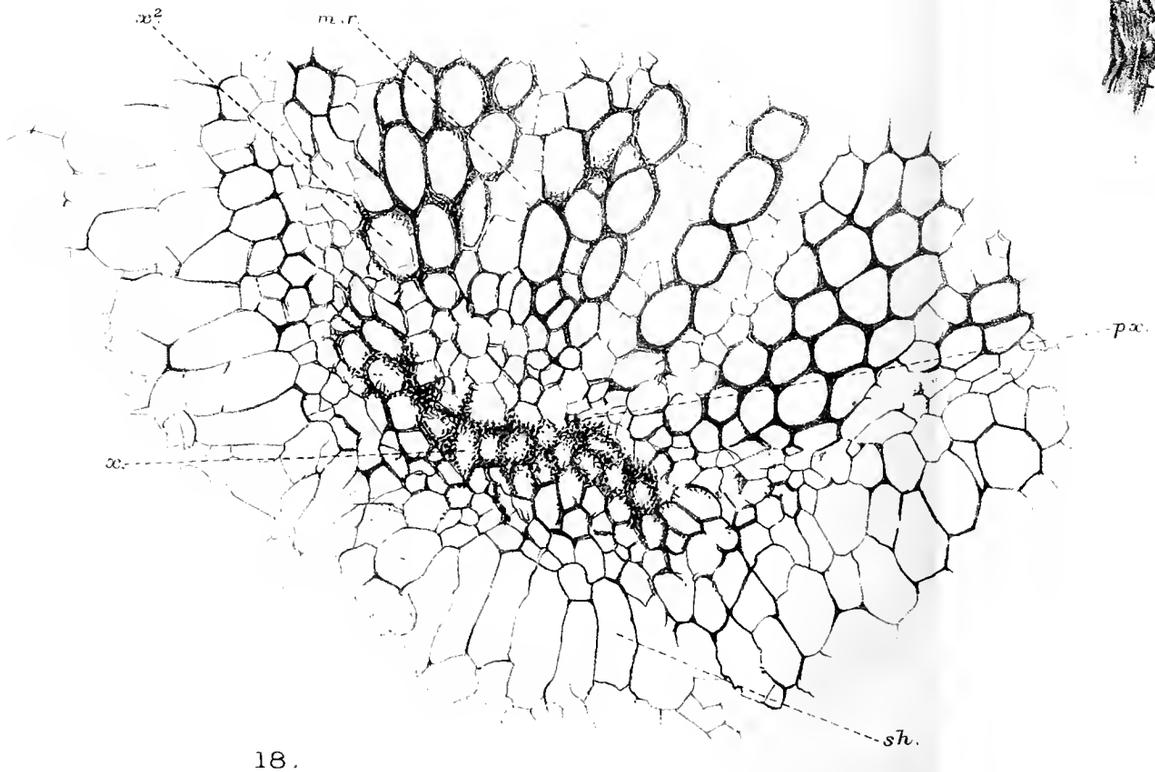

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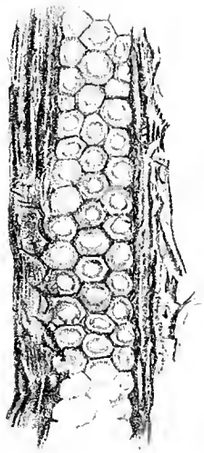

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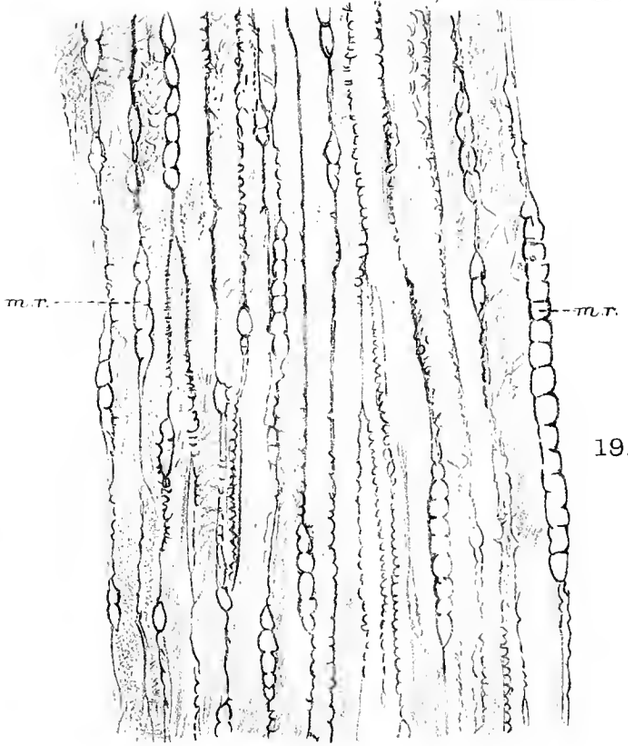

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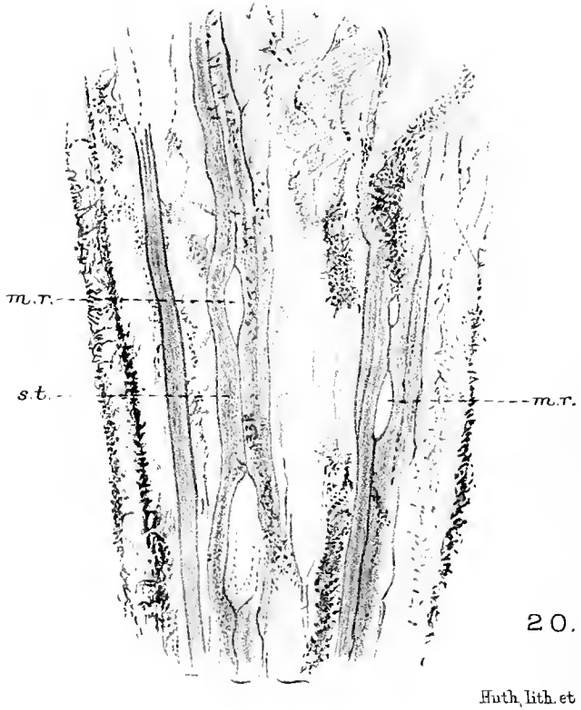

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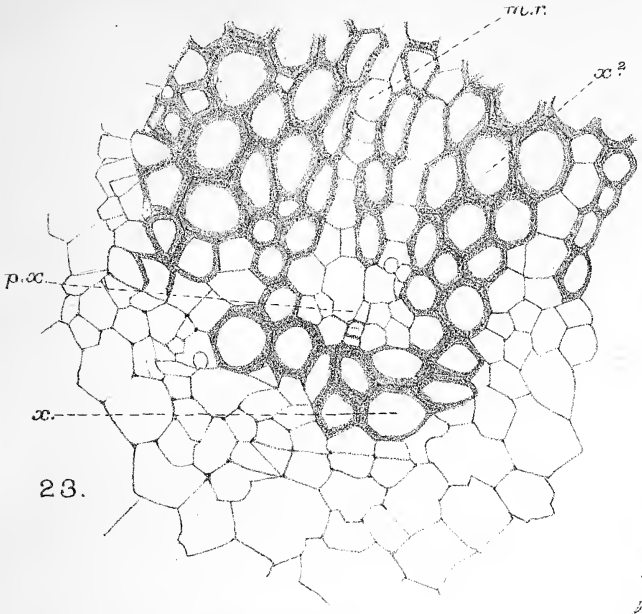
SCOTT — MESOXYLON LOMAXII.



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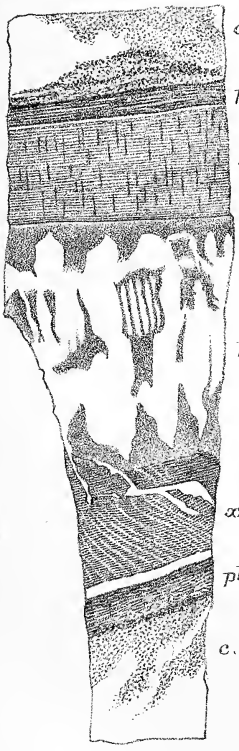

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SCOTT — MESOXYLON POROXYLOIDES.

On the Structure and Affinities of Sutcliffia, in the Light of a Newly Discovered Specimen.

BY

E. DE FRAINE, D.Sc., F.L.S.

University of London; University College.

With Plates XCI and XCII and nineteen Figures in the Text.

CONTENTS.

I. INTRODUCTION.

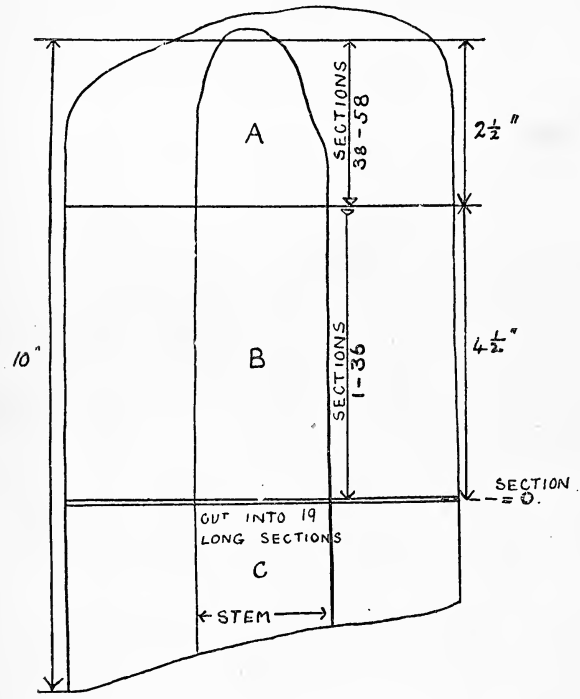
THE fossil plant described in the present paper was obtained from the colliery at Dearnley near Littleborough in Lancashire in 1910, and is of Lower Coal Measure age. It occurred in a nodule from the roof of the workings, as is shown by the presence of Goniatite shells scattered through the sections, and probably was obtained from the same seam as the type specimen, *Sutcliffia insignis*, which came from a roof-nodule from the adjacent mine at Shore Littleborough.[1] The specimen, which was about 10 inches long, was cut by Mr. James Lomax, and thanks are due to the 'Committee of British Fossil Plants' of the British Association for the Advancement of Science for a grant which partially defrayed the cost entailed in cutting the specimen. In September, 1911, the series of fifty-eight transverse and nineteen longitudinal sections, labelled 'new Medullosa, probably Sutcliffian type', was handed over to me by Professor F. W. Oliver, F.R.S., for investigation. I cannot allow this opportunity to pass without gratefully acknowledging the never-failing help and advice which

[1] Scott, D. H.: IV. On *Sutcliffia insignis*, a New Type of Medulloseae from the Lower Coal Measures. Trans. Linn. Soc. London, vol. vii, Pt. 4, ser. 2. Sept. 1906.

he has extended to me during the progress of the work. Dr. Scott has also kindly examined three of the sections, and I have thus had the advantage of his valuable opinion as to the identification of the specimen.

My thanks are also due to W. H. Sutcliffe, Esq., who kindly lent me three of his sections of *Sutcliffia insignis* for purposes of comparison.

Though the fragment of stem was, as stated, about 10 inches long (25·5 cm.), the series of transverse sections only cover a distance of about 7 inches (18 cm.). The maximum transverse dimensions were $9\frac{1}{2} \times 3\frac{1}{2}$ cm.; it is, however, most probable that the form has been considerably altered by lateral pressure, though it is doubtful whether it ever possessed a circular contour. A comparison with the stem described by Scott [1] shows the size to be distinctly less than in his fossil, in which the dimensions were 12 × 6·5 cm. The specimen was cut into three blocks, and the further treatment of each of these is shown in Text-fig. 1.



TEXT-FIG. 1. Block A represents the upper end of the specimen, as will appear during the course of the paper.

Owing to the complexity of the vascular structures present in the stem, it appeared desirable to construct a model to better elucidate the course of the strands. In preparation for this the outline of the different xylem-strands (including both primary and secondary tissues) was traced from the sections, and the tracings were then enlarged to twice the size by the pentagraph. The average distance between each section was determined, and wax plates of this thickness were prepared,[2] two sheets being used for each section, so that the correct proportions as far as possible were maintained. The method employed in building up the model from these wax plates was,

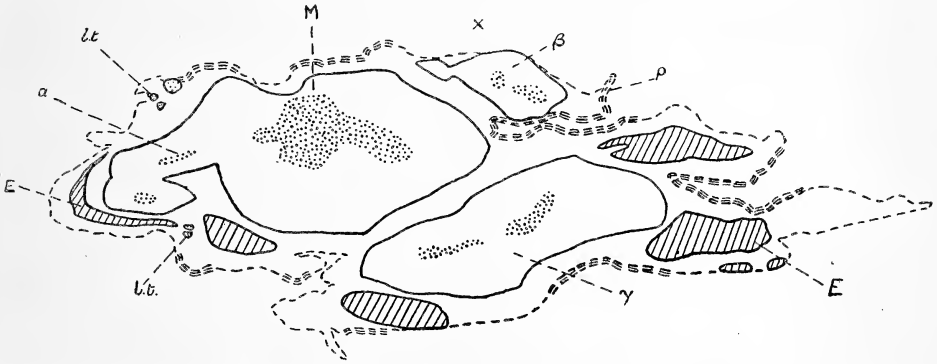
[1] Loc. cit., p. 46.

[2] Bees-wax softened with vaseline until the consistency desirable was attained was used; the dental wax commonly employed for this purpose being inappropriate on account of the thinness of the sheets.

in other details, similar to that described by Farmer and Hill,[1] so that further description is unnecessary. In the preparation of this model great assistance was given me by Mr. T. G. Hill, who not only suggested the method, but helped me during the progress of the work; I should also like to express my appreciation of his unstinted help in connexion with some of the diagrams which illustrate this paper.

II. General Structure.

The specimen appears to consist of a protostele associated with 'meristeles',[2] in all of which considerable secondary thickening has taken place; a few small leaf-trace strands are also present. Extrafascicular strands of wood and bast surround the protostele and 'meristeles', and the whole of the vascular tissues are invested by a discontinuous sheath of secondary



Text-fig. 2. Outline tracing of the vascular strands of Section O. Dotted areas represent the primary wood, shaded areas the extrafascicular strands, the broken line the limit of the tissues, and the triple broken lines the arcs of secondary cortex; phloem is omitted. M = protostele; α, β, and γ = 'meristeles'; *l.t.* = leaf-traces; ρ = secondary cortex; E = extrafascicular strands.

cortex. The basal portion of the stem is represented by Section O, the apical section being LVIII; the structure is, on the whole, best seen in the lowest sections of the series.

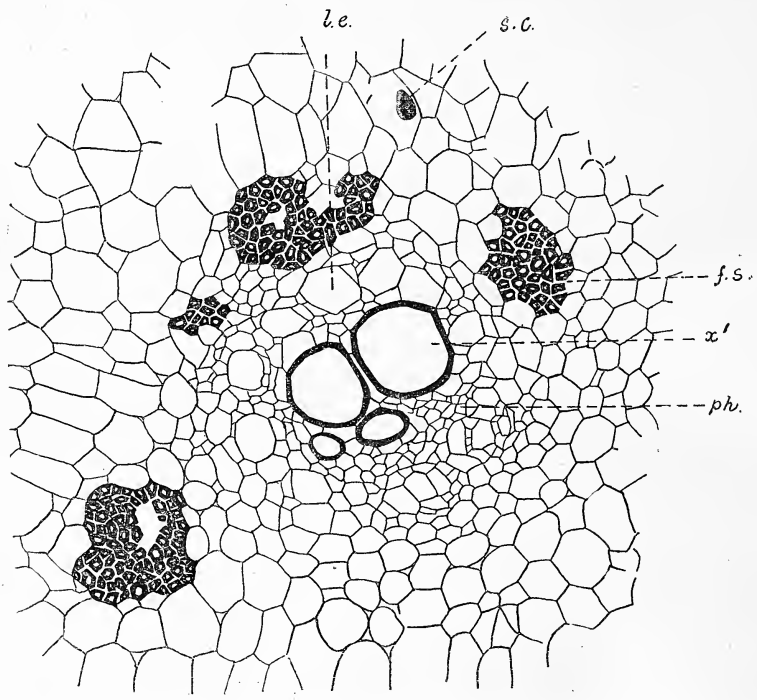
Practically nothing but the vascular system is represented, and it appears probable that in places this is not present in its entirety (at x, Text-fig. 2, it is almost certain that some strands are missing).

In the lower sections a somewhat discontinuous zone of periderm-like tissue occurs at a short distance outside the vascular strands. The entire absence of cortex (except in small isolated patches) and the lack of any

[1] Farmer, J. B., and Hill, T. G.: On the Arrangement and Structure of the Vascular Strands in *Angiopteris evecta* and some other Marattiaceae. Ann. of Bot., vol. xvi, 1902, p. 375.

[2] The term 'meristele' is used in order that the descriptive parts of the present paper may conform to the terminology employed by Scott in his description of *Sutcliffia insignis*, so that comparison may be facilitated. No morphological significance is attached to the term by the present writer.

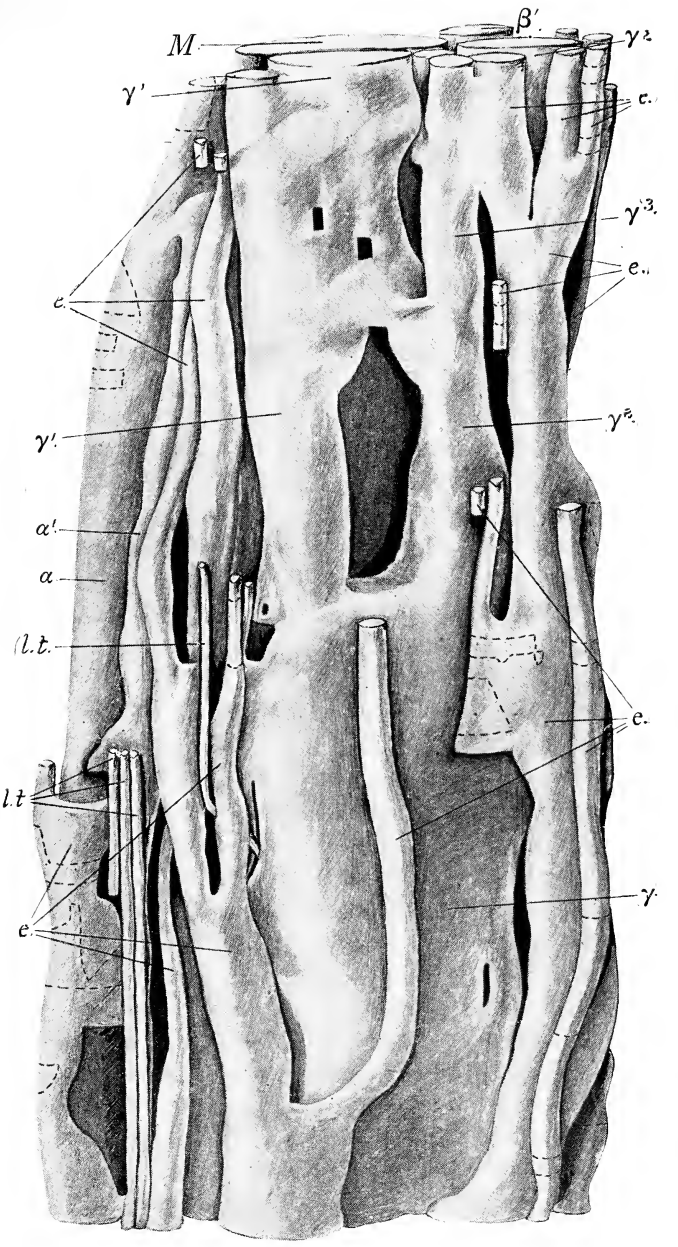
trace of the decurrent leaf-bases which form so distinctive a feature in the specimen described by Scott are, in all probability, due to the formation of cork, which resulted in the sloughing off of the exterior tissues. In the upper sections of the series the beginning of cork cambium formation can be traced (see Text-fig. 18, A and B), but in this region, as in the lower part of the specimen, the outer tissues are absent. It is possible, of course, that as the fragment of stem drifted out to sea,[1] the vicissitudes of the journey partly destroyed and tore the more delicate outer tissues, and there can be no doubt that some parts of the stem have been injured in this way ;



TEXT-FIG. 3. Transverse section of a very small concentric leaf-trace strand. x^1 = primary wood; *ph.* = phloem; *l.e.* = large elements of the phloem; *f.s.* = fibrous strands; *s.c.* = secretory cell. × 70.

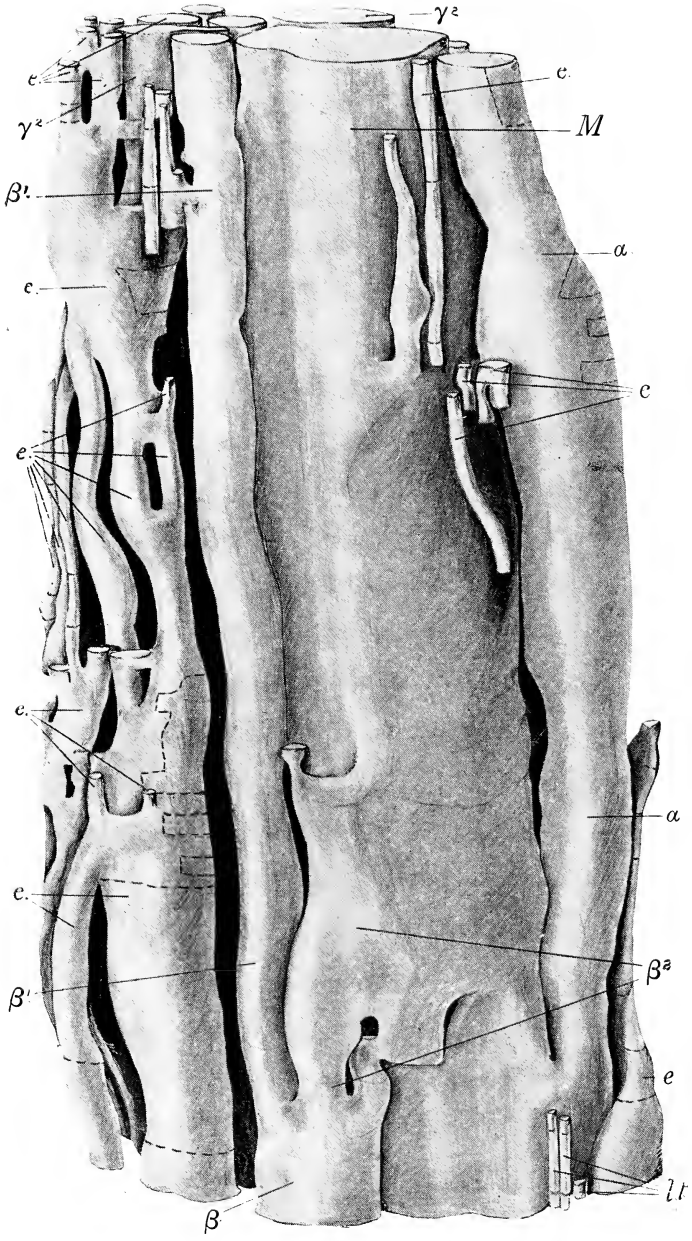
at the same time it appears probable that some part of the cortex has been lost naturally. The suggestion is put forward that the phellogen was a short-lived tissue, and was replaced by successively deeper seated cambiums, a phenomenon which is not uncommon in many vascular plants at the present day. Where traces of the cortical tissue are found, here and there throughout the series, it is seen to be composed of delicate, thin-walled parenchyma cells, with numerous secretory elements scattered

[1] Stopes, M. C., and Watson, D. M. S.: On the Present Distribution and Origin of the Calcareous Concretions in Coal Seams, known as Coal 'Balls'. Phil. Trans. Roy. Soc. London, Ser. B, vol. cc, 1908, pp. 204 and 211.



TEXT-FIG. 4. View of one side of the model of the vascular system of the new *Sutcliffia*. The model faithfully embodies the actual data, except that in places where these were obviously defective the missing parts have been restored. Such restorations are in all cases indicated by dotted lines on the drawing. Nat. size.

e = extrafascicular strands; *α*, *β*, and *γ* = 'meristeles' (compare with Text-figs. 8 and 9); *l. t.* = leaf-trace strands; M = protostele.



TEXT-FIG. 5. View of the other side of the model of the vascular system of the new *Sutcliffia*. Nat. size. Lettering as in Text-fig. 4.

throughout, the resemblance to the internal cortex of *Sutcliffia insignis* being very close. Small leaf-trace bundles occur in groups of two or three sparingly scattered throughout the series; the extraordinarily few bundles present compared with the size of the stem are accounted for by the fact that, owing to the deep-seated nature of the periderm, the bundles become cut off immediately they enter the cortex. In all cases where the phloem is preserved the leaf-trace strands are seen to be concentric in structure (Text-fig. 3).

Each of these ultimate leaf-trace bundles is partially surrounded by three or four strands of fibrous elements, differing in size, and where in the upper sections of the series the cortex is preserved, similar strands occur. In intimate connexion with the sclerenchyma strands secretory elements are frequently found; these appear to be of the same type as those prevalent in the ground tissue. A similar association of secretory elements with fibrous strands is seen in *Sutcliffia insignis*,[1] and has also been described by Seward as characteristic of *Sutcliffia Williamsoni*[2] (= *Rachiopteris Williamsoni*).

The vascular system of the stem is essentially similar to that of *S. insignis*; there are, however, two important additions. The main stele and all the vascular strands with the exception of the small leaf-trace bundles are characterized by a wide zone of secondary wood and bast. In addition to this numerous extrafascicular arcs of wood and bast encircle the vascular system proper. The preservation of these arcs leaves much to be desired in many cases, but there is little doubt that in life they formed a complete, irregular, anastomosing network around the cylinder, strongly recalling the extrafascicular strands of certain genera of Cycads (Pl. XCI, Fig. 1, and Text-figs. 4 and 5).

The single main stele, which is roughly triangular in outline, varies but slightly in size throughout the series, except where vascular strands are given off, or where fusion of a neighbouring strand with the main stele is taking place.

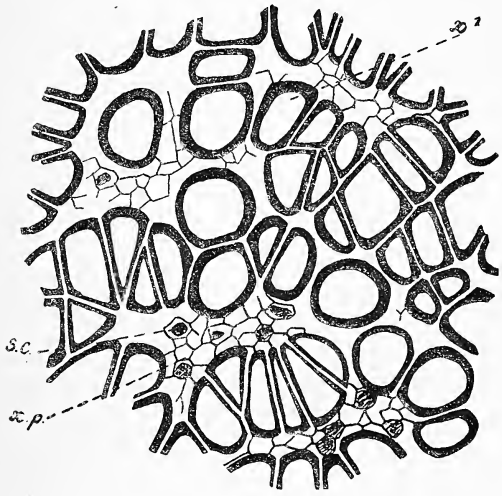
The primary wood is in all essentials precisely similar to that of *Sutcliffia insignis*, that is, the tracheides mixed with parenchyma extend to the centre, and the protoxylem occupies the exarch position (cf. Text-figs. 6 and 7).

The dimensions of the primary wood are somewhat smaller than in Scott's specimen, as would be expected from the smaller size of the fossil as a whole. The maximum dimensions of the primary wood of the new specimen are 1·4 × 1·1 cm. as compared with 4·7 × 1·8 cm. in *S. insignis*. Secondary growth has added considerably to the thickness of the wood, so that the total wood measurement amounts to 2·9 × 1·9 cm.

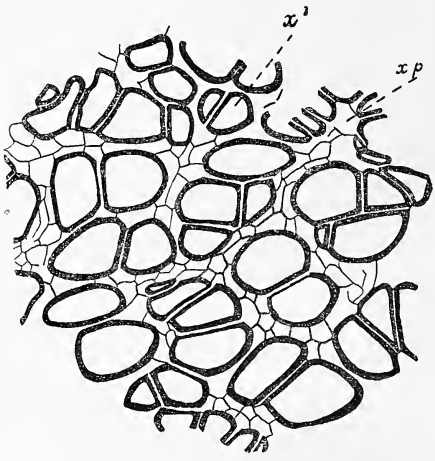
[1] Scott: loc. cit., p. 48.

[2] Seward, A. C.: On *Rachiopteris Williamsoni*, sp. nov., a New Fern from the Coal Measures. Ann. Bot., vol. viii, 1894, Plate XIII, Figs. 3 and 8.

The vascular masses subsidiary to the main stele, called by Scott 'meristeles', are far less numerous than in the original specimen, a fact which may be again accounted for by considering the small size of the new stem compared with the old; they are moreover distinctly smaller (cf. Pl. XCI, Fig. 1, with Pl. VII of Scott's paper). As very considerable crushing and collapse has taken place—so that except in occasional patches, where the preservation is excellent, the elements are more or less flattened in the plane of the long axis—the dimensions given for the meristeles are



TEXT-FIG. 6. Transverse section of the primary wood of *Sutcliffia insignis*. University College, London, Collection (Scott's section V). × 37. x^1 = primary wood; *x.p.* = xylem parenchyma; *s.c.* = secretory elements.



TEXT-FIG. 7. Transverse section of the primary wood of the new *Sutcliffia*. × 37. x^1 = primary wood; *x.p.* = xylem parenchyma.

only approximately correct. Disregarding the secondary tissues, so that more ready comparison may be made with Scott's figures, the following results were obtained for the dimensions of the meristeles figured in Text-figs. 8 and 9:

$\gamma = 7 \times 1{\cdot}5$ mm. $\quad \alpha^1 = 2{\cdot}5 \times 1{\cdot}5$ mm.
$\beta^1 = 5 \times 1{\cdot}5$ „ $\quad \beta^2 = 2 \times 1{\cdot}5$ „
$\gamma^2 = 4 \times 2{\cdot}5$ „ $\quad \gamma^3 = 2 \times 1{\cdot}5$ „
$\alpha = 2{\cdot}5 \times 1{\cdot}5$ „

The measurements made by Scott[1] in *Sutcliffia insignis* were $\alpha = 7 \times 4{\cdot}5$ mm. and $\beta = 14 \times 4$ mm. The structure of the 'meristeles' is, in all cases, precisely similar to that of the main stele, with the exception that the zone of secondary thickening does not attain to so great a thickness, and occasionally does not entirely surround the primary wood. It

[1] Loc. cit., p. 49.

may be remembered that in Scott's fossil the structure of the 'meristeles' was similar to that of the main stele, and, like it, sometimes showed a slight indication of the beginning of secondary growth.

III. Course of the 'Meristeles'.

In order to elucidate the behaviour of the 'meristeles' as completely as possible, the course of three strands, designated α, β, and γ, will be traced. In Text-fig. 2, taken from the lowest section of the series, the main stele (M) has a large hook-shaped projection at its left-hand side (α); it consists almost entirely of secondary wood, but embedded within it are two groups of primary xylem (Text-fig. 8, Fig. II); these groups are respectively labelled α and α^1.

In III[1] a slight indentation appears at the extreme left of this projection; it occurs immediately opposite a deep incision present on the opposite side of the mass, and lies just above one of the groups of primary wood; the end of the hook is evidently preparing for detachment. In IV the process is almost entirely complete, while in V α^1 is quite independent (Text-fig. 8, Figs. IV and V, and Pl. XCI, Fig. 1). Very rapidly, secondary wood almost, but not quite, encloses the detached end of α^1, while α soon shows no sign that disturbance has taken place. No further change occurs in α^1—it never becomes entirely surrounded by a zone of secondary wood—until Section XXXIX, $3\frac{3}{4}$ inches higher up (9·5 cm.).

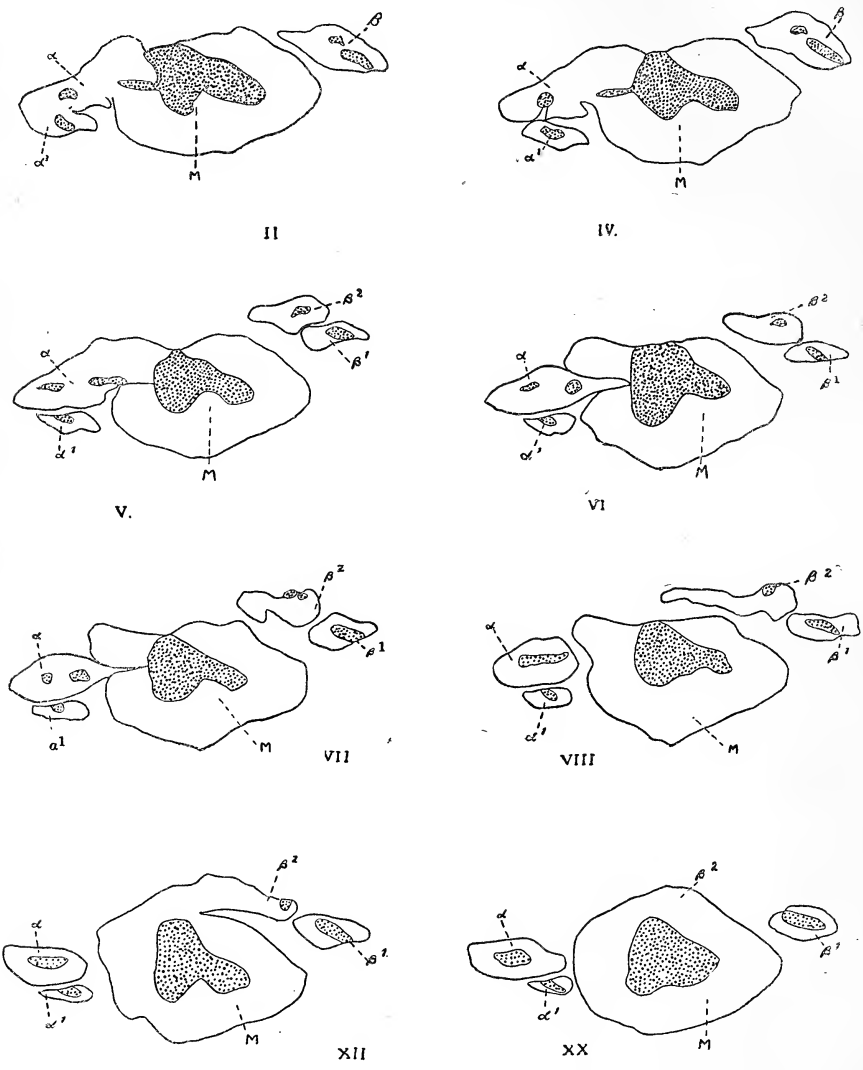
At this level its primary wood is beginning to divide into two portions, a division which is completed by XLVI. It is difficult to say with certainty what is happening in this upper part of the stem, for the tissues have been subjected to considerable crushing and the preservation is poor; but in Sections XLV to XLIX, though the 'meristele' α^1 appears to be fusing with α, the appearance is in all probability deceptive and due to mechanical pressure;[2] moreover, α^1 is dividing up into ultimate leaf-trace strands.

Returning to the 'meristele' α, in II a group of primary xylem elements can be traced passing from the stele M to join the isolated cluster already present; in V it has passed nearly through the secondary wood of M and the 'meristele' is detaching itself from the main stele (Pl. XCI, Fig. 1); it becomes entirely free in VIII (Text-fig. 8, Figs. II–VIII). As soon as these changes are completed the secondary wood immediately closes round it, and also round M; this condition is maintained for a distance of $3\frac{1}{4}$ inches (8·2 cm.) (Text-figs. 4 and 5). From Section XXXVIII upwards the sequence of events cannot be given with absolute certainty owing to the crushing which has taken place, but the primary xylem of α undoubtedly divides into two parts; the smaller portion is passing out through the

[1] The Roman numerals refer to the number of the slide.

[2] In the model this strand is necessarily represented as fusing with α (Text-fig. 4).

secondary zone, but the sections are insufficient to trace the behaviour of the larger. The larger agrees, so far as the size and appearance are concerned, with the radially symmetrical leaf-traces which will be described later (p. 1047), while the smaller is probably a leaf-trace strand of the



TEXT-FIG. 8. Tracings showing the breaking up of the 'meristeles' α and β. The dotted areas indicate the primary wood; only xylem is included in the tracing. Slightly under nat. size. The numbers refer to the numbers on the slides. M = main stele. All vascular tissues except the main stele, and the 'meristeles' α and β, are omitted.

unilateral type. The evidence is not absolutely satisfactory, but it would appear to justify the conclusion that the hook-shaped projection seen in Section O is an outgoing foliar strand, which divides into smaller strands,

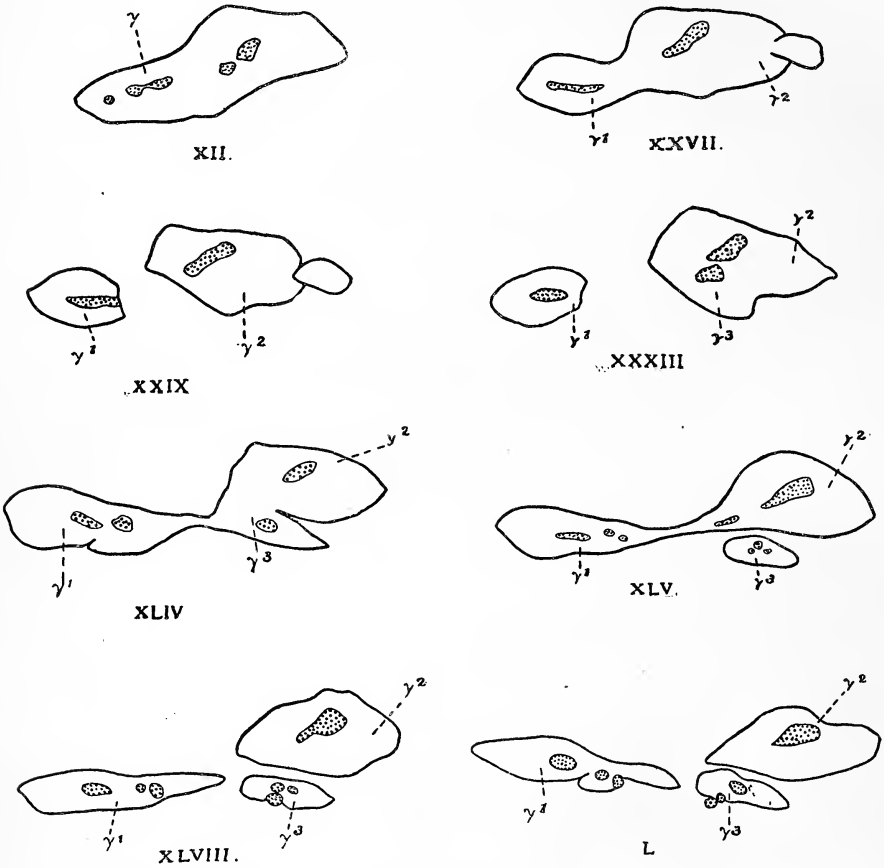
which in turn break up completely into leaf-trace bundles. Further evidence in favour of this conclusion is provided by the behaviour of the other 'meristeles'.[1]

The course of the 'meristele' β will next be traced; in the lowest section of the series (see Text-fig. 2) it is already entirely free from the main stele M; it lies on the side of the stele remote from the 'meristeles' α and α^1 which have just been described. It is entirely though somewhat unevenly surrounded by secondary tissues, and contains two groups of primary tracheides. In IV the 'meristele' is preparing to divide into two parts, each containing one primary xylem group; this division is completed in V (see also Pl. XCI, Fig. 1). One of these two parts, β^1, shows no secondary tissues for some considerable distance on the side remote from β^2 (Text-fig. 8); this is probably due almost entirely to injury in this region. β^1 shows no further change until XXVIII, when a portion of the primary wood at its upper end shows signs of detachment; this separation is completed by XLI, and the rounded mass of primary xylem β^3 has passed out from the secondary wood. It cannot be traced above this level, so that its exact significance cannot be stated definitely. From its size, and the position of the protoxylem elements, β^3 probably represents a radially symmetrical leaf-trace strand such as was indicated as occurring during the breaking up of the 'meristele' α. Returning to the other half of the divided 'meristele' β, namely β^2 (Text-fig. 8, Fig. V), almost immediately after its separation from β^1 the secondary wood on the outer side opens out, exposing the primary xylem. This 'opening out' of the 'meristele' is undoubtedly partly the result of injury, but there can be little doubt that some movement of the vascular mass took place in life, in order to prepare for the fusion with the main stele M, which occurs immediately above this level. The course of events is shown in Text-fig. 8, Figs. II–XX; comparison with Pl. XCI, Fig. 1, shows that the portion of the stele M with which β^2 finally fuses has been considerably injured, though how far the absence of secondary wood at this part of M is a natural feature it is difficult to say—in all probability a break in the secondary tissues of M occurred preparatory to the entrance of β^2, thus allowing for the fusion of the secondary and primary tissues of the former with the corresponding ones of the latter. During the process of fusion, a small arc of secondary wood with two or three primary tracheides attached to its margin became isolated. The ultimate fate of this arc could not, however, be determined. This instance of fusion of a 'meristele' with the stele, the only one occurring in the series, is of considerable interest; it is unparalleled in *S. insignis*, for Scott decided that what appeared to be a case of re-fusion in that stem was due to

[1] The ultimate breaking up of the 'meristeles' α and α^1 is not shown in Text-figs. 4 and 5. The model ends with Section LIV, for the last four sections were too fragmentary to include.

mechanical pressure;[1] he was unable to trace a genuine case of re-fusion of a 'meristele' with a stele.

