ANNALS OF BOTANY

EDITED BY

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ASSISTED BY OTHER BOTANISTS

VOLUME XXV
With Eighty-seven Plates, and One Hundred and Seventy-eight Figures in the Text

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On the Structure and Affinities of the Palaeozoic Seeds of the Conostoma Group.

BY

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AND

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Quain Student of Botany in University College, London.

With Plates I–III and thirteen Figures in the Text.

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[Annals of Botany, Vol. XXV. No. XCVII. January, 1911.]

I. Introduction.

THE object of the following paper is a twofold one. In the first place, to present a detailed account of a small and homogeneous group of palaeozoic seeds, the hitherto practically unknown Conostoma group; in the second place, closely to compare these newly-described seeds with forms already familiar, viz. with the Pteridospermic seeds, *Lagenostoma* and *Physostoma*, and with *Gnetopsis*. Thanks to the kindness of Professor C. E. Bertrand and the extreme courtesy of Professor H. Lecomte of the Musée d'Histoire Naturelle at Paris, we have had at our disposal for comparison the superb and unique series of type specimens of *Gnetopsis elliptica* from the Renault collection. We are thus in a position to state our conclusions after consulting at first hand the whole of the preparations which bear on the subject.

The little-known genus *Conostoma* was founded by Williamson in 1877[1] for the reception of three of the smaller palaeozoic seeds, viz. *C. oblongum*, from the Gannister beds of the Lancashire coalfields, and *C. ovale* and *intermedium* from the Calciferous Sandstone Series of Burntisland. With the Burntisland seeds we have no concern here, as they are under reinvestigation at the hands of Miss Benson. We understand, however, that the two species are not really distinct and are being reduced to one, for the reception of which, in view of its structural peculiarities, Miss Benson is founding a new genus to be named *Sphaerostoma*.

Conostoma oblongum is one of the very rarest of Coal Measure seeds, and apart from a passing notice by one of us,[2] has not, so far as we know, found mention in the literature of Palaeobotany since the publication of Williamson's brief description in 1877. At intervals during the last nine years, however, specimens from Mr. James Lomax have been added to the University College Collection; these, together with one from Mr. W. Hemingway and two kindly lent us for description by Mr. D. M. S. Watson, form the whole of the new material at our disposal. One of Williamson's two type specimens is available for reference in the Williamson Collection;[3] his other type, the more valuable of the two, we have been unable to consult. It is probably in the Butterworth Collection, but we have not been successful in tracing the section.

During the course of our reinvestigation of *Conostoma oblongum* occasional sections of what appears to be a closely allied but undescribed species came into our hands. The first sections (received in 1904) were from Shore and Dulesgate; more recently a series of four transverse sections of a single

[1] W. C. Williamson: On the Organization, &c., pt. viii, Ferns, Gymnospermous stems and seeds. Phil. Trans., 1877, p. 243.

[2] F. W. Oliver: On *Physostoma elegans*. Ann. of Bot., vol. xxiii, pp. 99, 105, and 110.

[3] Nos. 1443 and 1444.

specimen were cut from a small block obtained many years ago by Dr. Kidston at Langendreer in Westphalia, and again this year another specimen has been found at Shore.

These specimens by themselves would have been inadequate for satisfactory description had they not been supplemented by Dr. J. W. Jongmans of Leiden, who, hearing that we were at work upon *Conostoma*, placed at our disposal with the greatest liberality both the sections from Duisburg, in Rheinpreussen, that were already in his collection, and uncut blocks from the same locality which have yielded us additional specimens of this undescribed seed. Thus it comes about that whilst the earliest and latest specimens are from Shore, Littleborough, the majority, and certainly the most valuable preparations, are derived from the nodules of Langendreer and Rheinpreussen. In view of this double source of type specimens we propose to name our new seed *Conostoma anglo-germanicum*.

The two members of the Conostoma group with which we deal fully in this paper are *C. oblongum* and *C. anglo-germanicum*.

Before proceeding to the detailed descriptions, it will be convenient briefly to outline the methods on which we have placed reliance in the reconstruction of these two seeds.

With objects preserved as petrifications in coal-balls accurate knowledge of form mainly depends on the interpretation of sections cut at different heights and at varying angles. When the object under investigation is of convenient size it is usually possible to procure series of sections cut parallel to one another at regular intervals. Such series are readily drawn or photographed, and models in wax or other plastic material constructed, so that the object can be faithfully reproduced on any desired scale. In the case of small objects only a few millimetres in length and of complex structure, like our seeds, this direct method is not available. For in virtue of its smallness such an object is apt to evade detection in the matrix until a chance section reveals its presence. Moreover, for the same reason, even when detected before cutting, its very smallness places a limit to the number of sections which can be cut through it. In other words, the ordinary methods of the palaeobotanist break down when applied to the investigation of minute objects.

Our experience with *C. oblongum* affords a good example of what must be a common predicament. In all, sixteen sections were available, cut at varying angles through sixteen distinct specimens. Of these sixteen sections fourteen were oblique longitudinal sections through the body of the seed, one a transverse oblique, and one transverse across the stalk.

In one respect our task was sensibly lightened at the outset, for, owing to the histological peculiarities of the testa, no serious doubt was ever entertained as to whether a given section belonged to our seed or not.

Our object was to reconstruct our seed in the form of an enlarged model upon which the planes of the various sections could be plotted, and then, by cutting along the appropriate plane, any given section could be reproduced. We began by plotting a sketch of a provisional median longitudinal section, using for the purpose such data, not exaggerated by obliquity of plane, as could be derived from the most favourable preparations available. As each successive section was handled the sketch of course underwent gradual modification.

Thus, every section contributed something; and in the end, after repeated correction, a sketch was obtained upon which all our sections could be approximately plotted. This 'ideal' section could at best be no more than an approximation, because the sections upon which it was founded had been cut from a large number of specimens which from the nature of the case would show some variability of dimensions, even if the extreme assumption were made that they all belonged to the same developmental phase.