As neither of the meristeles α or β, which have hitherto been considered, give really conclusive evidence of the breaking up of the 'meristeles' into foliar strands, for this purpose the changes undergone by the remaining 'meristele', γ, will be described. The 'meristele' γ is a vascular mass lying on the remaining free side of the stele M and almost equalling it in



TEXT-FIG. 9. Tracings showing the breaking up of the meristele γ. Slightly under nat. size. A slight restoration has been necessary in Sections XLIV–L.

size (Pl. XCI, Fig. 1), giving the stem the appearance of having two almost equal steles in the lower half of the series, but that γ is only a large 'meristele' and not a stele is abundantly proved by its subsequent behaviour.

The primary wood in this 'meristele' occurs in two masses, one at either end, and the secondary wood extends completely to the middle in the central region. In Section O a small strand of primary wood shows evidence of detachment,[2] in XVIII it begins to pass outwards through the

[1] Loc. cit., p. 52.

[2] Shown still more clearly in Plate XCI, Fig. 1.

secondary wood, and in XXI it has become entirely free from it. The secondary xylem closes up immediately after the exit of this bundle, and none of it accompanies the strand during its outward passage. Immediately it becomes free from the 'meristele' it turns vertically upwards and can be traced as far as XXXI (Text-fig. 4); after this level it is no longer present in the series. The strand consists of a small group of primary tracheides mixed with parenchyma, and only one protoxylem group appears to be present. Its preservation is far from satisfactory, but it is certainly concentric in structure, though how the phloem was gained could not be ascertained. In the upper sections in which it appears the phloem has associated with it the ring of the large elements which are so characteristic a feature of the phloem of the leaf-trace bundles; bundles of fibres also occur immediately beyond the phloem. There can be no doubt that this strand is a leaf-trace bundle of the unilateral type, passing out directly and prematurely from a 'meristele'.

In tracing the course of the 'meristele' γ the central region of it shows signs of injury quite early in the series, and in XIX there is a complete break at the middle part. A division of the 'meristele' into two portions in all probability occurred in life, but the appearance in the fossil is considerably emphasized by crushing and injury (Text-fig. 9, Figs, XII, XXVII, and XXIX). A small portion, γ^3, is soon cut off from γ^2, and subsequently γ^1 and γ^2 appear to fuse. The evidence for this re-fusion is however doubtful, for considerable crushing has taken place in the upper portions of the stem, and added to this there is further injury in preservation at the critical region. In any case, by the time XLVIII is reached (Text-fig. 9, Figs. XXXIII–XLVIII) γ has divided into two larger and one small portion, γ^1, γ^2, and γ^3. Above this level the primary wood of γ^1 is dividing up into smaller portions, which do not leave the 'meristele' in the course of our series; so far as one can judge from this stage, the 'meristele' γ^1 is dividing into radially symmetric leaf-trace strands. Division also begins in γ^3, and three small strands of primary xylem—each with a single protoxylem group—are produced; they begin to pass out through the secondary wood, but they cannot be traced to the stage where they gain the phloem zone. On this account the unqualified statement that the 'meristele' γ^3 is used up entirely in the production of unilateral leaf-trace strands cannot be made, but comparison with the similar bundle given off by the 'meristele' γ in Section XVIII leaves little room to doubt that it is correct.

In summarizing the evidence provided by the course of the three 'meristeles' α, β, and γ, it appears evident that large masses of vascular tissue are cut off from the central axis, that these run parallel to the latter for some distance, giving off leaf-traces and ultimately dividing up into smaller strands, often unequal in size, and that the primary wood of these strands was ultimately entirely used up in the production of leaf-trace

bundles either radially symmetric or unilateral in type. It could not be determined whether the radially symmetric foliar strands ever divided further to produce the smaller unilateral traces. Unfortunately neither Scott's fossil nor the new stem enables the question to be definitely settled as to whether the whole vascular mass which separates from the main stele is completely used up in the production of leaf-traces, but the great probability that they were has already been shown by a comparison of the behaviour of the 'meristeles' α^1, γ^1, and γ^3.

The very interesting cases recorded by Scott of the 'occurrence of fusion between meristeles probably of quite distinct origin' do not occur in the new stem, though instances of re-fusion of 'meristeles' of similar origin, and of a 'meristele' with the main stele, have been already described. The distribution of the 'meristeles' around the central strand appears to be in the form of parallel cords of vascular tissue rather than in the state of 'a kind of network round the stele';[1] the network in the new stem is provided by the extrafascicular arcs which will be described later (compare Text-figs. 4 and 5). One further question still remains to be considered. Are the 'meristeles' to be considered as a leaf-trace system, or are they to be considered as part of the stelar system proper to the stem? In discussing this question Scott points out the marked agreement between the meristeles of *Sutcliffia insignis* and the large concentric strands which leave the stele in *Medullosa anglica*; in both there is the same close agreement between the steles and the large strands given off from them. In *M. anglica* these main foliar strands, which are concentric at first, break up entirely into collateral bundles which enter the bases of the leaves, that is, they represent a leaf-trace system only. Scott concludes that they cannot 'be directly compared to the meristeles of *Sutcliffia*' because in *Medullosa anglica* 'there is no evidence of fusion with neighbouring strands, nor any indication that part of the strand remained behind in the stem after the foliar bundles were given off', and for these reasons he interprets the vascular structure in *Sutcliffia* as a protostele giving off a peripheral system of subsidiary steles which form the points of departure of the actual leaf-traces. The discovery of a portion of a second stem tends to show, though the evidence is unfortunately not absolutely complete, that the meristeles were completely used up in the formation of leaf-traces; in all probability none were left behind exclusively cauline in nature; further, fusion of neighbouring strands was not of common occurrence, and may have been merely a consequence of the crowding together of numerous 'meristeles'. In connexion with this point, moreover, it must be remembered that fusion of leaf-trace bundles is a not uncommon occurrence in the Medulloseae. The 'meristeles' of our stem appear to offer a close comparison with the leaf-trace strands which leave the stele in *Medullosa anglica*; in both a large

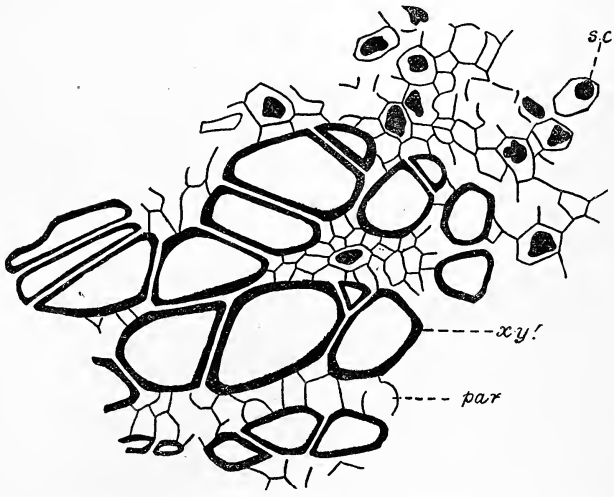
[1] Scott: loc. cit., p. 53.

concentric bundle, partially or completely surrounded with secondary tissues, leaves the stele—the main stele repeated on a small scale. In the new *Sutcliffia* this strand divides more or less unequally into smaller strands, of which the primary wood breaks up ultimately into concentric leaf-trace bundles, the secondary tissues being lost during the process. In *Medullosa anglica* the strand passes gradually outwards, and then divides up into collateral leaf-trace bundles, the secondary tissues also being lost during the breaking-up process.[1]

On the grounds of the additional evidence afforded by the new stem it would appear justifiable to regard Sutcliffia *as possessing for its main vascular axis a protostele, from which was given off vascular strands varying in size, but in every case similar in structural details to the protostele; these strands divided irregularly into smaller bundles which were ultimately completely used up in the production of leaf-trace bundles. This interpretation of the structure connects the fossil more closely with the genus* Medullosa *through such a type as* M. anglica, *and still further supports Scott's view of its primitive position in the Medulloseae.*

IV. Histology.

1. *Structure of the Wood.* The structure of the primary wood is in all essential characters similar to that of *Sutcliffia insignis*, as can be seen by comparing Text-figs. 6 and 7, which represent portions of the primary wood of each drawn on the same scale. The tracheides are of the ordinary elongated form, with multiseriate bordered pits on the radial walls; they are of large size, varying in diameter from 216 to 400 μ, the average being about 281 μ; their size is thus slightly greater than in *S. insignis*, in which, though the diameter may reach 350 μ, the range lies between 170 and 320 μ.

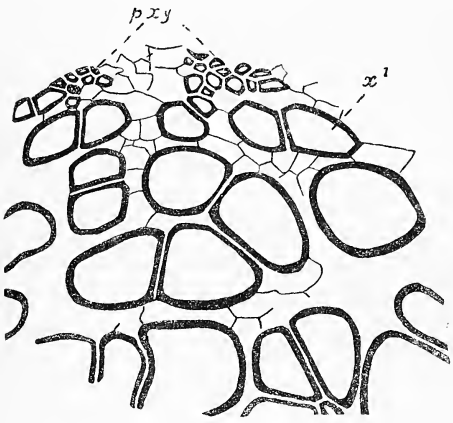


Text-fig. 10. Part of a radially symmetrical leaf-trace bundle. × 70. *s.c.* = secretory elements; *xy'* = primary xylem; *par.* = parenchyma.

[1] Scott, D. H.: On the Structure and Affinities of Fossil Plants from the Palaeozoic Rocks. III. On *Medullosa anglica*, a New Representative of the Cycadofilices. Phil. Trans. Roy. Soc., Ser. B, vol. cxci, 1899, p. 92.

Thin-walled xylem parenchyma forms a continuous network among the primary tracheides; its cells are elongated transversely parallel to the tracheide walls just as in Scott's fossil (compare Pl. XCI, Fig. 2, with Pl. IX, Fig. 13, of Scott's paper). On the whole the parenchyma is less abundant than in the latter stem, giving an appearance of somewhat greater 'woodiness' to the new fossil. A very characteristic feature in *S. insignis* is the presence of numerous elements with dark contents scattered throughout the xylem parenchyma (Text-fig. 6); in the new stem these secretory elements are apparently absent except in the case of one 'meristele' (Text-fig. 10), in which such elements occur.

The xylem parenchyma is, in most places, very badly preserved, so that the apparent absence of secretory elements may be due to this cause. Here and there, however, in both transverse and longitudinal series, are elements of a lighter colour, some giving an appearance of vacuolation; these may quite possibly represent the secretory cells, so abundantly present in the much better preserved wood parenchyma of *S. insignis*.



TEXT-FIG. 11. Transverse section of part of the primary xylem of a meristele, showing the 'paired' protoxylem groups. × 70. *p.xy.* = protoxylem; x^1 = primary xylem.

The position of the protoxylem is always exarch, and it is very common for the prominent protoxylem groups to run in pairs (Text-fig. 11. Compare with Scott's photograph,[1] Pl. IX, Fig. 11). In longitudinal sections the protoxylem is seen to be composed of spirally thickened elements which are succeeded towards the inside of the stele or 'meristele' by denser spiral and then multiseriately pitted elements, the latter composing the bulk of the wood. In all the vascular strands the arrangement is similar (Pl. XCI, Fig. 3).

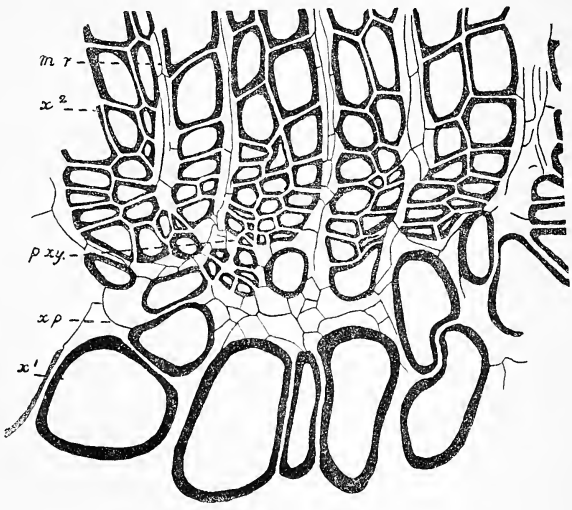
A characteristic feature of the fossil is the presence of a broad zone of secondary wood and bast, which entirely surrounds the primary wood in the main stele, and either entirely or partially encircles the 'meristeles'. The thickness varies in the stele and 'meristeles' of different sizes; in the former it may attain a breadth of 0·9 mm., but in the latter its dimensions are usually much smaller. The secondary tracheides are arranged in regular radial series, separated by medullary rays; frequently one series of tracheides only lies between two rays, though as many as 3–5 may occur. The average breadth of the medullary rays is from 3 to 4 cells, but their vertical

[1] Scott: *Sutcliffia insignis*, loc. cit.

height is considerable. The principal rays extend from the parenchyma of the primary wood to the external limits of the secondary phloem, but shorter secondary rays may arise in the secondary wood. In some places the secondary xylem may be locally very parenchymatous in nature, the tracheide rows or files being 1–2 cells thick, while the medullary rays are 5–6 elements in breadth. The rays are made up of thin-walled parenchyma cells, in which the longest diameter lies in the radial direction.

The tracheides of the secondary wood are of the same multiseriate type as those of the primary xylem, though they are arranged with much greater regularity; their average diameter is distinctly smaller, being only 159 μ. The diameter of the first-formed secondary tracheides is much less than that of the later formed ones, being only from 33 to 67 μ, the full size of the elements being only gradually attained (Text-fig. 12, and Pl. XCI, Fig. 1).

Owing to the kindness of Dr. Scott, I had the opportunity of examining a section of *Sutcliffia insignis* in which secondary growth was just beginning, and it is of great interest to note that the size of the secondary tracheides which are there forming agrees exactly with the similar elements in the new stem.[1]



TEXT-FIG. 12. Transverse section of part of the wood of the stele, showing the small size of the first-formed secondary elements. × 70. *p.xy* = protoxylem; x^1 = primary xylem; x^2 = secondary tracheides; *m.r.* = medullary ray; *x.p.* xylem parenchyma.

No trace of cambium could be distinguished, but the preservation was not perhaps sufficiently good to warrant such an expectation.

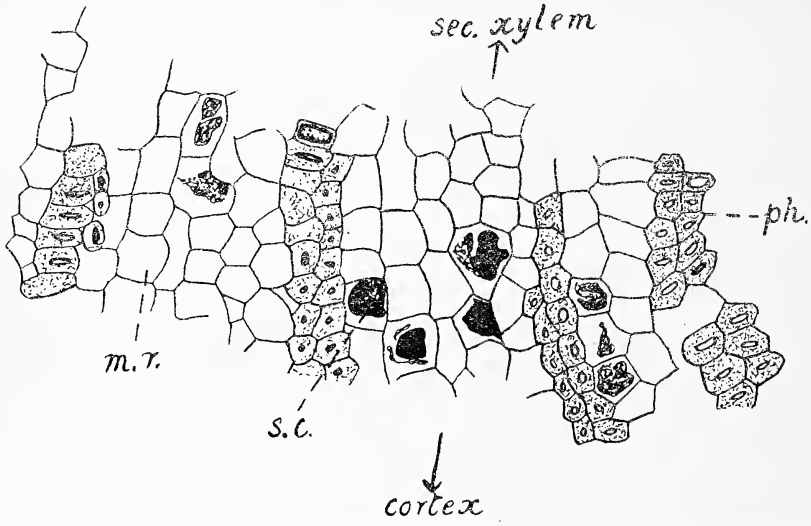
It frequently happens that the continuity of the secondary wood is interrupted, the radial seriation of the elements starting afresh at a certain distance outwards, so that the new series no longer corresponds with the cells previously formed; the size of the elements after the break is always considerably smaller than before. At these places there is produced a superficial resemblance to an annual ring, but that it is merely superficial is clear from the fact that the phenomenon is purely local, and never extends completely round the stele; it is evidently due to some local irregularity in cambial activity (Pl. XCI, Fig. 1). It is interesting to note that

[1] Cf. Scott, *Sutcliffia insignis*, Plate VIII, Fig. 10.

an exactly similar peculiarity has been described by Scott[1] as occurring occasionally in the secondary xylem of *Medullosa anglica.*

2. *Structure of the Phloem.* The primary phloem is not preserved around the steles and 'meristeles', but a broad zone of secondary bast, usually very badly crushed, extends entirely or almost entirely round them; here and there the preservation is sufficiently good to enable some details of its structure to be observed. In most cases the zone appears to have attained considerable thickness, though lateral crushing has undoubtedly exaggerated the appearance to some extent.

The phloem elements are arranged with great regularity, the radial rows corresponding with the tracheidal series of the wood, while the medullary



TEXT-FIG. 13. Transverse section of secondary phloem. × 105. *ph.* = thick-walled elements of phloem; *m.r.* = medullary ray; *s.c.* = secretory element.

rays are an extension of those present in the xylem. The phloem elements resemble those described by Solms-Laubach[2] as occurring in the secondary phloem of *Medullosa Leuckarti*, and by Scott[3] in *M. anglica*; they appear to have thick cell-walls, so that the lumen in transverse section appears as a dot (Text-fig. 13). For reasons detailed in his paper, Solms-Laubach regards these elements as sieve-tubes, and this conclusion Scott considers as probably correct. The latter observer offers two suggestions to account for the thick-walled appearance presented by the elements.

The cell-walls may have been thin walled during life, swelling up greatly under the influence of maceration and decay in the period before petrifaction;

[1] Scott: *Medullosa anglica,* loc. cit., p. 90.
[2] Solms-Laubach: Über *Medullosa Leuckarti.* Bot. Zeit., 1897, p. 179.
[3] Scott: *Medullosa anglica,* loc. cit., p. 90.

or, the apparent 'middle lamella' may represent the whole thickness of the wall, the thick-walled appearance being delusive and depending on some change in the cell contents. In the new stem the 'middle lamella' usually appears to be quite distinct from the remainder of the apparent 'thick wall', so that Scott's second suggestion would certainly appear the more probable explanation in this case. In longitudinal section the phloem is seen to be composed of strands of long, narrow, tapering elements (Pl. XCI, Fig. 4), and the short-celled parenchyma of the medullary rays; in the latter secretory elements frequently occur.

Immediately beyond the phloem lies a somewhat narrow zone of very badly preserved tissue containing numerous large, secretory sacs; this tissue appears to be sharply marked off from the cortex and may possibly represent the pericycle.

3. *Structure of the Leaf-trace Bundles.* The number of leaf-trace strands occurring in the series is extraordinarily small, and it is evident, considering the length of stem under examination, that the leaves could only have occurred at long intervals. It is known that some of the Permian Medulloseae were fair-sized trees,[1] and doubtless *Sutcliffia* should be included among them, though it is just possible that the suggestion put forward by Göppert and Stenzel [2] that some members of the family were climbers may apply here, but much more evidence needs to be brought forward before any definite statement can be given.

Where leaf-trace strands occur in the series they are always in clusters of two or three closely associated together, giving further weight to the suggestion that their derivation is by the breaking up of a 'meristele'; the only exception is the isolated trace derived from γ, but this is probably to be regarded as a premature phenomenon and not as a normal feature. In one case two adjacent, distinct leaf-traces appeared to be approaching preparatory to fusion, but whether this actually occurred cannot be stated, for the leaf-traces disappeared from the series before the critical region was reached. As in *Sutcliffia insignis*, two types of foliar bundles exist; the larger have several protoxylem groups distributed around the wood, and are thus radially symmetrical; the smaller have either one or a twin protoxylem group and are unilateral; in both cases the wood is exarch (Text-figs. 3, 14, and 15).

The zone of ground tissue is too narrow to determine whether the radially symmetrical strands bifurcate during their outward passage, producing unilateral foliar bundles; from the fact that in *S. insignis* [3] the former occurred near the 'meristele' zone, and the latter in the leaf-base, it is probable that this takes place.

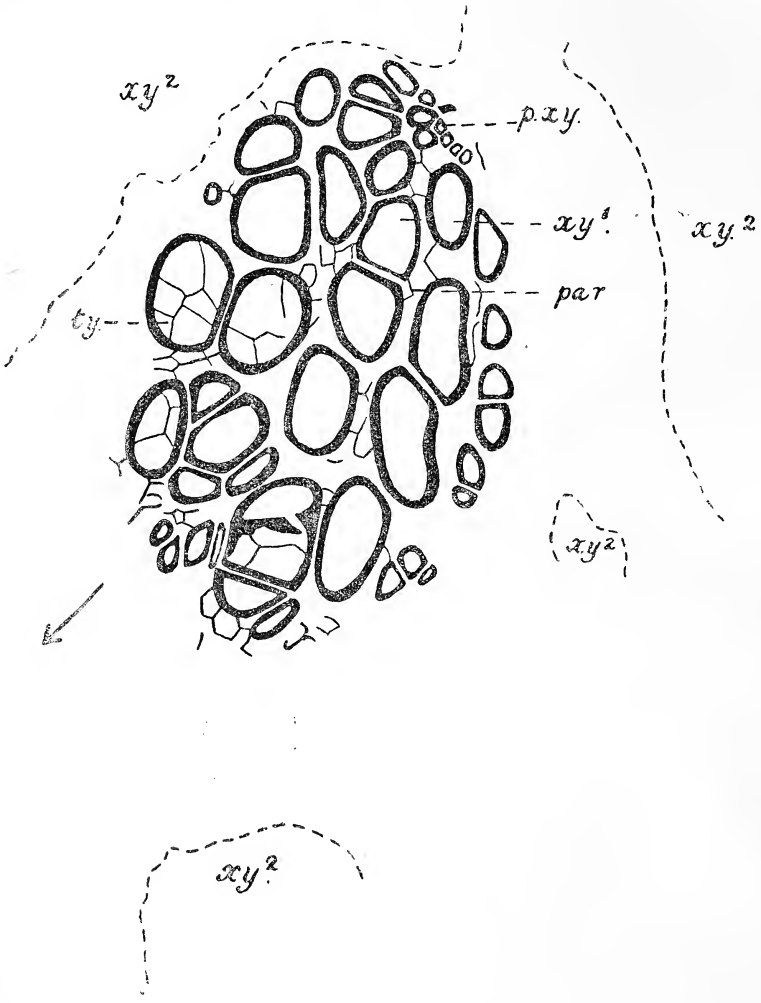
In both types of bundle the tracheides are mixed with parenchyma as

[1] Scott, D. H.: Studies in Fossil Botany, 2nd ed., Part II, p. 443.

[2] Göppert und Stenzel: Die Medulloseae, eine neue Gruppe der fossilen Cycadeen. Palaeontographica, 1881.

[3] Scott: *S. insignis*, loc. cit., p. 57.

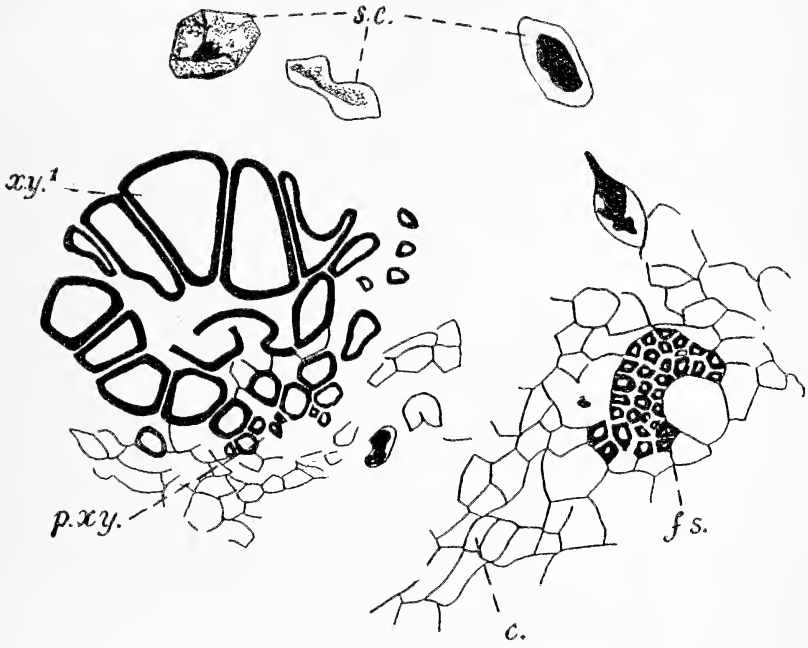
in *S. insignis*. The phloem round the radially symmetrical bundles is unfortunately always absent, though its preservation is almost perfect in some of the unilateral strands. When present, the bast forms a complete zone around the wood, though the distribution may be somewhat unequal, the thickness being greater on the side remote from the fibrous strands



TEXT-FIG. 14. Transverse section of a radially symmetrical leaf-trace bundle, just passing out through the secondary wood xy^2. × 55. The arrow indicates the direction in which the bundle is moving. *p.xy.* = protoxylem; xy^1. = primary xylem; *par.* = wood parenchyma; *ty.* = tyloses. (Compare with Scott's *S. insignis*, Pl. IX, Fig. 14.)

(Text-fig. 3). A very characteristic feature of the phloem of the leaf-trace strands is the presence of a ring of large elements, usually occurring in the outer zone of the bast, though it may in places abut on the wood (Text-fig. 3). These large cells may occur singly, or two or three together, in the

latter case they are separated from one another by a delicate cell-wall. Their size varies somewhat, the larger cells being usually found on the side nearest the fibrous strands. The elements in question are precisely similar in appearance to those described and figured by Scott in *S. insignis* (p. 58, Pl. IX, Figs. 14 and 15, and Pl. X, Fig. 22) and by Seward[1] as occurring in *S. Williamsoni* (p. 211, and Pl. XIII, Figs. 6, 7, 8, 11, and 12). Seward in his fossil described various stages in the organization of these elements, and concluded that 'the characteristic receptacles which accompany the vascular bundles of *Rachiopteris Williamsoni* are of the nature of small secretory

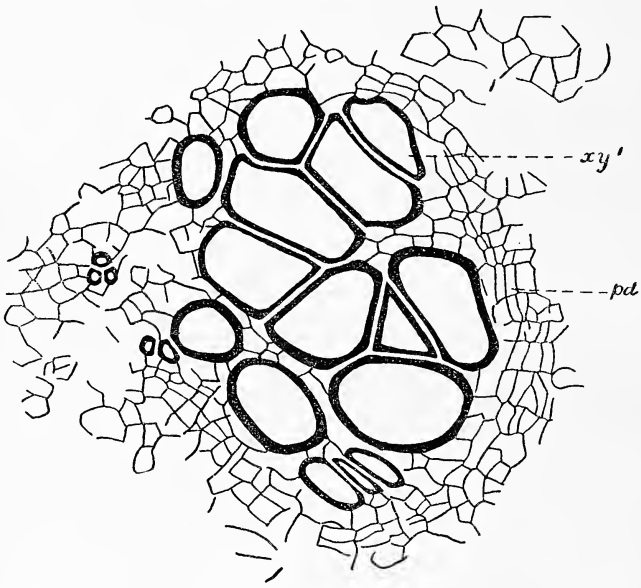


TEXT-FIG. 15. Transverse section of a unilateral leaf-trace bundle. × 55. *xy*'. = primary xylem; *p.xy.* = protoxylem; *f.s.* = fibrous strand; *c.* = cortex; *s.c.* = secretory element. (Compare with Scott's *S. insignis*, Pl. IX, Fig. 15.)

canals, formed as the result of a schizolysigenous process. In one slide there is a solitary instance of a bundle-canal containing dark-coloured contents similar to that in the larger canals of the fundamental tissue, and possibly representing the remnants of secretion.' Scott, on the other hand, is 'inclined to regard the large elements of the phloem as sieve-tubes', and expresses some doubt as to 'the interpretation of these structures as developing canals'. In the new specimen the ring of small cells surrounding the scattered elements, which shows so clearly in *S. insignis*, does not usually occur, but the large elements sometimes show in the interior evidence of broken-down walls, giving the impression that the ring of small

[1] Seward, loc. cit.

cells did exist, and is being disorganized ; no sign of the early stages of development, such as are figured by Seward, were to be traced. In all the secretory elements so abundantly scattered throughout the specimen evidence of the secretion is seen either in the form of black carbonaceous matter, light coloured contents, or the appearance of vacuolation, but in no case have any of these signs of contents been observed in the large elements of the phloem. Longitudinal sections were not available for examination, but from Scott's observations on *S. insignis*, they are known to be long tubes. On the whole the new specimen does not materially add to the evidence which would elucidate the question of the nature of the elements in question. The absence of any sign of secretion and the distribution of the elements in the



TEXT-FIG. 16. Transverse section of a leaf-trace bundle showing periderm formation. × 70. *pd.* = periderm; xy^1. = primary wood. (Xylem slightly restored.)

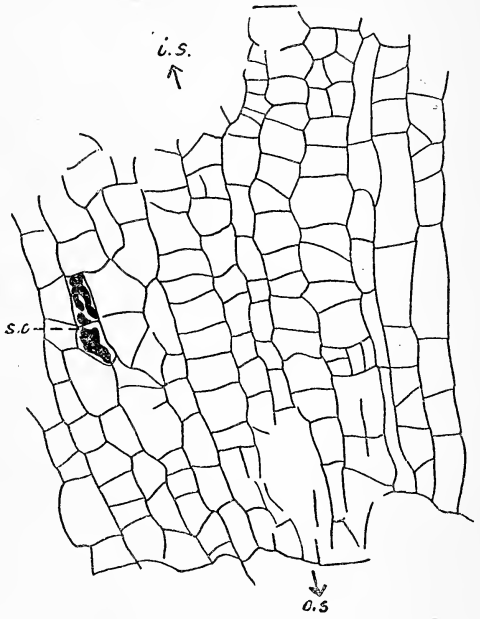
phloem (where the presence of such numerous secretory elements appears to be unparalleled) would incline to the view that they represent sieve-tubes.

An interesting feature occurs in two of the leaf-traces at the upper end of the stem. The bundles are of the unilateral type, and no phloem is present; but immediately surrounding the primary wood a zone of periderm occurs (Text-fig. 16). The tracheides of the primary wood have obviously been injured in some way, probably by fungal growth, and the leaf-traces in consequence are being isolated by means of a periderm formation. A precisely similar case of isolation of a vascular strand which had been injured by a fungal attack has been observed in the hypocotyl of a seedling of *Allionia albida*.

V. The Ground Tissue and the Secondary Cortex.

The description given by Scott of the ground tissue of *Sutcliffia insignis* applies equally well in the case of the new fossil, for where patches of the cortex are preserved, 'the short-celled ground-parenchyma contains numerous sacs with carbonaceous contents, and is traversed by secretory canals and strands of sclerenchyma.' The latter are in almost every case grouped round the leaf-trace strands, where they occur in an arc of three or four groups, varying in size, and lying on one side of the bundle only.

A distinctive feature of the stem is the presence of a wide zone of tissue, which usually forms the limiting layer of the fossil, and which may attain a thickness of 15 to 20 elements in some parts. The elements composing this layer are clearly of secondary origin, for they are arranged with some regularity in radial series. Examination of longitudinal and transverse series shows the tissue to be composed of somewhat rectangular shaped cells, thin walled, and empty of contents, while occasional secretory elements run between them (Text-fig. 17).

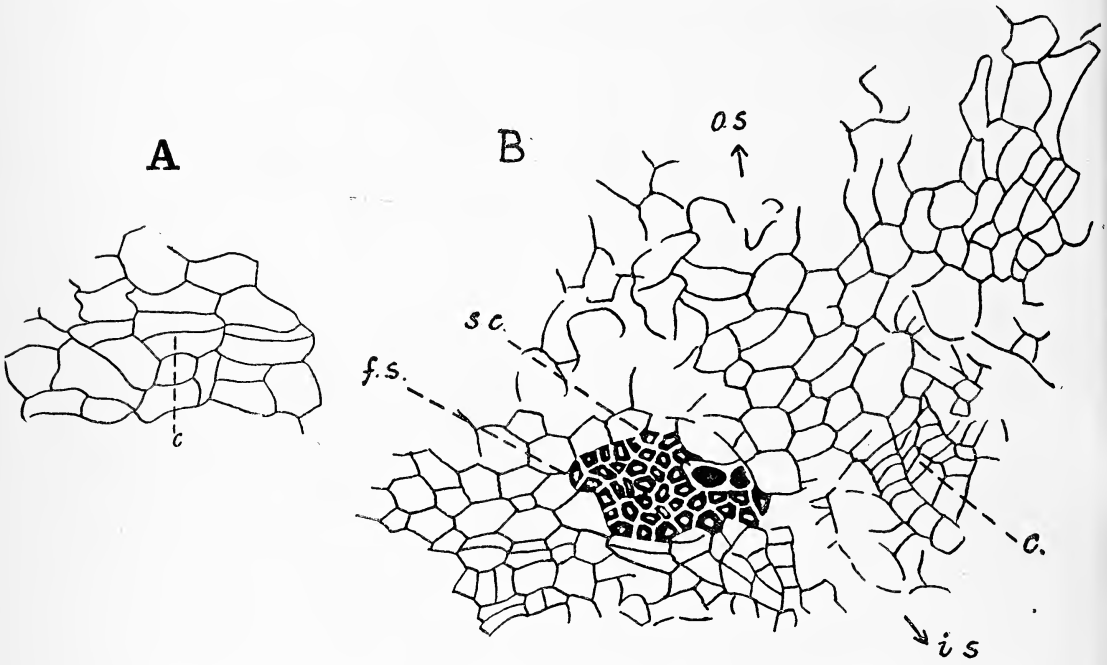


Text-fig. 17. Transverse section of secondary cortex. × 70. *s.c.* = secretory elements; *i.s.* = direction of the stele; *o.s.* = direction of the cortex.

The development of this tissue can be traced in the upper part of the series. In the delicate tissues of the inner region of the cortex, here and there partially preserved, certain cells show obvious signs that a tangential division has just been completed (Text-fig. 18); in others further divisions have taken place. There can be little doubt that periderm formation is here beginning. A comparison of the various stages of development between this beginning of periderm formation and the wide zone of tissue of secondary origin found lower in the stem has led to the conclusion that the latter represents only the secondary cortex produced by the phellogen. Support is given to this view by the fact that no sign of cambial activity can ever be detected on the inner margin of the tissue; moreover, the cells do not show the characteristic features usually associated with cork, and finally it appears improbable that secretory elements would

occur in the midst of dead suberized tissue. If this be the case, then, the cork, and in some cases deeper seated tissues also, must have suffered destruction.

In any one section the zone of secondary cortex never extends in a continuous band round the vascular structures, but is found in arcs of greater or less extent. The fact that, in the upper region of the series, cork-cambium formation can be traced in what is obviously an old stem suggests that the phellogen may have had only a comparatively short period of activity before it was replaced by a new, more deeply placed arc



TEXT-FIG. 18. A. Beginning of phellogen formation. × 70. B. Later stage of cork cambium development. × 55. *c.* = cambium; *s.c.* = secretory element; *f.s.* = fibrous strand; *o.s.* = direction of cortex; *i.s.* = direction of stele.

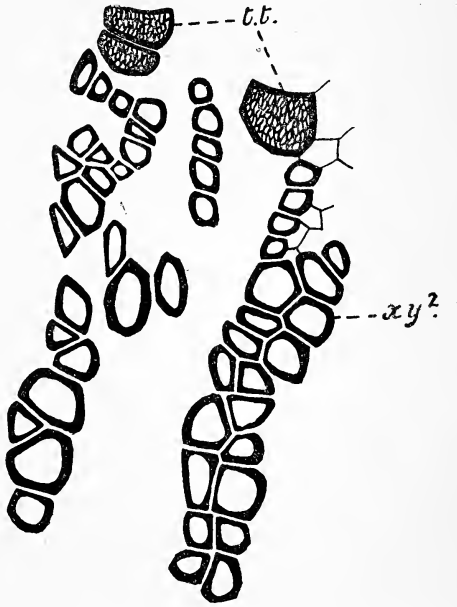
of meristem; so that by successively formed layers of cork, each more deeply placed than the last, the stem would in time be composed only of the vascular cylinder and the last formed cork formations, a condition arrived at in the new stem of *Sutcliffia*, where practically all the cortex and the leaf-bases have been sloughed off as bark.

It is of great interest to record in this connexion, that Dr. Scott,[1] who very kindly examined two or three of my sections, remarked, 'I sometimes find very distinct bands of meristem in the cortex of my *Sutcliffia*, but they look more like the beginning of periderm than of vascular tissue. The inner cortex generally is very delicate and fit to originate meristems.'

[1] In a letter to Professor F. W. Oliver on June 22, 1912.

VI. The Extrafascicular Strands.

One of the most interesting features in the vascular anatomy of the new stem is the presence of numerous extrafascicular strands around the vascular system proper. In life it is probable that these strands formed a complete anastomosing network around the steles and 'meristeles', for in the fossil they extend round three sides; their absence on the fourth is accounted for by the fact that considerable injury occurred on this side in the course of petrifaction. The strands appear to cluster at the ends of the long diameter of the stem, but this appearance is perhaps partly exaggerated by the lateral pressure to which the stem has been subjected (Text-figs. 3 and 4). The arrangement of these extrafascicular strands varies considerably; usually they take the form of normally orientated bands of secondary wood and bast, frequently they assume a fan-shaped appearance, incompletely concentric structures may occur, rarely nearly perfectly concentric bundles are found, while in one case a band-like strand showed inverted orientation of the vascular tissues. The size as well as the arrangement of these strands shows great diversity (Text-fig. 2 and Pl. XCI, Fig. 1). In every case, no matter what the size, arrangement, or orientation of the strands, there are found on the side of the wood remote from the phloem a number of short isodiametric tracheides (Text-fig. 19).



Text-fig. 19. Transverse section of part of an extrafascicular strand, showing isodiametric tracheides (*t.t.*) on its inner margin. xy^2 = secondary xylem. × 84.

These short broad tracheides have, as a rule, reticulate markings on their walls, though occasionally pitted elements seem to be present. Their average maximum diameter is about 100 μ; their length, which is slightly greater, averages 66·6 μ. They are clearly marked off from the long, multiseriately pitted tracheides which compose the bulk of the wood of the extrafascicular strands, for the diameter of these elements only reaches about 58 μ, although in some strands the dimensions are greater and may reach 115·8 μ;[1] it is very difficult to determine clearly the short

[1] They are thus somewhat smaller than the secondary tracheides of the stele and 'meristeles', the average diameter in the latter case being 159 μ.

tracheides of the more or less concentric strands owing to the crushing which has usually taken place.

Scattered throughout the series in the band of tissue immediately beyond the secondary phloem of the 'meristeles' (probably the pericycle) are found clusters of isodiametric tracheides with reticulate markings exactly resembling those which occur on the inner margin of the wood of the extrafascicular strands (Pl. XCI, Fig. 5). It is suggested that these strands of short tracheides may have been directly derived from the delicate parenchyma cells of the inner cortex to facilitate the storage of water. Strands or clusters of storage tracheides once having been formed may possibly have provided a starting-point or 'nucleus' around which cambial activity being initiated secondary formation of xylem and phloem began; the ultimate product of the secondary growth being the diversely organized extrafascicular arcs which form so characteristic a feature of the fossil. It may perhaps be pointed out here, though the question will be discussed later, that these strands are regarded as entirely secondary structures and are not considered to have any phylogenetic connexion with the stele or 'meristeles'.

The short tracheides at once recalled the somewhat similar, pitted, isodiametric tracheides described by Seward[1] in *Megaloxylon*, but in that genus they compose the bulk of the primary wood, and they certainly have no connexion with that tissue in *Sutcliffia*. The occurrence of parenchymatous tracheides, reticulately marked but with scattered bordered pits, has been described by Rothert[2] as present singly or in groups in species of *Cephalotaxus* where they represent modified elements of the pith. As in the case of *Megaloxylon*, the function of water storage is assigned to them, and a comparison is instituted with the similar cells found in *Nepenthes*, *Salicornia* sp., *Crinum*, and certain members of the Orchidaceae. Thus the conversion of parenchymatous cells into short tracheides for the purpose of water storage appears to be a not uncommon phenomenon.

In this connexion it may be noted that Lang[3] observed the occurrence of short tracheides in a species of *Ophioglossum*; they were mixed with the parenchyma of the pith and 'apparently derived by the conversion of some of the medullary cells into tracheides'. A similar phenomenon may occur in *Botrychium*. Lang notes, however, that in both cases the stimulus to the development of the tracheides may have been traumatic.

The fact of the greatest interest in connexion with these extrafascicular

[1] Seward, A. C.: Notes on the Binney Collection of Coal-Measure Plants. Part II, *Megaloxylon*, gen. nov. Proc. Camb. Phil. Soc., vol. x, Part III, 1899.

[2] Rothert, W.: Über parenchymatische Tracheïden und Harzgänge im Mark von *Cephalotaxus*-Arten. Sonder-Abdruck aus den Berichten der Deutsch. Bot. Gesellsch., Jahrgang 1899, Bd. xvii.

[3] Lang, W. H.: On the Interpretation of the Vascular Anatomy of the Ophioglossaceae. Mem. and Proc. of the Manchester Lit. and Phil. Soc., vol. lvi, Part II, 1912.

strands is the extraordinarily close parallel they afford with the successive vascular rings characteristic of certain genera of the Cycads, namely, *Cycas*, *Encephalartos*, *Macrozamia*, and *Bowenia*. Not only is the arrangement of the secondary strands in arcs, fan-shaped masses, and occasionally concentric bundles closely similar in *Sutcliffia* and in the above-mentioned genera of the Cycads, but comparison with the descriptions and figures given by Worsdell [1] in his series of papers on Cycadean anatomy shows that the intimate structure is practically identical also. Large isodiametric tracheides occur at the edge of the wood in both cases, the great development of the medullary rays results in a very parenchymatous xylem in both living and fossil stems, while in both occasional inversion of the strands is found.

One further point still remains to be considered, namely, the origin of the extrafascicular strands of the Cycads. Worsdell (loc. cit., 1900, p. 452) considers the large, irregularly-shaped, reticulately thickened tracheides of the extrafascicular rings are homologous with the reticulate tracheides of the central region of the cortical concentric strands of the stem and leaf of *Cycas*, and in this genus he was able to observe their development. In *Cycas revoluta*, Thunb., he found 'the first-formed vascular elements arose by tangential division of the large, rounded or angular, cortical or pericyclic cells resulting in the large, isodiametric, irregularly-shaped tracheides of precisely similar shape to those cells. After one or more such elements have been cut off, the parenchyma cells begin to divide radially as well as tangentially, in this way forming smaller and smaller elements with each centrifugal division, until at length the majority of the tracheides are of the same size and shape as those of the central cylinder or stele.' The displacement of the large first-formed tracheides by pressure of surrounding tissues, and the great difference in size between them and the later-formed ones, render 'the determination of their mode of origin, at an older stage of the same vascular tissues, utterly obscure. The similar reticulate tracheides in the cortical concentric strands . . . in all probability arise in precisely the same way.' [It is of interest to note that in an isolated, concentric strand in the cortex of the upper regions of the hypocotyl of one seedling of *Macrozamia spiralis*,[2] the central core of the bundle consisted of short tracheides which were surrounded by a zone of cambium and cambiform cells, the few immature xylem-elements present were developed centrifugally from the centre of the bundle.] Quotation at length has been made from Worsdell's paper

[1] Worsdell, W. C.: I. The Anatomy of the Stem of *Macrozamia* compared with that of other Genera of Cycadeae. Ann. of Bot., vol. x, 1896. II. The Comparative Anatomy of Certain Genera of the Cycadaceae. Journ. Linn. Soc., vol. xxxiii, 1898. III. The Comparative Anatomy of Certain Species of *Encephalartos*. Trans. Linn. Soc., Oct., 1900. IV. Contributions to the Comparative Anatomy of the Cycadaceae. Trans. Linn. Soc., Sept., 1901. V. The Structure and Origin of the Cycadaceae. Ann. of Bot., vol. xx, April, 1906.

[2] Hill, T. G., and de Fraine, E.: On the Seedling Structure of Gymnosperms. III. Ann. of Bot., vol. xxii, 1909, p. 442.

because the description given by him appears to explain in a satisfactory manner the way in which the similar strands in *Sutcliffia* may possibly have arisen from parenchyma cells. It is, however, impossible to state the mode of origin with certainty, as no stages between the clusters of tracheides formed from cortical or pericyclic cells and fully formed arcs or strands were observed.

The investigations of Weber and Sterzel [1] have shown that in *Medullosa stellata*, var. *gigantea*, and probably also in *M. Solmsii*, var. *lignosa*, extrafascicular zones of vascular tissue occurred outside the system of steles, and that these zones were very similar to those found in Cycads of the present day. Scott [2] also found that in *Medullosa anglica* irregular bands may occur in the stem which are 'probably best regarded as extrafascicular new formations, comparable to . . . the irregular strands which sometimes occur in the extrafascicular region of *Macrozamia*'.

There still remains one further structure of interest to be described, a strand which appears in a few sections only, and which lies beyond the other vascular tissues. It consists of a concentric bundle of fair size, the maximum diameter being about 2 mm., the whole being slightly elongated tangentially (Pl. XCII, Fig. 6). Short isodiametric tracheides with reticulate markings form the bulk of the central region of the strand (Pl. XCII, Fig. 7), and round them lies a zone of secondary wood, completely encircled by secondary phloem; the structure of the latter appears precisely similar to that surrounding the stele and 'meristeles'. All the wood elements are smaller than those of the extrafascicular strands: the average diameter of the secondary tracheides only reaches $33{\cdot}\dot{3}\ \mu$, whereas in the former they are usually $115{\cdot}8\ \mu$. The short tracheides have a diameter nearly twice as great as the elements of the secondary wood, and are $58{\cdot}\dot{3}\ \mu$ broad.

The resemblance of this strand of the accessory vascular strands of *Medullosa anglica* is very striking. Scott instituted a comparison between these latter and the cortical bundles of *Cycas*, and states that he regards them, as also the extrafascicular bands, as 'characteristic Cycadean anomalies', which comparison and interpretation might well be extended to *Sutcliffia* also.[3]

VII. Attribution of the Specimen.

The fossil stem here described has been attributed to the genus *Sutcliffia* on account of the close resemblance it exhibits to the only other stem of the genus at present known, viz. *Sutcliffia insignis*. The general structure of the vascular system is identical in the two fossils, for both have a simple

[1] Weber und Sterzel: Beiträge zur Kenntnis der Medulloseae, p. 116, and Taf. viii, Fig. 2.

[2] Scott, *Medullosa anglica*: loc. cit., p. 98.

[3] Compare Scott, *Medullosa anglica*, loc. cit., p. 98, Pl. XII, Fig. 18, with Pl. XCII, Fig. 6 of this paper and with Worsdell, loc. cit., 1898, Figs. 6–8, 1896, Figs. 2, 9, and 10.

protostele [1] surrounded by large, irregular masses of vascular tissue, detached from or in connexion with the main stele. The origin and subsequent behaviour of these vascular masses ('meristeles') is very similar; in both cases they are derived by the separation of portions from the protostele, and in both these detached portions undergo further irregular division. In *S. insignis* the meristeles 'form the points of departure for the actual leaf-traces',[2] while in our specimen evidence is afforded that they are entirely used up in the production of foliar bundles.

Fusion between 'meristeles, probably of quite distinct origin', occurs only in the type specimen, but re-fusion of 'meristeles' with the meristeles of similar origin, and with the protostele, has been described for our fossil (p. 1039). In both cases the 'meristele' repeats the structure of the protostele on a small scale.

The leaf-trace strands form a further feature of comparison between the two specimens, for in both stems the foliar traces are concentric and the structure is exarch, and, moreover, two types of leaf-trace bundles are present in both, namely, the unilateral and the radially symmetric.

Finally, the agreement in histological details between our stem and that of *S. insignis* is very striking. The structure and composition of the primary wood is very similar in the two cases, and the beginning of secondary growth in *S. insignis* agrees closely with the first formed secondary tracheides in the new specimen. The primary phloem surrounding the leaf-trace bundles shows the same peculiarities in both stems. The strands of fibrous elements with the closely associated secretory elements, and the great abundance of secretory cells throughout the tissues, are characteristic features common to both.

Thus, a consideration of the general structure of the stem, the origin and behaviour of the 'meristeles', the leaf-trace bundles, and the close agreement in histological details in the two stems leaves little room for doubt that the relationship between them is exceedingly close. The question next arises whether the differences which occur are sufficient to warrant specific distinction, or whether they can be explained on any other grounds.

The outstanding feature which characterizes our stem is the great development of the secondary tissues around the stele and 'meristeles', coupled with the network of extrafascicular zones and accessory vascular strands; the absence of any leaf-base forms a further feature of difference. The latter is undoubtedly to be correlated with the formation of periderm; in *Sutcliffia insignis* cork was absent, though, as Scott has pointed out,[3] arcs of meristem were in process of formation; so the absence of leaf-bases may therefore well be attributed to the greater age of the stem. It appears

[1] Using the term in the sense in which it is employed in Tansley's 'Lectures on the Evolution of the Filicinean Vascular System'. New Phyt., 1908, p. 138.

[2] Scott: loc. cit., p. 54.

[3] See footnote, p. 1052.

extremely probable that the presence of the secondary tissues and the extrafascicular strands may be explained on the same grounds, for in *S. insignis* slight secondary growth had taken place around the stele and 'meristeles' in the lower parts of the specimen, and the resulting tracheides agree absolutely with those in the new stem.

The apparent absence of secretory elements from the xylem parenchyma constitutes the most serious difficulty in assigning the fossil to *Sutcliffia insignis*, but in one 'meristele' such elements can be readily detected (Text-fig. 10), and, moreover, it appears very probable that under more favourable preservation similar elements would be found generally distributed among the primary tracheides (see p. 1044).

Until further evidence is available it would appear desirable, therefore, to attribute, provisionally, the new specimen to *Sutcliffia insignis*; on this view Scott's specimen would be the stem in its young state, whereas the present fossil would show the complexity[1] attained in the older, though smaller, plant.

VIII. Affinities.

In the original description of the genus, *Sutcliffia*[2] is included in the family Medulloseae, and as the reasons for this conclusion are so fully stated there, it is quite unnecessary to add further remarks on this question. The additional data afforded by the new specimen only serve to bring into still closer relationship the two genera, *Sutcliffia* and *Medullosa*, through some such forms as *Medullosa anglica*, the simplest and geologically oldest known member of its genus.[3] The protostele of *Sutcliffia*, with its wide zone of secondary tissues, bears a very close resemblance to one of the three steles of *M. anglica*, the main difference depending upon the mesarch nature of the protoxylem in the latter species compared with its universally exarch structure in the former. Extrafascicular strands of wood and bast and accessory vascular bundles are present in both; and finally there appears to be no serious objection to the view that the 'meristeles' of *Sutcliffia* are homologous with the leaf-trace strands which leave the stele in *M. anglica*, for both appear to be entirely used up in the formation of foliar bundles.

It remains to be considered whether the new specimen throws any further light on the affinities of the Medulloseae with other groups. Scott expressed the view that *Sutcliffia* is 'the most primitive member of the Medulloseae yet discovered', though he continues, 'it is doubtful, however, whether it lay on the direct line of descent of any of the more complex types with which we are acquainted.' He considered that its structure 'has not advanced very far beyond the simple protostelic condition' of such a form

[1] Cf. Scott, *Sutcliffia insignis*: loc. cit., p. 54. [2] Scott, *Sutcliffia insignis*: loc. cit., pp. 63–5.

[3] Scott, *Medullosa anglica*: loc. cit., pp. 114–15. Studies in Fossil Botany, Part II, 1909, p. 428.

as *Heterangium*; moreover, he suggests that 'the whole course of evolution from the protostele to the more elaborate dialystelic type may have been gone through within the family', i. e. 'the Medulloseae are their own interpreters.' Finally, Scott suggests the probability of a common origin for the Lyginodendreae and Medulloseae 'from a point not very far below the level of stems such as those of *Sutcliffia* and *Heterangium*'.[1] The great possibility of the origin of the Medulloseae from a group of Fern-like ancestors has thus received full treatment; it is on the probable affinities in other directions that the new specimen appears to give further evidence.

The question of the relationship between the Medulloseae and the Cycadaceae has been so fully discussed that it would seem superfluous to reopen the question, were it not for the fact that the new specimen of *Sutcliffia* appears to throw additional light upon it. Two main theories have been held with regard to the origin of the anatomical peculiarities of the Cycads. Scott[2] considers that 'so far as the anatomy of the stem is concerned, *Lyginodendron* appears to come near the Cycads, for the general organization is of a similar character, and the mesarch structure of the bundles is still retained in the peduncles of the cones of some recent Cycads as well as in the leaves'. He regards the Medullosean stem as anatomically different from that of recent or Mesozoic Cycadophyta 'in being polystelic (except in the protostelic *Sutcliffia*, which does not affect the question)'. The extrafascicular cylinders found in certain Cycadean genera are 'local peculiarities in the vascular system ... due to anomalous distribution of the cambium' and are not to be regarded as of ultimate phylogenetic significance.

A second theory deriving the Cycads from the Medulloseae has been advanced by Potonié,[3] and independently stated and elaborated by Worsdell.[4] The view of Matte[5] differs from these observers in certain respects, for he considers 'les Cycadacées comme dérivées de Lyginodendrées ou d'une famille voisine par l'intermédiaire des Médullosées'. In a recent account of the Pteridospermae Chodat[6] summarizes his views of the relation between Medulloseae and Cycadaceae as follows: 'Les Médullosées nous apparaissent donc comme des Protocycadacées'; and he further states that 'il nous est impossible de trouver dans l'anatomie des *Lyginodendron* la moindre analogie avec celle des Cycadacées'. The main reason for the last state-

[1] Scott: loc. cit., p. 64. Compare Studies in Fossil Botany, 2nd ed., Part II, p. 464.

[2] Scott, D. H.: Studies in Fossil Botany. 2nd ed., Part II, pp. 648–50.

[3] Potonié, H.: Lehrbuch der Pflanzenpalaeontologie. 1899, footnote on p. 168.

[4] Worsdell, loc. cit. The paper of 1906 contains a full résumé of his views and the evidence on which they are based.

[5] Matte, H.: Recherches sur l'appareil libéro-ligneux des Cycadacées. Caen, 1904, p. 211.

[6] Chodat, R.: Les Ptéridopsides des temps paléozoïques; étude critique. Arch. des Sci. phys. et nat., t. xxvi, 1908, p. 38 and p. 17.

ment centres round the nature of the primary strands, for in agreement with Bertrand and Corneille,[1] Chodat regards these in *Lyginodendron* as 'divergeants du type *Osmunda* en ω renversé, les deux ailes du divergeant se rebattent en arrière et finissent par se réunir par leurs pôles'; this is a not uncommon type in the Ferns, whereas in the stem of Cycads no 'divergeants' occur, the stem, as in other Gymnosperms, being composed of a 'couronne de faisceaux endarques'. The views of Chodat thus summarized have been considered at some length by Weiss,[2] who concludes that the criticism seems 'sufficiently weighty to demand a careful reconsideration of the structure and affinities of *Lyginodendron*'.

The theory which would derive the Cycadaceae from the Medullosean line has been most fully discussed by Worsdell.[3] He regards the normal vascular ring in the Cycad as composed in reality of 'the one-sided remnants of a number of steles', while the extrafascicular arcs and concentric bundles characteristic of some Cycadean genera are 'remnants of some ancient structure' which 'consisted of rings or layers of concentric vascular strands'.[4] The evidence adduced by Worsdell in favour of the Medullosean ancestry of the Cycads is based upon structure observed (1) in the cotyledonary node, and (2) in the 'flowering' axis. It is not intended to enter here into a full discussion of the evidence afforded by the 'flowering' axis, but it would appear that the incurved and horseshoe-shaped bundles of *Stangeria* described by Worsdell have had far too weighty phylogenetic significance attached to them;[5] it has yet to be proved that far-reaching conclusions of such a type can be based, legitimately, upon evidence afforded by a structure such as the 'peduncle', in which special circumstances[6] and physiological needs must play an important part.

The evidence brought forward as supplied by the cotyledonary node is that given by the concentric structures around the main axis in certain species of *Cycas*, *Encephalartos*, *Macrozamia*, and *Bowenia*, which give the seedling an appearance of 'polystely' in this region. The strands are

[1] Bertrand, E. C., et Corneille, F.: La masse libéro-ligneuse élémentaire des Filicinées actuelles. Travaux et Mémoires de l'Université de Lille, 1902, t. x, Mém. no. 29.

[2] Weiss, F. E.: Presidential Address to the British Association for the Advancement of Science, Section K, Portsmouth, 1911.

[3] Worsdell: loc. cit., 1906, p. 137.

[4] The supporters of the Medullosean ancestry for the Cycads had not, at the time they wrote, the advantage of considering a relatively simple member of the group such as *Sutcliffia*.

[5] It may be pointed out that Scott considers that the mesarch bundles which occur in the peduncles of the cones in certain genera of the Cycads, e.g. *Stangeria*, represent the retention of a primitive character by the 'floral axis'. Scott, D. H.: The Anatomical Characters presented by the Peduncle of the Cycadaceae. Ann. of Bot., vol. xi, 1897; and Studies, pp. 365–6.

[6] The observations of Thoday on the inflorescence axis in *Gnetum* sp. are of considerable interest in this connexion. Thoday (Sykes), M. G.: Note on the Inflorescence Axis in *Gnetum*. Ann. of Bot., vol. xxvi, 1912, p. 621.

described in detail by Worsdell,[1] Gregg,[2] Matte,[3] and Hill and de Fraine,[4] and are regarded by the first-named observer as giving additional weight to his view. The case of the seedling stem of *Encephalartos Barteri* is also brought forward as further evidence of its original polystelic ancestry. Matte [5] found in one individual only of this species three practically independent, distinct steles in place of the usual endarch cylinder ; this both he and Worsdell compare with *Medullosa anglica*, and regard as of the highest significance. This anomalous structure is undoubtedly of great interest, more especially when it is compared with a somewhat similar abnormal case of seedling anatomy described by Shaw [6] as occurring in a single specimen of *Araucaria Bidwillii*. This observer correlated the abnormality with the tuberous habit of the seedling, and it is interesting to note, therefore, the somewhat similar tuberous appearance presented by the seedling of *Encephalartos Barteri* (Matte, Pl. XVI, Fig. 257). The question remains to be considered whether the cotyledonary node supplies reliable data for the elucidation of phylogenetic problems. The attention of several investigators [7] has been directed of late years to the answering of this question, and, so far as one can judge from the evidence at present available, it is inadvisable to apply characters of seedling anatomy to solve broad phylogenetic questions. Only the main points of the evidence brought forward by Worsdell have been outlined, and no attempt at a complete discussion has been made, but it is hoped that sufficient indication has been given to show that the origin of Cycads from ' polystelic ' ancestors is not yet satisfactorily proved. It is fully agreed that the probable origin of the Cycadaceae is along the Medullosean line, but from monostelic rather than from ' polystelic ' forms. It is suggested that the central cylinder of a Cycadean stem was probably derived from a protostelic form, such, say, as occurred in the genus *Sutcliffia*, by the gradual disappearance of the internal tracheides. Extrafascicular zones and accessory cortical strands occur in precisely the same form in *Sutcliffia*, *Medullosa*, and recent Cycads. What the origin of these structures may be it is impossible at present to say, but there is no

[1] Worsdell : loc. cit., 1906, p. 147.

[2] Gregg : Anomalous Thickening in the Roots of *Cycas Seemanni*. Ann. of Bot., vol. i, 1887.

[3] Matte : loc. cit., p. 185, &c.

[4] Hill, T. G., and de Fraine, E. : On the Seedling Structure of Gymnosperms. III. Ann. of Bot., vol. xxiii, 1909, p. 442.

[5] Matte : loc. cit., p. 201.

[6] Shaw, F. J. F. : The Seedling Structure of *Araucaria Bidwillii*. Ann. of Bot., vol. xxiii, 1909, p. 327, &c.

[7] Hill, T. G. : On the Seedling Structure of Certain Piperales. Ann. of Bot., vol. xx, 1906. de Fraine, E. : On the Seedling Structure of Certain Cactaceae. Ann. of Bot., vol. xxiv, 1910. Hill, T. G., and de Fraine, E. : On the Seedling Structure of Certain Centrospermae. Ann. of Bot., Jan., 1912. Compton, R. H. : An Investigation of the Seedling Structure in the Leguminosae. Journ. Linn. Soc. (Bot.), vol. xli, June, 1912. Lee, E. : Observations on the Seedling Anatomy of Certain Sympetalae. I. Tubiflorae. Ann. of Bot., vol. xxvi, 1912. Hill, T. G., and de Fraine, E. : On the Influence of the Structure of the Adult Plant upon the Seedling. New Phyt., Oct., 1912.

conclusive evidence to show that they were in any way connected with the original central cylinder; it is possible that they have arisen later and independently of it. Their function is evidently to provide an increased water-storing and conducting tissue for the plant, and it is suggested that as radial increase progressed in response to the increased physiological requirements, the increased vascular needs were provided for by extrafascicular arcs and strands which arose independently in the cortex, and which are not therefore to be derived by reduction from a system of steles. From such a type as *Sutcliffia*, which may be regarded as the most primitive member of the Medulloseae at present known, it is suggested that two divergent lines may have arisen. The one advanced with increasing complexity in the direction of multiplication of the number of steles, through some such form as *Medullosa anglica*, and ended blindly in the more complex Medulloseae, the extraordinary complexity of the series of steles and the anastomosing medullary and cortical 'star rings' possibly proving too cumbrous and not sufficiently plastic to allow of further evolution along these lines. The other maintained the protostelic condition, and advanced by further modification of the single vascular cylinder, and perhaps by the elaboration of the extrafascicular arcs and accessory vascular strands of the cortex, in the direction of the Cycadales.

It is to be hoped that the discovery of further fossil forms may shed additional light on this question, for the evidence connected with the reproductive organs which is at present available, tends to show that the Medullosean seeds such as *Trigonocarpus* and *Stephanospermum* are in substantial agreement in certain respects with the genera of living Cycads.[1] And in this connexion it may be noted that the recent investigation of the ovule of *Bowenia spectabilis* by Kershaw[2] shows that as regards structure, and development of the pollen-chamber, 'the closest parallel to the Cycads among fossil seeds is to be found in seeds of the *Trigonocarpus* affinity, and there seems to be no evidence in either of them that this structure had resulted from simplification of a more complicated type, such as that of the Lagenostomales.' Kershaw further points out that not only in the pollen-chamber, but also in respect of the integument and vascular supply, there is a very close connexion between Cycadean seeds and those of the Medulloseae.

Further, as Scott[3] has already shown, 'the structure of the petiole and the organization of the leaves generally are very similar in Medulloseae and Cycadaceae.'

[1] Oliver, F. W., and Salisbury, E. J.: On the Structure and Affinities of the Palaeozoic Seeds of the *Conostoma* Group. Ann. of Bot., vol. xxv, 1911, p. 40.

Scott, D. H., and Maslen, A. J.: The Structure of the Palaeozoic Seeds, *Trigonocarpus Parkinsoni*, Brongniart, and *Trigonocarpus Oliveri*, sp. nov., Part I. Ann. of Bot., vol. xxi, 1907, p. 115.

[2] Kershaw, E. M.: Structure and Development of the Ovule of *Bowenia spectabilis*. Ann. of Bot., vol. xxvi, July, 1912.

[3] Studies, loc. cit., p. 649.

Hence, at the present time, the balance of evidence drawn from both the vegetative and reproductive features favours the view of the Medullosean ancestry of the Cycads,[1] through some protostelic form.

IX. Summary.

1. *Structural facts* (Sections II–VI, pp. 1033–56). The specimen consisted of a stem of large size and considerable complexity of structure. The vascular system was composed of a protostele, from which large leaf-trace strands ('meristeles') were given off. These large leaf-traces divided up into smaller ones and were finally completely used up in the formation of either unilateral or radially symmetrical concentric foliar traces.

The vascular strands of all orders, with the exception of the ultimate foliar traces, were surrounded by a wide zone of secondary tissue.

Extrafascicular bands of wood and bast formed a network round the stele and large leaf-trace strands; they showed occasional inverse orientation. Accessory vascular bundles also occurred.

A wide, irregular, discontinuous zone of secondary cortex surrounded the greater part of the stem; it lay immediately outside the vascular structures.

The primary xylem consisted of tracheides intermixed with xylem-parenchyma; the protoxylem, which frequently occurred in paired groups, was in all cases exarch.

The primary and secondary tracheides both showed multiseriately arranged bordered pits on the radial walls; the protoxylem consisted of spirally marked elements.

Short, broad, isodiametric tracheides occurred on the inner edge of the xylem of the extrafascicular strands, and occupied the central region of the accessory vascular bundles.

The phloem of the leaf-traces contained a ring of large elements, probably of the nature of sieve-tubes. The secondary phloem in all cases consisted of strands of narrow, apparently thick-walled elements, embedded in parenchyma.

The 'pericycle' and cortex have abundant secretory elements varying very much in size; in some cases the contents are preserved in the form of black carbonaceous matter, others present a vacuolated appearance.

The concentric foliar bundles were partially surrounded by strands of fibrous elements.

2. *Attribution* (Section VII, pp. 1056–8). The stem is included in the genus *Sutcliffia* on account of the general structure of the vascular

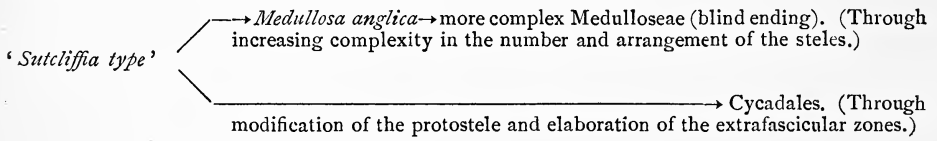
[1] Compare with the tentative statement in Studies, p. 650.

system, the origin and behaviour of the 'meristeles', the leaf-trace bundles, and the close agreement in histological details.

It is provisionally attributed to *S. insignis* because the features which characterize the stem (i. e. the secondary tissues, the extrafascicular arcs, the secondary cortex, and the absence of leaf-bases) are considered to be most probably due to age of the plant.

3. *Theoretical conclusions* (Section VIII, pp. 1058–63). It is suggested that the origin of the Cycadaceae is to be sought along the Medullosean line from some such genus as the protostelic *Sutcliffia*, the central cylinder of the Cycads being probably derived from the protostele by the gradual disappearance of the primary internal tracheides. The extrafascicular arcs and accessory vascular strands are regarded as homologous in *Sutcliffia*, *Medullosa*, and the Cycads; they are not considered to be derived by reduction from a system of steles, but to have arisen independently of the central cylinder, possibly in response to increased physiological requirements.

It is suggested that two divergent lines may have arisen from some such primitive type as *Sutcliffia*, as follows:



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——————: see Farmer and Hill.

—————— and de Fraine, E.: On the Seedling Structure of Gymnosperms. III. Ann. of Bot., vol. xxiii, 1909.

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EXPLANATION OF PLATES XCI AND XCII.

Illustrating Miss de Fraine's paper on *Sutcliffia.*

The photographs were taken by Mr. F. Pittock, of the Zoological Department, University College, London.

PLATE XCI.

Fig. 1. Transverse section of the stele and some of the surrounding tissues, from Section V. M = stele; α = meristele still attached to M; α^1 = meristele just detached from α; γ, β^1, and β^2 = meristeles; *e* = extrafascicular strands. × $2\frac{2}{5}$.

Fig. 2. Longitudinal section from the primary wood of stele, to show xylem-parenchyma, *par.* *p.xy.* = primary tracheides. From Section 66. × 55.

Fig. 3. Longitudinal section through part of the wood, from Section 76. *p.xy.* = spiral elements of protoxylem; *xy.* 1 = primary xylem; *xy.* 2 = secondary xylem. × 55.

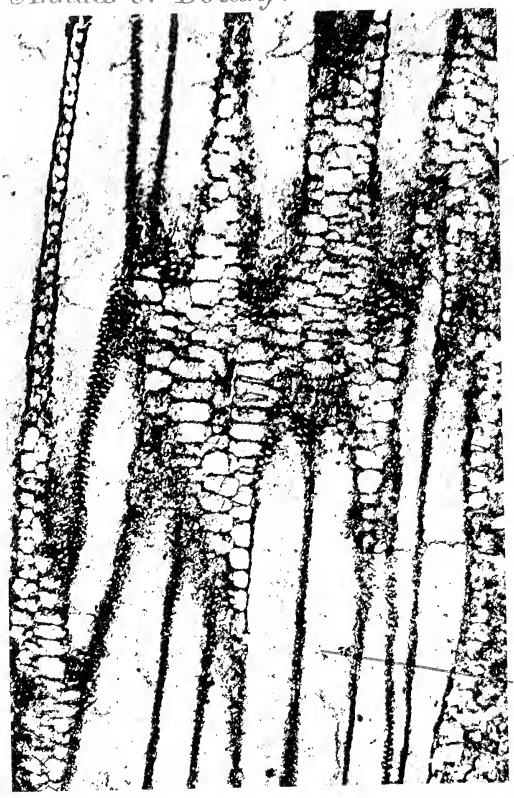
Fig. 4. Longitudinal section through part of the secondary tissues, showing the long, tapering elements of the secondary phloem, *s.ph.* xy^2 = secondary xylem; *m.r.* = medullary ray. From Section 71. × 55.

Fig. 5. Longitudinal section through cortical region, showing strand of isodiametric tracheides, *i.t.*, and parts of ordinary secondary tracheides, *o.t.* From Section 59. × 220.

PLATE XCII.

Fig. 6. Transverse section of accessory vascular strand, cut somewhat obliquely, from Section 40. *s.ph.* = secondary phloem; *xy.* 2 = secondary wood; *i.t.* = isodiametric tracheides. × 55.

Fig. 7. Part of central portion of Fig. 6, enlarged to show the isodiametric tracheides, *i.t.* × 220.



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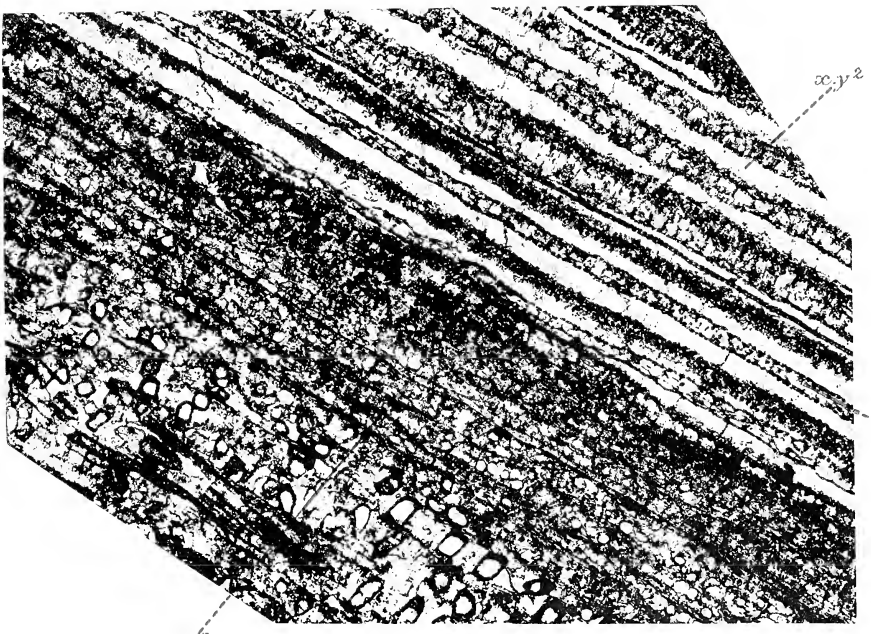
DE FRAINE—SUTCLIFFIA.



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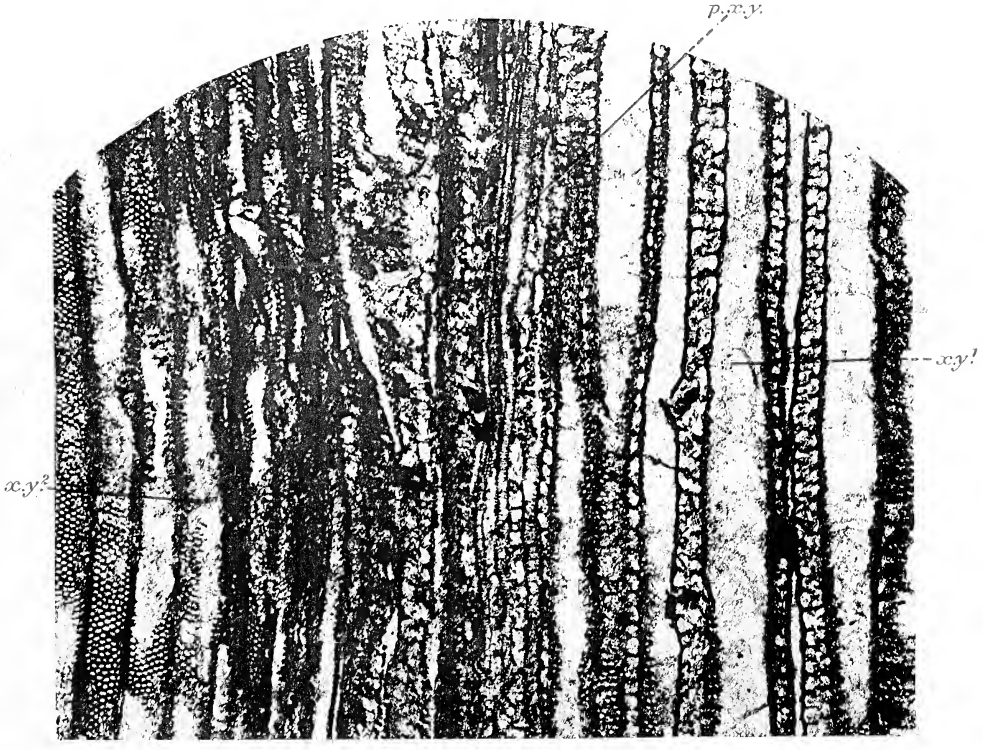

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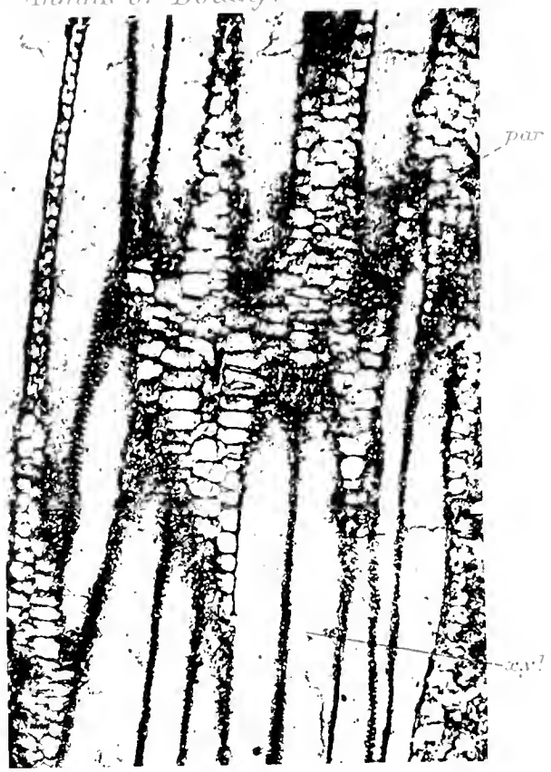

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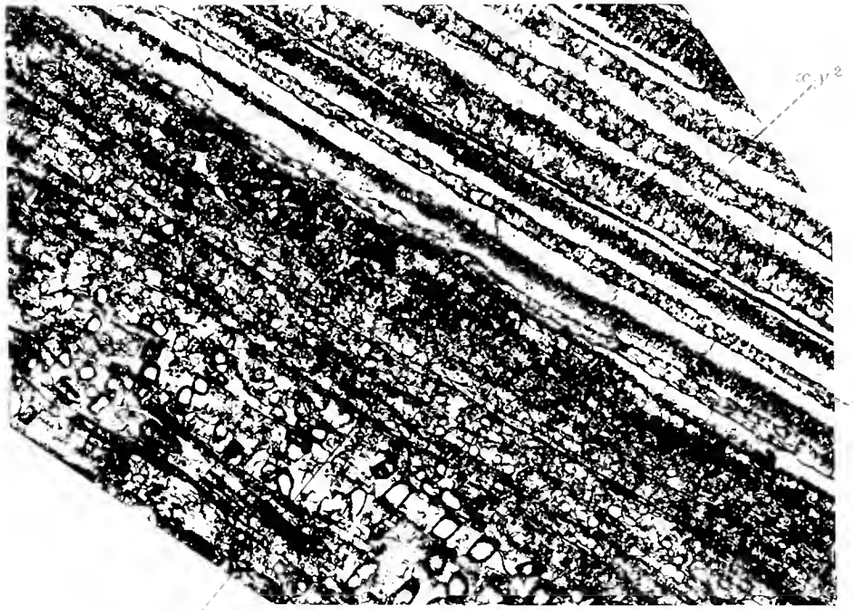

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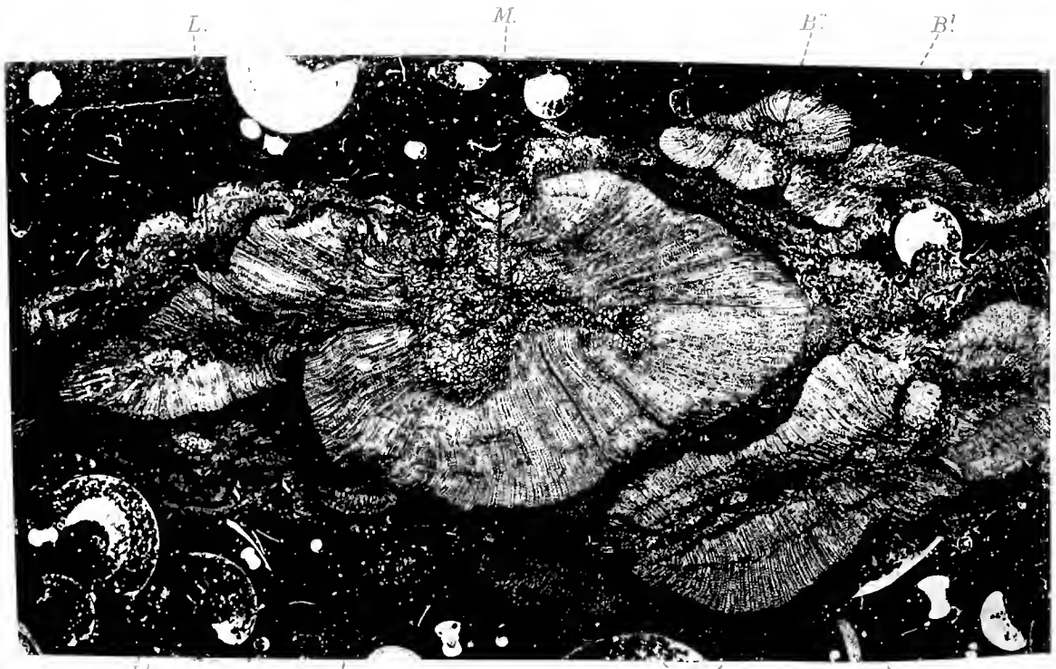

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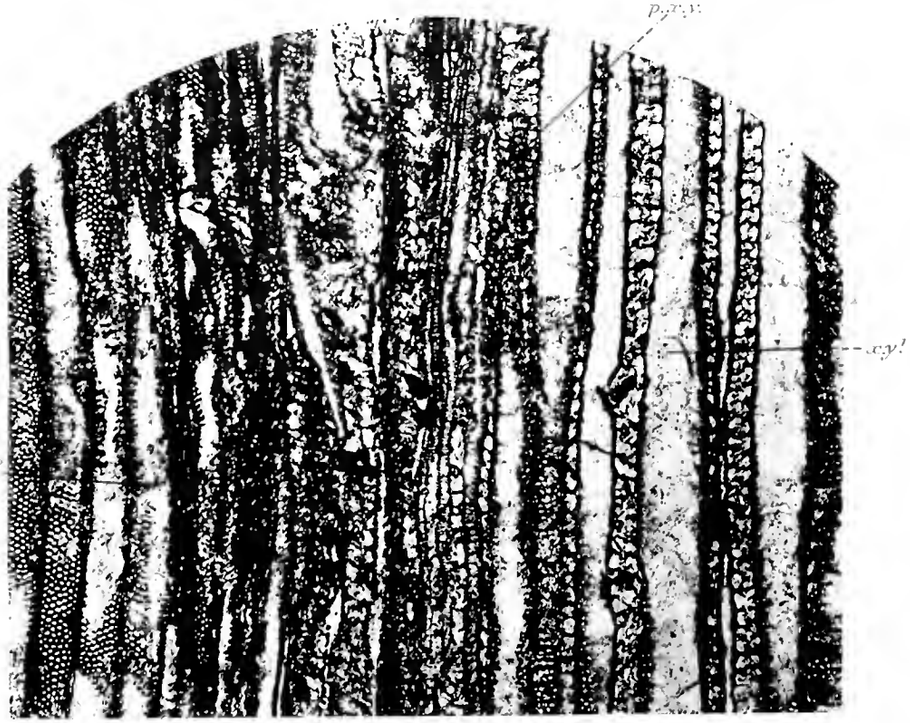

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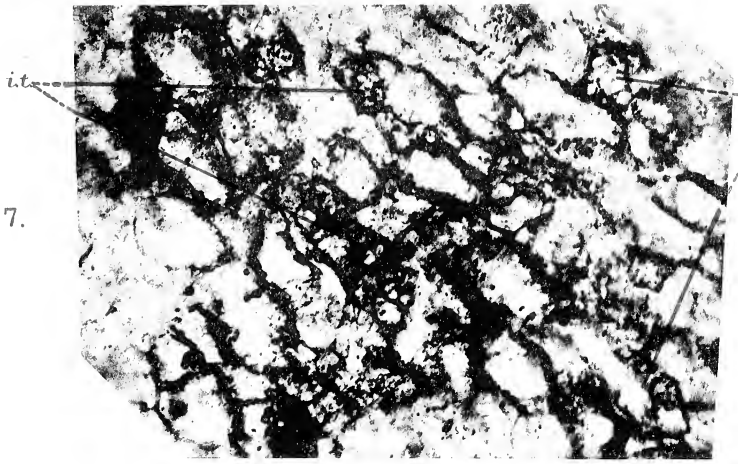
DE FRAINE—SUTCLIFFIA.