With the 'ideal' longitudinal section as basis, a model of the halved seed on a scale of 40:1 was constructed in papier mâché, together with a number of identical plasticine models of the complete seed on the same scale from which to reproduce our original sections. The papier mâché model, which gave internal details as well as surface relief, was of course of great utility in orientating the planes to be followed in sectioning the plasticine models. For accuracy in cutting the sections of the models a simple guillotine or microtome was devised. It consisted essentially of an oblong frame hinged at its narrower end on to a board which formed the support on which the model was placed for sectioning. The plasticine model projected through this frame (which could be adjusted at any required angle), and the section was cut by sliding a taut copper wire along the smooth upper surface of the frame. By the use of this contrivance we have found it possible to reproduce in essentials any given section of our series. Repeated trials have often been necessary, for the slightest differences in either the plane of cutting or in the relation of the point of entrance of the stretched wire to the surface bring about very marked differences in the contours of the sections produced. In other words, form, as expressed in a section, is an extremely sensitive thing, subject to very striking fluctuations from apparently trivial causes. What is true of the form of an object like a seed holds also in the case of minute histological detail.

When reliance has to be placed on the study of oblique sections (an everyday occurrence in palaeobotanical work) we have found wrong inferences, as to the three-dimensional figure of the elements cut, to be almost inevitable without checking by means of models.

In the case of the seeds described in this paper, the plotting of almost every section has been verified by following the method outlined above, and

as a result we are able to publish our results and reconstructions with much more confidence than would otherwise have been the case.

We now pass on to the detailed description of *Conostoma oblongum* and *anglo-germanicum*. This is followed by comparisons with allied seeds and by a series of diagnoses, including those of the genus and species; the paper concludes with a general discussion on points arising out of the work.

II. Conostoma oblongum.

1. *Enumeration of specimens.*

Our account of this seed is based on the following preparations, all from the seam nodules of the Lower Coal Measures of Lancashire and Yorkshire.

University College Collection.

		When received.
R. 110	Dulesgate	Feb., 1905
R. 111	Shore	May, 1906
R. 112	Shore	1906
R. 113	Halifax	Dec., 1907
R. 114	Halifax	Dec., 1902
R. 115	Shore (contains two specimens)	1906
R. 116	Shore	Dec., 1905
R. 117	Shore	Oct., 1904
R. 118	Shore	1906
R. 119	Shore (contains two specimens)	1908
R. 120	Shore	Feb., 1906
R. 121	Shore	Apr., 1907
R. 122	Shore	May, 1909
R. 123	Deighton, Yorks. . . .	1910

Mr. D. M. S. Watson's Collection.

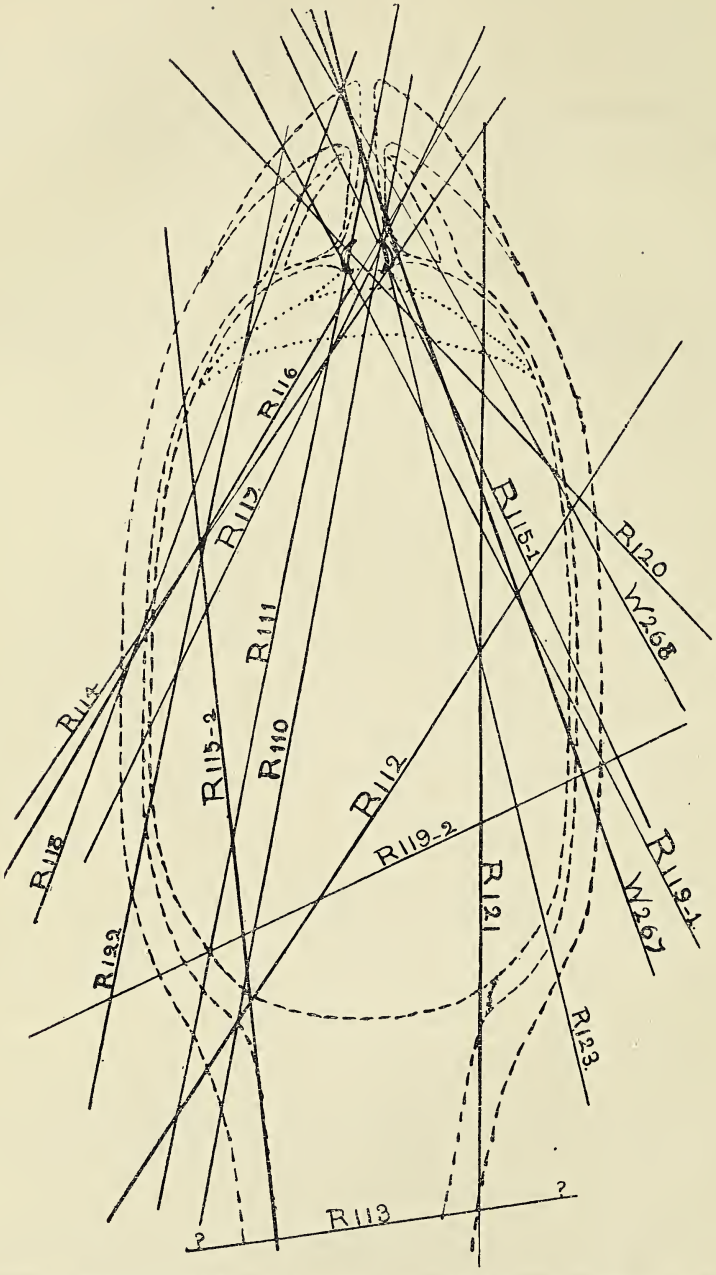
W. 267	Shore	1908
W. 268	Shore	1908

Williamson Collection.

Nos. 1443 and 1444 Oldham.

The only other specimen that we know of is that figured by Williamson in his 8th Memoir (1877), Pl. XII, Fig. 86. It was lent him by the late John Butterworth, and has not been seen by us.

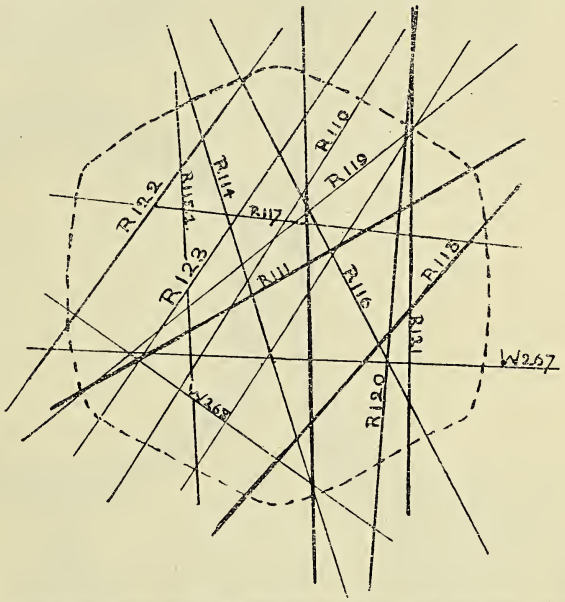
The positions of these sections, other than those in the Williamson Collection, are plotted in relation to the median longitudinal section of the seed in Text-fig. 1, and in relation to the transverse section just below the level of the tapetal septum in Text-fig. 2.