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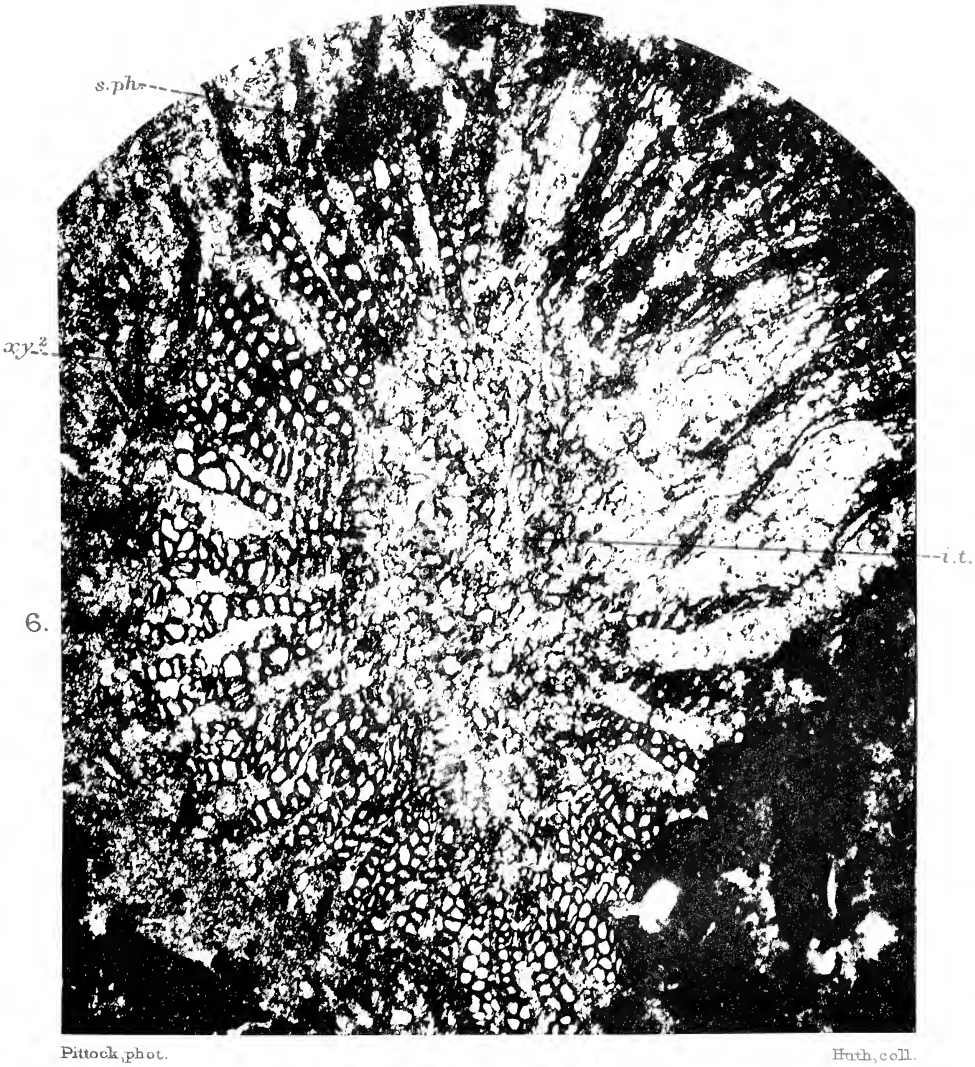

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Pittock, phot. Huth, coll.

DE FRAINE—SUTCLIFFIA.

The Structure and Development of the Haustorium of Striga lutea.[1]

BY

EDITH L. STEPHENS, B.A., F.L.S.

With Plate XCIII.

STRIGA LUTEA, Lour.[2] (the Witchweed or Rooibloem—'Red Flower') is a semi-parasitic annual belonging to the Order Scrophulariaceae. It occurs in scattered localities throughout Zululand, Natal, and the Transvaal, on various native grasses and also on *Zea Mays*, which is an important South African food-crop. Its life-history is now being studied by Professor Pearson, to whom I am indebted for the supply of material and for much helpful information as to its life-history and mode of growth. Most of the material investigated was collected by him on the maize crops in the Transvaal; the remainder was obtained from cultures of *Striga* on the maize reared in this laboratory.

The Plant.

Striga lutea is a root parasite which passes a portion of its life-history underground in the form of a slender white shoot bearing scales, leaves, and numerous adventitious roots. Later, the shoot grows above ground into a slender green-branched stem, 2 to 18 inches high, bearing decussating pairs of linear leaves and spikes of bright red flowers.[3] The adventitious roots arising from the underground portion of the shoot branch freely and come into contact with the root-system of the maize plant on which the seedling parasite has established itself. The roots of host and parasite form a network in which those of the *Striga* plant are readily distinguished by the absence of root-hairs, by their transparency and paler colour (the endodermis of the maize showing through the cortex and giving the root a yellowish tinge), and often also by the presence of small patches of a reddish secretion (giving reactions for mucilage) on their surface. At points where roots of the two come in contact or approach one another

[1] This investigation was assisted by a grant from the Union Government.

[2] Fide Flora Capensis, vol. iv, sect. 2, p. 445.

[3] See figure of plant, Burtt Davy ('04), Plate LXXIV A.

closely, haustoria grow out from the *Striga* root and penetrate that of the maize. In Fig. 1 is shown (in optical section) a root of *Striga* which has run parallel to a maize root for a short distance, and has developed a row of haustoria penetrating the maize. Fuller figures[1] part of a shoot of *Striga* showing the connexion between its underground root-system and that of the maize, but his figure makes the *Striga* shoot apparently end in a tap-root, an error which is corrected in Burtt Davy's copy[2] of Fuller's plate. Fuller also gives[3] several diagrammatic sketches of sections of the haustoria, but no details of their structure or development have yet been published. It is the object of the present investigation to supply these details.

The Haustorium.

(*a*) General Structure.

Compared with the haustoria of other phanerogamic parasites, that of *Striga* is comparatively simple in structure. Typically, it is almost globular in outline (Pl. XCIII, Fig. 1), and consists mainly of a mass of transparent nucleated cells which, following the usage of other writers, I will refer to as the 'nucleus'[4] (*n.*, Figs. 1, 5, 6). This is surrounded by a cortex of varying thicknesses (*c.*, Figs. 1, 5, 6). Down the centre of the 'nucleus' runs a strand of tracheides, linking the vascular system of the parasite to that of the host (*a. s. t.*, Figs. 1, 5, 6). This strand can be most clearly seen in Fig. 5, which shows an *optical* longitudinal section of a young haustorium which has penetrated into a maize root as far as the endodermis. This haustorium still retains the lenticular shape characteristic of its earlier stages, being at the most only six cells deep back to front. Its transparency thus enables its structure to be studied in optical section, though the outlines of the cells of the 'nucleus' at the median focus of the figure are obscured by those lying above, so that only a few were seen with sufficient clearness to be drawn in. They can be better seen in Fig. 6, which shows an older haustorium (which has attained its full size and globular outline) cut in a plane transverse to both mother root and haustorium. This section is slightly oblique to the axis of the haustorium, so that the connexion of the axial strand of tracheides with the vessels of the mother root is not seen.

(*b*) Origin and Early Development.

The chief interest attaching to these haustoria lies in the fact that the very delicate and transparent nature of the parent root and young haustorium enables the origin and development of the latter to be studied

[1] Fuller ('01), Pl. V. [2] Burtt Davy ('04), Pl. LXXIV A. [3] Fuller, loc. cit.

[4] Barber ('06), Benson ('10). Fraysse ('06) refers to this tissue as 'noyau méristématique central' or 'masse méristématique centrale'. Other writers give it no special name.

quite clearly in optical section up to the stage of Fig. 5. This is of interest as throwing light on the much-debated point as to whether the haustoria of root-parasites in general are to be regarded as much-modified lateral roots, or as special exogenous outgrowths. In the case of *Striga* the haustoria show no homologies with lateral roots, being markedly exogenous in their origin, which is from the cells of the subhypodermal or (more rarely) the hypodermal layer. It cannot of course be said with certainty that every haustorium has originated from one of these layers, but even in the older stages it is clear that it has taken its rise in one of the layers between epidermis and endodermis. The haustoria may arise on roots of any age—e. g. the young haustoria of Figs. 3 and 4 are growing from roots decidedly older than in the case of the more mature haustorium of Fig. 5. As to the cause of their originating at any particular point, it may perhaps be of significance that the early stages of development have only been seen on roots which were in close proximity to young roots of maize still covered with root-hairs, and that the length of the haustorium before it penetrates the host (e. g. such a haustorium as that of Fig. 5) is about that of a maize root-hair. These facts lead me to put forward the purely tentative suggestion that stimulus due to contact with a hair of the maize root may be the determining cause of their development.

Superficially, the haustorium first appears as a slight elevation on the surface of the root, elongated parallel to its axis. In a side view (in optical section) this elevation is seen to be due to the elongation of several (3–5) cells of the subhypodermal or hypodermal layer (Fig. 2). These cells are situated in the same row, parallel to the axis of the root, and the developing haustorium is therefore elongated in that direction. They begin to divide by transverse and longitudinal walls, pushing up the overlying layer or layers still further to form a protuberance which is cone-like in section (Fig. 4) but elliptical in surface view. Thus the young haustorium of Fig. 3 is only 3–4 cells deep back to front (i. e. above the level of the mother-root), and that of the later stage shown in Fig. 4 is only 4 cells deep. This later stage is formed from that of Fig. 3 (if this has not by now come in contact with a maize root) by its growing out still further into a blunt finger-like process (Fig. 4), which may be twice as long as the root from which it develops is broad. It never seems to develop further than this without contact with the root of the host. Only one case of self-attachment was seen ; in this the haustorium was still of the size and general shape of Fig. 4, and had penetrated to the depth of about three cells into the cortex of the *Striga* root, with which its cells then gradually merged. None of the developments which are seen when the haustorium touches a foreign root had taken place.

The cells of the developing haustorium have the appearance of ordinary cortical cells, and it never assumes the small-celled and meristematic

appearance characteristic of the developing root, which has here an ordinary endogenous development. Numbers of young lateral roots and haustoria have been seen arising side by side on the mother root, and it is clear that there is no resemblance between them in origin, development, or structure.

(*c*) Connexion with Host.

Penetration of the soft cortical tissues of the maize root is rapidly effected when once the haustorium comes into contact with it. Stages in which the tip of the haustorium has not already penetrated to the endodermis are rarely found. When the epidermal cells of the haustorium touch the host root they begin to elongate, forming papillae of a finger-like form and arranged in palisade rows, which bore their way between and through the cortical cells of the host. Their passage is effected by a ferment they secrete, which swells and ultimately dissolves the cell-walls which it touches. The margin of the advancing haustorium is marked by an irregular clear bright yellow or yellow-brown line, presumably this ferment plus the dissolved tissues of the host, and the same yellow line is often seen round individual papillae which have grown beyond the level of the rest of the haustorium. This line is represented (marked *f.*) in Figs. 5–8. It stains with safranin, but not with haematoxylin ; microchemical tests to determine its nature have yielded no results. The walls of the cells of the host to a distance of several cells from the haustorium may become swollen with the action of the ferment, when they also take on this yellow colour and stain in a similar way (cf. the walls of the endodermal and cortical cells bordering the line of ferment in Fig. 7). The pressure due to growth assists in the passage of the haustorium through the tissues of the host, but to a much less degree, as is shown by the fact that very little collapsed tissue is seen in the path of its advance. Growth pressure is in evidence when individual papillae have to penetrate a hard-walled vessel, as in Fig. 7, where a papilla has evidently had considerable difficulty in penetrating into the central vessel of the maize stele. The wall of the latter has been pushed in, and the length of time that the papilla has taken to effect an entrance can be seen by the spiral thickening formed nearly down to its tip.

A series of longitudinal sections through a haustorium soon after penetration (or an optical section such as Fig. 5) shows that the central papillae have reached the endodermis, and have flattened their ends against it to form several regular palisade layers. With regard to Fig. 5, it must be remembered that it is a *median* optical section ; focusing slightly above or below the level of the section, the papillae in front of and behind the layer figured as abutting on the endodermis can be seen as it were crawling over its surface trying to effect an entrance. As soon as the ferment touches the endodermis, the contents of the cells of the latter disappear, and their outer walls swell (Fig. 7). In Fig. 8 is seen an endodermal cell in longitu-

dinal section, with several papillae touching its outer wall. This has become so swollen and dissolved by the ferment they have secreted that only its inner line is distinguishable; and the inner wall has also begun to swell. The walls of the endodermal cells in the maize are thicker than those of the cortical cells, the inner considerably so, and the haustorium may enlarge to almost its mature dimensions while the papillae are trying to penetrate them.

Meanwhile, the strand of elongated cells forming the axis of the young haustorium has become converted into the line of tracheides seen in Fig. 5, running from the vessels of the parasite down through the centre of the haustorium to the stele of the host. Sometimes this line of tracheides begins to form almost immediately after the haustorium has begun to penetrate the tissues of the host; sometimes it is only laid down after some amount of cell-division has taken place in the haustorium. The latter seems to be the case when parasite and host lie close together, so that a comparatively short haustorium is formed; the former when the haustorium has had to grow out to some length, and thus is further from the water supply of its mother root. In any case, this line of tracheides is formed in the same way, and apparently always before the haustorium has penetrated through the endodermis, which it takes some time to do. Spiral markings appear in the cells of the two or three layers which lie between the vessels of the mother root and the base of the line of elongated cells forming the axis of the haustorium. The spirals run in the same direction as those of the vessels, and the tracheides thus formed make a little hump on the vascular strand, which links it with these axial cells. (These connecting tracheides can be seen at the junction of the vascular system of the mother root with the axial strand of tracheides in Fig. 5. The two lines of tracheides which appear in the figure to end blindly in the nucellus close to the vascular strand are seen at a slightly higher focus to link on to it by similar connecting tracheides.) In the lowest of these axial cells, i.e. the one contiguous with the connecting tracheides, spiral thickening now appears, and each successive cell in the same longitudinal row becomes converted into a spirally thickened tracheide. Later on, other tracheides are added to this strand by the appearance of spiral markings in the papillae that are striving to pierce the endodermis of the host, and in the cells running up behind them (cf. Fig. 8). A few tracheides have been added in this way to the axial strand of Fig. 5. Comparing it with that of Fig. 6, it will be seen that as these additional tracheides follow the line of the maize stele, the central strand is lenticular in shape, although the haustorium when mature is almost globular. Occasionally in the case of a large haustorium a branch of the axial strand may be formed later on in the 'nucleus' (cf. the left-hand haustorium in Fig. 1).

While this axial strand has been forming, active division has been

taking place in the cells surrounding it. These divide up to form a mass of small transparent nucleated cells—the 'nucleus'. It is interesting to note that this appears to be formed entirely by the renewed division of cells derived from the original subhypodermal layer of the mother root. The two outer layers of the haustorium (derived from the two outermost layers of the root) merely divide to form a cortex several layers in thickness (*c.*, Figs. 1, 5, 6; in Fig. 5 the cortex is still only two layers thick in the lower portion of the haustorium). In the younger stages, as Fig. 5, this cortex is still clearly marked off from the small meristematic cells of the 'nucleus', but later on the outer cells of the latter enlarge, lose their slightly rounded outlines, and pass over into ordinary cortical cells (cf. Fig. 6). Around the axial strand, however, they seem to remain active until the death of the haustorium, and perhaps play some part in the transmission of elaborated food-stuffs from host to parasite.

By the time the 'nucleus' is well formed, a line or group of papillae (those which were the first to penetrate the host root) have bored their way through or between the endodermal cells, and are now tapping the vessels of the stele. They penetrate to various depths; in Figs. 6 and 7 one is just entering the large central vessel. Their ends flatten against the vessels as they did against the endodermal cells, and the ferment they secrete dissolves the wall of the vessel. Sometimes the papilla then grows on and penetrates through the opposite wall into the next vessel (cf. Fig. 7), sometimes instead its tip becomes dissolved together with the wall, so that it forms a side pipe tapping the vessel. One vessel may be tapped in this way by a longitudinal row of papillae. Spiral thickenings run up the papillae to their tips, and the tracheides thus formed constitute an efficient conducting system between the xylem of the host and that of the parasite. The further development of the haustorium is the result of the continued division of the cells of the 'nucleus', which causes the haustorium to grow down on either side of the host root till it may come to resemble an india-rubber ball with a stick (the maize root) pressing it in along one side. Several cases have been seen in which the lips of the haustorium had closed round the maize root and destroyed its cortex till its stele appeared to be almost embedded in the haustorium.

Examination of a large range of material has shown that the course of development outlined above is followed in practically every case. The very few exceptions that have been seen will be discussed later in connexion with the comparison of the *Striga* haustorium with that of other plants. The development of haustoria on the underground shoot may be mentioned here; this is occasionally seen when a maize root comes in contact with the shoot. No early stages of development of these haustoria have been found, but sections through several mature haustoria have shown them to be of the usual type, except that they are almost embedded in the

cortex of the stem, and that the tracheides, lacking the definite point of development afforded by the vascular system of the root, run in scattered lines through the 'nucleus' instead of in a central strand. The 'nucleus' seems to grow backwards through the stem, and the lines of tracheides run back with it till they meet and link on to the vessels of a leaf-trace. If they do not meet a leaf-trace, they simply end blindly in the 'nucleus'.

(*d*) Mechanism of Nutrition.

The whole question of the nutrition of this parasite, especially during the underground portion of its life-history, is obscure. While the water-carrying vessels of host and parasite are connected so as to allow of a free passage of water and salts, there is no such conducting system for assimilated food. No sieve-tubes are formed in the haustorium, none have been found in the mother roots, and there is apparently no connexion with the phloem of the host. It is of course possible that the parasite may absorb food by osmosis from the sieve-tubes of the host where its thin-walled cells come in contact with these, as is suggested by Peirce[1] for *Arceuthobium occidentale*. But as the haustoria fasten only upon the younger roots of the maize, in which the sieve-tubes are not easily distinguished or have not yet been formed, I have not been able to ascertain whether the conditions for such a transference are found here. Until the subaerial shoot is formed, the plant thus apparently has to depend for its supply of assimilated food on the reserve stores passed over from the phloem to the xylem of the host, and on what the haustoria may absorb from the cells dissolved by its ferment. Many microchemical tests have been made to find out what food-stuffs are transferred from host to parasite, but without result. There is no storage, even temporary, of food such as has been recorded for the haustoria of other parasites[2]—a fact which may be connected with the relatively short life of the haustorium in this case. It is not known just how long an individual haustorium may live, but the fact that they are never seen on the older roots of the full-grown maize shows that their life is not a long one.

Discussion.

The fact that the haustorium of *Striga* may be regarded as comparatively simple in structure has already been noted. No glands are formed to assist in penetration;[3] there are no arrangements of strengthening tissue;[4] and no 'collapsed layers' are seen, such as are generally found in haustoria which have to penetrate the woody cylinder of dicotyledonous roots. All these facts may be correlated with the comparative ease with which the haustorium

[1] Peirce ('05), p. 109. [2] e. g. Barber ('06), Fraysse ('06), Benson ('10), &c.
[3] As formed in, e. g., *Santalum album* (Barber, '06).
[4] As seen in, e. g., *Osyris alba* (Fraysse, '06) and *Krameria canescens* (Cannon, '10).

can make its way through the cortex of the maize, and, once the endodermis is passed, into the comparatively slightly lignified vessels of the young root. Here it is interesting to note that several haustoria were found trying to penetrate an unknown dicotyledonous root which possessed a layer of cork about six cells thick round the stele. The haustoria had entered the root as far as the cork, and were striving to penetrate it with the secretion of much ferment, but they had not got half-way through. Though they had grown to the size of the haustorium of Fig. 6, the axial strand of tracheides had not yet been formed, confirming the opinion stated above that this is only formed after the haustorium has penetrated to the stele of the host.[1] In this case, a line of 'crushed tissue' had been formed down the centre of the 'nucleus'.

The chief interest of this investigation lies in the exactitude with which the origin and development of the haustoria can be followed up through the transparent tissues of the mother root, and in the light which is thus thrown on the disputed question of whether haustoria are to be regarded as organs *sui generis* or as much modified lateral roots, a point on which investigators have differed even in regard to the same species.[2] My first-hand knowledge of the literature of parasitism is not sufficient to enable me to add to the many discussions [3] on this point, beyond recording the fact that in *Striga* all the lateral haustoria are clearly exogenous in origin, and show no homologies with the lateral roots. Very rarely a case is found in which the tip of a *Striga* root has apparently met a maize root and formed a terminal haustorium. I use the word 'apparently' because in every such case but two a new growing point had been formed near the apex of the haustorium (as is described for like cases in *Santalum*,[4] *Exocarpus*,[5] and *Krameria*[6]). When this new root had grown on, the haustorium would present the appearance of an ordinary lateral one; but it seemed clear in the half-dozen cases seen that the haustorium was originally terminal. It might of course still have had an exogenous origin from one of the layers of the very simple root-tip, but the appearance of the haustorium would suggest that the cells of the root-tip simply grew out as papillae into the host, the vessels extended down to form the central strand of the haustorium, and the inner cortical cells divided up to form the 'nucleus'. Unfortunately, no young stages in the development of such a haustorium have been seen. It may be noted that these haustoria were all formed at the ends of fairly long roots; it might have been expected that in the case of two roots running parallel, as in Fig. 1, penetration by the lateral roots of the haustorium might occur, but no such case was found.

[1] Fraysse ('06) remarks of the haustoria of *Osyris alba* (p. 28): 'Dans aucun cas, l'appareil vasculaire n'apparaît complètement formé avant l'installation définitive du parasite. Dès que la pénétration est suffisante, les trachées se différencient et s'insinuent jusqu'aux divers vaisseaux . . . '

[2] Fraysse ('06), p. 23. [3] Peirce ('93 and '05), Goebel ('05), Fraysse ('06), &c.

[4] Barber ('06). [5] Benson ('10). [6] Cannon ('10).

The fact that the haustoria here are clearly exogenous, and apparently organs *sui generis*, does not of course necessarily mean that in other parasitic plants they are never formed by the modification of root primordia, but it leads one to wish for more exact and detailed descriptions of their origin and development than are generally given, before coming to a conclusion on the point as regards any one plant.

Summary.

1. *Striga lutea* is a semi-parasitic South African annual which grows as a root-parasite on native grasses and on the maize. It consists of a slender shoot, the underground portion of which bears many adventitious roots on which haustoria arise.

2. These haustoria are markedly exogenous in origin, being formed by the division of cells of the subhypodermal or hypodermal layer of the root, which push up the overlaying layers to form a finger-like protuberance.

3. When this meets the root of a maize plant, its epidermal cells grow out as papillae and bore their way into the host, by secreting a ferment which dissolves its tissues.

4. Down the centre of the haustorium a line of tracheides is now formed, linking the vessels of root and parasite, and by division of the cells surrounding this axial strand a 'nucleus' of parenchymatous cells is formed, which gives the haustorium a globular outline.

5. The mode of nutrition of the parasite is obscure. There is free passage of water and salts from the host, but although the parasite passes a portion of its life-history underground as a total parasite, no system is formed for the conduction of assimilated food.

6. The haustoria of this plant are exogenous in development, and are probably to be regarded as organs *sui generis*.

Botanical Laboratory,
South African College,
April 24, 1912.

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EXPLANATION OF PLATE XCIII.

Illustrating Miss Stephens's paper on the Haustorium of *Striga lutea*.

c. = cortex; *n.* = 'nucleus'; *a.s.t.* = axial strand of tracheides; *f.* = line of ferment secreted by the haustorium, with the disorganized tissues of the host dissolved in it.

Fig. 1. Optical median longitudinal section of root of *S. lutea* running alongside root of maize. Four haustoria have penetrated the maize root. × 23.

Fig. 2. Optical median longitudinal section of cortex of a root of *S. lutea*, showing three cells of the hypodermal layer elongating; these will give rise to a haustorium. × 140.

Fig. 3. Optical median longitudinal section of portion of root of *S. lutea*, showing a young haustorium originating from cells of the subhypodermal layer. × 140.

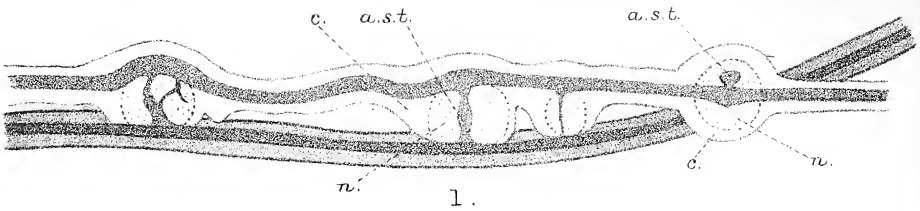
Fig. 4. Optical median longitudinal section of root of *S. lutea*, showing a later stage in the development of the haustorium. × 140.

Fig. 5. Optical median longitudinal section of a haustorium of *S. lutea* which has penetrated as far as the endodermis of a root of maize. Only such of the cells of cortex and nucleus are drawn in, as could be seen with distinctness at the focus of the section. × 140.

Fig. 6. Transverse section of a haustorium of *S. lutea* which has penetrated into the stele of a root of the maize. × 280.

Fig. 7. Portion of Fig. 6 enlarged, showing penetration of papillae of haustorium into vessels of the maize stele. × 760.

Fig. 8. Longitudinal section of a single cell of the endodermis of the maize, showing papillae of the haustorium secreting ferment which has swollen and dissolved the inner wall of the cell till only its inner limit is perceptible. The outer wall has also begun to swell. × 1100.



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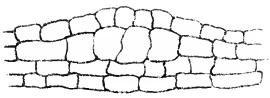

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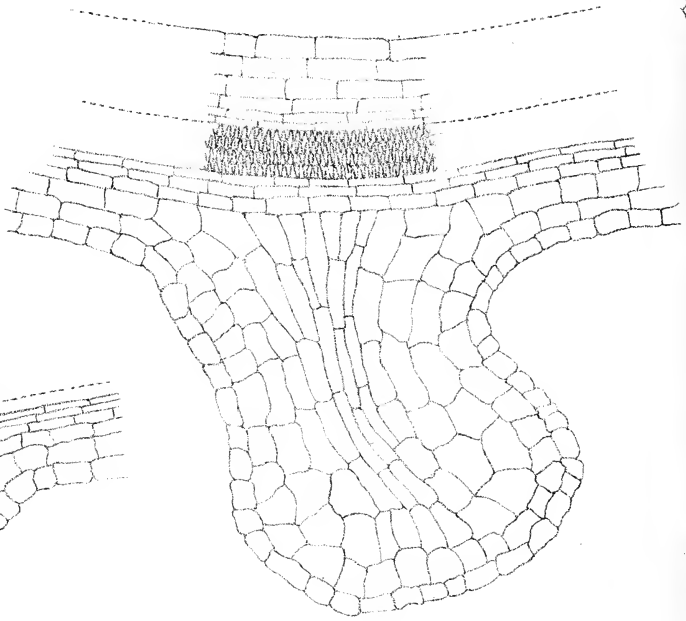

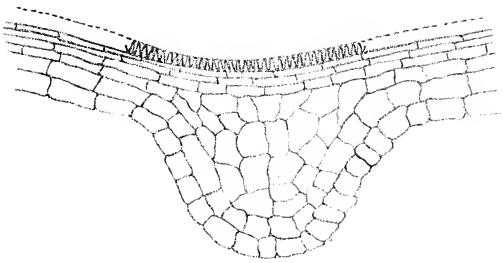

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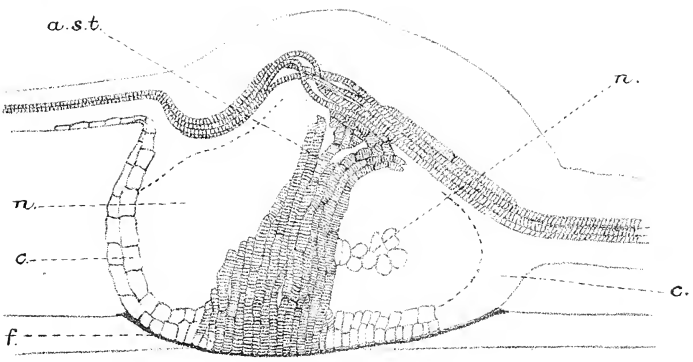

STELE OF MAIZE

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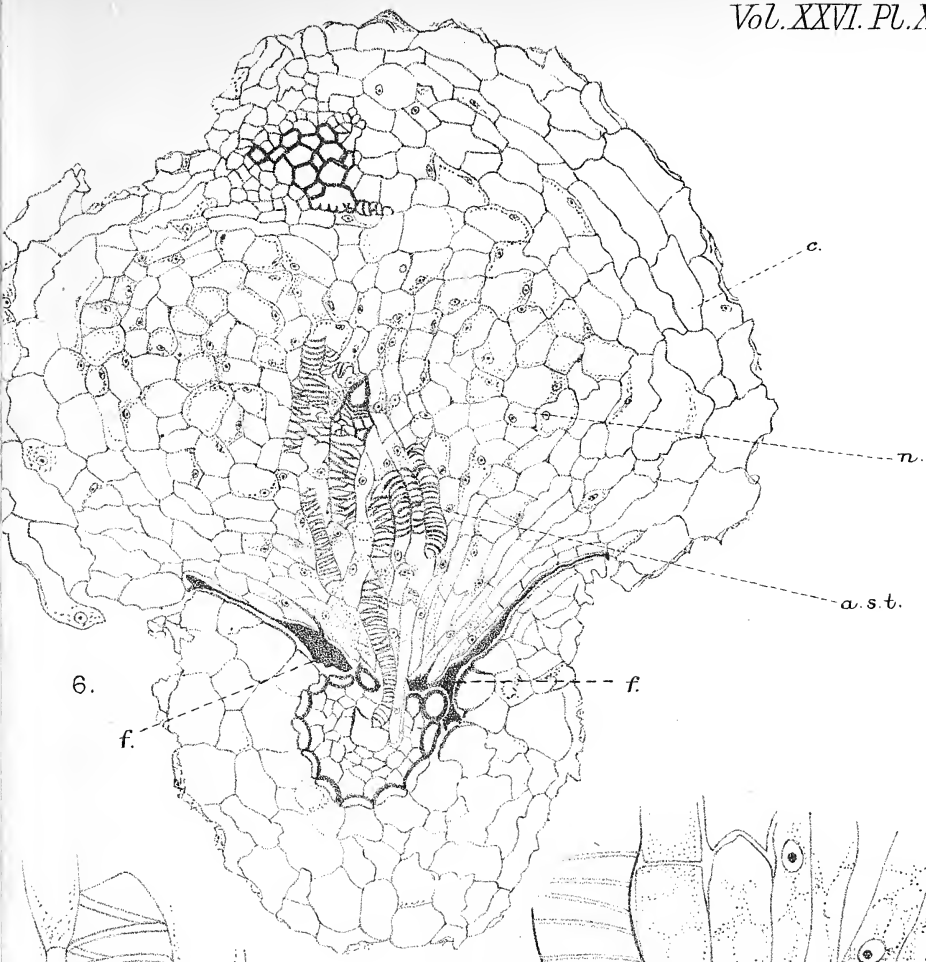

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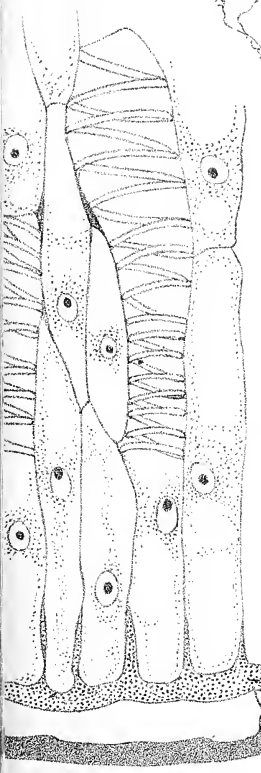

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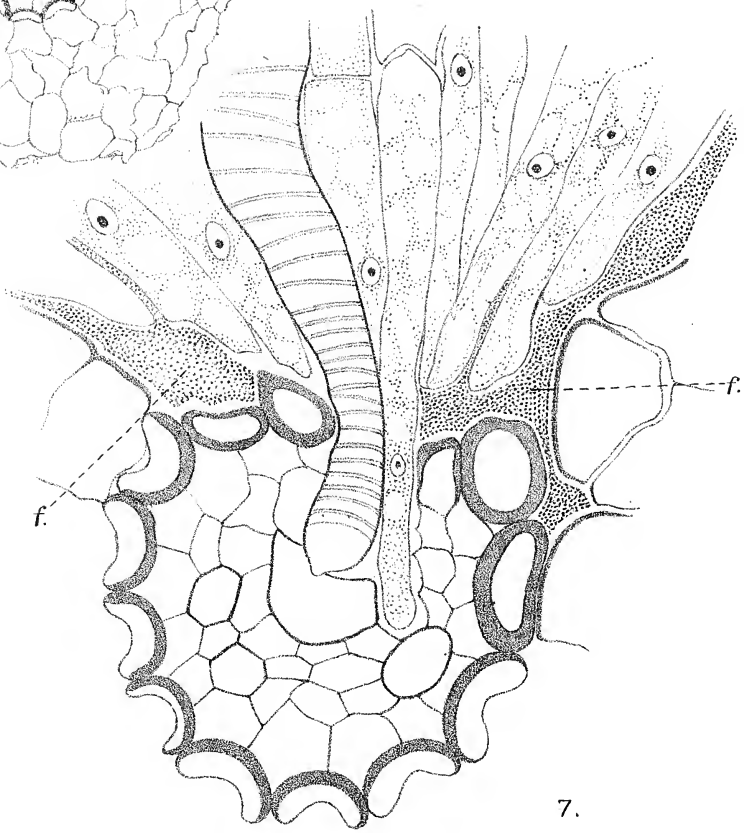

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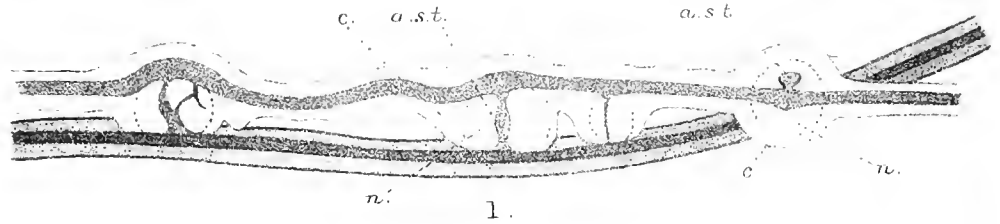

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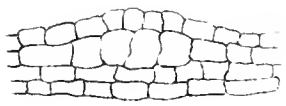

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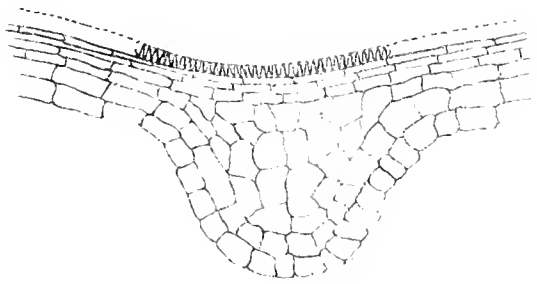

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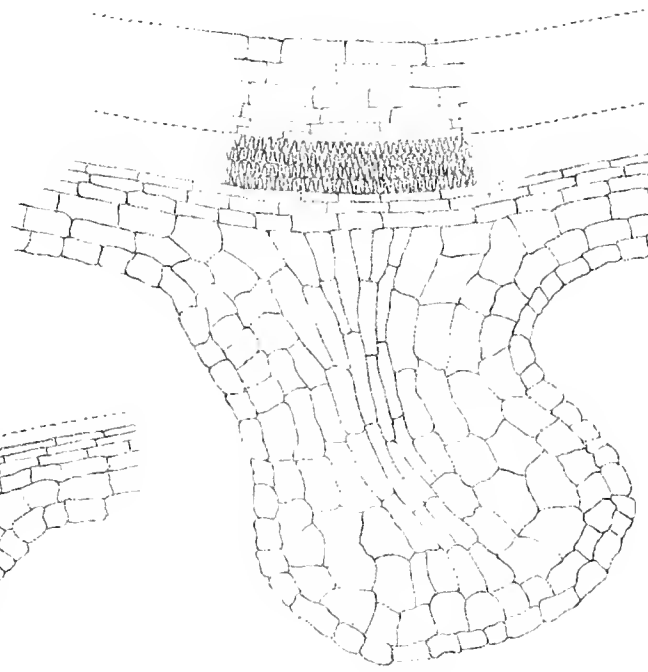

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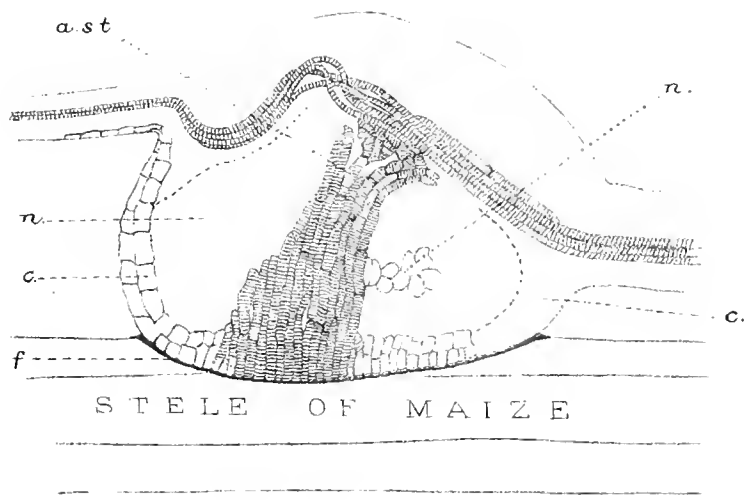
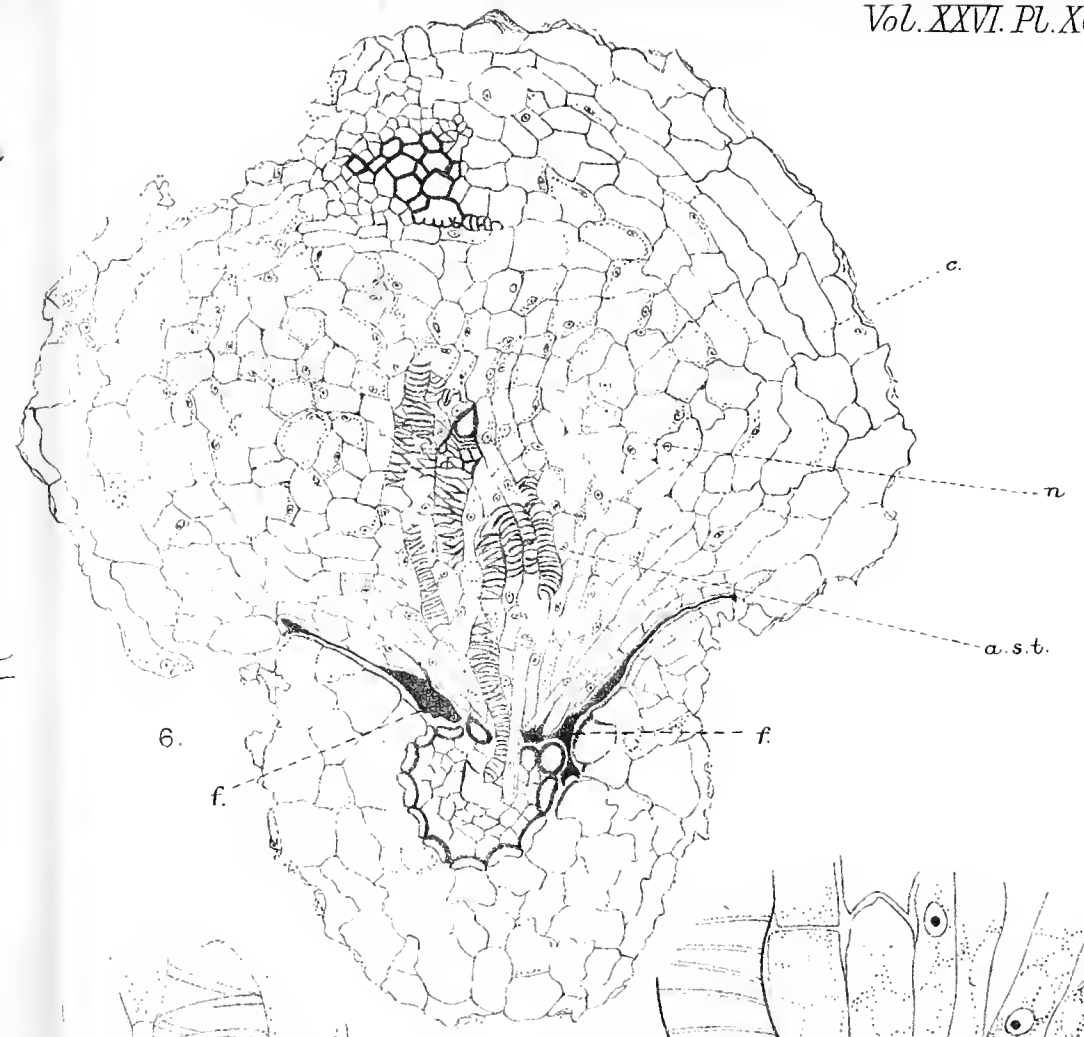


STELE OF MAIZE

5.

STEPHENS—STRIGA.



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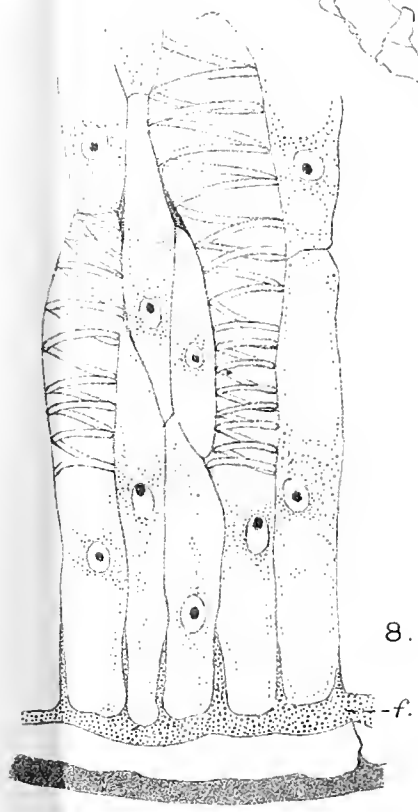

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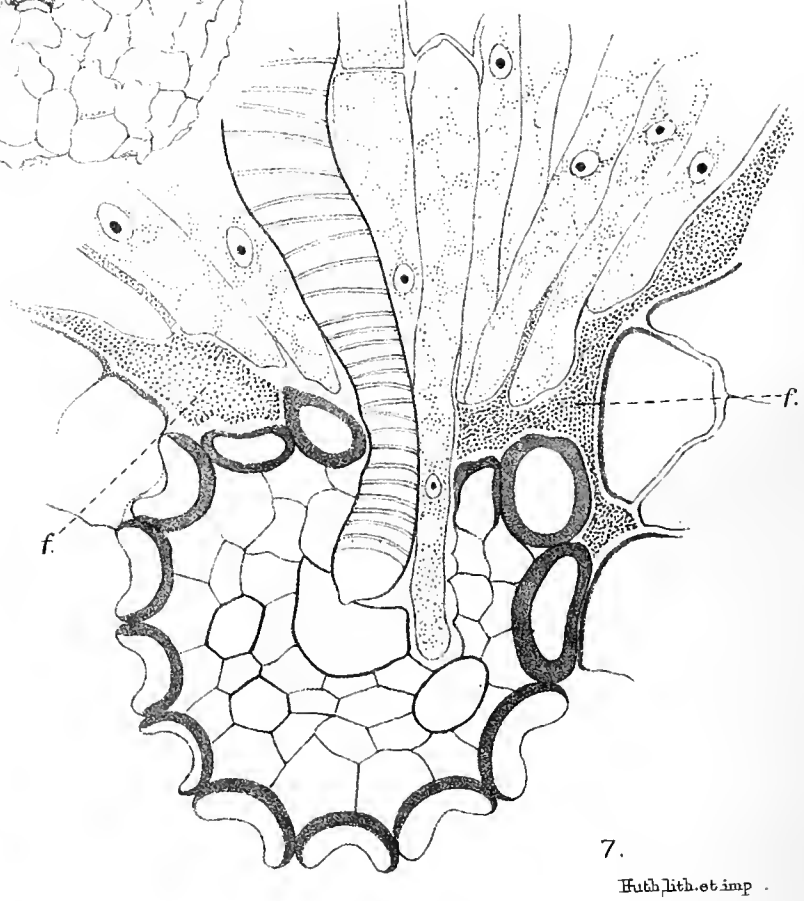

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The Anatomy and Relationships of the Gnetales.

I. The Genus Ephedra.[1]

BY

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With Plates XCIV–XCVII and two Figures in the Text.

THE question of the true affinities of the Gnetales, alluring in itself and yet hitherto so baffling, is being drawn into fresh prominence as morphologists turn to the great question of the origin of the Angiosperms. It is recognized that, aside from its own interest, a settlement of the Gnetalean affinities would mark a strategic advance in the attack on the ancestry of the dominant group of our modern seed-plants. Hitherto the chief attention of those who have added to our knowledge of the Gnetales has been focused on the reproductive structures, although even in this field much still remains to be done. The anatomical features have usually been dismissed with a bare reference to the occurrence of true vessels in the wood. There is accordingly great need of systematic study of the anatomy of the whole group, especially in accordance with the methods which have recently proven so fruitful of phylogenetic results when applied to the Conifers and lower Dicotyledones.

The results of such an anatomical study of the most primitive member of the family, the genus *Ephedra*, are given in the present contribution. It is hoped soon to complete the study of the other genera. In studying *Ephedra* the writer has had material of several species, including *E. altissima*, *californica*, *distachya*, *fragilis*, *Gerardiana*, *monostachya*, *trifurca*, *viridis*, and *vulgaris*. There is little specific variation, so that all the species may be treated together and important differences mentioned under the features concerned.

With regard to the history of the subject, it is contained chiefly in scattered references to individual features, particularly in general texts on anatomy. These will be mentioned as occasion arises.

[1] Contributions from the Phanerogamic Laboratory of Harvard University, No. 51.

[Annals of Botany, Vol. XXVI. No. CIV. October, 1912.]

Pith.

The pith of *Ephedra* is relatively very large, as may be seen in Pl. XCIV, Fig. 1, which is a photograph of a transverse section of an entire young branch. It is composed of dead, fairly thick-walled cells, many of which are filled with a hard brown substance. This substance is either homogeneous or of a foam-like structure (see Fig. 1). Often the cell-walls in certain places disappear, so that the dark masses become continuous, and, when the stem is broken, project as definite strands. This material is lacking at the node of the adult, and in both node and internode of the seedling. In a few species the pith also contains thick-walled fibres resembling those of the bast, which are confined to its periphery.

In certain places, notably in proximity to the nodes, the cells of the pith adjacent to the primary vascular bundles become differentiated. They are smaller and strongly lignified, and appear as definite strands extending inward from the protoxylem cluster. Fig. 2 gives their appearance in *E. trifurca.* It is plain that they might readily be mistaken for centripetal wood. Longitudinal sections, however, reveal their true character as lignified pith cells. It is possible, however, as Worsdell[1] holds for similar cells in *Welwitschia,* that they may represent modified centripetal wood. In *Ephedra* there is little evidence for such a view. The conditions just described emphasize the necessity for exercising the greatest care in identifying centripetal wood.

Another peculiar feature of the pith is the presence of a diaphragm of periderm-like cells a short distance above the node. In Fig. 3 the node is to be recognized by the constriction in the pith and the layer of thick-walled cells. Some distance above the node, the diaphragm may be seen extending completely across the pith and into the wood. The diaphragm is composed of vertical rows of three or more cells apparently formed by secondary divisions. It is sharply differentiated from the rest of the pith by the arrangement, size, and contents of its cells. Very frequently the branch breaks just above the node, and this layer always forms the line of separation. It serves no doubt to protect the surface exposed after the break. But on account of its extension into the wood it would also appear to be a device for cutting off young branches after the manner of an abscis periderm in leaf-fall. It recalls strikingly the similar structure found above the node in *Equisetum* and the Calamites.[2] In these forms, however, it is confined to the centre of the pith, not ordinarily reaching the periphery nor the surrounding wood. In the Calamites there was a diaphragm both above and below the node. Of course these peridermal diaphragms in

[1] Vascular Structure of the Flowers of the Gnetaceae. Annals of Botany, 1901.
[2] Williamson and Scott: Phil. Trans. Roy. Soc., 1894.

Ephedra and the Equisetales can have no evolutionary connexion, but they furnish an interesting case of parallel development in plants of similar habit.

Primary Vascular Bundles and Leaf-Traces.

The bundles of the primary wood, though not large, are rather conspicuous in transverse sections. Fig. 1 gives their appearance in *E. monostachya*. They may be seen to consist of two pairs of large bundles and two groups of three smaller ones. The groups of large and small bundles alternate regularly. The number and arrangement are constant for each species, but vary in the different species. The reasons for the appearance which they present in transverse sections are readily understood when one follows their course by means of a series of sections.

1. Course.

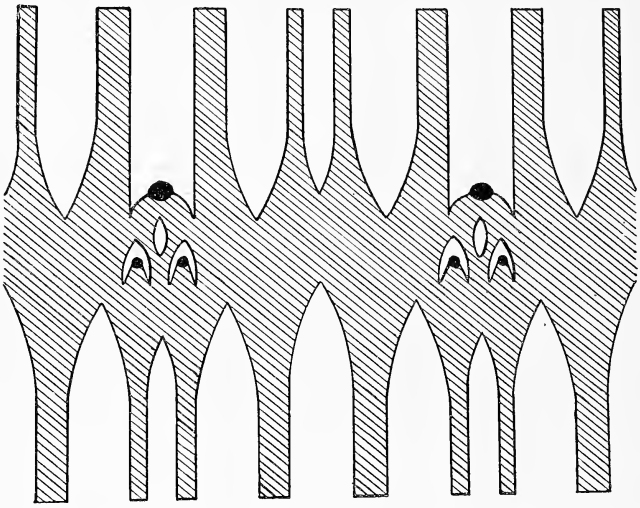
The simplest condition is found in *E. distachya*, and is represented diagrammatically in Text-fig. 1, which is a reconstruction drawn after a study of complete series of celloidin sections through the node.[1] As we shall see, it differs considerably from the conceptions arrived at by former investigators, but owing to the exactness of the method and the number of repetitions the writer feels certain of its accuracy. The ring of bundles is represented as split and turned into one plane. In the internode there are two pairs of large bundles as in *E. monostachya*, but the smaller bundles are also in pairs instead of groups of three. There are therefore two pairs of large bundles alternating with two pairs of small ones. As the node is approached, the smaller bundles flatten tangentially, and short tracheides appear between them. By this means they are soon united, so that a continuous band of primary wood results. The appearance of this band in another species is shown in Phot. 4. The original bundles are near the end of the band. Very soon the adjacent large bundle on each side becomes included in the same way. Meantime a similar band has been formed on the opposite side of the stem. Finally, these two bands become united by the extension of their ends. There results a complete girdle of nodal wood broken only by the exit of the leaf-traces. Often the position of the internodal bundle is indicated by a slight thickening of the girdle.

The leaf-traces, of which there are two at every node to supply the pair of opposite leaves, are in all cases double. The double character is shown in *transverse* section in Figs. 5 and 6, and in tangential section in Fig. 7. In the latter figure the two strands of the trace are below the centre of the field which is occupied by the large branch gap. The transverse sections

[1] The sections as they came from the microtome were arranged in order on a large slide and mounted in glycerine in which they were kept indefinitely for further study. By this means the accuracy of the work is rendered just as great as with the similar paraffin series used for soft material.

also show that the two strands are inserted separately. Each arises directly above one of the smaller internodal bundles, a piece of the girdle at this point simply turning out to form a strand (see Text-fig. 1). Sometimes a thickening of the girdle runs up from the small bundle and out with the trace, indicating that the small bundle as a whole originally formed the trace.

That part of the girdle between the two strands of each trace continues up as a definite bundle, so that in transverse sections the two strands are separated by a block of wood. This feature is shown in Fig. 5. The same bundle is seen in tangential section in Fig. 7. Above the traces it divides, and each part unites laterally with the girdle to form the outgoing branch.



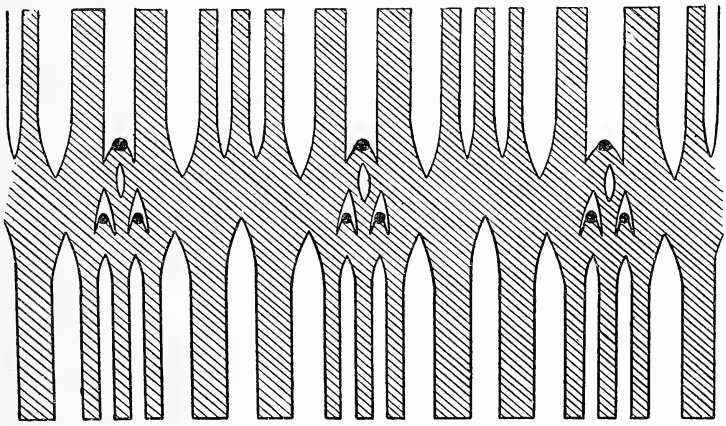
TEXT-FIG. 1. *E. distachya.* Reconstruction of the course of the primary fibro-vascular bundles.

The bundles of the succeeding internode are organized from the nodal wood in a definite manner and position with respect to those of the lower internode. On each side of both branch gaps a large bundle is organized. The two pairs of large bundles of each internode are thus accounted for. Between the pairs of large bundles two small ones are then formed, and the two pairs of small ones are then accounted for. The result is the same organization as in the lower internode, except that the large bundles are now in the position of the lower small ones and vice versa. At the succeeding node the story is repeated, except of course that the leaves alternate with those below.

It is obvious that the bundles of the upper internode, although their position with respect to the lower ones is definite, cannot be regarded as continuations of them. Nor can it be said that any bundle of the internode passes out definitely as a leaf-trace. It is true that in some specimens

the continuous thickening of the girdle would indicate that this was originally the case. But as a rule it may be said that the leaf-traces are organized from the ring of nodal wood.

Another species possessing this organization of the primary vascular bundles is *E. vulgaris*. In *E. trifurca* the leaves are in whorls of three, so that in each internode there are three pairs of large bundles alternating with three pairs of small ones. The girdle and traces, however, are usually organized in the same way as in *E. distachya* and *E. vulgaris*. Occasionally, however, the intercalary bundle between the two strands of the leaf-trace is absent. Such a trace with its two strands leaving the stele side by side is photographed in Fig. 6. The condition here is similar to that of other plants which have the double leaf-trace. It is no doubt the primi-



TEXT-FIG. 2. *E. altissima*. Reconstruction of course of primary fibro-vascular bundles.

tive condition, for it obtains exclusively in the traces to the reproductive bracts.

A more complicated condition of the whole primary system is found in such species as *E. altissima*. Text-fig. 2 is a reconstruction of the bundle course in this species. As the leaves occur in whorls of three, the internode has three pairs of large bundles alternating with three groups of smaller ones. But in this case each of the smaller groups contains three bundles instead of two. Accordingly the total number of bundles is fifteen. The nodal ring is organized in the same way as before. The most striking difference is that the strands of the leaf-trace arise, not above the small bundles of the internode, but above the spaces between them. The intercalary bundle is then directly above the central small bundle of the group, and the traces arise over the spaces which separate the central bundle from its fellows. In this case, at least, the traces cannot be considered as continuations of the internodal bundles. In addition to the origin of the leaf-traces, the most striking difference is the presence of three small bundles

instead of two in each group. This is no doubt a specialized feature, for in the seedling (see Pl. XCVI, Fig. 27) one finds the simpler type of organization.

The observations of former investigators differ considerably from those just outlined, and also differ materially from each other. Geyler,[1] the pioneer in this sort of work, observed the girdle at the node in *E. equisetiformis*, but did not consider it of importance. He believed that the bundles were continuous through two internodes, the leaf-traces attaching themselves laterally at that distance below their exit. In reality they run through only one internode where they are formed from the girdle. He also made the mistake of considering the large internodal bundles as the leaf-trace bundles of the succeeding node. Van Tieghem [2] observed the intercalary bundle between the leaf-trace strands which Geyler failed to see. He thought that it was derived from the traces and divided into two to give the proper number of bundles for the next internode. Strasburger,[3] while considering that the bundles ran through two internodes, correctly observed the origin of the intercalary bundle from the girdle. All these investigators examined species, *E. distachya*, *vulgaris*, and *equisetiformis*, which belong to the simplest type described. As we have seen, the conditions existing in many other species are quite different, although intermediate stages exist.

In spite of the disagreement in regard to details the course of specialization may readily be traced. There are indications that in the ancestral form the internodal bundles were as in the simpler type, and the small bundles ran out intact to form the leaf-traces. The intercalary bundle was absent. From this condition the girdle increased, the intercalary bundle developed, and the traces lost their connexion with the internodal bundles. Finally, the third small bundle of the internode developed, and the traces came to alternate with the internodal bundles.

Some of the features just described are of importance from the standpoint of relationships. The small number and simple regular course of the bundles through the internode are quite different from the conditions in the Cycads and Bennettitales, with which there has recently been a tendency to connect the Gnetales. It is well known that all the Cycadophyta had their primary vascular system arranged in the form of an irregular trellis from which the leaf-traces departed in a haphazard manner. The conditions in *Ephedra* resemble much more closely those of the Coniferales and Dicotyledones.

The double leaf-trace is a feature on which much stress has been laid. There appears to be general agreement as to its primitive character, for it is present in the ancient Cycadofilicinean forms. In *Ephedra* it differs from the double trace of many Gymnosperms in that the two strands of which it

[1] Gefässbündelverlauf und Laubblattregion d. Coniferen. Pringsheim's Jahrbücher, 1867.
[2] Anatomie des Fleurs des Gymnospermes. Ann. de Sci. Nat., Bot., 1889.
[3] Die Coniferen und die Gnetaceen, 1872.

is composed do not unite before entering the stele, but are inserted separately. In this respect it resembles the Cycads and *Ginkgo*. It is doubtful, however, whether any importance can be attributed to this point, for even in many of those ancient forms of which it is characteristic the two parts unite before entering the stele (*Lyginodendron*), while in others they remain separate (*Poroxylon*). On this basis the primitive Conifers can claim just as close an affinity to *Ephedra* as can the Cycads, for they also have a double trace. Furthermore, the Bennettitales, which on account of their floral organization have a much stronger claim than their modern representatives on the Gnetalean ancestry, had only a single trace, as Wieland [1] has shown. This fact has been overlooked in much recent work. For example, Miss Sykes,[2] in her work on *Welwitschia*, considered its double leaf-trace indicative of Cycadean affinities. It is obvious that the argument which involves the floral organization is quite contradictory to that which involves the double leaf-trace, and one or the other must be abandoned.

The bundle intercalated between the strands of the leaf-trace is a feature not found in other Gymnosperms. This fact and its absence in certain cases in *Ephedra* indicate that it is a specialization. It is possibly another condition in which *Ephedra* approaches the Dicotyledones in which the several traces to a single leaf arise at various points in the circumference of the young stele and are separated by masses of wood.

The girdle of tracheides at the node is a special feature with no significance in respect to relationship. It recalls strikingly the nodal wood of *Equisetum*, but of course this can be only a case of parallel development. This feature and the medullary diaphragm of both forms are probably correlated in some way with their similarity of habit.

2. Structure.

Each primary vascular bundle is roughly triangular, the innermost angle being occupied by the smallest protoxylem elements (Pl. XCIV, Figs. 1 and 2). The succession of elements from this point outward to the secondary wood is of the usual type. There is no centripetally developed wood, so that the bundles are of the endarch type. As has been noted above, a band of lignified elements which might easily be mistaken for centripetal wood often extends from the protoxylem for some distance into the pith (Fig. 2), but in no case was true centripetal wood found in the internode.

At the node, however, centripetally developed tracheides are occasionally to be found. They occur in the pith about the level of the exit of the leaf-traces. In this position they are not of the usual type, but bear a close resemblance to transfusion tracheides of the leaf. Fig. 8 shows one

[1] American Fossil Cycads. Carnegie Institute, 1906.

[2] Anatomy and Morphology of the Leaves and Inflorescences of *Welwitschia mirabilis*. Phil. Trans. Roy. Soc., B., 1910.

such element separated by at least two pith cells from the xylem. By following a series of sections one finds that they occur in strands attached to the protoxylem below, but diverging centripetally above. The lowermost elements are most like tracheides, but the upper ones rapidly assume the characters of transfusion cells. It is probable that they represent the original centripetal wood which was present universally in the ancestral types, and has been lost from the internode as in so many other plants, but in this case retained in a modified condition at the node.

At the edges of the primary bundles of the internode one often finds in *E. monostachya* and *E. vulgaris* typical transfusion tracheides like those of the leaf. They are adjacent to the pith, and often reach across from one bundle to the next. In Fig. 9 two primary bundles may be seen at the extreme right and left, and between them three or four of the scattered transfusion tracheides with their characteristic bordered pits and thickening bars. Their presence is probably due to the fact that the cortex of the young plant functions as a leaf.

Having described the origin of the leaf-traces in connexion with the course of the primary bundles, it may be well at this point to take up its structure. In all cases the trace is very small, consisting of but a few elements. Its tracheides are nearly all spiral or scalariform, although occasionally one may find a pitted element. In all cases the arrangement is endarch, i. e. the protoxylem elements are on the adaxial side of the bundle, and the metaxylem is all centrifugal. The endarch character may be distinguished in Fig. 7, in which the protoxylem elements of the trace are uppermost. In no case was centripetal xylem found. The structure is then identical with that of the trace of the Angiosperms and Conifers. It is vitally different from that of the Cycads and older Gymnosperms. In the Cycads, as is well known from the work of Scott and the earlier studies of Mettenius, there is always a large amount of centripetal wood which is considered to be retained from a primitive condition in which it occurred in the stem as well as in the leaf. At the same time the centrifugal primary wood is very small in amount and diminishes in the petiole. In *Ephedra* the conditions are exactly reversed ; the centripetal wood has been completely lost and the centrifugal wood developed. In regard to the structure of the leaf-trace, therefore, *Ephedra* is as remote as possible from the Cycads, and is in agreement with the Conifers and Dicotyledones. In view of the general agreement as to the importance of the leaf-trace as a seat of ancestral characters, this is a point which should be emphasized in all phylogenetic studies.

Secondary Wood.

It has long been known that the secondary wood of *Ephedra* consists of tracheides, vessels, wood parenchyma, and rays. But an intimate study of each of these constituents is necessary, especially in the light of recent

similar studies on the wood of Conifers and Dicotyledones. A transverse view in which the general characteristics and arrangement of all the elements may be distinguished is shown in Fig. 10.

1. Tracheides.

The pitting of the tracheides is usually uniseriate with the individual pits well separated (see Fig. 11). In this respect it agrees with that of all the Conifers except the Araucarians, and is sharply distinguished from that of the Cycads and Bennettitales. In the latter group the pits were almost always of the very primitive scalariform type,[1] while in the Cycads the multiseriate pitted condition is the dominant one, although the scalariform condition is not uncommon.

The structure of the individual pits presents additional points of difference. The pit mouths in *Ephedra* are circular, as in the majority of the Conifers, whereas in the Cycads they are more or less slit-shaped. The torus is also very well developed (Fig. 11), while in the Cycads it is absent.

Although the pits are usually well separated, conforming thus to the 'Abietinean' type, one often finds the mutually compressed type or 'Araucarian' condition. This is represented in Fig. 12. It is most frequent at the ends of the tracheides. Often also the more typical Araucarian condition with two alternating rows of compressed pits is to be found. Rarely are more than two rows present.

In association with the Abietinean pitting the tracheides possess a feature which has been shown recently to be of primary importance, namely, the so-called bars of Sanio. These structures, named in honour of their discoverer, are horizontal bars or folds of cellulose crossing the tracheide wall between the pits. Stained in haematoxylin they appear as dark bands. They may be distinguished in the central tracheide of Fig. 11. Miss Gerry[2] has shown that they are present in all Conifers except the Araucarians, i. e. wherever the Abietinean type of pitting occurs. Outside the Conifers they are to be found only in *Ginkgo*, which also has uniseriate separated pits. Naturally they cannot exist where the pits are closely compressed. Their presence in *Ephedra* serves to distinguish this genus sharply from the Cycadales, and associate it rather with the Coniferales and Ginkgoales.

The bordered pits and bars of Sanio are not confined to the radial walls of the tracheides as in most woods, but are found also on the tangential walls, as may be seen in Pl. XCV, Fig. 13. In the Conifers tangential pits, when present at all, occur only in the summer wood, but in *Ephedra* they are to be found throughout the year's growth. They are usually somewhat smaller than those of the radial wall.

[1] Wieland: loc. cit.

[2] Distribution of the 'Bars of Sanio' in the Coniferae. Annals of Botany, Jan., 1910.

The tracheide walls sometimes show tertiary spiral striations such as are characteristic of *Taxus* and *Pseudotsuga*, and according to Bailey[1] are of sporadic occurrence in all the Pineae. Fig. 14 gives their characteristic appearance in *E. californica*. They appear to be of very sporadic occurrence. Their presence is not a specific character, nor are they more frequent in young wood, as Bailey found to be the case in the Pineae. They supply one more point of resemblance to the tracheides of the Conifers.

Another type of spiral thickening to which Boodle and Worsdell[2] has called attention is photographed in Fig. 15. These thickenings are broader and less numerous than those of the other type. Boodle and Worsdell observed that they appear to be continuations of the mouths of the pits. The relation to the pits is plainly shown in Fig. 15, especially in the upper part of the field. In many places the relation is not so obvious on account of the fact that the section may not include the underlying pit. It is plain that these thickenings have no connexion whatever with those of the usual type. They have also been observed in the genus *Widdringtonia*, one of the Cupressineae.

Still another feature which occurs sporadically in the tracheides of the Coniferales and only in that family is present in *Ephedra*, namely, the so-called trabeculae. By this term is meant a series of lignified septa occurring all in the same horizontal line in a single radial row of elements. When seen in radial section (Fig. 16), they present the appearance of a continuous row of lignified bars crossing the field. Their occurrence is so uncommon and sporadic in any wood that little account is taken of them in the literature of wood structure. They serve, nevertheless, as another addition to the list of Coniferous characters possessed by *Ephedra*.

There is just one other point in connexion with the tracheides of *Ephedra* to which attention should be called, namely, the frequent presence of resin plates. Resin is often deposited in the tracheides in the form of spool-shaped plates. In Fig. 16 the small tracheide at the left of the large central vessel contains several of them. Although this is the prevailing form, the resin is sometimes deposited in globules or larger masses. The spool-shaped plates bear a strong resemblance to those of the wood of certain Conifers, notably the Araucarians, in which they become a characteristic feature.

All the characteristic features of the tracheides of *Ephedra* are, then, also characteristic of the tracheides of the Conifers; the arrangement of the pits, the structure of the individual pits, the bars of Sanio, tertiary spirals, trabeculae, and resin plates. No single family of the Conifers possesses all these features, so that it would be difficult to connect *Ephedra* from the

[1] The Structure of the Wood in the Pineae. Bot. Gaz., July, 1909.
[2] L. A. Boodle and W. C. Worsdell. Annals of Botany, 1894.

standpoint of evolution with any special members of that family. It would seem rather to be related to some generalized ancient form.

2. Vessels.

The fact that *Ephedra* possesses true vessels is perhaps its best known anatomical feature. It is also well known that the perforations of those vessels correspond to the bordered pits of tracheides. The further fact that there are interesting transitions between bordered pits and perforations has been emphasized by Strasburger [1] and by Boodle and Worsdell.[2] The writer's study has shown that the transitions between tracheides and vessels are much more complete than was suspected, and has revealed additional structural features. For the sake of completeness some facts already known will be briefly recapitulated.

The lumina of the vessels vary from that of a tracheide to many times that size (Pl. XCVI, Figs. 27 and 29). The pits on the lateral walls are identical with those of the tracheides, though arranged more often in two rows. In addition the vessels show the bars of *Sanio*, tertiary spirals, and trabeculae characteristic of the tracheides. The perforations of the end walls are seen in transverse section (Pl. XCIV, Fig. 10) to be simply enlarged bordered pits in which the border has become narrow and the torus has disappeared. In the radial section (Fig. 12 and Pl. XCVI, Fig. 25) these points are also visible, although the border is not so clear. The perforations may be in two or three rows which are opposite or alternate. Perhaps the clearest ideas may be obtained from the tangential section which has been photographed in Pl. XCV, Fig. 17. In this figure the slanting end wall, the open communication between the conterminous elements, and the border of the perforations are clearly shown.

From the appearances presented in these figures the conclusion is natural that the perforations represent modified bordered pits. This assumption is rendered a certainty by the fact that one frequently finds true bordered pits in series with the perforations (see Fig. 20). The latter in such cases occupy the position of the bordered pits of a normal tracheide. This condition is shown in tangential view in Fig. 18, in which the two bordered pits at the bottom of the end wall have well developed borders and tori.

The transitions between tracheides and vessels are in fact remarkably complete, especially in the first few annual rings. In Fig. 19 is represented an element which differs from a tracheide only in the absence of the torus and narrowness of the border of *one* of its enlarged central pits. The other pits, while showing a slight decrease in the border, are still true bordered pits. A further stage is represented in Fig. 20, and many other figures could have been presented showing all stages up to the completed vessel of Pl. XCVI,

[1] Über den Bau und die Verrichtungen, &c. [2] loc. cit.

Fig. 25. But a single vessel is often sufficient to show clearly the gradual disappearance of the torus and border. In Pl. XCV, Fig. 20, at the end of the vessel the pits have a typical border and a deeply stained, well-marked torus. Towards the bottom of the field, i. e. towards the centre of the end wall of the vessel, the torus becomes gradually fainter until it disappears, and there results an open communication through the pits. The decrease in breadth of the border is equally gradual. Usually the conditions are not so diagrammatic as this. Sometimes the torus and border do not disappear at the same rate, but the latter may be quite well developed when the former has completely vanished. Diagrammatic cases are sufficiently numerous, however, and the various stages presented by different elements are so often repeated that there can be no doubt as to the origin of the perforations, in *Ephedra* at least.

In those cases in which the torus is only partly developed, it may have become so in one of two ways: either in the course of ontogeny it has ceased to grow at that stage in its development, or it may have continued to the complete condition, and later have been partially resorbed. The writer was unable to determine which of these alternatives is correct.

If the vessels have developed in the manner indicated it might be expected that the seedling would present primitive conditions approaching tracheides. As a matter of fact the seedling possesses very few vessels of any kind, the mass of wood consisting almost exclusively of tracheides. This fact is shown strikingly in Pl. XCVI, Fig. 27, which is a photograph of a transverse section through the base of the seedling stem of *E. altissima.* Very few vessels can be recognized, and even those which can are extremely small. The absence of vessels is also shown in longitudinal view in Fig. 28. Of course, as one examines the upper parts of the seedling it is found that they gradually increase in number. Moreover, the vessels which are present have only a single row of perforations, and these are usually of the transitional type. Such facts furnish another striking example of the recapitulation in the seedling of ancestral characteristics.

It should also be noted that in the first annual ring of adult branches the few vessels which are present usually have a single row of perforations which are often transitional to bordered pits. Evidence is thus added to that which has recently been accumulated showing that this region is also the seat of retention of ancestral features.

Boodle and Worsdell[1] state that the node also lacks vessels. For the species which the writer has examined this statement does not appear to hold good, although the number in that region may be slightly less than in the internode. The presence of vessels in the node of *E. altissima* is shown by photography in Pl. XCIV, Fig. 7, especially at the right.

We have here seen the structure of the primitive vessels of *Ephedra*

[1] loc. cit.

and of the typical ones; there remains a more highly developed condition which is occasionally to be found, but which has not yet been described. It is illustrated in Pl. XCV, Fig. 21, which is a photograph from *E. distachya*. The upper two perforations appear from their shape to have resulted each from the horizontal fusion of a pair of perforations. This inference is confirmed by the condition of the pair of opposite perforations below, for the latter are separated by an extremely thin part of the wall (compare with Fig. 20). It is clear, therefore, that a fusion of perforations sometimes occurs, resulting in the formation of still larger perforations. The resulting vessels bear a much closer resemblance to those of the Angiosperms, especially to the ones with scalariform end walls which are characteristic of the lower Dicotyledones.

In spite of this resemblance, however, it must be admitted that the vessels of *Ephedra* differ essentially from those of the Angiosperms. The perforations of the Angiospermic vessels by no means correspond to modified bordered pits. It may be stated here, however, that the vessels of *Gnetum* to a large extent clear up the difficulty.

3. Wood Parenchyma.

Wood parenchyma is very abundant in *Ephedra*, the number of its elements often nearly equalling the number of tracheides. It is sometimes scattered irregularly throughout the wood (Pl. XCIV, Fig. 10), but is more often arranged in definite tangential bands. The banded arrangement is visible in Pl. XCVI, Fig. 29, in which the parenchyma cells appear dark owing to their deeply staining protoplasmic contents. There are usually two rows of tracheides separating the successive bands of parenchyma cells, although often, especially in root wood, only a single row of tracheides intervenes. Sometimes, indeed, the bands of parenchyma cells themselves become two or three rows in thickness.

The individual cells are identical with tracheides in length and shape (Pl. XCV, Fig. 23). They are also lignified in the same way and to the same extent as tracheides. Moreover, in the vast majority of instances they have no cross-walls as do those of Conifers. Therefore, the only features which serve to distinguish them from tracheides are the character of the pitting and the possession of protoplasmic contents. When in dry material the protoplasm disappears, the distinction is often difficult to make.

As for the other distinguishing feature, namely, the character of the pits, it is usually simple. This is always true in the case of pits between adjacent parenchyma cells. In some cases, however, where the parenchyma cell adjoins a tracheide or vessel the pits show a small border on the parenchyma side as well as the usual large one on the tracheide side. The small border is shown in Fig. 22. The parenchyma cell adjoining the vessel

on the right is charged with a black resinous mass which has penetrated through the pits into the lumen of the vessel, where it remains as little globules. The deep colour of this material emphasizes sharply the doubly bordered character of the pits as well as the fact that the border on the vessel side is wider than that on the side of the parenchyma cell.

A peculiar fact in connexion with these wood parenchyma cells is that they are often multinucleate. Fig. 23 shows a parenchyma cell in the wood of *E. monostachya* which in the short extent shown in the photograph obviously contains two nuclei. This condition obtains quite frequently, although it is often hard to demonstrate. Three and four nuclei have been observed in a single parenchyma cell. It would seem that the distance from the centre to the ends of such an extremely long living cell is too great to enable the single nucleus to perform its proper functions. Accordingly the original nucleus divides and the daughter nuclei become scattered along the cell. In fact, in the proper material it is possible to observe that the nucleus is actually undergoing division. Such a case is photographed in Fig. 24. The details of nuclear divisions were impossible to distinguish.

In a few cases, as Strasburger [1] has pointed out, the parenchyma cells are further distinguished from tracheides by the presence of cross-walls such as exist in the parenchyma of most other woods. Such a wall is shown in Pl. XCVI, Fig. 25, at the centre of the field. The inclination is typical. As a rule, however, septa are rare, and in some species were never seen. They are most abundant in *E. monostachya*. In no case was more than one septum seen in a single parenchyma cell, a condition which is in sharp contrast to that of the parenchyma of most woods.

The typical wood-parenchyma cell of *Ephedra* is then, except for its pitting, merely a tracheide which has retained its protoplasmic contents. And in some cases we have seen that even its pitting resembles that of a tracheide. From these facts, and also from the entire absence of wood parenchyma in the ancient Gymnosperms, it may be concluded that the wood parenchyma of *Ephedra* has been derived from tracheides. Each parenchyma cell would therefore represent a modified tracheide.

It is to be noted that this type of parenchyma differs essentially from that of the Conifers. The latter consists of short cells, a number of which together give the shape and size of a tracheide. In fact, Bailey [1] has recently brought forward evidence to show that this type was also derived from tracheides. The real difference then consists in the presence of numerous septations in the Coniferous cells. We have seen that in *Ephedra* a single septum is sometimes present, so that the distinction is not really so profound. The Conifers appear to have carried the process of septation much further.

The cells which resemble them most closely, however, are to be found in the Angiosperms, where they have been called fibrous cells or substitute

[1] loc. cit.

fibres. In fact, de Bary [1] concluded that they fell naturally into this category. It should be noted, however, that the Angiospermic fibrous cells, as their name indicates, resemble the woody fibres rather than tracheides. They are much longer and have thicker walls. Although the lack of septa is common to the two, it is doubtful whether they are homologous structures.

4. Wood Rays.

The ray structure of *Ephedra* has hitherto been passed over with the simple statement of Strasburger [2] that they are multiseriate. In reality the conditions which they present are no less significant from an evolutionary point of view than those of the vessels to which the whole attention has been devoted in the past. The fact that they are multiseriate is in itself of supreme importance, for in connexion with other facts it emphasizes their close relationship to those of the Dicotyledones.

The multiseriate condition is shown in transverse section in Pl. XCIV, Fig. 10 at the left and in tangential section in Pl. XCV, Fig. 13. The number of rows of cells varies from one to ten, but the average number is four or five. There is considerable specific and regional variation in this respect. In tangential section they are found in most cases to be solid homogeneous masses which extend vertically to considerable heights. They are not so high, however, as those of the Oak, but have a more fusiform outline (Pl. XCVI, Fig. 32).

The individual cells are extremely variable in size and shape. They may be isodiametric or elongated radially or vertically. As these variations may be found within a small area, they give the ray a peculiarly irregular appearance, as is shown in Fig. 26, and especially in Pl. XCV, Fig. 13. Furthermore, in old wood, all the cells of the marginal rows often become elongated obliquely to the course of the ray, a condition which no doubt results from the slow growth of the ray in comparison to that of the surrounding wood.

Each cell is strongly lignified, resembling in this respect the ray cells of Conifers and Dicotyledones and differing from those of the Cycads and Bennettitales. The simple pits, like those of the Dicotyledones, occur on all walls and are very small, being merely fine pores. Their character may be distinguished in Pl. XCVI, Fig. 26, especially at the top of the field. All these features give an appearance in radial section strikingly like that of a large ray of the Dicotyledones.

The conditions of the rays in the young stem are quite different from those just described for the adult. At the pith one finds only uniseriate rays similar to those which are characteristic of the Conifers (Figs. 27 and 29). From this condition the broad ray of the adult is produced in two

[1] Comparative Anatomy of Phanerogams and Ferns.

[2] Über den Bau, &c.

ways, first by the enlargement of the uniseriate rays, and secondly, by their fusion. The first process, that of enlargement, is itself effected in two ways. The cells of a uniseriate ray may simply divide radially to produce a biseriate one, which in turn continues the process, or the adjacent tracheides may be transformed into ray parenchyma. The latter method is much more common, and indeed many rays originate by this transformation in the wood and do not extend to the pith. During the process the tracheides retain their contents and become septated irregularly. As one examines the sections from the pith outwards one finds that the compartments into which the tracheides are divided become gradually shorter and arranged irregularly. Fig. 28, which is a photograph of a tangential section near the pith of *E. altissima*, shows the formation of a number of rays in this manner. The ray cells are all very much elongated vertically, and some of them are obviously segments of tracheides. Indeed, in some places, it is difficult to distinguish the limits of the rays.

The second method in which the broad rays are formed is the significant process of fusion of small rays. Two or more small rays become joined into one large one, while the intervening fibres are either pinched out or transformed into ray parenchyma. Later, other rays may be added laterally in the same way, so that rays of various sizes are found in the compounding area. Fig. 29 shows the compounding process taking place in *E. monostachya*. Two of the large rays are obviously in the process of formation by a fusion of similar ones. Fig. 30 shows the same thing taking place in *E. californica*. The completion of the process usually requires a number of annual rings. Often it is very slow, and the included fibres remain intact for a long distance. Indeed, in some cases the fusion appears never to become complete, for tangential sections may reveal the presence of fibres and even vessels in the rays of very old wood. Such a tangential section of *E. californica* is represented in Fig. 31. The numerous included fibres show that this broad ray has resulted from the fusion of smaller ones. A similar condition from *E. trifurca* is shown in Fig. 32. Broad rays such as these containing fibres are homologous with the so-called false rays of many lower Dicotyledones, e. g. *Alnus*, *Carpinus*, *Betula*.