TEXT-FIG. 1. Diagrammatic sketch of a median longitudinal section of *Conostoma oblongum* upon which are plotted the approximate positions of the planes of section of all preparations used in this paper. The reference letters and numbers given with each section on the figure are the designations under which the preparations are cited in the explanation to the plates. R = University College Collection; W = Mr. D. M. S. Watson's Collection.

2. *General Features.*

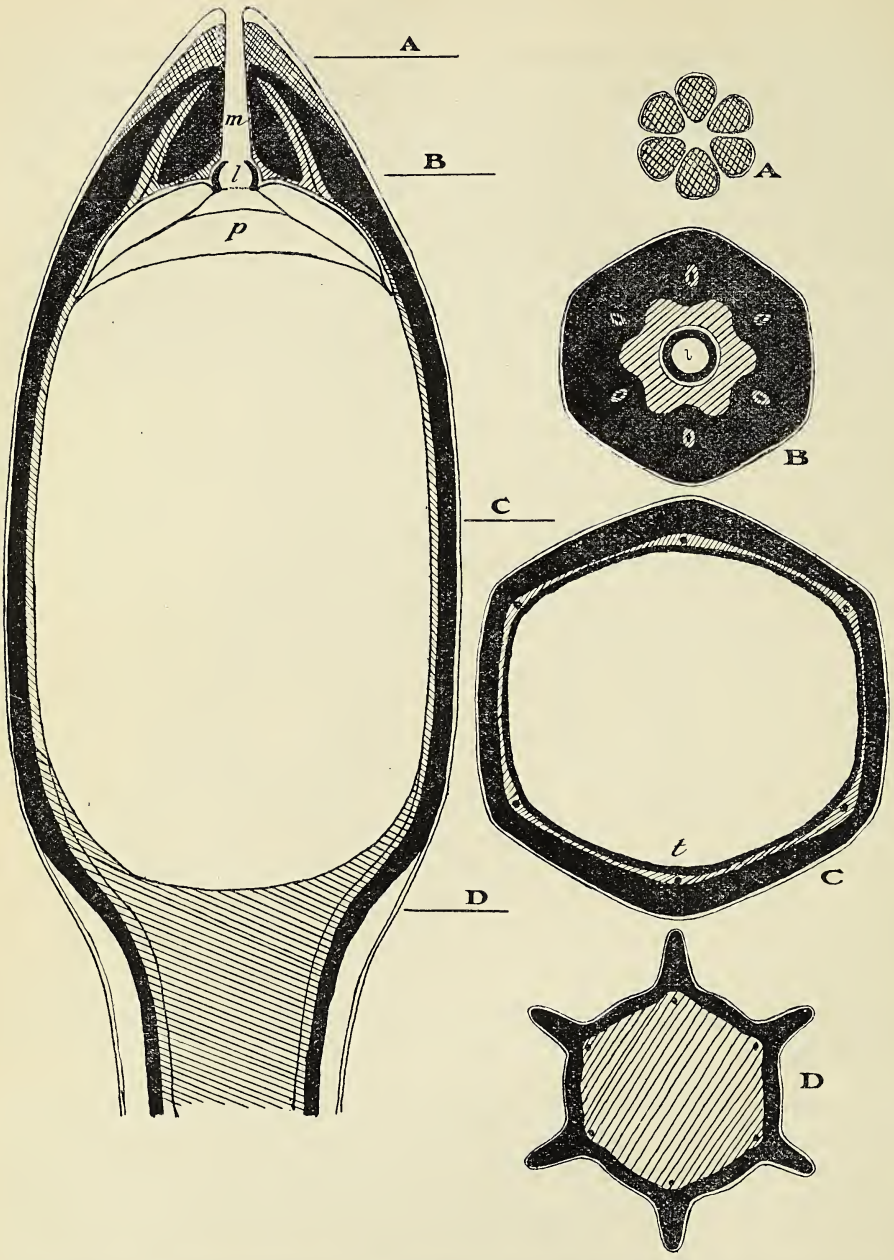
Conostoma oblongum is a straight, cylindrical, or obscurely prism-shaped seed, tapering gently to a blunt point above, where it is perforated by the micropyle, whilst below, in the chalazal region, it narrowed more abruptly to its insertion on a fairly thick stalk (Text-fig. 3 and Pl. I, Fig. 1). The average length was 5 mm. and the maximum diameter 2·3 mm. The seed-base bore six salient ridges, which, running up from the peduncle, died out almost at once, reappearing, however, at the apex to form a crown around the micropyle. On the main body of the seed these ridges are represented by a slight angling of the surface, hardly prominent enough to be called ribbing. Perhaps the most interesting feature in the form of this



TEXT-FIG. 2. *Conostoma oblongum.* Diagrammatic transverse section of seed cut about 1 mm. below the lagenostome, with plottings of the sections used. References as in Text-fig. 1.

seed is the existence of a slight bilateral symmetry, only trifling in amount, but sufficient to rank it technically with the 'platysperms' (Text-fig. 3, C).

The internal organization of the seed resembles that of a *Lagenostoma* in so far as the more general relations of integument, nucellus, and pollen-chamber are concerned, though it differs from that genus in many important particulars. As in *Lagenostoma*, the single integument or testa is coalescent with the nucellus from the seed-base to within about 1·3 mm. of the apex. It formed a hard shell to the seed about 0·1 mm. in thickness, and exhibited a very characteristic histological structure. At the summit it undergoes marked thickening to form a conical 'canopy' surrounding the micropyle.



TEXT-FIG. 3. *Conostoma oblongum.* Restorations. The longitudinal section passes through two opposing ribs; in the middle region the vascular bundles and nucellar wall are represented by a single line except in the transverse sections of the latter; A and D are in part hypothetical. The hard testa is in black, parenchymatous tissue shaded, and the soft apical tissue cross-hatched. The ribs and 'blow-off' layer are in white and the lagenostome in black. *m*, micropyle; *l*, lagenostome; *p*, plinth; *t*, tapetum (omitted from longitudinal section). × about 25.

The canopy shows conspicuous lobing, each of the six lobes corresponding to a ridge. The six vascular strands which enter the seed at the chalaza traverse the soft lining of the hard shell of the testa below the ridges and angles, and, continuing to the apex, enter the lobes of the canopy. The surface layer of the seed appears to have undergone mucilaginous degeneration, thus recalling the condition of *Lagenostoma*. Curiously enough, the actual tip of the seed was succulent, thus contrasting in a striking way with the otherwise sclerotic texture of the integument. This unique feature may well have been correlated with secretory activity at the moment of pollen reception.

The nucellus, which, as usual, stood erect in the axis of the seed, had a length of 3·7 mm. over all. Its lower 3·1 mm., coalescent throughout with the testa, were occupied by the megaspore cavity, which possessed a well-marked tapetal lining or jacket. The free summit of the nucellus, which was closely ensheathed by the lining layer of the integument, was dome-shaped, the dome resting on the tapetal septum which stretched across the nucellus at this level. Above, in the centre of the convex extremity, was a low depression or dimple on which rested the smallest pollen-chamber we have seen in an English seed. This pollen-chamber —or lagenostome, as we prefer to call it—was a tiny, truncated, globular body, open above, and possessed a one-layered wall of characteristically sculptured cells. Its mouth lay immediately below the micropylar tube of the integument, which was clamped to the rim of the lagenostome by a ring-like flange (Text-fig. 5, *u.f.*, p. 17). In this way the efficient transport of pollen would be amply secured. Pollen-grains when present in the seed have never been found in the lagenostome, which would appear to have served in this case merely as a vestibule to a more spacious lower chamber occupying the interior of the plinth. Into this lower chamber the unusually large pollen-grains were conveyed through the collapse of the floor of the lagenostome. The details of this curious mechanism, so far as we apprehend them, will be fully set forth in the sequel.

The main points in the structure of this interesting seed which merit full description are (1) the testa with its wings, succulent tip, and highly specialized micropyle; (2) the free part of the nucellus closely invested by the lining of the integument and consisting of a two-storied appliance for the reception and maturation of the pollen.

We embody in the accompanying Text-fig. 3 an attempt to reconstruct the median longitudinal section of this seed, together with transverse reconstructions at the several heights indicated.

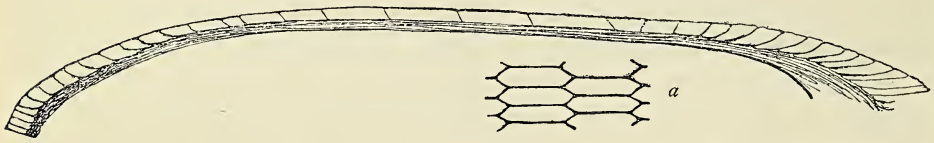
3. *The Testa.*

The hard part of the testa which gave form to the seed was of a rounded, hexagonal shape, tapering with a steep curve towards the micro-

pyle, and passing into the stalk below more abruptly. Thus, disregarding the peduncle, the seed, as seen in longitudinal section, was approximately boat-shaped—the apex representing the prow and the base the stern (R. 111, Pl. I, Fig. 1).

There is only a single transverse section, somewhat distorted in form, owing to its obliquity both to the axis of the seed and to the two ridges first cut; but if reliance can be placed on this solitary section, the seed was flattened, to a very slight extent, in a plane passing symmetrically between the angles, two of which were thus rendered more obtuse (Text-fig. 3, C). At these angles the sclerotesta was thickest, whilst it was thinnest at the other four (R. 119, 2, Pl. I, Fig. 5, *ma.*, *mi.*). At the apex the angling of the middle region passes into definite ridges which end around the micropyle in six not very prominent lobes (Text-fig. 3, A).

In the basipetal direction the angles develop as prominent ridges (R. 110, Pl. I, Fig. 4) and must have extended downwards, giving a winged



TEXT-FIG. 4. Cells of palisade layer of the testa cut obliquely, showing the forward slope at the apex and the backward slope at the base (left of fig.). R. 122. *a*, cells of testa in surface view.

character to the seed stalk. (In the instance referred to they are exaggerated by reason of the obliquity of the plane of section.) Although six ridges appear to have been the general rule, variations, which are so marked a feature of the ribbing in *Physostoma*, no doubt occurred, if only in the direction of increase. The instance is shown in R. 110, Pl. I, Fig. 10, r^7, where a seventh rib is seen on the left. In one other case, although the number of ribs remains constant, two of them were abnormally close together.

The internal form of the testa was, in the main body of the seed, similar to that of the exterior. The absence of transverse sections at the base leaves the lower limits of the interior doubtful; at the apex the cavity was dome-shaped, passing into the funnel-like lower end of the micropyle. The dome-shaped apex of the cavity and the acute apex of the exterior thus rendered the testa around the micropyle of considerable thickness (0·57 mm.) as compared with that of the general body of the seed (0·13 mm.).

In detail, the testa, like that of *Lagenostoma Lomaxii*, consisted of two kinds of elements, viz. an outer palisade layer and an inner hypoderm. Seen in transverse section the palisade layer presents the form of a continuous investment of radially elongated elements about 70 μ in

width, whilst near the apex it attains a much greater radial thickness (114 μ).

The vertical measurement appears to vary considerably, reaching a maximum of about 323 μ in the body of the seed, though adjacent elements cut in the same sense may scarcely reach a quarter of that length. Where the plane of section passes through the curved surfaces of the apex and base, the cells of this layer appear to curve respectively forwards and backwards (Text-fig. 4). Since tangential sections of this layer are wanting, no direct evidence is available as to their external form; but the appearances described can be satisfactorily explained if we assume the exterior wall to have been a hexagon, in which the sides *parallel* to the main axis of the seed were longest, and that these cells were arranged in vertical rows (Text-fig. 4, *a*). Models based on these assumptions, when cut, reproduced with striking fidelity the appearances in the various sections. The invariable obliquity of our preparations will account for the variations of the palisade cells with regard to their vertical dimensions; the maximum will correspond to cells cut in the direction of the long axis of the hexagon, and the minimum to cells cut across the corners.

If a cylinder with longitudinal ribs be cut obliquely it will be found that the ribs appear in the sections to slope forwards at the apex and backwards near the base; this is exactly what we find to be the case with the palisade cells; though here the effect is exaggerated both by their tapering ends and the fact that their outer surfaces are slightly more extensive than their inner. Similarly in the oblique transverse section where the palisade cells are cut through their tapering ends, these show varied trapezial forms, in some places even appearing two-layered.

The carbonized contents of these cells are usually of a uniform dense brown with much lighter or almost colourless walls. In some specimens the cells near the apex show a dark body occupying a nearly central position and surrounded by a lighter portion resembling a vacuole. In general, they resemble the structures seen in the cells of the testa of *Stephanospermum*, only far less well defined (R. 114, Pl. I, Fig. 2, *t*).