The formation of broad rays by the compounding process is exactly what Eames[1] has recently described as occurring in the oak. He has shown that in the seedling and in the young branch of some species the characteristic broad oak ray is not present, but develops later in a manner exactly similar to that just outlined for *Ephedra*. From these facts and from fossil evidence he concluded that the broad type of ray found in Angiosperms is not a primary structure representing the gap between the original primary vascular bundles, as has been supposed, but is really derived secondarily from an aggregation of uniseriate rays of the Coniferous

[1] Eames, A. J.: Origin of Broad Ray in *Quercus*. Bot. Gaz., March, 1910.

type. Bailey[1] emphasized the transitions in the compounding process furnished by the false rays of the Cupuliferae which, as we have seen, likewise have their counterpart in *Ephedra*. He further showed that the compounding took place primarily around the leaf-trace. The development of broad rays is obviously an adaptation for the storage of assimilates manufactured in the leaf, and the most convenient place for their storage is in the stem adjacent to the leaf-trace. From their original position around the trace he considers that the broad rays have spread throughout the wood.

Not only in the possession of broad rays, therefore, but also in their origin does *Ephedra* resemble the lower Dicotyledones. It should be noted, however, that the compounding process bears no obvious relation to the leaf-traces, but may give rise to rays which do not touch a trace at any point. In fact, it seems to be quite independent of the leaf-trace, and is likely to result in the formation of a broad ray on any radius of the stem. It is true that the trace is accompanied by a broad ray, as may be seen in Pl. XCIV, Fig. 7, but this ray is neither so broad nor so high as the usual type of compound ray. Moreover, it rarely shows the fusion process. In fact, it represents rather the leaf-gap of other woods. The reason for its poor development is probably to be found in the non-functional character of the leaves In practically all cases the leaves are merely small scales which have little or no chlorophyll, and have given over their function of assimilation to the cortex of the young plant which is abundantly supplied with chlorophyll and stomata. Not the restricted area around the leaf-trace, therefore, but the whole circumference of the branch would feel the need for storage. Of course it is quite possible that the original position of ray development was in the vicinity of the leaf-traces, and that with the reduction of the leaves and the functioning of the cortex in assimilation the rays may have lost their original connexions. Indeed, the occasional occurrence of compounding around the trace and the constant presence of a ray in that position would justify such an inference. In any case the resemblance both in regard to the history of development and adult form between the rays of *Ephedra* and those of the lower Dicotyledones is remarkably close.

Just as in many oaks the compounding process is visible only as a passing phase in the seedling, the broad rays of other regions extending right to the pith, so in certain regions of *Ephedra* the broad ray has worked back almost or quite to the pith. This is particularly true of those regions in which storage is greatest, namely, in roots and underground stems. In these places the broad ray develops at a very short distance from the pith, and may indeed be quite continuous with the pith. Pl. XCVI, Fig. 33, which represents a transverse section of a subterranean stem of

[1] Bailey, I. W.: Relation of the Leaf-trace to the Origin of Compound Rays in the Lower Dicotyledons. Ann. Bot., Jan., 1911.

E. monostachya, shows a very early development of several broad rays. Some of them which lack cell contents are to be distinguished as areas in which there are no vessels. As a rule, however, the broad rays do not appear until a considerable zone of wood has been formed.

With the increase in circumference of the young stem the rays become proportionately broader. If the increase went on indefinitely the rays would soon become too broad for the proper organization of the stem tissues. In *Ephedra* the stems rarely become large enough to cause this difficulty, but when they do it is met by the secondary obliquely vertical division of the rays. In tangential view the rays are found to be divided by oblique strands of fibres into a number of segments which then become scattered horizontally through the wood, as the strands of fibres increase in size. This division of broad rays with the increase in diameter of the stem is similar to that described by the writer [1] as occurring in certain of the Fagaceae and Casuarinaceae. Zilztra [2] has observed the same phenomenon in several Dicotyledonous plants. It was used in the former contribution in support of the theory that the smaller multiseriate rays of the Dicotyledones have been derived from the broad aggregate ray by the diffusion of the latter throughout the wood. In *Ephedra* the stems rarely become large enough to make the dissection process a necessity, but its occasional presence shows that the same rule holds as in the Dicotyledones.

Bailey [3] has shown that oak wood formed immediately after an injury is likely to present primitive conditions of ray structure. In such areas one may find only uniseriate rays, and these later undergo the compounding process to form broad rays again. These phenomena are in agreement with the well-established principle that traumatic areas tend to revert to primitive conditions. In *Ephedra* such a reversion is extremely rare, although it is occasionally to be seen. For the most part, however, wounding has very little effect on ray structure, the rays continuing about the same size as before the wound. Occasionally, indeed, the wound causes the enlargement of rays already present, and even the introduction of new ones. As Bailey has suggested for a similar phenomenon in the oak the wound appears in these cases to stimulate the development of broad rays. On the whole, however, the effect of wounding is very indefinite.

One other respect in which the rays of *Ephedra* resemble those of the Dicotyledones is their retarding effect on the growth of the stem. In the oak the growth is slower in the vicinity of the broad ray, and the result is manifested in a distinct 'dip' in the contour of the annual rings which

[1] Thompson, W. P.: Origin of the Multiseriate Ray of the Dicotyledons. Ann. Bot., October, 1911.

[2] Die Gestalt der Markstrahlen im sekundären Holze. Recueil des Travaux Botaniques Néerlandais, vol. v, 1908.

[3] Bailey, I. W.: Reversionary Characters in Traumatic Oak Woods. Bot. Gaz., Nov., 1910.

increase with the diameter of the stem. As Bailey [1] has shown this has had an important effect on the structure of the stem, for it has resulted in the production of the so-called depressed segments alternating with projecting ones. The depressed segments had formerly been considered to represent the 'interfascicular' wood. Fig. 30 shows the presence of the dip in *E. californica*, and its increase in the successive annual rings. Owing to the lack of association between broad rays and leaves in *Ephedra* the depression has not the regularity which it exhibits in the oak.

To summarize the important features of ray structure: The most primitive condition is obviously that presented by the first-formed secondary wood which has uniseriate rays, the individual cells of which are lignified. In both these respects, the uniseriate condition and the lignification, the rays differ from those of the Cycads and Bennettitales, and resemble those of the Conifers. For the rest the relationship is with the lower Dicotyledones, as is manifested by the process of compounding, the ultimate multiseriate condition, the presence of false rays, their retardation of the growth, the form and arrangement, and especially the pitting of the individual cells. A condition typically Dicotyledonous has therefore been derived from a condition typically Coniferous in a method which is to be expected from a study of Dicotyledonous anatomy.

The different constituents of the secondary wood which we have been considering show very little specific variation, so that a diagnosis on the basis of wood structure would be very difficult to make. What variation exists is mainly one of degree. Thus *E. monostachya* has more septated wood parenchyma cells than the others; *E. Gerardiana* and *E. monostachya* exhibit a greater tendency towards the ring-porous arrangement of the vessels; *E. californica* usually presents a larger number of 'false' rays, &c. But these features vary greatly in different pieces of wood and in different regions of the same plant. Accordingly, it is very difficult to make a specific diagnosis with any degree of certainty. But this difficulty is only in keeping with the recognized conservatism of vascular structures.

Bast.

The bast of *Ephedra*, unlike the wood, shows little indication of Angiospermous affinities. Indeed, except for the presence of broad rays, it is typically Gymnospermous.

Fig. 34 represents a transverse section of the bast as well as the cambium and a little secondary wood. The biseriate ray in the centre of the field is seen to broaden out in a characteristic manner. In addition to the ray parenchyma there are other bast parenchyma cells which are arranged in radial rows and elongated vertically. They do not bear the

[1] loc. cit.

relationship of companion cells to the sieve-tubes, but are arranged rather in groups.

The sieve-tubes themselves are visible in this photograph as smaller cells which lack contents and are somewhat collapsed. They are never present in abundance, but, as shown here, are usually confined to small patches. In very large stems of the American species which have been examined they become proportionately much more numerous. In longitudinal section they are found to lack well-defined end walls, but to be long and tapering, as is typical of Gymnospermous sieve-tubes. This feature is shown in the tangential section photographed in Fig. 35. The sieve areas are not confined to the end walls of the tubes, but are scattered rather uniformly along the lateral radial walls, as may also be distinguished in Fig. 35. The same condition is shown in radial section in Fig. 36, in which the sieve areas appear in face view.

Outside the zone of actively functioning bast many of the cells collapse, but others become strongly sclerified. The latter are scattered singly or in small groups. They represent part of the parenchyma of the living bast.

The bast of *Ephedra*, then, in all the characters of its sieve-tubes and in most of those of its parenchyma, possesses no Angiospermous affinities, but is typically Gymnospermous. Strasburger[1] states that in its general appearance and arrangement it resembles most closely the bast of *Araucaria*.

Cortex, Epidermis, etc.

As may be seen in the transverse section of the entire young branch (Pl. XCIV, Fig. 1) the external surface is strongly ribbed. Each rib marks the position of a bundle of sclerenchymatous fibres immediately below the epidermis (Fig. 1 and Pl. XCVII, Fig. 37). The fibres are extremely hard, and do not take the usual lignin stains. Others of the same type are scattered either singly or in groups through the rest of the cortex, and large groups occur in contact with the primary phloem. The outermost living cells are arranged more or less in the form of a palisade and are abundantly supplied with chloroplastids. Beneath this layer the tissue is more irregular and contains air-spaces. Thus the structure of the cortex is remarkably like that of a leaf, whose function it performs.

The epidermis is thick-walled and heavily cutinized. It is abundantly supplied with stomata like a leaf, but these are confined to the furrows between the projecting ridges (Fig. 37). The guard cells are strongly lignified, and deeply sunken under overarching accessory cells (Fig. 37).

The periderm formation which eventually throws off the cortex begins just outside the soft bast and inside the primary bast fibres. Its radial rows of cells are in line with those of the bast.

[1] loc. cit.

Root.

The root of *Ephedra* presents few features of special interest. The primary wood is diarch and small in amount. In the large primary root near the base of the stem it is arranged in two triangular masses whose outermost apices are occupied by the two protoxylem groups, and whose broad bases are separated by a lignified pith. In the secondary roots the primary wood is very small in amount and extremely difficult to recognize. Indeed, in most cases it appears to have completely disappeared, as has the fundamental parenchyma, so that the centre of the root is occupied by a solid mass of secondary wood.

This secondary wood is in all essentials like that of the stem, differing chiefly in the relative amounts of the various constituents. Thus the wood parenchyma is more abundant and the rays larger, features which are to be anticipated from the function of the root in storage. The vessels are also more frequently of the primitive type, and the bars of Sanio more conspicuous.

Leaf.

The leaves of *Ephedra* occur in opposite decussating pairs or in whorls of three according to the species. They are extremely reduced, in most cases being small brown functionless scales a few millimetres in length. In seedlings and young plants they may be much better developed, probably reverting to an ancestral condition. They then consist of a sheathing base and long needle-shaped extremity.

A section through the sheathing base of one of the best developed leaves is represented in Fig. 38. Only the thickest part of the leaf in the region of the vascular bundles is included. Laterally, where it joins its fellow of the opposite side the leaf is only two or three cells in thickness. Stomata are exceedingly rare, but when present are deeply sunken like those of the stem. The mesophyll is not differentiated into layers and contains at best very little chlorophyll.

Such a leaf as this is enormously developed in comparison with the ordinary adult leaves, which are only three or four cells thick. Moreover, the cells soon become thick-walled and perish. In most cases sclerenchymatous fibres are scattered throughout the substance and may occasionally be arranged in bundles as in the stem.

The tissue of the leaf is early separated from that of the stem by the development of a layer of absciss periderm. The whole of the sheathing base is involved. In Fig. 42 the dark band across the centre represents the periderm layer, below which is the tissue of the cortex and leaf-trace, and above is the empty thick-walled mesophyll of the leaf. The presence of the absciss periderm is a distinctly Angiospermous character.

The vascular system of the leaf consists of two bundles, the continuations of the double trace whose course we have already examined. The two strands continue distinct and unbranched to the apex of the leaf. They are visible at low magnification in Fig. 38, and one of them is shown more highly magnified in Fig. 39. Each bundle is without a sheath of any kind and is flattened in a dorsiventral direction. The tracheides, which are very few in number, are arranged in radial rows. There is no trace of centripetal wood, all the larger elements being developed centrifugally to the protoxylem. The phloem is very poorly developed. At the edges of the bundle, as Strasburger [1] has noted, there is an occasional transfusion tracheide, one of which can be distinguished in Fig. 39 by its bordered pits and thickening bars. The bundle figured is better developed than is usually the case, for as a rule the tracheides are very few in number, the bast practically absent, and the transfusion tracheides rare. Such a bundle with its lack of centripetal wood and possession of transfusion tracheides is exactly like a reduced Coniferous bundle, and quite unlike that of the Cycads with its large development of centripetal wood.

In their basal portion the long needle leaves of the seedling have the same vascular organization as the typical adult leaves. But in their tips this organization becomes modified. The secondary wood of the two bundles diminishes (Fig. 40) until it quite disappears (Fig. 41). Meanwhile, the transfusion tissue increases laterally, and also develops centripetally. In the latter position it increases in amount towards the tip until it forms a solid mass between the two fast vanishing groups of centrifugal wood (Fig. 40). As it increases in amount, the form of its cells also changes; they become longer and more like true tracheides. Finally, in the extreme tip of the leaf the centrifugally developed tracheides have completely disappeared, and only a group of the centripetal ones remain (Fig. 41). Some of the latter are like typical transfusion tracheides, and some of them are more like ordinary tracheides, and might for this reason be considered centripetal wood.

The conditions just described might be used to support Worsdell's contention [2] that the transfusion tissue has been derived phylogentically from the centripetal wood of the ancient Gymnosperms. The transitions between centripetal wood and transfusion tissue which he observed in several forms, notably in *Cycas* and *Araucaria*, are duplicated here. The evidence is thus the same as he presents. It should be emphasized that the leaves in which these conditions are found are the abnormal seedling ones, and that the elongated tip to which the centripetal wood is confined is not ordinarily present.

The structure of the vascular bundles of the leaf strongly indicate Coniferous affinity. The development of centrifugal and typical absence of

[1] Über den Bau, &c.

[2] Origin of Transfusion Tissue. Trans. Linn. Soc., 1895.

centripetal wood are quite like the conditions in Conifers, and quite unlike those in Cycads. The small amount of centripetal wood in the abnormal seedling leaves has its counterpart in several of the Conifers.

Conclusions and Relationships.

A complete discussion of the affinities of the Gnetales as indicated in an anatomical study is deferred until the other members of the group have been subjected to a similar study. Certain facts brought out in this investigation, however, may be emphasized here.

As we have already noted, there is an increasing tendency at the present time to regard the Gnetales as derived from Cycadalean stock. The opinion has no doubt sprung from the conviction of many botanists that the Angiosperms have had the same origin, namely, from the Bennettitales, and that there is a real affinity between the Angiosperms and Gnetales. The fullest expression of this idea has been given by Arber and Parkin,[1] whose conclusions have been reached almost entirely from a consideration of floral organization. They maintain that these two great groups have developed along parallel lines from a common ancestor which was in turn derived from forms like Bennettitales. Deferring the consideration of the Angiospermic relationship, let us see what bearing the results of the present study have on the idea of a Bennettitalean origin for the Gnetales.

It may be stated at once that on the anatomical side there is very little evidence for connecting the Bennettitales with *Ephedra*, although this genus, being the most primitive of the Gnetales, is the one where the evidence ought to be found. The only notable feature of resemblance is the possession by both of multiseriate rays, but, as we have seen, those of *Ephedra* have undoubtedly been developed from a condition quite different from the Bennettitalean one, namely, from a uniseriate lignified condition. Therefore the only real anatomical point of resemblance proves valueless in establishing a connexion.

On the other hand, there are a great many differences so vital as to make any real affinity extremely doubtful. Among those differences should be mentioned the course and arrangement of the primary bundles, the pitting of the tracheides, bars of Sanio, tertiary spirals, wood parenchyma, primitively lignified uniseriate rays, adult rays derived by fusion, double leaf-trace, absence of centripetal wood, and development of centrifugal wood in the leaf-trace. Thus almost every tissue presents grave obstacles to this view. All these differences constitute too great a mass of evidence to be overlooked, especially since there appear to be no anatomical resemblances on which to base a relationship.

[1] Studies in the Evolution of the Angiosperms: The Relationship of the Angiosperms to the Gnetales. Ann. Bot., 1908.

With regard to the more recent Cycads they possess one feature of resemblance lacking in the Bennettitales, namely, the double leaf-trace whose two strands were inserted separately in the stele. But as the double leaf-trace is common to so many groups of Gymnosperms it might be used with equal force to connect any of them with the Gnetales. As for the fact that the two strands are inserted separately in the stele, we have seen that this is a variable feature, even the ancient Gymnosperms showing both a condition of separation at the stele (*Poroxylon*), and one in which the strands unite before entering (*Lyginodendron*). Miss Sykes, in her study of *Welwitschia*, brought forward other points of resemblance to Cycads, namely, the occurrence of centripetal wood in the peduncle and of inversely oriented and concentric bundles in the reproductive axis. A preliminary study of the reproductive organs of *Ephedra* has failed to reveal any of these features. On the whole it may be said that the differences between *Ephedra* and the modern Cycads are almost as strongly marked as between *Ephedra* and the ancient Bennettitales. Moreover, if the Gnetales have been derived from Cycadalean stock, most botanists would agree from the evidence of floral organization that it must have been from the Bennettitales, so that any advantages of the modern over the ancient members of that alliance should not have weight.

With the growth in favour of the idea of Cycadalean affinity the older view of the Coniferous relationship of the Gnetales is being supplanted. The present study has led the writer to conclude that the older view had much more in its favour than the more modern one. Every one of the points enumerated as opposed to the Cycadalean relationship may be used in an argument for the Coniferous relationship. To emphasize only the strongest points one should mention the arrangement and structure of pits of the tracheides, bars of Sanio, tertiary spirals, trabeculae, primitive uniseriate and lignified rays, lack of centripetal and development of centrifugal wood in the leaf-trace, and the structure of the vascular bundles of the leaves. These points of remarkable resemblance do not prove a descent from any modern group of Conifers. Indeed, such a descent seems difficult to establish. Opposed to it is the very generalized character of the secondary wood combining many characters found in the various groups of Conifers, such as Araucarian and Abietinean pitting, bars of Sanio, tertiary spirals, trabeculae, resin plates, and wood parenchyma. These points would rather indicate an affinity with that Abietinean-Araucarinean stock which it is now generally conceded gave rise to the modern Conifers. Of course it is possible that the Gnetales represent a line of development paralleling to a considerable extent that of the Conifers. But, however opinions may differ as to the exact point of origin of the Gnetalean line, there is a large mass of evidence to indicate an affinity with the base of the Coniferous line of descent.

Evidence from other sources supports this view. Hill and de Fraine,[1] from their study of the vascular system of the seedlings, conclude that in this respect *Ephedra* strongly resembles *Araucaria* and the Podocarps. Miss Sykes states that the transition phenomena between root and stem in *Welwitschia* strongly resembles that in *Araucaria.*

If the anatomical evidence leads to these conclusions the gametophytic evidence is just as conclusive. According to Land's [2] description the male gametophyte is typically Coniferous in all details. The female gametophyte differs from that of Coniferales only in the long neck of the archegonium and presence of a pollen-chamber, features which he explains on a physiological basis.

Thus all the essential features, both gametophytic and anatomical, indicate some relationship to the Conifers. Opposed to these and in favour of the Cycadalean affinity we have so far only the evidence of floral organization.

With regard to the Angiospermous relationship *Ephedra* presents several points of interest. Prominent among these is, of course, the possession of vessels. But, as has been pointed out, the vessels of *Ephedra* exhibit vital differences from those of the Angiosperms, although the rare occurrence of fusion of perforations lessens this difficulty. An equally striking feature and one which has been overlooked is the possession of broad rays like those of the lower Dicotyledonous woods, and especially their origin by compounding. This is really a feature of first importance. Among those of lesser value one should recall the separation of the leaf-traces on the stem and the presence of an absciss periderm in the leaf.

Summary.

The pith presents two striking features in the presence of a peridermal diaphragm at the base of each internode and occasional patches of lignified cells simulating centripetal wood.

The primary vascular bundles run regularly through one internode and lose themselves in a nodal girdle of tracheides. Throughout the internode their structure is endarch, but at the node occasional elements like transfusion tracheides are present in a centripetal direction. Transfusion tracheides also occur laterally to the bundles in the internodes of some species.

The leaf-traces are double and the two strands are inserted separately, usually with a vascular bundle between. In structure they are endarch throughout.

The tracheides of the secondary wood are characterized by the arrangement of the pits in both the Abietinean and Araucarinean fashion, structure

[1] On the Seedling Structure of Gymnosperms, IV. Gnetales. Ann. Bot., 1910.

[2] Spermatogenesis and Oogenesis in *Ephedra trifurca.* Bot. Gaz., 1904.

of pits typically Coniferous, tangential pits, bars of Sanio, and occasionally by tertiary spirals, trabeculae, and resin plates.

The vessels possess all the features just enumerated for the tracheides, and have also perforations which represent bordered pits. The transitions between tracheides and vessels are remarkably complete, all stages in the disappearance of torus and border being visible even in single elements. The vessels are very few in number and of a primitive character in the seedling, and more numerous though of the same character in the first-formed secondary wood of the branches. Fusion of perforations may be observed rarely.

The wood-parenchyma cells, which occur abundantly either scattered or in tangential rows, resemble tracheides in size, shape, lignification, and sometimes in pitting, and have probably been derived from tracheides. They are often multinucleate. They appear to resemble most the so-called fibrous cells of Angiosperms.

The medullary rays of the first-formed secondary wood are uniseriate, and from these the broad rays of the adult are derived either by simple enlargement, addition through the transformation of tracheides to ray parenchyma, or by compounding. The latter process is the same as in Dicotyledones. False rays are common. The individual cells are lignified and pitted like those of Dicotyledones. The broad rays have a retarding influence on the growth of the surrounding wood.

The bast is typically Gymnospermous.

The cortex is abundantly supplied with chlorophyll and functions as a leaf. The stomata in the epidermis of the stem are numerous, and are confined to furrows between projecting ridges due to hypodermal bundles.

The leaves are small and non-functional, except a few on the seedling. The vascular bundles are two in number, small and endarch. Transfusion tracheides are common. At the tip of the seedling leaves the transfusion tracheides develop centripetally and become more like centripetal wood.

The idea of Cycadalean and Bennettitalean affinity receives little support from the anatomy of *Ephedra*. On the other hand, there are many points which are opposed to it, and in favour of Coniferous relationship: the arrangement of the primary vascular bundles, double leaf-trace, arrangement and structure of pits on the tracheides, bars of Sanio, tertiary spirals, trabeculae and resin plates, primitive uniseriate lignified rays, wood parenchyma, and endarch vascular bundles of the leaf. The Gnetales do not appear to have arisen from any modern group of Conifers, but rather from or close to the base of the Coniferous line.

An Angiospermous affinity is indicated by the possession of true vessels, broad rays, formation of broad rays by fusion, and separation of the leaf-traces on the stem.

This investigation has been carried on by the writer in the Phanero-

gamic Laboratories of Harvard University, as an 1851 Exhibition Science Research Scholar of the University of Toronto. He is indebted to Professor E. C. Jeffrey for material and advice.

EXPLANATION OF PLATES XCIV–XCVII.

Illustrating Mr. Thompson's paper on the Gnetales.

PLATE XCIV.

Fig. 1. *E. monostachya*: transverse section of entire young branch. × 60.

Fig. 2. *E. trifurca*: transverse section, showing group of pith cells simulating centripetal wood. × 125.

Fig. 3. *E. altissima*: radial section through region of node, showing peridermal diaphragm. × 30.

Fig. 4. *E. altissima*: transverse section through lower part of node, showing girdle of primary tracheides. × 125.

Fig. 5. The same: section through node at exit of trace, showing the double character and separation of strands by a block of wood. × 125.

Fig. 6. *E. trifurca*: nodal section, showing double trace whose strands are not separated by wood. × 100.

Fig. 7. *E. altissima*: Tangential section through node, showing two strands of leaf-trace separated by wood. × 125.

Fig. 8. *E. vulgaris*: centripetal transfusion tracheide at node. × 667.

Fig. 9. *E. monostachya*: transfusion tracheides in internode. × 667.

Fig. 10. *E. monostachya*: transverse section of secondary wood. × 500.

Fig. 11. *E. trifurca*, root: radial section, showing Abietinean pitting and bars of Sanio. × 667.

Fig. 12. *E. distachya*: compressed Araucarian pitting and normal vessel. × 500.

PLATE XCV.

Fig. 13. *E. trifurca*, root: tangential pits, bars of Sanio, and broad ray. × 500.

Fig. 14. *E. californica*: tertiary spirals. × 667.

Fig. 15. *E. Gerardiana*: thickenings around pits. × 500.

Fig. 16. *E. trifurca*: showing trabeculae. × 500.

Fig. 17. *E. altissima*: tangential section, showing perforations in end wall of vessel. × 500.

Fig. 18. *E. Gerardiana*: tangential section near pith, showing perforations in association with bordered pits. × 500.

Fig. 19. *E. gerardiana*: tracheide showing first stage in transition to a vessel. × 667.

Fig. 20. *E. monostachya*: vessel showing gradual loss of torus and border. × 500.

Fig. 21. *E. distachya*: vessel showing fusion of perforations. × 667.

Fig. 22. *E. californica*: parenchyma cell whose pits are slightly bordered. × 667.

Fig. 23. *E. distachya*: wood-parenchyma cell with two nuclei. × 500.

Fig. 24. *E. trifurca*: wood-parenchyma cell, showing nucleus dividing. × 500.

PLATE XCVI.

Fig. 25. *E. monostachya*: septated wood-parenchyma cell and normal vessel. × 500.

Fig. 26. *E. distachya*: radial section of medullary ray. × 500.

Fig. 27. *E. altissima*, seedling: transverse section. × 60.

Fig. 28. *E. altissima*, seedling: tangential section near pith, showing formation and enlargement of medullary rays by transformation of tracheides. × 125.

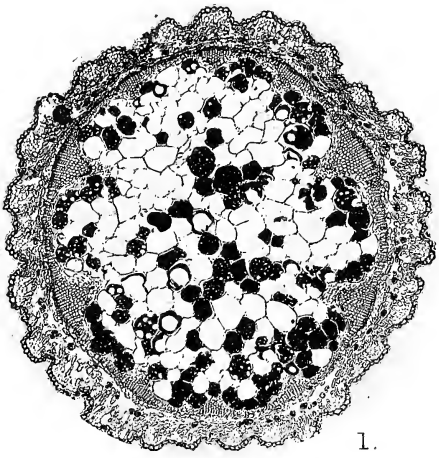
Fig. 29. *E. monostachya*: transverse section, showing compounding of rays and dip in annual rings. × 30.

Fig. 30. Transverse section of wood of *E. californica*. × 10.

Fig. 31. *E. californica*: tangential section of false ray. × 125.
Fig. 32. *E. trifurca*: 'false' ray. × 250.
Fig. 33. *E. monostachya*: subterranean stem, showing broad rays extending to pith. × 250.
Fig. 34. *E. Gerardiana*: bast and cambium. × 125.
Fig. 35. *E. altissima*: tangential section of bast, showing tapering sieve-tubes and lateral sieve-plates. × 250.
Fig. 36. *E. altissima*: radial section of bast, showing sieve-tube with sieve-plates in face view. × 667.

PLATE XCVII.

Fig. 37. *E. monostachya*: young stem showing epidermis and cortex. × 330.
Fig. 38. *E. altissima*: leaf-base. × 60.
Fig. 39. The same: vascular bundle with transfusion tracheide. × 667.
Fig. 40. The same: seedling leaf, showing centripetal transfusion tissue and bundles disappearing. × 500.
Fig. 41. The same: seedling leaf-tip with group of centripetal tracheides. × 667.
Fig. 42. *E. Gerardiana*: longitudinal section of leaf-base, showing abscis periderm. × 500.



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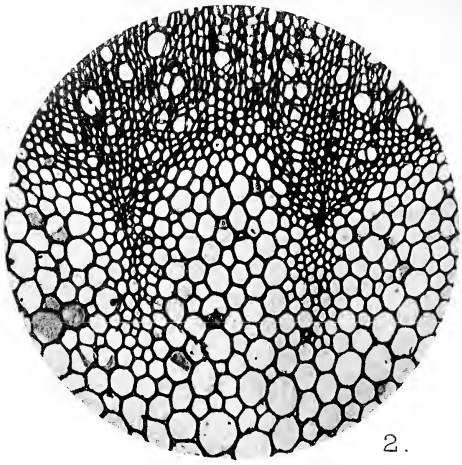

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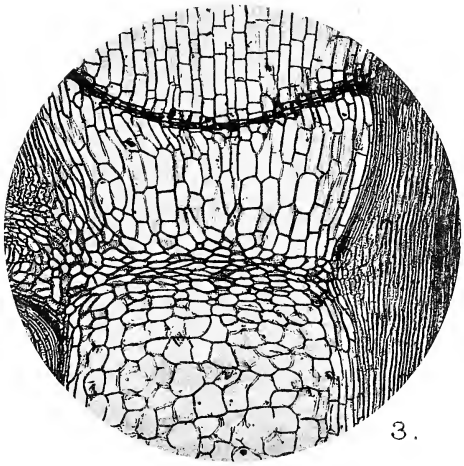

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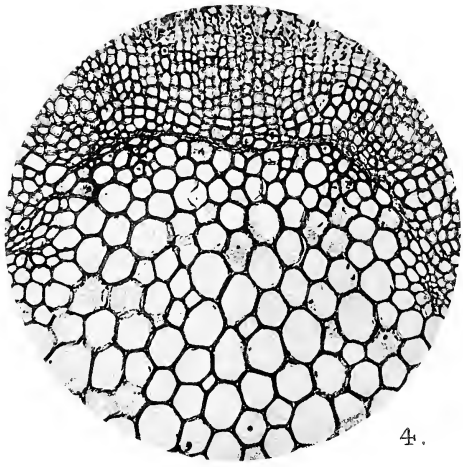

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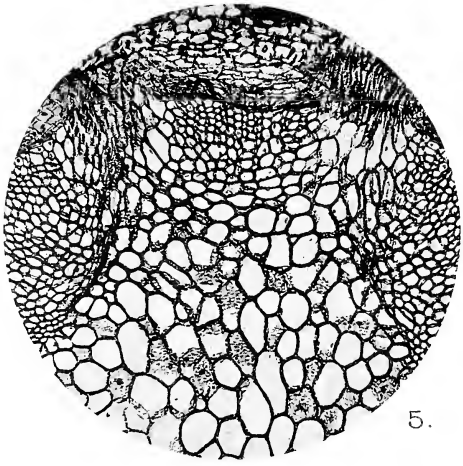

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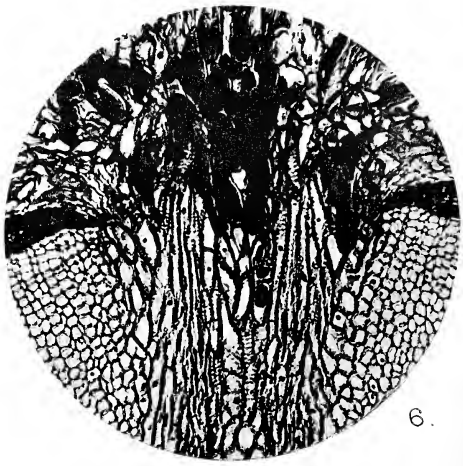

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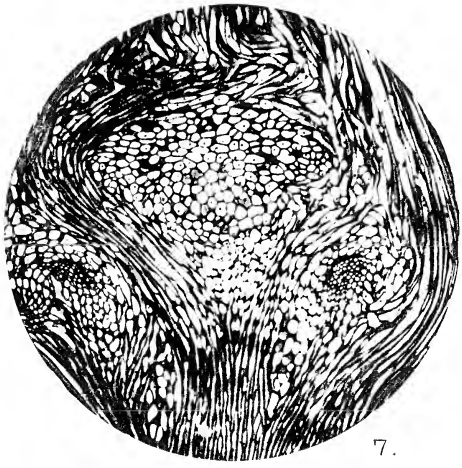

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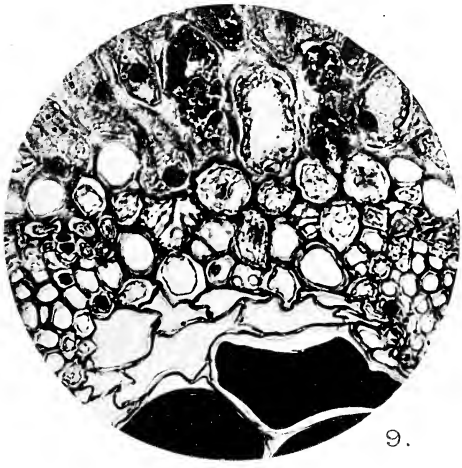

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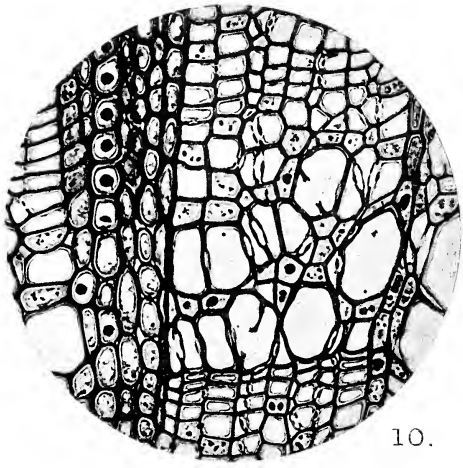

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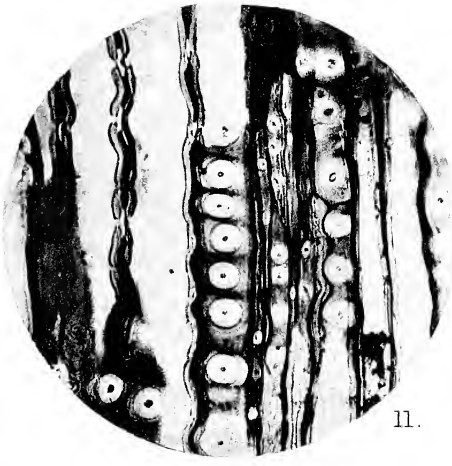

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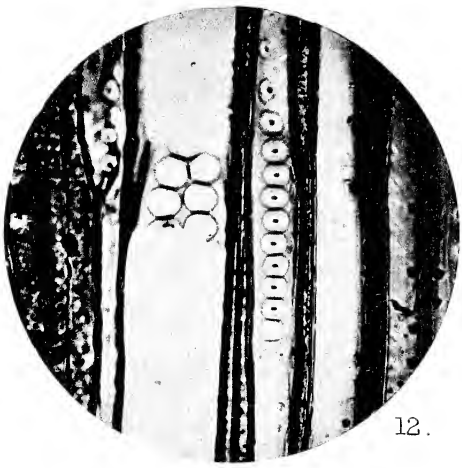

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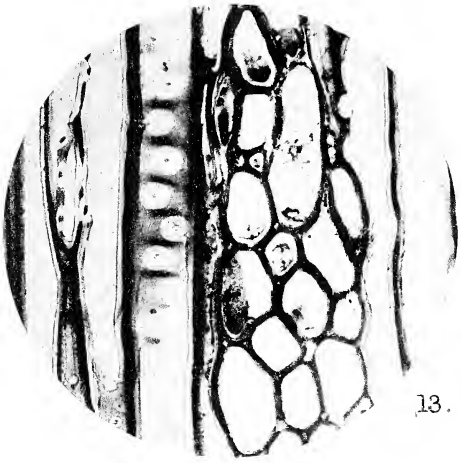

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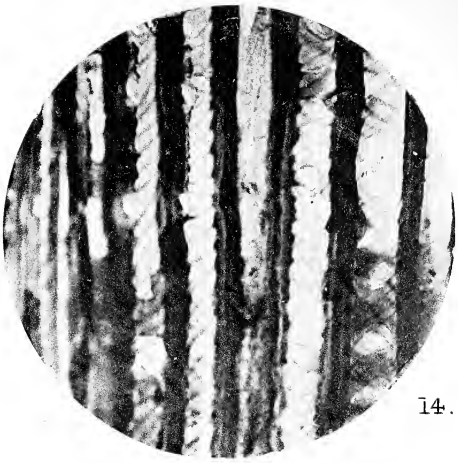

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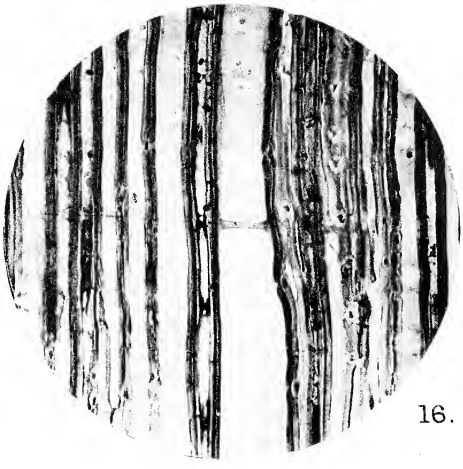

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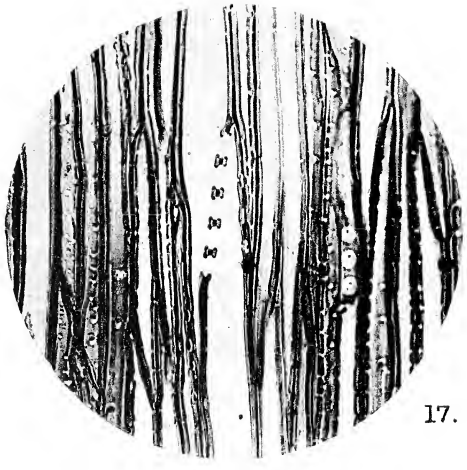

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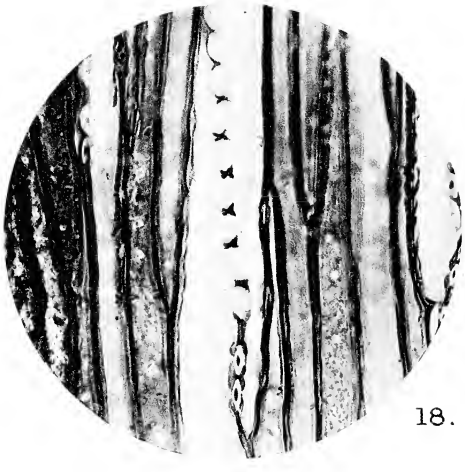

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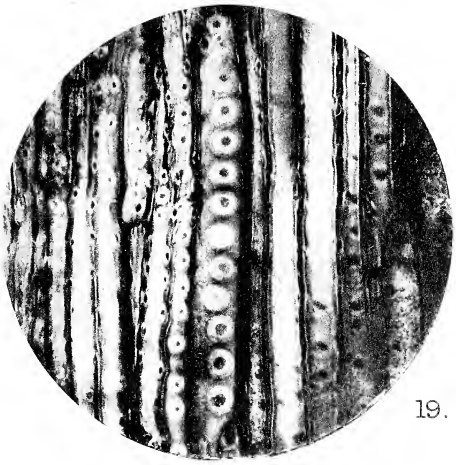

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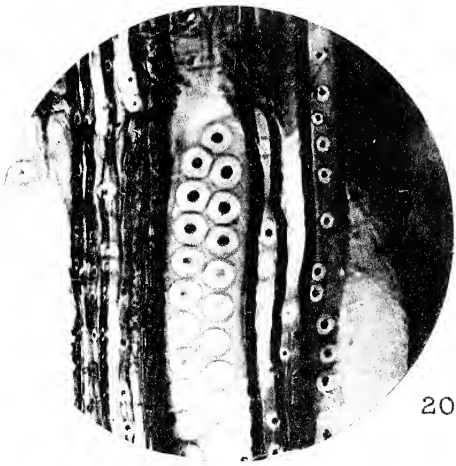

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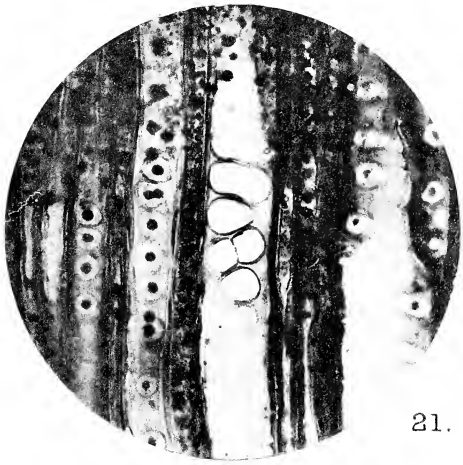

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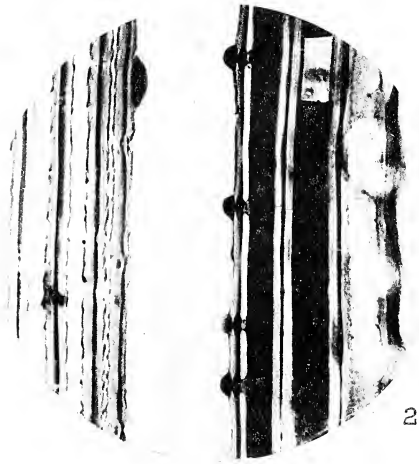