Abutting internally upon the palisade cells were three to four layers of fibrous elements constituting the hypoderm; these were ellipsoidal in transverse section, the flattening being in the radial direction. So far as can be judged from oblique sections, their length varied considerably. In the body of the seed this layer reached a thickness of from 57–76 μ, and if the single transverse section is to be relied upon, was thickest at the two major angles and thinnest opposite the four minor. It is chiefly due to increase of this tissue that the hard testa attains its greater thickness around the micropyle. In contents and nature of the cell-wall the elements of this layer resemble those of the palisade. The sections all show the testa cut obliquely, the

most nearly vertical being R. 111, Pl. I, Fig. 1. The others fall into two categories with regard to their direction, viz. :—

1. Sections in which the plane of symmetry *passes through* a rib or angle (e. g. R. 110, Pl. I, Fig. 10) will taper above and below—the two ends appearing longer and more pointed as the plane of section approaches the vertical, shorter and more rounded as it approaches the transverse.

2. Those in which the plane of symmetry *falls between* two ribs, the sections appearing boat to coffin shaped according as they pass through the apex or not.

Asymmetrical variations of these two types are the rule, due to the median plane of the section falling between the planes of symmetry (the median plane being that radial plane of the seed which is cut at right angles), or more strictly—regarded as variations of these two groups—due to obliquity of the plane of section to one of these planes of symmetry.

Section R. 119, 1 (Pl. I, Fig. 11) is a good example of such a variation of the second type. The outline is roughly that of a coffin with sloping ends, the upper half foreshortened owing to the tapering of the apex ; as will be seen from the plotting on the transverse diagram (Text-fig. 2) the plane of section stands asymmetrically with regard to the two ribs first cut (r^1 and r^2). For convenience of description, such sections may be termed *doubly oblique*. In the case of a perfectly cylindrical seed doubly oblique sections are not possible, since the plane of any section is always at right angles to a plane of symmetry. In a ribbed seed, the number of planes of symmetry being only twice that of the ribs, where these, as in the present instance, are few, it is only rarely that a section presents a regular outline.

It appears highly probable that sclerization of the hard testa took place in an acropetal direction. This is suggested by two of the seeds which on general grounds we regard as immature. In the first of these (R. 122, Pl. I, Fig. 3) the soft-celled tissue (*s.t.*) which constituted the apical cap is not sharply delimited from the hard tissue below (*t.*), as is the case in the mature condition, where the demarcation was sufficiently abrupt to have fissured, in some cases, along this line (W. 267, Pl. I, Fig. 8, *s.t.*). This seed (R. 122) would furthermore appear to be young, since the 'blow-off' layer is mostly *in situ*, and also the plinth, which having regard to the plane of section should have been cut across, is not present—a fact which seems to indicate the later development of this structure.

The second example of a young seed (R. 114) also shows less sclerization of the testa cells—their contents, usually so obscure, being here comparatively well defined (Pl. I, Fig. 2). The presence of the pad of tissue beneath the lagenostome with well-preserved cells (*ls.*), the exhibition of cellular structure by the integumental lining, and a plinth only half the height of those in the mature seeds, all seem to point in the same direction.

4. *The Vascular System.*

Corresponding in position to the six ribs of the testa were six bundles which occupied the internal angles. These passed up just outside the nucellus till they reached a level slightly above the base of the lagenostome, where they turned upwards and entered the sclerotesta, accompanied by a strand of soft-celled parenchymatous tissue (R. 117, Pl. II, Fig. 16, *loc.*); thence they again curved inwards, probably ending at the limit of the hard testa close to the micropyle; here the accompanying parenchyma, which gradually narrows towards the apex, dies out (Text-fig. 3, between B and A).

Around the micropyle the hard testa was slightly lobed, the lobes corresponding in number and position to the vascular strands within; to this lobing is due the internal asymmetry of the apex seen in some of the sections where they pass through a ridge on one side of the micropyle and through a 'furrow' on the other (R. 110, Pl. II, Fig. 14; R. 123, Pl. II, Fig. 19, *i.r.*).

The apex of the hard testa in *Conostoma* was therefore very like the canopy of *Lagenostoma Lomaxii*, where likewise the lobes corresponded in number and position to the vascular bundles which passed into them. They did not, however, agree in position with the external ridging of the apex, which, as has been pointed out before,[1] was associated with the radial septa. The chief point of difference in *Conostoma* lies in the reduction of the soft tissue which occupied the loculi of the canopy to mere strands accompanying the bundles. The vascular bundles themselves consisted of four to five elements (each about 10 μ across) with delicate scalariform thickenings which occasionally exhibited fusion between adjacent bars.

5. *The Soft Integument.*

Surmounting the apex of the hard testa was a cap of soft-celled tissue (R. 122, Pl. I, Fig. 3, *s.t.*) which was thickest around the micropyle, where it formed six free lobes (Mr. Watson's specimen 268, Pl. II, Fig. 12, *l.*) corresponding to the obscure lobing of the hard tissue below, and thinned out in the basipetal direction, ending just above the shoulder of the seed (Text-fig. 3). The only evidence that the vascular supply extended into this region is a very doubtful vascular bundle in one of the preparations. This soft tissue may have been of a secretory nature, supplying in part the necessary fluid for the process of pollination, by furnishing a drop mechanism as in *Taxus*. The specialized epidermis around the micropyle, to be described later, somewhat resembles the protective epidermis

[1] Oliver: On *Physostoma*. Ann. of Bot., vol. xxiii, p. 105.

('lip') surrounding the glands of *Polygonum*, &c., and may even have served a similar purpose.[1]

Lining the micropyle and continuous with this soft tissue was a single layer of cells, of which only the membrane forming the micropylar tube is preserved (R. 111, Pl. I, Fig. 7, *m.m.*). This latter consists of several tiers of longitudinally elongated elements, which here and there show faint spiral markings; the cells of each tier are of approximately equal length, and, in the middle region, stand directly above those of the tier below. In width they were nearly the same throughout (17 μ), the increased circumference of the micropyle at the base entailing a corresponding increase in their number. At the level of the lagenostome this lining layer of cells suddenly thickened to form a triangular flange, which rested on the upper edge of that organ. A similar flange occupied the shallow sinus where the lagenostome rested upon the plinth. In section the flanges appear as angular projections into the micropyle (R. 117, Pl. II, Fig. 18, *u.f.* and *l.f.*). Below the lagenostome the membrane closely ensheathed the dome-shaped plinth, at the base of which the nucellus and integument become fused. In two of the sections the sinus between this membrane and the plinth is seen cut across at the base (R. 110, Pl. II, Fig. 14, *s.*, and R. 117, Pl. II, Fig. 16, *s.*). Below this point the nucellus and testa were connected by soft-celled tissue, through which the vascular bundles passed. In section R. 114 (Pl. I, Fig. 2, *pl.j.*) the single layer of cells forming the lining membrane of the micropyle is seen cut across between the flanges. From this we see that the lagenostome and plinth, although free, were closely ensheathed by the integumental membrane, which by means of the flanges held the lagenostome in a tightly fitting socket, thus ensuring perfect continuity between the passage of the micropyle and the cavity of the lagenostome; to such an extent was this the case that the contraction of the nucellus previous to fossilization, which usually takes place, has not only caused the lagenostome to descend, but has in some cases brought down the micropylar tube with it, causing rupture near the apex (R. 111, Pl. I, Fig. 7). So long as these were the only sections available, the tube appeared to belong to the lagenostome, but the sections which have more recently come to hand show clearly that its true nature is integumental (Watson's 268, Pl. II, Fig. 12, *m.m.*, and R. 123, Pl. II, Fig. 19, *m.f.*), as the continuity with the soft tissue at the apex is completely shown.

6. *The 'Blow-off' Layer.*

Investing the whole external surface of the seed was a differentiated epidermis, which we shall term the 'blow-off' layer. This, together with

[1] E. J. Salisbury: On the Extrafloral Nectaries of the Genus *Polygonum*. Ann. of Bot., vol. xxiii, 1909, p. 239.

the soft-celled tissue of the apex, formed a kind of sarcotesta around the seed. The 'blow-off' layer consisted of longitudinally elongated cells with an almost uniform radial dimension of about 20 μ. Near the apex, however, they reached a maximum of about 80 μ. These cells, as preserved, stand in marked contrast with the palisade cells upon which they rest. Their interior is almost colourless and their walls are dark and slightly thicker towards the exterior. Just below the apex of the seed the cells of this layer reached a maximum radial extension (Watson's specimen 268, Pl. II, Fig. 12, *bl.*), bevelling off suddenly towards the micropyle, so that the extreme end of the seed was covered by an undifferentiated epidermis.

The chief point of interest is the exfoliation which the 'blow-off' exhibits: in only one other section besides W. 268 has it remained *in situ* (R. 122, Pl. I, Fig. 3, *bl.*). Elsewhere, the layer stands away from the hard testa as if forced off, and in most cases only the basal portions of the radial walls remain to indicate its former presence. These latter appear as projecting pegs from the margin of the testa, to which they give a crenated appearance (R. 110, Pl. I, Fig. 4, *bl.w.*)

The method by which this exfoliation took place is shown in R. 111 (Pl. II, Fig. 13, *pe.*) near the apex, where the cuticle-like layer, formed by the outer walls, is raised up by cones of mucilage which bear a remarkable resemblance to those already described for *Lagenostoma Lomaxii*.[1] But whereas the cell-walls in *Lagenostoma* were raised up as separate entities, in *Conostoma* they formed a continuous layer, which was removed as a whole, although it underwent fission, due to its rigidity and lack of elasticity, points well shown in one section (R. 116, Pl. I, Fig. 6, *bl.*) where the exfoliated 'blow-off' at the apex still retains the lobing of the tissue beneath, but, owing to the increased circumference, has fissured along the grooves.

A section which is perhaps correctly allocated to this seed, viz. R. 113 (Pl. I, Fig. 9), is presumably an oblique transverse section passing through the stalk. It was winged with a central cavity (*c.s.*), probably lysigenetic, and a narrow, angled band of hard tissue following the outline of the exterior (*scl.*). Its attribution rests upon the general appearance of its tissues, bounded at the exterior by an epidermis which closely resembles the 'blow-off' layer as seen in certain of the sections. Its prominent wings, to the number of seven, corresponding to seven vascular bundles (*v.b.*), might well have been the base of such a seed as R. 110, which, as we have already pointed out, varied in this respect from the normal number of ribs, the hard tissue being perhaps the basal limit of the testa. The chief difficulties in the way of its acceptance are (*a*) the rapidity with which the bundles are passing out, and (*b*) the presence in the wings of secretory passages (*s.p.*), one in each. In any case it seems possible that the bundles of *Conostoma* remained distinct for some distance below the seed-base,

[1] Oliver and Scott: On *Lagenostoma Lomaxii*. Phil. Trans., B, vol. 197, p. 206.

perhaps to converge suddenly later to a single strand. Another section (R. 115–2) which gives food for speculation passes tangentially through the stalk. This shows what may have been a cupule containing a vascular strand. A peculiar feature of this preparation is that the plane of section should theoretically (if the seed be borne vertically on a straight stalk) pass through the axis of the stalk, but actually it cuts the stalk or cupule, as the case may be, tangentially and passes out on the same side; therefore either the stalk in this instance was bent or else the seeds in this species were borne in a cupule in a similar manner to the closely allied seed *Gnetopsis elliptica*, where the insertion of the seeds and cupule is oblique to the main axis.[1] The continuity is not convincing, but the fact that the possible cupule contains secretory canals suggests that the section R. 113 may have been cut through the stalk at the base of the cupule, which would account for both the difficulties of its acceptance. In any case we ourselves regard the present evidence as quite inadequate for a definite decision.

7. *The Nucellus.*

This, the central body of the seed, requires detailed description in view of the unusual and possibly significant elaboration of structure shown by its distal portion which was concerned in the reception and storage of the pollen.

The nucellus falls into three regions:—

(1) The *lagenostome*, a structure evidently corresponding with the pollen-chamber of such a seed as *Lagenostoma Lomaxii*. It lies in a saucer-like depression at the summit of (2) the *plinth*, which forms the truncated continuation of (3) the main body of the nucellus or megaspore chamber.

The lagenostome and plinth, though closely invested by the integumental lining ('micropylar funnel' and 'plinth jacket'), were free from the integument; the megaspore chamber, which extends from the chalaza to the level at which the apical tapering of the seed begins, shows on the other hand complete coalescence with the testa.

The horizontal septum separating the plinth cavity from the megaspore chamber is formed by the tapetum, and may for convenience of reference be termed the tapetal septum (cf. Text-fig. 3).