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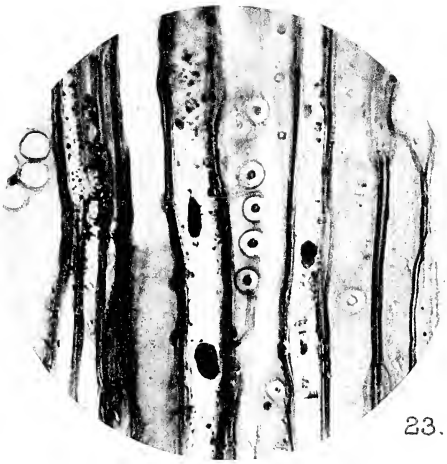

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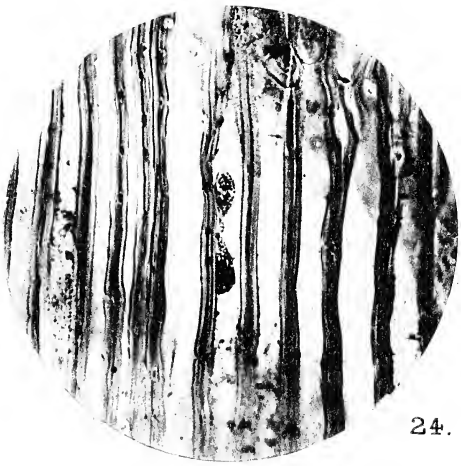

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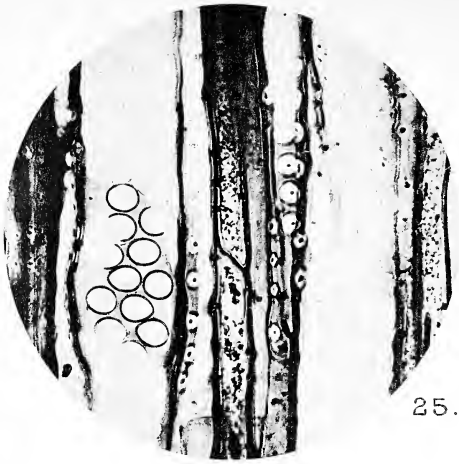

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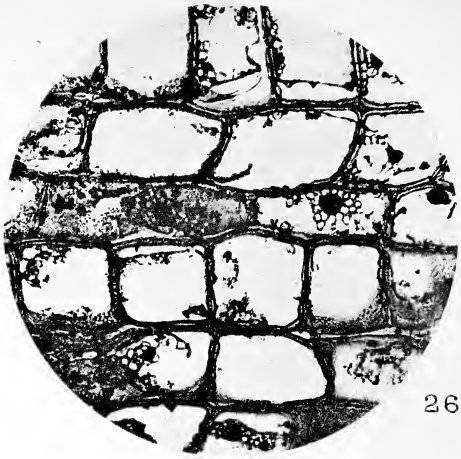

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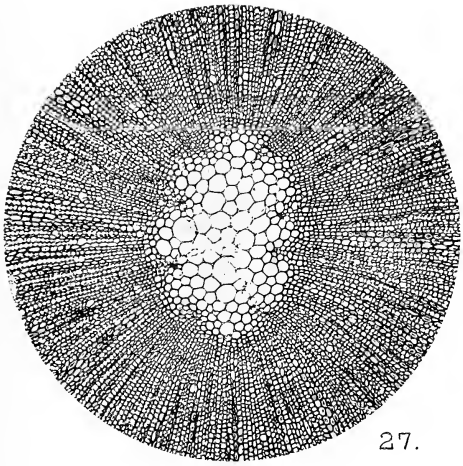

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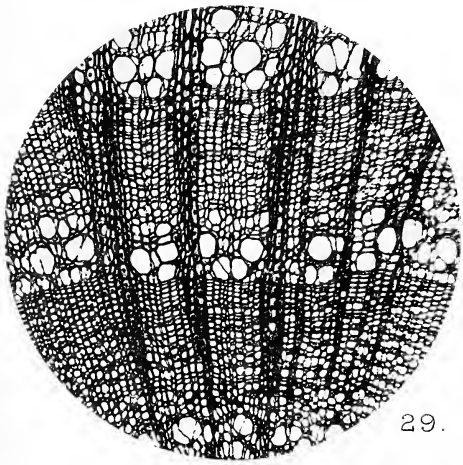

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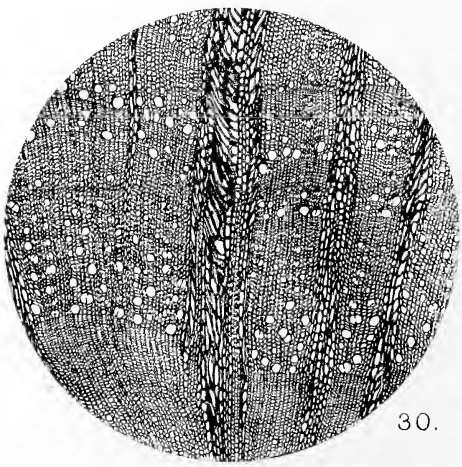

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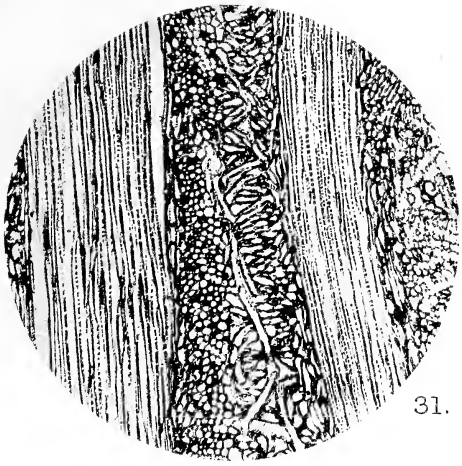

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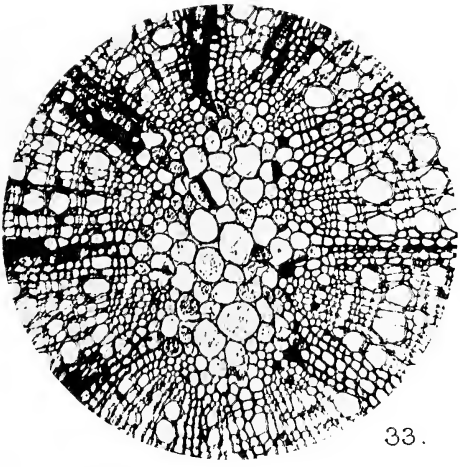

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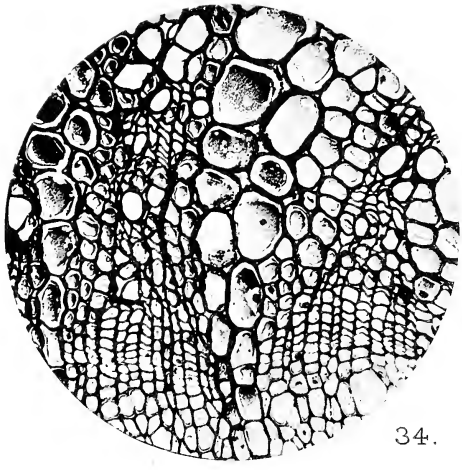

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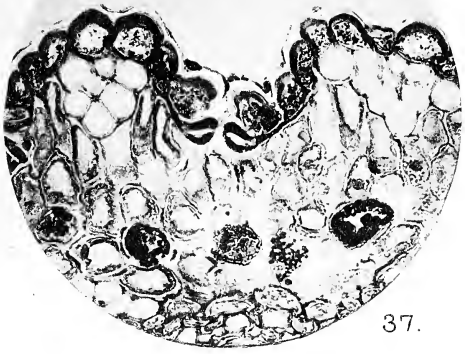

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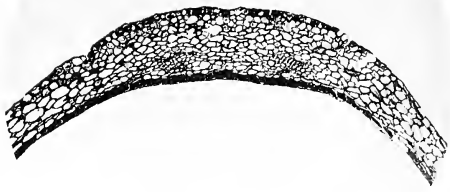

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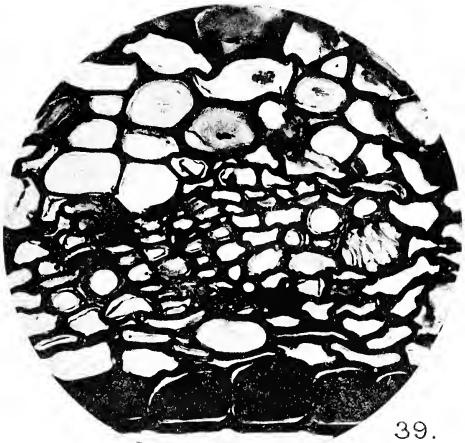

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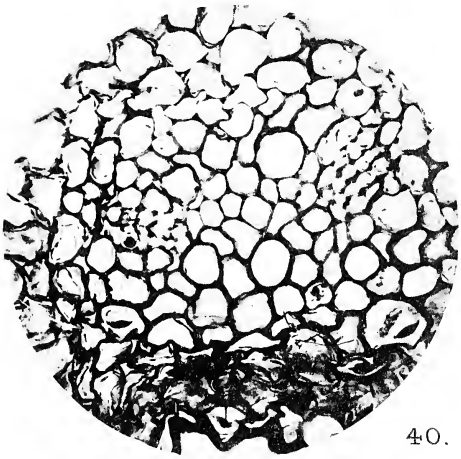

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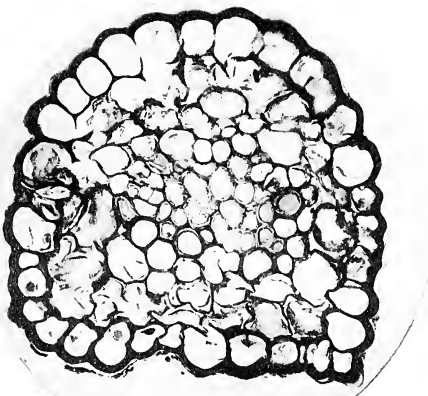

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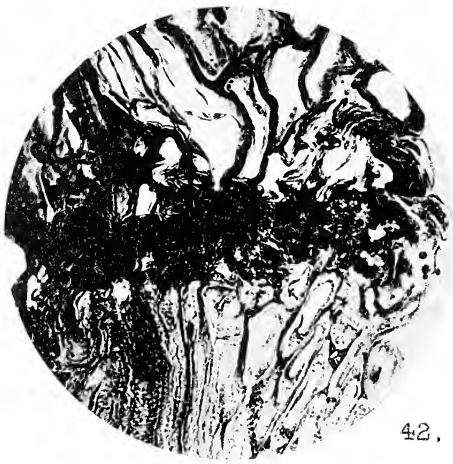

42.

THOMPSON.—EPHEDRA.

Some Plant Formations from the Arid Regions of Western China.

BY

F. KINGDON WARD, B.A., F.R.G.S.

THE arid or semi-desert regions to which the following notes apply comprise several deep trench-like valleys, trending for the most part north and south, which dissect the complicated mountain systems of far western China in the provinces of Kansu, Ssü-chuan, and Yun-nan, or as this country may be comprehensively termed, Chinese Tibet.

As examples to which I shall have occasion to refer we may instance the headwaters of the Min river in Southern Kansu, the T'ung-ho, and the Kin-sha-kiang (or upper Yangtze) in Ssü-chuan, the upper Mekong in North-West Yun-nan, and the upper Salween in South-East Tibet, north of lat. 28°. The arid nature and peculiar vegetation of these gorges in the midst of a region of copious rainfall, certain historical questions which obtrude themselves in connexion with one of the most characteristic components of the open formation met with, and the morphological adaptations of the flora, are interesting points which deserve to be considered in turn; but at the outset it will be well to explain how these barren gorges have come into existence in the first instance.

The rivers referred to here flow at altitudes varying from about 6,000 to perhaps 10,000 feet above sea-level, while the mountains immediately overshadowing them rise to very much greater altitudes, more especially in the case of the Mekong and Salween rivers.

Apart from the xerophytic nature of the vegetation, the fact that these rivers have been able to cut their way straight down between their investing walls is sufficient evidence for the dryness of the climate in the valleys themselves, but the big alluvial cones debouching into the main valleys speak just as eloquently of a furious rainfall above.

Where the rock is limestone clean-cut gorges are indeed always formed, owing to natural jointing; but even where the rocks are of granite, or of metamorphic origin, these rivers have managed to cut their way straight down, often leaving cliffs of rubble as much as 700 feet high.

Through these funnel-like valleys a daily wind blows up from south to north throughout the summer months with the regularity of a trade wind, springing up shortly before mid-day and dying away after sunset.

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I have remarked this scorching blast in all the valleys alluded to, particularly on the three biggest rivers, the Kin-sha, Mekong, and Salween, strongest of all perhaps on the Mekong, where it is more confined than anywhere else. Throughout the day it increases in ferocity, till about four o'clock in the afternoon it reaches a climax, then gradually calms down towards nightfall; and though rather of the nature of a sirocco, it is always extremely welcome after the intense heat of the day.

This wind is of course caused by the cold air sweeping down from the surrounding peaks to fill the partial vacuum caused by the great heating of the shut-in valleys during the day, and it greatly intensifies such desert conditions as are already imposed owing to the proximity of the mountains, which act the part of rain-screens. It is indeed a peculiarly desiccating wind, since it has already rid itself of its moisture, and throughout the summer it comes up the valleys like the breath from a hot furnace, till the withered vegetation on the cliffs seems gasping for water. Thus the severity of the conditions increases automatically as one travels northwards, for the intensity of the wind itself increases with the dryness produced by its action.

On the Salween and Mekong rivers an abrupt transition from a rainy region to one of extreme aridity corresponds to a general elevation above the snow-line of the dividing watersheds; the only break in the dreary scene is where a mountain torrent has flung out an alluvial cone which, terraced, and green with cultivation, forms a little oasis in the wilderness.

Travelling up the Mekong valley I have frequently noticed a long ribbon of blue sky faithfully following the valley, while the clouds, though perhaps clear of the mountain peaks, are massed in great puffs of cumulus on either hand, showing plainly enough that columns of hot air are ascending from the valley.

Once in the Mekong valley I watched the clouds in the south, from which snow and rain were actually falling at the time, trying to force their way up the valley into the arid region beyond, where blue sky prevailed; however, they failed signally to cross the dividing line, and it was as though some invisible barrier was forcibly holding them back, the truth being that the hot air rising from the bare rocks prevented any cloud existing as such.

Again, in the Salween valley I have watched the clouds gathering black in the west over the Irrawaddy, watched them sweep slowly over the valley, and reinforced still further, burst again over the Salween-Mekong divide to the east, a few scattered drops hissing on the scorched rocks of the Salween valley alone announcing their passage of that river.

It is abundantly clear then that the arid valleys of Western China owe their inception to just those causes which, on a much larger scale, give rise to continental deserts.

The scarcity of rain, which probably does not exceed five inches in a year, would of itself be a serious hindrance to plant life, and this is

aggravated by the scorching winds which blow throughout the vegetative season; consequently we here meet with an open formation of stunted shrubs and herbs more or less specialized to combat the hostile conditions.

Characteristic shrubs are *Sophora viciifolia*, *Bauhinia densiflora*, and several other Leguminosae, *Clematis Delavayi*, *Ceratostigma Griffithii*, species of *Pertya*, *Wikstroema*, and so on, none of them rising more than two or three feet from the ground.

A good deal of bare rock is necessarily exposed—indeed viewed from any distance these gorges often look quite devoid of life, except for the cultivable oases above referred to.

It is worth noting that the usual structure of river valleys is completely reversed in the case of mountain streams entering these arid gorges, and this is particularly true where the rock is of limestone. A river as a rule flows through ravines and gorges near its source, its valley gradually growing broader, till finally the stream is wandering sluggishly across a flat flood-plain. Here, however, a stream having its source high up in the mountains flows in a comparatively broad valley, winding through alpine pastures; gradually the valley contracts, passing from a typical U-shape in cross section to a pronounced V, the stream plunges down a long stairway, and finally cuts its way abruptly through a deep gorge to join the main river.

In this case the phenomena of reversed valley structure is emphatically a function of the climate apart from the nature and jointing of the rocks. I have drawn attention to it because it accounts satisfactorily for the fact that, while the main valley presents nothing but a dreary waste of rock, scattered amongst which are withered herbs and dwarfed spiny shrubs, the side ravines are often densely choked with vegetation, fresh and green.

This is not due to the fact that the stream itself supplies water to the cliffs and precipices of the gorge, which it certainly does not, but to the circumstance that the sunlight is kept out nearly all day, and consequently the heavy dews in a region of intense radiation are able to supply the requisite amount of water, a point I noticed frequently in the Mekong valley. Future travellers in these regions may however note that this reversed valley structure makes a journey up the Salween or Mekong one of the most appalling nightmares possible.

Though the bigger torrents enter the main valleys through richly vegetated gorges, the smaller and more intermittent ones throw out alluvial cones, and it is under these circumstances that irrigation by means of terracing becomes possible, so that villages occur only where a small stream debouches from the mountains.

Since in South-Eastern Tibet the rain-bearing winds pass over the mountains from the west, the valleys as we go east receive less and less rain, so that the most abrupt transition from a rainy to an arid climate

occurs, not in the easternmost valley, which is already shut out of a great part of its normal rainfall, but in the most westerly—the valley of the Salween ; nothing indeed could be more striking than the abrupt change from semi-tropical jungles in the southern rainy part of the valley, to semi-desert further north, a change rendered all the more striking by a correlated change in the people, from the tribesmen of the jungles to the Tibetans of Tsa-rüng in the north.

Granite occurs frequently in these valleys, and can be recognized from a distance by the clumps of Prickly Pear (*Opuntia vulgaris*) which grow amongst the 'tors' and boulders of the weathered rock; it apparently grows nowhere else but on granite rocks, and, conversely, wherever granite occurs there also will be found the Prickly Pear. Its interest lies in the fact that *Opuntia vulgaris* is a native of Mexico or California, and it is worth while inquiring how it found its way into Western China. I have traced it from Kansu through Ssü-chuan to South-East Tibet and Southern Yun-nan, cultivated of course for the sake of its fruit, and such a wide, if discontinuous, distribution at that distance from its original home is curious.

It is not of course the only plant which has wandered far from its home under circumstances hitherto unexplained, or merely conjectured ; we need only instance the maize, also said to be a native of Mexico, now cultivated throughout the world. But a cereal of such general utility as maize, in common with rice and other staple grains, was probably distributed over the globe from the very earliest times, whereas such is not likely to be the case with *Opuntia*, which after all is of no very great importance as a source of food.

Two suggestions present themselves,—the first that it was brought across the Pacific by the Chinese themselves, the second that it was introduced from Europe after it had been brought into the Mediterranean region from across the Atlantic ; a third alternative, that it was quite recently introduced by the Jesuit missionaries who came from America to China about the time of the fall of the Spanish Empire, is hardly tenable in view of its present wide distribution in Western China.

There can be little doubt that the Chinese visited California long before Columbus or possibly even the Norsemen discovered America, being carried across the Pacific accidentally by the Kuro Shwio, and voyaging across it when the intrepid Chinese mariners sailed those seas to the Indies, to Japan, perhaps even to Australia. It is equally well established that there was considerable intercommunication between Europe and Asia, particularly between Greece, India, and China, in very ancient times ; and if during this intercourse ideas on art and religion were exchanged, it is highly probable that other things were also, more especially useful plants and the ordinary commodities of trade.

Let us lastly consider the xerophytic flora in general, noting that one of its most constant features is the 'rosette' habit.

It is well exhibited by *Didissandra lanuginosa* and other species (10,000–12,000 feet), *Androsace Bulleyana* (9,000–11,000 feet), *Saxifraga candelabrum* (10,000 feet), and even *Eremurus chinensis* (9,000 feet) with its rosette of stiff spear-like leaves.

It is scarcely necessary to point out that the 'rosette' habit is not peculiar to xerophytes, being common for example on lawns (*Taraxacum*, *Bellis*), where it is doubtless brought about by the necessity for exposing as much surface as possible in a limited space, or to mutual pressure of parts.

But when the most successful plant of all found growing under abnormal conditions adopts a peculiar habit, and when moreover this identical habit is found in several other plants growing under similar conditions, we are justified in believing that this habit is in fact an adaptation to withstand those conditions.

Thus the most characteristic plant of all, growing in the semi-desert and arid valleys down as low as 7,000 feet, is *Selaginella involvens*, which covers the rocks in its thousands.

The 'rosette' habit protects the plant to a considerable extent against undue transpiration, the small, closely packed and overlapping leaves shading one another and forming an admirable protection for the growing point; moreover, the resulting dwarfed habit implies a minimum exposure of the plant to the desiccating winds, and is doubtless largely due to the brilliance of the direct and reflected light.

But the *Selaginella* referred to amply protects itself throughout the driest weather by rolling up into a ball like a hedgehog, thus exposing only the under surface of the leaves, which is silvery. Almost exactly the same device is adopted by the Fern *Cheilanthes farinosa*, each frond of which curls up into a little ball during the dry season, exposing a brightly silvered surface. It will probably be found that this surface, by reflecting the light and heat rays, reduces still further the transpiration from the under sides of the leaves.

Other familiar adaptations are fleshiness, found in several species of *Sedum* (stem and leaves), besides *Opuntia*, and the very thick cuticle of *Eremurus*; we need say nothing about them. But it may be noted that the 'rosette' habit often carries with it a biennial existence, e. g. *Androsace Bulleyana* and *Saxifraga candelabrum*, the first year being employed in storing the rosette with reserve food for the final effort of flowering in the second.

Finally, we may point out that there are not a few plants characteristic of the higher arid valleys which present none of the above obvious peculiarities; such are *Amphicome arguta* and the twining *Dregea sinensis*. But it is only fair to add that these are not found at the bottom of the deep

trenches where flow the Salween and Mekong rivers in the real semi-desert regions. On the whole perhaps the arid regions of Western China present features more interesting to the geologist than to the botanist, since we find practically the same adaptations to combat the hostile conditions here as are found in waterless regions elsewhere; nevertheless, a comprehensive survey of the flora and its various morphological peculiarities, with a knowledge of all the physical conditions which react on the vegetation, would be most instructive when we come to deal with distribution. For it is just these deep semi-desert valleys which intervene between the Himalayan flora and the flora of Western China.

On the Comparative Anatomy of the Genera Ceraria and Portulacaria.[1]

BY

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With Plate XCVIII and four Figures in the Text.

THE three species of the genus *Ceraria* are all typical xerophytes, well adapted to the dry situations in which the plants grow. The material for this investigation was obtained by Dr. Pearson in South-West Africa. As both species were found during the flowering season, which does not coincide with the period of greatest vegetative activity, some interesting points connected with the structure of the stem could not be determined with certainty. *Ceraria gariepina* was found on granite hills in Bushmanland, on the same slopes as *Pachypodium namaquanum*, in January, 1909 (Text-fig. 1).

Ceraria namaquensis, material of which was obtained in the Richtersveld in January, 1911, had previously been found by Atherston and Wyley in undefined localities in Namaqualand.[2] Both these species are small trees, averaging about twelve feet in height. Until recently *C. namaquensis* was known as *Portulacaria namaquensis*, under which name it appears in the 'Flora Capensis'.[3] It has now, on account of important characters of ovary and fruit, been placed in a separate genus, *Ceraria*,[4] of which two other species are known, *C. gariepina* and *C. fruticulosa*. *Portulacaria afra*, now the only species in the genus, is a large shrub common in the Karroo.

On account of the close systematic relationship between the two genera it was thought that a comparative study of the anatomy might prove interesting. The stem of *Ceraria* is covered with thick leathery bark, interrupted at regular intervals by the prominent nodes, each of which bears several minute sessile leaves. The plants were only seen during the flowering period, when the number of leaves was small. At other seasons the leaves are probably more numerous. They are extremely small (2–4 mm. in length), obovoid in shape, and extremely fleshy. The branching

[1] Percy Sladen Memorial Expedition in South-West Africa. Report No. 17.
[2] Pearson ('11), p. 191 (2).
[3] Sonder, pp. 385–6 (7).
[4] Pearson and Stephens ('12) (3).

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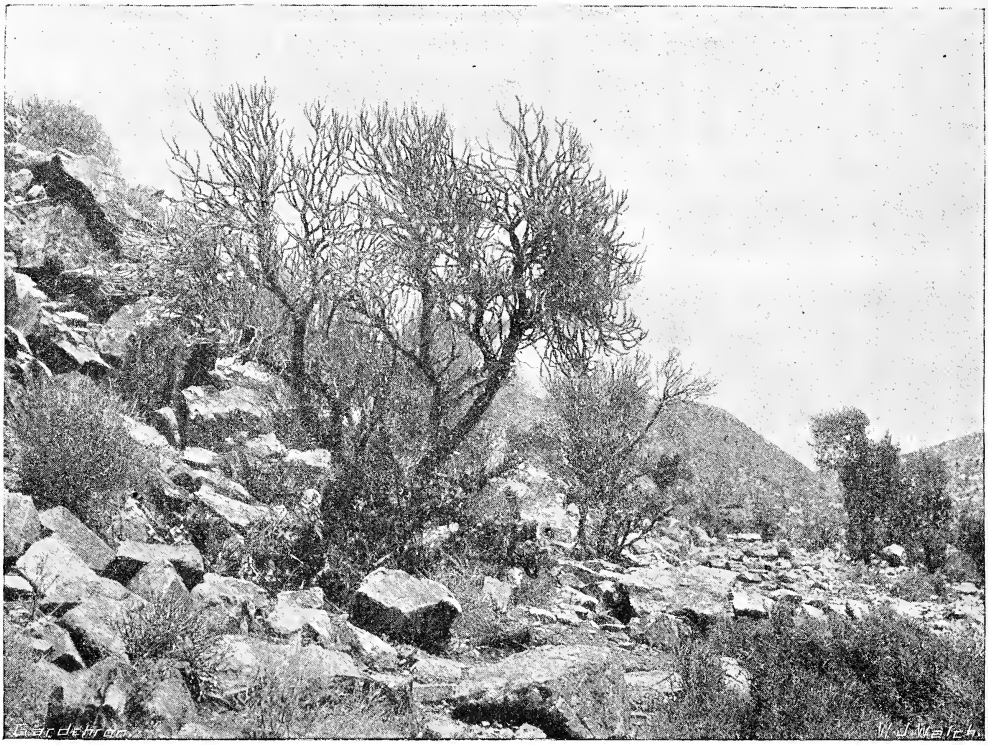
in this genus is pseudo-dichotomous,[1] therein differing from that in *Portulacaria*, where the branching is of the ordinary lateral type.

Solereder gives a brief account of the anatomical features of the Portulaceae,[2] but little work appears to have been done with regard to this order. Mention is made of a few of the outstanding characters of *Portulacaria afra*. None of the features of this order, upon which Solereder lays stress, is absent in *Ceraria*, although in a few minor details this genus seems



TEXT-FIG. 1. *Ceraria gariepina*. Photograph taken at Aggenys in Bushmanland in 1909 by Dr. H. H. W. Pearson. From the 'Gardeners' Chronicle'.

to have no parallel in the other genera. The arrangement of the mucilage cells, and the occurrence of calcium oxalate in the intercellular space beneath a stoma, may be quoted as instances of this.

ANATOMY.

A. Leaf.

C. namaquensis.

The structure of the leaf corresponds very closely with that of *Portulacaria afra*, to which genus *Ceraria* is closely allied. Proceeding from without inwards, it is found that the epidermis has its outer and inner walls cutinized to a very great extent (Pl. XCVIII, Fig. 1). The radial walls are

[1] Pearson and Stephens ('12) (3).

[2] Solereder ('08), vol. i, pp. 111–13 (6).

only to be seen with difficulty, and with a low-power objective the outer and inner walls alone are visible. The guard cells (Pl. XCVIII, Fig. 2) are spherical in section, and are accompanied by two subsidiary cells (Pl. XCVIII, Fig. 3) placed parallel to the pore. A peculiar feature of the leaf is the occurrence of calcium oxalate crystals in the intercellular space beneath several of the stomata.

De Bary [1] and Sachs [2] both cite a few cases in which calcium oxalate is present in the cell-walls of the epidermis, though the formation of crystals of this substance in intercellular spaces is unrecorded by them. According to Sachs the occurrence of calcium oxalate in the cell-walls is rare among Angiosperms, only having been observed by Solms-Laubach in certain species of *Mesembryanthemum* and in *Sempervivum calcareum*. Pfeffer has recorded the occurrence of these crystals in the cell-walls of some Dracaenas, and in these cases calcium oxalate is not confined to the epidermis, but is found also in cells lying deeper in the tissue. On the authority of Solms-Laubach it is stated that these crystals occur commonly in the Gymnosperms, being formed in the middle lamellae of the cell-walls. No indication of a similar origin of the crystals in *Ceraria* is given. No definite palisade tissue occurs, the outer cells of the mesophyll containing numerous chloroplasts. These decrease in number as the centre of the leaf is approached, though none of the central cells is devoid of them. Below the epidermis, large brown mucilage cells are found in which there are no chloroplasts. In this species the mucilage cells occur scattered in the first two rows of cells beneath the epidermis. The vascular bundles are small in section and vary but little in size. There are from seven to eleven bundles in a leaf, and these are deeply embedded in the tissues, so being invisible from the outside. The appearance in transverse section is that of a parallel-veined leaf, but it can be shown by cutting a section in the plane of venation that the bundles form a loose reticulum. Each bundle leaves the primary vein at a small angle, and almost immediately assumes a direction parallel to that of the central bundle. At the tip all the bundles branch and anastomose, so giving rise to a complex network.

Clustered crystals of calcium oxalate occur in the mesophyll. There are two kinds of these crystals. The first has a rough sandy appearance, due to the fact that all the crystals in a cluster are not of the same length and some project beyond the others, so giving the whole mass, when highly magnified, a spiny appearance. These crystals resist the action of nitric acid longer than the large crystals which are found beneath some of the stomata. This second kind of cluster is made up of a large number of slender wedge-shaped crystals, radiating from a centre and so forming a hemispherical mass.

[1] De Bary ('84), p. 102 (1). [2] Sachs ('82), p. 66 (4).

C. gariepina.

This species resembles the former in all its outstanding features. The following differences, however, must be noted:

(*a*) The mucilage cells are less numerous, and only occur in the cell row immediately below the epidermis.

(*b*) The crystals of calcium oxalate are more numerous, and the larger clusters occur in the cells of the mesophyll as well as below stomata. Crystal-blocked stomata are more frequently found in this species, about 20 per cent. of the stomata possessing the crystals.

The leaf of *Ceraria* affords a good example of the xerophytic structure so common in plants growing in dry regions. The size and shape of the leaf are to be noticed in this connexion. In *Ceraria* the xerophytism is more pronounced than in the allied genus *Portulacaria*, and the differences which exist between the leaves of the two genera are correlated with this fact.

B. Stem.

C. gariepina.

The most striking feature in a transverse section is the broad band of dark-brown mucilage cells occurring in the cortex. This ring does not appear to be found in the other genera of this order.[1] The stem is surrounded with a thick bark, an account of which is given below.

Cortex. In the cortex there are three distinct bands of tissue (Fig. 4). Next the bark are large water-storing cells, separated from one another by radial bands of parenchyma. The mucilage band occupies the greater part of the cortex, and consists of large mucilage-containing cells interrupted here and there by small parenchyma cells. The innermost layer of the cortex consists of ordinary ground tissue with groups of sclerenchymatous cells immediately opposite the bundles.

Endodermis. The endodermis is not marked.

Stele. The vascular bundles are arranged in a ring, and are very similar to those of *Portulacaria afra,* the chief difference in the vascular cylinder being that in *Ceraria* three or four of the medullary rays are broader than the rest. There are about twenty open bundles of the usual collateral type, and in the oldest stem available three faintly marked annual rings could be distinguished (Fig. 6).

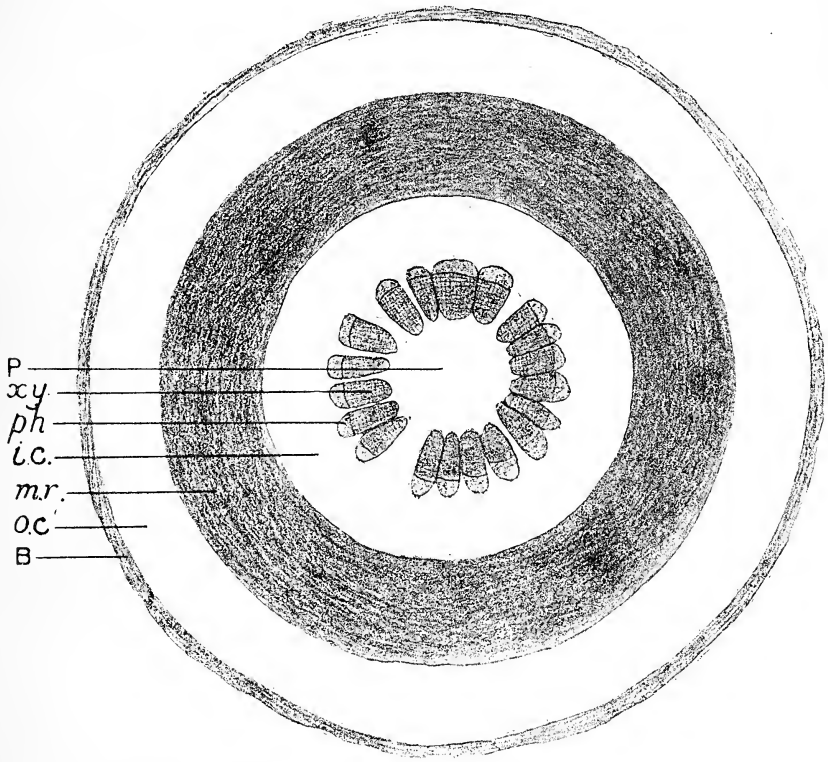
Phloem. The phloem is characterized by a great development of fibres on the outer side of each phloem mass. All elements of the phloem have an extremely small transverse diameter, and for this reason the separate elements are difficult to identify. The sieve plates occur on both the lateral and the end walls of the sieve-tubes, but owing to their small size, and the difficulty experienced in obtaining microtome sections, their

[1] Solereder ('08), p. 112 (6).

structure could not be determined (Pl. XCVIII, Fig. 5). A small amount of parenchyma occurs in the phloem, but companion cells have not been recognized with certainty.

Cambium. The xylem is separated from the phloem by a definite cambium, usually two or three cells wide.

Xylem. The most striking feature of the xylem is the entire absence of bordered pits,[1] simple pits being found in the vessels, tracheides, parenchyma, and fibres. The pits are often horizontally elongated, though



TEXT-FIG. 2. *Ceraria namaquensis.* Diagrammatic representation of a transverse section through the stem. P = pith; *i.c.* = inner cortex; *m.r.* = mucilage ring; *o.c.* = outer cortex; *b* = bark. × 16.

not enough so to justify the use of the term scalariform. Lignified parenchyma cells are present, but not to any great extent. Fibres are abundant as in the phloem, differing, however, in this case in being slightly pitted. The pits are more numerous on the walls adjoining the other xylem elements than elsewhere. Spiral tracheides, constituting the protoxylem, show no peculiarities.

Pith. The pith is small in amount, and consists entirely of parenchyma.

[1] Solereder ('08), pp. 111 and 113.

Clustered crystals of calcium oxalate are found in great abundance. In the large water-storing cells beneath the periderm, clusters occur which may extend over two or three cells. These clusters are larger, but otherwise resemble the second type described in the leaf (Pl. XCVIII, Fig. 7). Clusters of the first type are abundant in the parenchyma of the stem.

No starch is found in this species, probably owing to the fact that the material was obtained during the early part of the vegetative season.

Throughout the stem, drops of some fixed oil occur. These stain easily with either a 1 per cent. solution of osmic acid or an alcoholic solution of alkannin, but of the two alkannin is the more reliable stain. Certain organic compounds, as well as oil, are stained black with osmic acid; and as the mucilage ring stains easily with this acid, some organic compound must accompany the mucilage, which of itself is not affected by this reagent. The suberized cell-walls of the bark appear to be impregnated with some fat or oil, which, however, differs from the oil occurring in drops in being insoluble in ether, chloroform, or benzol. In the 'New Phytologist' an account is given by T. G. Hill of the use of 'Scharlack R' as a micro-chemical test for oil in plant tissues.[1] Any oil present stains pink, while other substances are not affected. In *Ceraria* this reagent only stains the oil in the cell-walls of the bark, leaving the oil drops colourless. As these drops stain easily with both the other reagents used, the result obtained with 'Scharlack R' cannot be considered a proof of their non-oily character. The cells of the innermost layers of the periderm have cellulose walls in which no oil is found. The oil appears simultaneously with the change in composition of the wall from cellulose to suber.

C. namaquensis.

The stem of *C. namaquensis* is very similar to that described above. The quantity of mucilage, however, is so great that sections cut and placed even in 70 per cent. alcohol swell immediately, and are unfit for staining. The chief differences are:

(*a*) The bands of parenchyma separating the large water-storing cells are in this case extremely narrow.

(*b*) Annual rings are absent, which fact, however, is not remarkable, as there are two rainy seasons a year in the regions in which this plant grows.

(*c*) The material of this latter species was gathered at the end of the principal vegetative season, and therefore it is not surprising that large quantities of starch are found in the parenchyma throughout the stem.

(*d*) The oil which is present in *C. gariepina* is found here as well, the only difference being that the quantity is considerably less. The suberized cells of the bark do not stain as deeply with alkannin in this species as they do in *C. gariepina*.

[1] Hill ('12), (10).

TEXT-FIG. 3. Bark of *Ceraria gariepina*. Photographed by J. A. Michell.

From what has been said it would appear that two distinct kinds of oil are found in the stem:

1. The drops of oil occurring throughout the stem in the cell cavities.
2. The oil permeating the corky walls of the bark.

It is possible that the oil-drops have been converted from starch into oil during the resting period, and that these plants are of the same nature as those designated by A. F. W. Schimper 'fat trees'.[1] The fact that no starch but much oil is found in *C. gariepina* which was gathered during the resting period, and that *C. namaquensis* which was obtained at the beginning of the resting period has a large quantity of starch and little oil, tends to support this hypothesis.

Periderm.

An interesting feature of *Ceraria* is the great development of leathery bark. This can easily be separated from the rest of the stem, and thus hollow cylinders of bark may be obtained in an unbroken condition (Text-fig. 3).

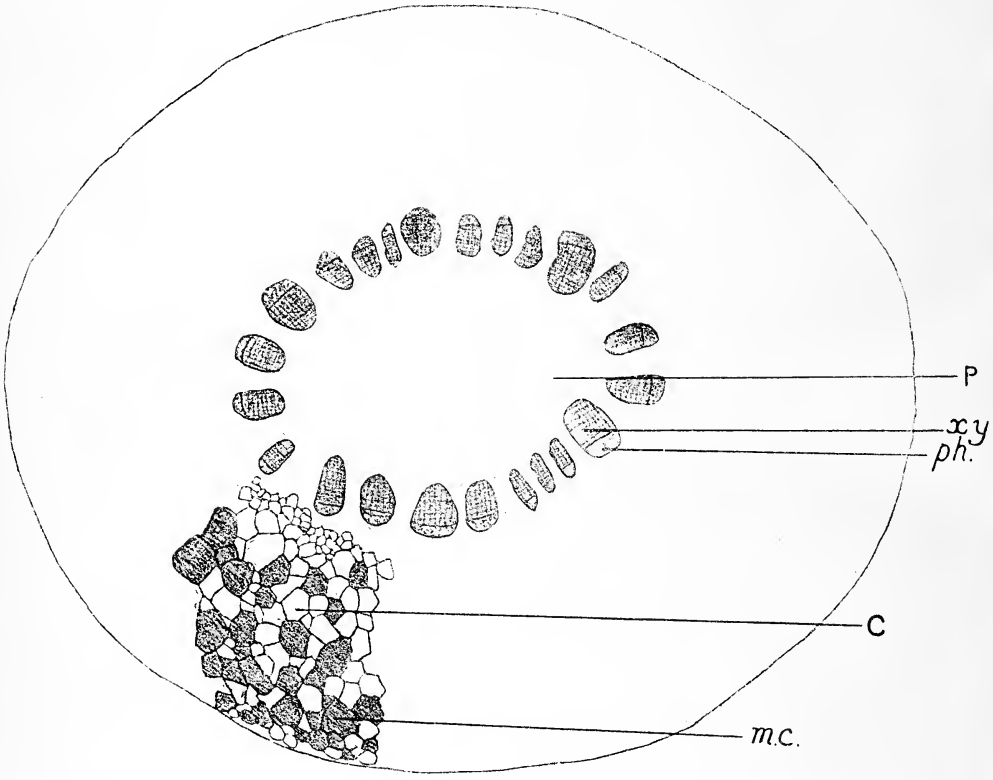
In the bark of *C. gariepina* there is some inflammable substance which does not appear to be present in as large quantities in *C. namaquensis*, and which causes the living plant to burn easily. The results of a chemical investigation of the constituents of the bark are not yet available. This phenomenon suggests a comparison with *Dictamnus albus*, the Candle-plant,[2] which secretes an extremely volatile oil. On calm hot days, if a match be applied the surrounding air takes fire and the plant itself burns.