8. *The Lagenostome and Plinth.*

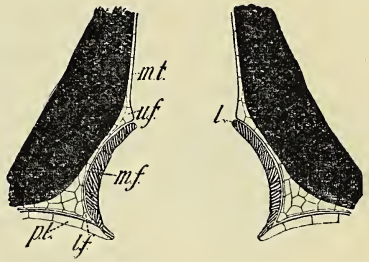
These two organs were so intimately related that it will be convenient to deal with them in the same section. The *plinth* is the tapering free end of the nucellus, on the flattened end of which the small urceolate *lagenostome* was inserted in a shallow, saucer-like depression. Apart from the remains of its internal filling tissue, to which reference will shortly be made, the plinth is as a rule in our specimens represented by its epidermis—

[1] Renault: Cours de Bot. foss., vol. iv, Pl. XX, Fig. 1.

a mere shell—usually completely carbonized and appearing as a continuous black, structureless crust (cf. specimen R. 123, Pl. II, Fig. 19). Occasionally, the individual cells of which this shell was composed are preserved (specimen R. 114, Pl. I, Fig. 2, *pl.*), showing it to have consisted of a single layer of flattened epidermal cells destitute of special sculpturing.

Tracing this layer down the steep slope of the plinth to the level at which the tissues of nucellus and integument became confluent (R. 110, Pl. II, Fig. 14), it is found to curve sharply outwards and upwards (below *s.*), returning on its course as the lining layer of the integument which fitted the plinth like a jacket (cf. p. 14).

Followed in the other direction the epidermis of the plinth dips slightly at the summit to form the depression in which the lagenostome was lodged. It does not, however, reach the central part of this recess, which, as sections across this region clearly prove, was perforated (specimen R. 117, Pl. II, Fig. 16). The epidermis of the plinth at the insertion of the lagenostome curves abruptly upwards and outwards, and at the same time, changing completely its histological character, becomes evaginated to form the sculptured wall of the lagenostome (Pl. II, Fig. 18). Thus we see that the epidermal layer of the interior of the seed is in complete continuity throughout and that the various regions termed the micropylar tube, micropylar funnel, plinth jacket, plinth and lagenostome walls, are merely different specialized portions of one and the same layer.



Text-fig. 5. *Conostoma oblongum.* Diagram of median vertical section of lagenostome to show how it was enclosed by the micropylar tube and funnel. *m.t.*, micropylar tube; *m.f.*, micropylar funnel; *p.l.*, wall of plinth; *u.f.* and *l.f.*, upper and lower flanges holding lagenostome (*l*) in place.

The lagenostome was a tiny goblet-shaped body, the cavity of which communicated with the plinth cavity. In shape it was slightly pyriform, its greatest horizontal diameter being about one-third up from its insertion. The mouth, which is rarely if ever shown in the sections, communicated directly with the micropylar tube, with which it closely engaged by a bevelled flange from the latter (Text-fig. 5, and Pl. II, Fig. 18). Unlike *Physostoma* and *Lagenostoma*, the mouth was unprovided with any tube or beak. In the former the mouth was placed on a low papilla (Text-fig. 12, p. 39), whilst in the latter the tapering tube of the pollen-chamber reached the surface of the seed (Text-fig. 12).

The principal dimensions of the lagenostome of *Conostoma oblongum* were as follows:—greatest horizontal diameter, 0·23 mm.; diameter at mouth, 0·12 mm.; height, 0·15 mm.

In *Physostoma* the lagenostome or pollen-chamber was 1 mm. high

× 1·2 mm. wide; in *Lagenostoma Lomaxii* the corresponding dimensions were 0·75 mm. × 0·7 mm.

The cells which formed the wall of the lagenostome were in lateral continuity by about a third of the depth of their radial walls, thus leaving their major exterior portions free. It is due to this peculiarity, together with the obliquity of the sections, that the misleading appearance of a two-layered wall is due. This feature, strikingly shown by the preparations R. 110 (Pl. II, Fig. 14, *lg.*) and R. 117 (Fig. 18, *lg.*), might readily give rise to the view that the lagenostome was a two-layered structure. The radial dimension of these cells ranges from 26 μ to 30 μ; the tangential horizontal is approximately 26 μ, and the tangential vertical 39 μ.

In form, the cells are roughly hexagonal as seen in tangential view. They were arranged in successive tiers with their shorter sides directed upwards and downwards. The wall seems to have been continued right up to the mouth without marked change in character, unless perhaps the appearance of thinning shown by preparation R. 117 (Pl. II, Fig. 18) at the top of the lagenostome be not merely the result of post-mortem shrinkage.

TEXT-FIG. 6. Sketch slightly diagrammatic, showing the sculpturing on the cells of the lagenostome.

A great feature of the cells of this layer was the elaborate, tracheid-like sculpturing of their walls—well shown in specimen R. 115, 1 (Pl. II, Fig. 20, *lg.*), and, on a much enlarged scale, in the adjacent text-figure (Text-fig. 6). These sculpturings, which were of the scalariform or slightly reticulated type, reach a maximum development on the tangential wall, i. e. the wall in contact with the integument. Other characteristic sections of the lagenostome are shown by specimens R. 111, R. 114, and R. 116 (Pl. I, Figs. 7, 2, and 6). These figures also illustrate the marked tendency of this cell layer to undergo degeneration prior to fossilization. Thus in preparation R. 111 (Pl. I, Fig. 7, *lg.*) the outer walls of one layer and the inner walls of the adjacent layer have perished, whilst in R. 116 (Fig. 6, *lg.*) solution has proceeded still further, so that little remains beyond the common radial walls of the obliquely cut cells.

Though these tracheid-like elements of the lagenostome were of delicate construction, they must, in virtue of their sculptured walls, have had a marked capacity to resist crushing forces. Occasionally the lagenostome is slightly retracted from the micropyle in consequence no doubt of post-mortem shrinkage (e. g. Fig. 7), but in all cases that have come under observation the form of the lagenostome is perfectly preserved.

The floor of the lagenostome did not consist of differentiated, sculptured cells, but was occupied by a pad of soft tissue which readily underwent displacement, leaving a clear orifice—well shown in preparations R. 117

and R. 116 (Pl. II, Fig. 18, and Pl. I, Fig. 6). The plug which formed the floor is only slightly displaced in R. 116 (Fig. 6, *ls.*), whilst in R. 110 (Pl. II, Fig. 14, *ls.*) the cushion of soft tissue which hangs suspended from the lower rim of the lagenostome appears to be still in position.