In *Ceraria namaquensis* the apex of the stem is protected by a number of scale leaves, the base of which consists of mucilage cells similar to those of the stem (Pl. XCVIII, Fig. 8). Immediately behind the scale leaves the epidermal cells appear to give rise to a cambium which persists throughout the life of the plants, cutting off cells on its outer side only. It cannot be stated with certainty that this is a cambium, although it seems probable. Only in three or four cases have cells been seen to have divided recently, but considering that the material was gathered at the end of the vegetative season, it would appear that the cambium had entered upon a period of rest. Support is lent to this supposition by an examination of two lots of material of *Portulacaria afra* obtained in the Cape Peninsula. Material (A) was collected in June at the commencement of vegetative activities, and this shows that a large number of cells of the phellogen had recently divided. Material (B) was gathered in January, during the resting period, and in this only two or three cells of the cambium had recently divided. There is no phelloderm formed and the number of cell rows separating the cambium from the large water-storing cells of the cortex is constant. As a rule only one row of these cells is present. The cambium first of all cuts

[1] Schimper ('03), pp. 436–7.

[2] Willis ('04), p. 350 (9).

off tubular thin-walled cork cells, and continues doing so until from fourteen to eighteen rows of cells have been formed. The cells which are now cut off have not the definite rectangular outline which characterizes the first type (Pl. XCVIII, Fig. 9) of cell formed, and they possess slightly thickened cellulose walls (Pl. XCVIII, Fig. 10). These cells become more and more crushed and stretched as new cells are cut off from the cambium. It is not, however, till their outline is hardly recognizable that they become suberized.



TEXT-FIG. 4. *Portulacaria afra.* Diagrammatic representation of a section through the stem. P = pith ; *xy.* = xylem ; *ph.* = phloem ; C = cortex ; *m.c.* = mucilage cells. × 16.

In the older parts of the stem it is these cork cells which constitute the bark, the first formed cork having worn away. In this no cell outlines are visible, and the lamellated appearance in section is due to the tangential walls of the original cells remaining distinct, while only traces of the radial walls are left. In *C. gariepina* the cork consists of a number of layers easily separated from one another, and in the young stem every alternate layer stains with safranin. Later on the cork stains uniformly, but the layers still remain distinct (Pl. XCVIII, Fig. 11). Possibly each of these layers represents the total amount of growth in one year. The fact that in

C. namaquensis, where the xylem shows no annual rings, the cork is not as distinctly layered as in *C. gariepina* favours this supposition.

It is quite probable that when suitable material is obtainable, phelloderm may be found to be present, and in that case the position assigned to the cambium will be incorrect.

Material for examining the apex of the stem of *C. gariepina* was not available, but as the formation of bark on the lower parts of the stem in this species is identical with that in *C. namaquensis* it is not probable that any differences of a serious nature are to be anticipated.

In *Portulacaria afra* the bark is less strongly developed, but in the older stems it shows the same structure as in *Ceraria*. The phellogen arises in the epidermis (Pl. XCVIII, Fig. 12) and cuts off cells on the outside only, as in *Ceraria*. These cork cells are thin-walled but are not rectangular in outline, and become crushed almost as soon as they are formed. No band of cellulose cells separates the cambium from the cork (Pl. XCVIII, Fig. 13). In *Portulacaria afra*, therefore, only one type of cork cell is found, while in *Ceraria* there are two. The stem apex is protected by two young foliage leaves and not by scale leaves as in *Ceraria*.

The anatomy of the stem agrees very closely with that of *Portulacaria afra* (Text-fig. 4). The points in which these two genera differ may be tabulated as follows:

CERARIA.	*PORTULACARIA.*
LEAF.	
1. Leaf club-shaped.	1. Leaf flat and obovoid.
2. Cuticle very marked.	2. Cuticle not conspicuous.
3. Palisade tissue slightly differentiated.	3. Tissues not differentiated.
4. Crystals beneath the stomata.	4. No crystals beneath the stomata.
STEM.	
1. Thick bark of a peculiar structure.	1. Bark thinner but of same structure.
2. Large water-storing cells in cortex.	2. No water-storing cells.
3. Mucilage cells in a ring and of a large size.	3. Mucilage cells forming a network in the cortex and of same size as rest of cortical cells.
4. Four medullary rays larger than the rest.	4. Medullary rays all of same size.
5. Vascular cylinder occupying about one quarter of total area of stem as seen in section.	5. Vascular cylinder occupying about one-third of total area of stem as seen in section.

6. No tangential bands of parenchyma in the xylem.	6. Tangential bands of parenchyma in the xylem.
7. Large quantities of oil present.	7. Very few oil-drops in the cortex.
8. Scale leaves protecting the growing regions during the resting period.	8. No scale leaves present.

Nodes.

The leaves fall off when the plant is preserved in spirit, and therefore the exact position of the leaves at each node could not be ascertained. A section through a node showed a large number of scale leaves, each consisting of mucilage cells at the base and a dark-brown suberized mass at the apex. A growing point seems to be situated at each node, and in a large number of cases in the material worked upon this has given rise to an inflorescence. In order to investigate this point thoroughly it is necessary to have materials obtained at the different seasons, and this is impossible at present owing to the long journey which must be undertaken to reach the home of these plants. The following suggestion may be made as to the changes taking place.

In the axil of each leaf a growing point arises protected by scale leaves. These fall off as soon as the need for protection ceases, and the bud gives rise to a short branch bearing a number of closely packed leaves. At the end of the vegetative season scale leaves again arise to protect the growing point, and, as before, these fall off and the short branch terminates in an inflorescence.

In *C. namaquensis*, of which stem tips were available, scale leaves exactly like those of *C. gariepina* were found on all the nodes and also surrounding the stem apex. *Portulacaria afra* has no scale leaves, and the foliage leaves arise in a decussate manner at the nodes. Structurally there is no great difference between *Ceraria* and *Portulacaria*, and in comparing the two plants one is struck with the resemblances rather than with the dissimilarities. From the anatomy of these plants, therefore, one is led to conclude that a close relationship exists between these two genera.

In conclusion I wish to express my thanks to Dr. H. H. W. Pearson for supplying the material, and for many helpful criticisms during this investigation.

I am also indebted to the publishers of the 'Gardeners' Chronicle' for the loan of the block from which Text-fig. 1 was taken.

Summary.

1. In *Ceraria* the nodes are arranged in four longitudinal rows, many leaves being borne at each node. *Portulacaria* has decussate leaves.

2. Scale leaves occur in *Ceraria* at the nodes and at the stem apex,

probably protecting the growing parts during the resting period. *Portulacaria* has no scale leaves.

3. The leaves of *Ceraria* and *Portulacaria* are very similar. They are fleshy, have no palisade tissue, and possess mucilage cells in the mesophyll.

4. Calcium oxalate is common in the stem and leaf of both genera. In the leaf of *Ceraria* alone, however, crystals occur in the intercellular space beneath a stoma.

5. In the stem of *Portulacaria* and probably of *Ceraria* the phellogen arises in the epidermis; apparently no phelloderm is formed.

6. A large mucilage ring occupies the middle of the cortex in *Ceraria*. In *Portulacaria* the mucilage cells form a network in the cortex.

7. In both genera the vascular bundles, which are numerous, are arranged in a ring and are separated by medullary rays.

8. Fibres occur in both xylem and phloem. Simple pits only are found.

9. Drops of oil occur in the cells of the stem of *Ceraria*. The cell-walls of the periderm are impregnated with some fat or oil insoluble in chloroform, ether, or benzol.

Botanical Laboratory,
South African College,
April, 1912.

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EXPLANATION OF PLATE XCVIII.

Illustrating Miss Michell's paper on *Ceraria* and *Portulacaria*.

Fig. 1. Transverse section of the leaf of *C. namaquensis*. *ep.* = epidermis; *mc.* = mucilage cells; M = mesophyll; *xy.* = xylem; *ph.* = phloem. × 20.

Fig. 2. Transverse section of a stoma, showing a clustered crystal of calcium oxalate in the intercellular space; (semi-diagrammatic) *C. namaquensis*. *g.c.* = guard cell; *s.c.* = subsidiary cell; *cu.* = cuticle; *C.O.C.* = clustered crystal of calcium oxalate; *i.s.* = intercellular space. × 440.

Fig. 3. Surface section of the leaf of *C. gariepina*, showing a stoma. × 620.

Fig. 4. Cortex of *C. gariepina*. *s.* = sclerenchyma; *i.c.* = inner cortex; *w.s.c.* = water-storing cell; *cp.* = cellulose cells of the periderm; *s.p.* = suberized cells of the periderm. × 100.

Fig. 5. Longitudinal section of *C. gariepina*, to show sieve-tubes. *st.* = sieve-tube; *sp.* = sieve-plate on lateral wall. × 1,100.

Fig. 6. Transverse section of *C. gariepina*, to show part of two annual rings. *m.r.* = medullary ray. × 440.

Fig. 7. Water-storing cells of cortex, showing large clustered crystals of calcium oxalate in *C. namaquensis*. × 220.

Fig. 8. Outline of stem apex of *C. namaquensis*. *v.c.* = vegetative cone; *s.l.* = scale leaf; *s.m.* = suberized mass; *p.* = periderm; *pa.* = parenchyma; *v.s.* = vascular strand. × 33.

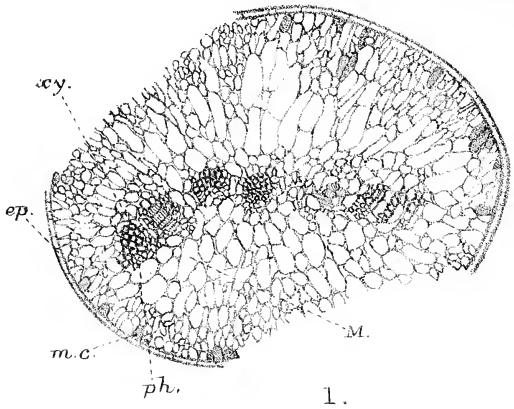
Fig. 9. Longitudinal section through the outer part of the cortex of *C. namaquensis* close behind the stem apex. *o.c.* = outer cortex; *c.c.* = phellogen. × 110.

Fig. 10. Transverse section through the outer part of the cortex of *C. gariepina*, showing a later stage in the development of periderm. *c.s.c.* = crushed suberized cells. × 147.

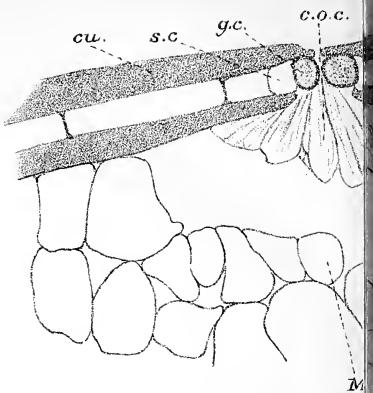
Fig. 11. Transverse section through bark of an old stem of *C. gariepina*. × 50.

Fig. 12. Transverse section of young stem of *Portulacaria afra*, showing origin of phellogen in the epidermis. × 440.

Fig. 13. Transverse section of an older stem of *P. afra*, showing formation of periderm. × 440.


xy.
ep.
M.
m.c.
ph.

1.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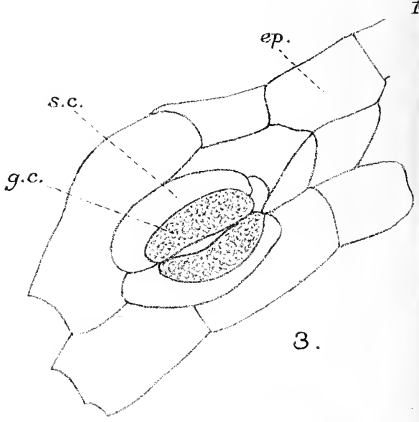
M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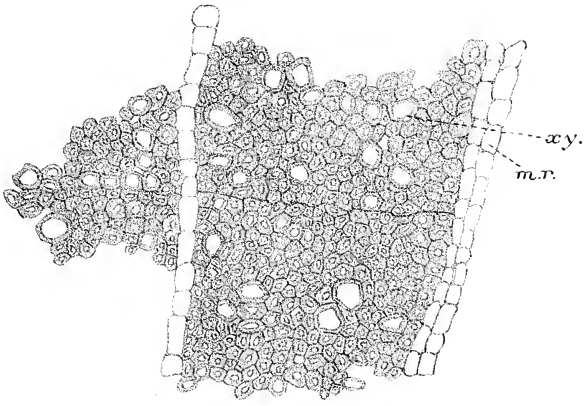
3.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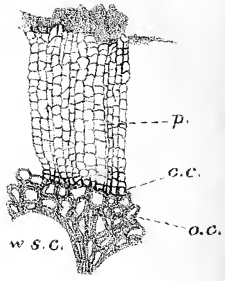
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o.c.
w s.c.

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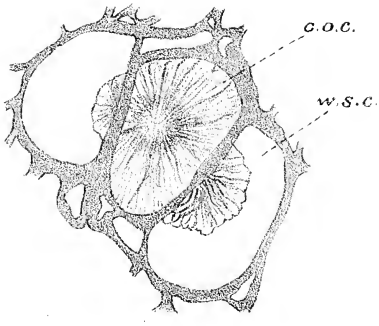
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w.s.c.

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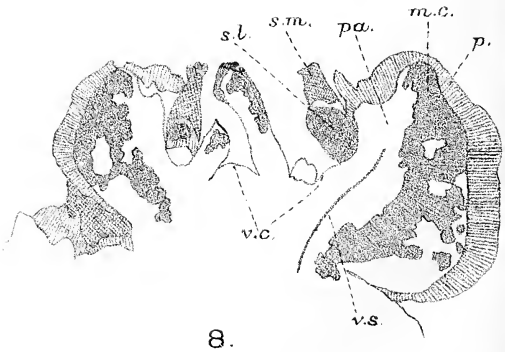
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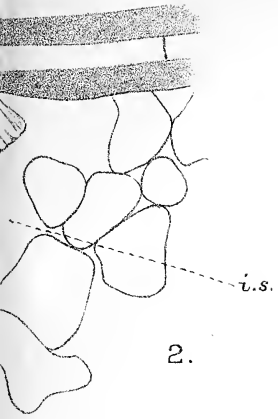

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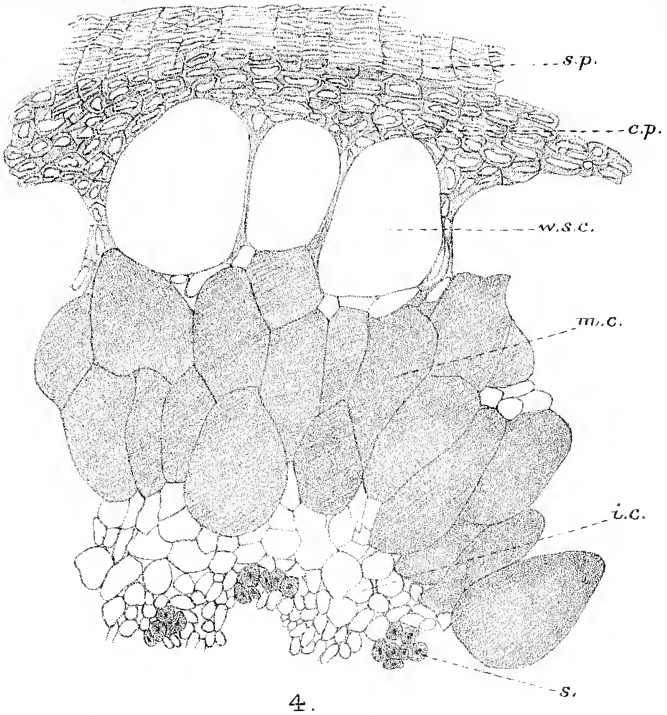

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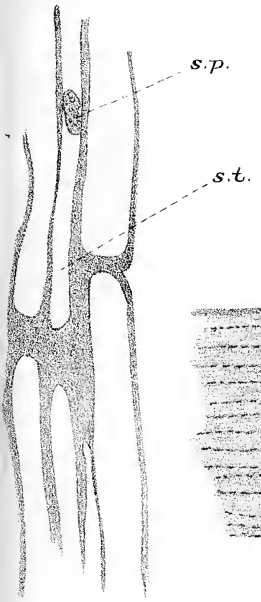

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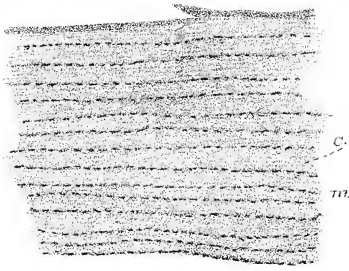

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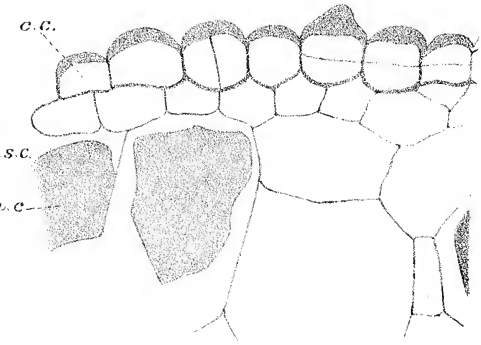

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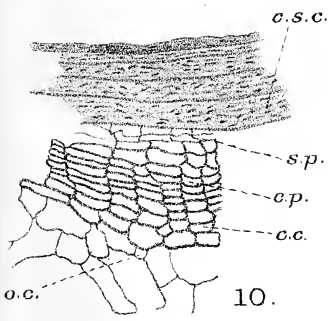

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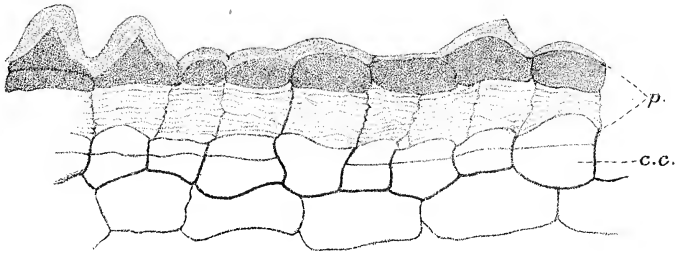

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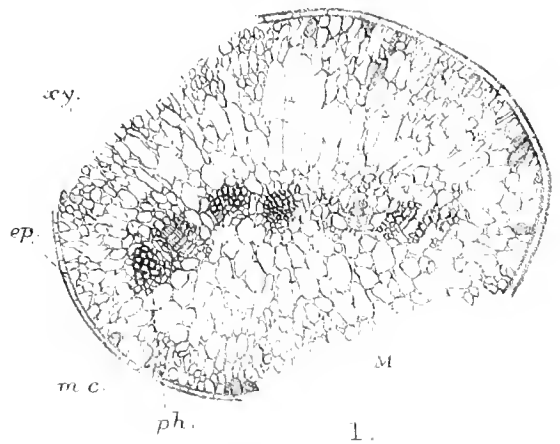

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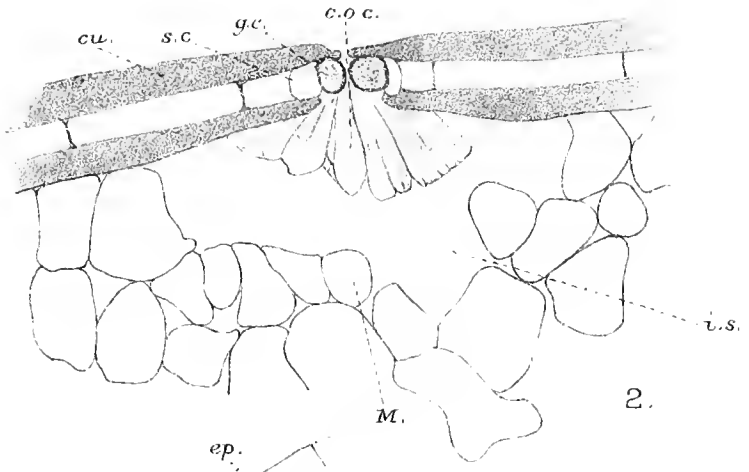

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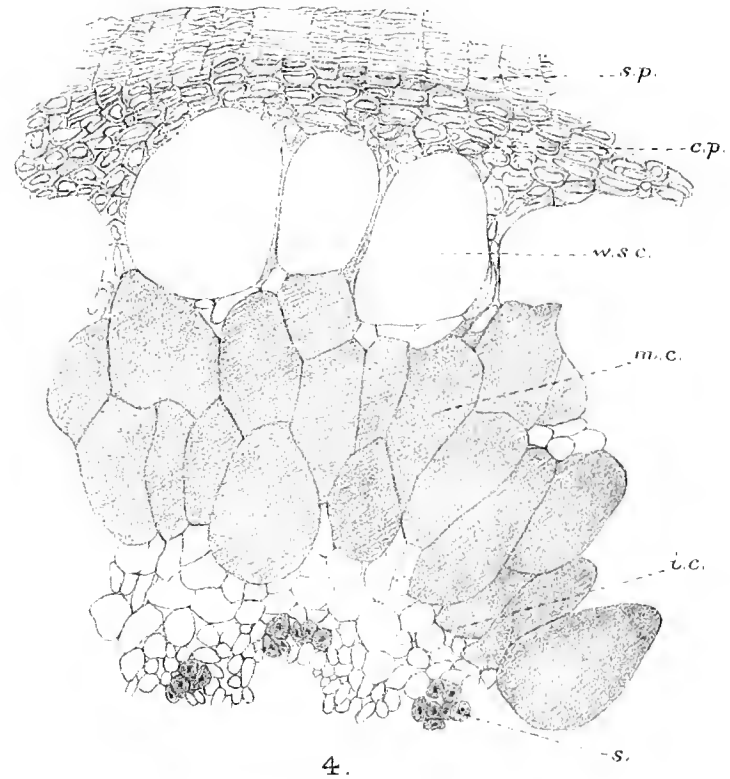

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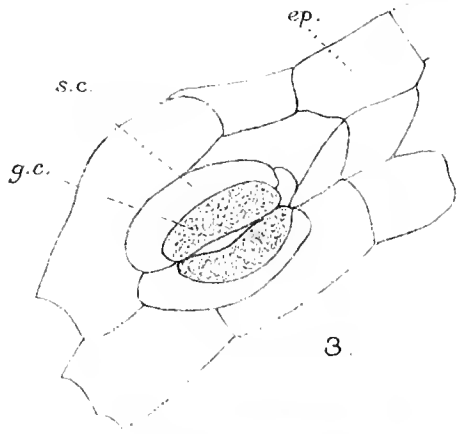

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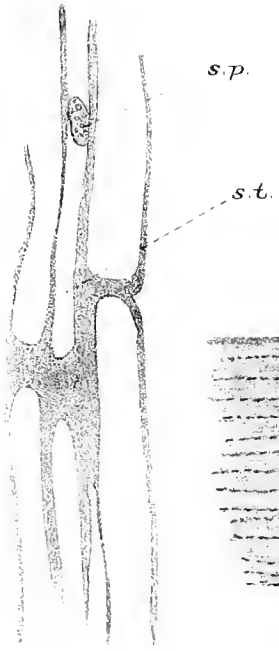

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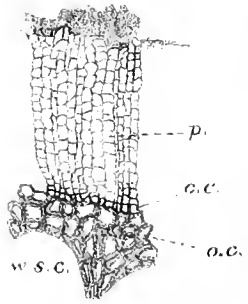

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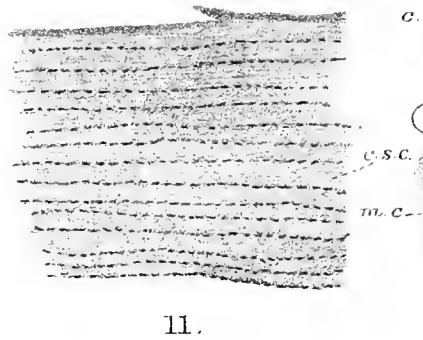

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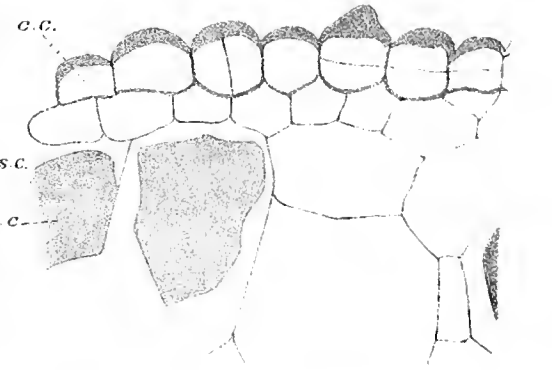

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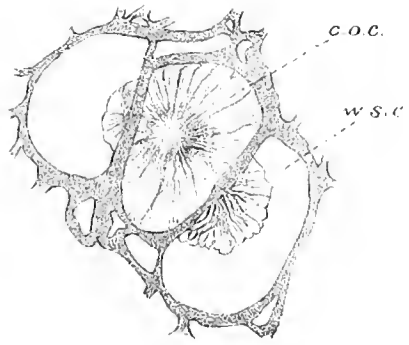

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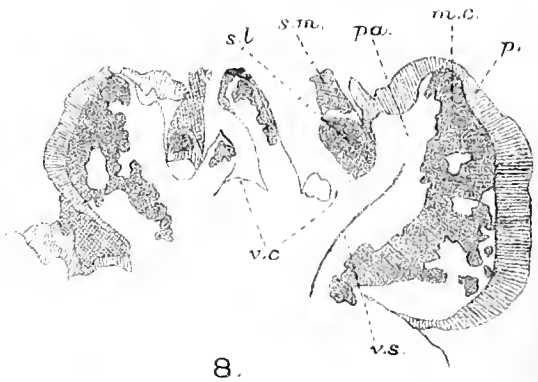

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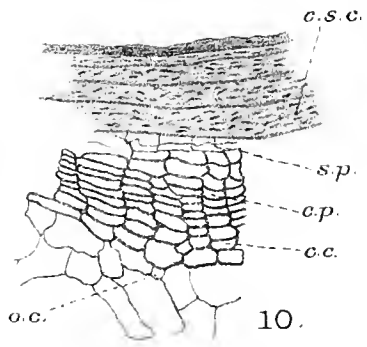

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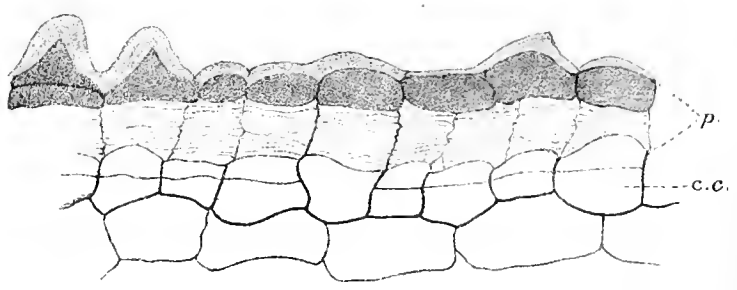

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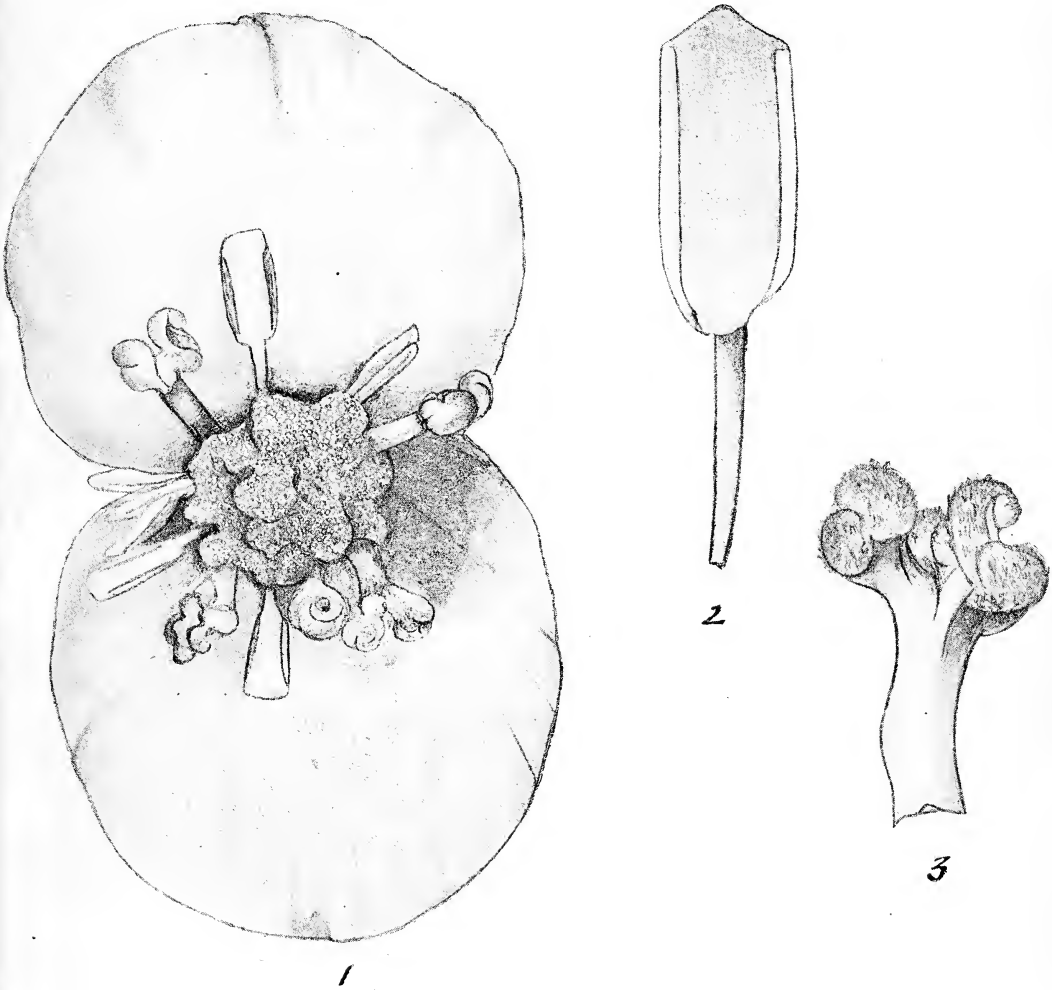
Huth lith et imp.

NOTES.

A BISEXUAL 'GYMNOSPERMOUS' BEGONIA.—Cases of teratologica interest, both floral and vegetative, are not infrequently exhibited by Begonias, but the instance here figured and alluded to is as unique as it is rare.



Begonia semperflorens, var. *gigantea*, Lemoine. 1. Abnormal flower × $3\frac{1}{2}$, showing stamens, styles, and exposed ovuliferous placentas. 2. Stamen with subacute connective. × 10. 3. Style. × 5.

It represents a bisexual flower of *B. semperflorens* var. *gigantea*, a floriferous garden hybrid (*B. semperflorens* × *B. Lynchiana*), having the gynaecium entirely

superior, while the ovules are wholly exposed owing to the disappearance of the protecting ovary-wall. This specimen was discovered among a batch of normally flowering plants in the Conservatory of the Royal Gardens, Kew, and proved to be the only example despite a careful search. The two petals had the colour and form usually associated with those of staminate flowers, the stamens numbering thirteen and being in every point a replica of normal ones, except that several of their connectives were subacute at their apices instead of truncate. Like the stamens, the styles originated from the base of the superior, exposed, ovuliferous lamellae, which latter appeared twisted and folded among themselves, and thickly studded with normally anatropous ovules of a dingy translucent grey. Three of the styles were perfectly normal, the remaining two exhibiting fusion with stamens. An instance of a somewhat similar nature [1] has been recorded and figured by Professor P. Magnus, but here the flower was undoubtedly of a more pistillate character, the gynaecium varying between a superior and an inferior condition, and the normal stamens being exceedingly few in number.

R. A. DÜMMER.

KEW.

THE MEDULLARY RAYS OF FAGACEAE.—Professor J. W. Moll has kindly directed my attention to a valuable paper that I overlooked in my recent work, namely, 'Die Gestalt der Markstrahlen im sekundären Holze,' written by K. Zijlstra ('Recueil des Travaux botaniques Néerlandais,' vol. v, 1908), who traced individual rays of *Fagus* and *Quercus* through numerous successive annual rings and was thus able to give a complete record of their change of shape and height. He confirmed L. Jost's observation that the tall primary rays of *Fagus sylvatica* undergo dissection in an outward direction. He also showed that when the secondary rays of this species are traced outwards they are seen to increase in height, to be joined by others which after linking become fused with them, and finally to fray out into more or less separate smaller rays. Zijlstra also demonstrated similar increase in height and outward fraying of the secondary rays of *Quercus Robur*. He thus proved that in Fagaceae the linking up of separate rays from within is not confined either to the annual rings of the seedling stem or even to primary medullary rays, and that both primary and secondary rays can fray outwardly into separate smaller ones.

A correction as regards fact is required in connexion with J. W. Bailey's paper occupying pp. 647–61 of this volume. Bailey refers to a specimen of wood that I described as belonging to '*Quercus* (*Pasania*) *fenestrata* (*Q. spicata*)' (*sic*), and goes on to write that he 'recently examined material of *Q. spicata* . . .' and is unable to agree with Professor Groom in stating that vessels are absent from the depressed segments between the approximated pairs of foliar rays'.

Bailey's attachment of the name *Q. spicata* to my material, and his statement as above quoted, imply that we both examined the same species of *Quercus*, and that the rays which I discussed were necessarily primary. Neither of these two implications

[1] Sitzungsb. Bot. Ver. Brandenburg, xxvi (1884), 72, f. 2.

is justified. As fully explained in my paper, the identity of my specimen (sent to me in log form direct from the forest) is dubious, and the evidence inclines against its belonging to *Q. spicata*. Hence the indication *prima facie* is that my specimen and Bailey's (obtained from Dehra Dun Research Institute) come from two different species. But the evidence is obviously incomplete, and the difference in structure of the two specimens does not necessarily imply that they come from two species, as in other species of *Quercus* transitional stages in the disintegration or integration of multiseriate rays demonstrate that vessels may occur in a region corresponding to one which is devoid of them in annual rings farther inwards or outwards.

PERCY GROOM.

NOTE ON THE ANATOMY OF STRIGA LUTEA, LOUR.[1]—A brief description of the general habit of this semi-parasite has been given in the present number of the 'Annals of Botany', in a study of its haustorium,[2] in connexion with which its anatomical structure has now been investigated. The plant in its mature (flowering) state shows in its aerial stem the general features—the dense, closed woody cylinder, the narrow lumina of the vessels, the absence of medullary rays, &c.—which characterize the majority of the Order Scrophulariaceae.[3] Its structure is almost identical with that of *Melampyrum pratense*, as described and figured by Hovelacque,[4] almost the only difference between the two being the presence of hairs in *Striga*, and the absence of the scattered 'trachées initiales' which are figured as occurring outside the protoxylem in *Melampyrum*. As in the latter, no cork or collenchyma is formed —a fact which may be correlated with its herbaceous habit. The hairy covering consists of both simple and glandular hairs. The former are large stiff glandular hairs, consisting of a single cell with thickened cuticularized wall, mounted on a base of several epidermal cells, and generally sharply recurved towards the apex of the stem. Among these are scattered the smaller glandular hairs, which consist of a short one- or two-celled stalk and a peltate head of three or four cells; similar hairs occur in a number of genera of the same order.[5] The transition from the aerial to the subterranean stem presents the usual features.[6] The stem becomes rounded instead of square in outline, the bast fibres disappear, and the width of the cortex increases, while that of the pith diminishes. The closed vascular ring breaks up into separate bundles, four main bundles and two leaf-trace bundles from the node immediately above.

[1] This investigation has been assisted by a grant from the Union Government.

[2] Stephens, Edith L.: The Structure and Development of the Haustorium of *Striga lutea*. Ann. Bot., vol. xxvi, p. 1067, 1912.

[3] Solereder, H.: Systematic Anatomy of the Dicotyledons, vol. i (English Translation). Oxford, 1908, pp. 583–9.

[4] Hovelacque, M.: Recherches sur l'appareil végétatif des Bigoniacées, Rhinanthacées, Orobanchacées et Utriculariées. Paris, 1888, pp. 384–90.

[5] Solereder, loc. cit., pp. 584–6.

[6] Costantin, J.: Tiges aériennes et souterraines. Ann. Sci. Nat., Bot., sér. 6, vol. xvi, 1883, p. 164.

The aerial leaf also presents no distinctive features. It is bifacial, with one or two rows of palisade parenchyma, and four to six of spongy parenchyma. Stomata are found on both surfaces, rather more abundantly on the lower. Hairs of the same character as those on the stem are found on both surfaces, the large simple hairs being always sharply bent backwards towards the apex of the leaf. In the transition to the tooth-like subterranean leaves, the mesophyll becomes less in amount and the palisade parenchyma is no longer differentiated. The stomata and hairs become much less frequent, and, as in the subterranean stem, the simple hairs are here thin-walled and point in any direction.

The root bears no root-hairs, which are seldom or never found on the roots of the Personate parasites.[1] The vascular bundle is diarch in structure; secondary thickening sets in very early. For figures showing the general structure of the root, those given by Hovelacque for the root of *Melampyrum cristatum*[2] may be consulted, as these show in all respects the same structure as that of *Striga*. I have been unable to demonstrate sieve-tubes in the root, though elongated parenchymatous cells, having the appearance of sieve-tube initials, are present. It is possible that these are sufficient for the purposes of translocation in the delicate tissues of the root, though of course it is equally possible that they may later on develop into sieve-tubes which have escaped notice.

It will be seen that *Striga* in its anatomical structure shows a general resemblance to other members of the order, and a close resemblance to certain other members of the section Rhinanthoideae. Its most distinctive feature is the comparatively feeble development of mechanical tissue, which may be correlated with its small size and partially subterranean habit.

EDITH L. STEPHENS.

South African College,
August, 1912.

[1] Hovelacque, loc. cit., p. 496.

[2] Hovelacque, loc. cit., pp. 473, 475.

ERRATA

P. 518, between lines 20 and 21 on the left *insert* Stalk in *Eupodocarpus*.
P. 525, line 11, *for* and *read* which
P. 529, line 29, *for* L *read* LI
P. 530, line 16, *for* 36 *read* 42
P. 534, line 35, *for* 77 *read* 76
P. 539, line 1, *for* 21 (second case only) *read* 20
,, line 20, *for* former *read* latter
P. 541, line 38, *for* come *read* comes
P. 542, footnote, line 6, for *elegans* read *septentrionalis*
,, ,, line 8, *for* more *read* less
,, ,, line 9, for *septentrionalis* read *elegans*
P. 555, line 9, *for* is *read* are
,, line 10, *delete* it is
P. 557, line 6, *insert* down *after* breaking
,, line 7, *for* usually *read* unusually
P. 568, Fig. 20, line 1, *for* tracts *read* bracts
,, Fig. 21, line 3, *for* tract *read* bract
P. 570, *insert* Fig. 57 *a*. Longitudinal section of the free apex of the integument, showing lignified cells. × 625.
P. 571, line 10, *for* 2–3 *read* 6–9

[Annals of Botany, Vol. XXVI. No. CII. April, 1912.]

FLORAL MECHANISM

By A. H. CHURCH, M.A., D.Sc.
LECTURER IN BOTANY IN THE UNIVERSITY OF OXFORD

The following statement has been drawn up by Professor Sydney H. Vines

THE object of this work is to provide the botanical student with a complete description of the development, morphology and mechanism of the principal types of flowers. Whilst giving the kind of information that is to be found in Payer's *Organogénie de la Fleur*, and in the late Professor Eichler's well-known *Blüthendiagramme*, it supplements this with an account of the ecology of the flower, including pollination and the formation of fruit and seed. Hence, when complete, it will be the most comprehensive treatise on the flower that has yet been published.

The general plan of the work may be gathered from Part I, which was published in 1908 as a royal 4to volume of 211 pages. In it are described the following twelve types of floral structure, selected from familiar garden flowers that bloom in the early part of the year (January–April):—

Helleborus niger . .	Christmas Rose.	*Viola odorata*	Sweet Violet.
Galanthus nivalis . .	Snowdrop.	*Narcissus Pseudo-Narcissus*	Daffodil.
Jasminum nudiflorum	White Jasmine.	*Erica carnea*	Heath.
Crocus vernus . . .	Blue Crocus.	*Ribes sanguineum*	Flowering Currant
Richardia africana .	White Arum Lily.	*Cydonia japonica*	Scarlet Cydonia.
Daphne Mezereum . .	Mezereon.	*Vinca major*	Greater Periwinkle.

In connexion with each type, two or three allied species are described for purposes of comparison.

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