The central cone of soft tissue that projected into the cavity of the pollen-chamber of *Lagenostoma Lomaxii* (Text-fig. 12, p. 39) does not appear to be represented in any of our specimens of *Conostoma*, though from analogy with that seed we regard it as certain to have been present in early developmental stages. Its absence from our specimens is best to be explained as a consequence either of very early deliquescence in development or imperfect preservation.

Before turning to the plinth cavity and its contents it will be convenient to mention a general character of this organ.

In occasional specimens of *Conostoma* we find that the plinth had not reached its full height, and as a rule lack of extension in this region seems to be correlated with other features which support the view that such specimens belonged to a younger stage of development than that usually found. Thus, in R. 114 (Fig. 2), where, having regard to plane of section, the plinth is only half the usual height, the testa is immature and shows progressive sclerization. Again, in R. 122 (Fig. 3), a very immature specimen as judged by the testa, no trace of the plinth is visible, though were the specimen a normal one this region should fall within the plane of section. In view of these data and of the well-ascertained fact that the plinth is undeveloped in the small-sized seeds of *Lagenostoma Lomaxii*,[1] there is good reason for supposing the plinth to have arisen as an intercalation at a relatively late stage in development. This extension of the nucellus just below the lagenostome must of course have been accompanied by a corresponding elongation of the free part of the testa, for, as we have seen, the relations between plinth and integumental lining were of the closest.

The cavity of the plinth now claims attention. It was a hemispherical chamber of variable height, as we have seen, extending from the tapetal septum, which formed its floor, up to the lagenostome. Its maximum height all over was 0·5 mm.; centre of floor to base of lagenostome, 0·3 mm. Its contents, which only partly filled it, included (1) remnants of the soft interior tissues of the plinth, (2) pollen-grains. So far as information is afforded by our preparations, pollen when present in the seed was invariably contained in the plinth cavity, not in the lagenostome.

The soft interior tissue of the plinth is mainly preserved in the form of a horizontal, circular cushion which in section has the form of a concavo-convex lens, the convex side being uppermost. This cushion or lens, which

[1] Oliver and Scott: On *Lagenostoma Lomaxii*. Phil. Trans., B, vol. cxcvii, p. 212, Pl. IV, Figs. 1 and 2.

was preserved in the form of a delicate tissue, is not as a rule high enough to fill the space between the tapetal septum below and the base of the lagenostome above.

As regards the position occupied by the lens we find two extreme states. (*a*) In some cases the lens is found attached by its convex upper surface to the under side of the saucer in which the lagenostome rested (specimen R. 110, Pl. II, Fig. 14, *ls.*), whilst its periphery hangs down into the plinth cavity—its edge being continued as a mere membrane which, often interrupted, can be traced obliquely downwards in the direction of the angle between the floor and sides of the plinth cavity, where it loses itself in the other tissues of the nucellus. Sometimes this membrane runs into the plinth wall just above the angle, sometimes it descends more steeply and strikes the floor just within the edge.

In addition to the lens, traces of plinth tissue are also found resting on the centre of the floor vertically below the lagenostome in the form of a hemispherical pad (R. 110, Pl. II, Fig. 14, *pd.*, and R. 111, Pl. I, Fig. 10, *pd.*), the constituent cells of which usually show very poor preservation. The relation of this pad to the lens above suggests that it has been derived and separated from the middle concave part of the lens as a result of an increase in the height of the plinth cavity, with which extension the tissue which doubtless originally filled it has not kept pace (Text-fig. 7, C).

(*b*) An example of the other extreme state is afforded by specimen R. 117 (Pl. II, Fig. 16), in which the whole of the plinth tissue (*pl.t.*) is found resting on the level floor of the plinth cavity. In this preparation what appear to be the same two portions of tissue are recognizable, viz. the lens tissue (*pl.t.*, Fig. 15) with good preservation of its cells, and the central projecting boss or pad (*pd.*) around which the former has collapsed.

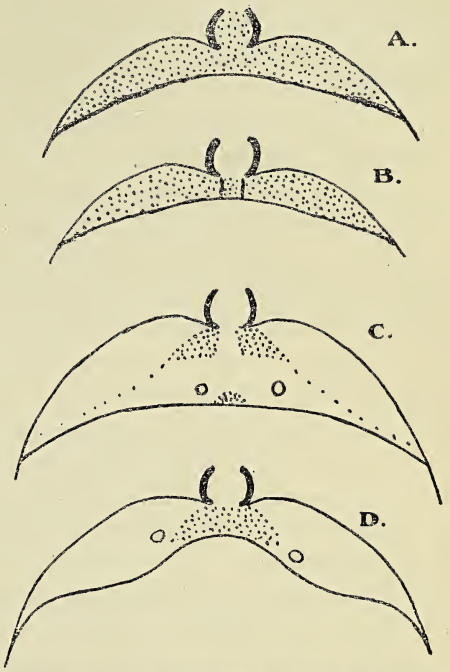
These two states are connected by intermediate conditions. Whilst in state (*a*) the convex summit of the lens is in position and still adheres to the base of the lagenostome, specimen R. 111 (Pl. I, Fig. 7, *ls.*) shows some slight separation which is a good deal more evident in specimen R. 116 (Pl. I, Fig. 6, *ls.*).

In addition to these cases, which include the majority of specimens, there are still others in which the parts seem to approximate to their original positions (R. 119 and 123, Pl. I, Fig. 11, and Pl. II, Fig. 19). It should be mentioned perhaps that in these two specimens evident traces of a prothallus are present in the megaspore chamber (Figs. 11 and 19, *pr.*), so that it may be conjectured that the stages in question are relatively old ones, whilst the presence of a pollen-grain in the closed plinth cavity of R. 123 (Fig. 19, *p.g.*) is a remarkable fact which requires explanation.

With a view to elucidating the significance of the various states of tissue distribution in the plinth cavity we shall now consider whether and

how far it is possible to reconcile them with the probable course of events in the history of ovular maturation.

At an earlier stage of development than any represented by our preparations, the tip of the nucellus must have been occupied by a soft internal tissue which filled both lagenostome and plinth, the tissue in question being continuous from the one to the other through the narrow orifice by which these structures communicated (Text-fig. 7, A). As the time of pollination drew near the tissue of the lagenostome doubtless underwent solution through the agency of appropriate enzymes spontaneously secreted. At this time the floor or septum which lay between the plinth and the megaspore cavity must have been arched right up so that it was separated from the base of the lagenostome by the thickness of the lens-shaped cushion of tissue which we find surviving in later stages. Peripherally this tissue must have extended considerably further than does its surviving remnant in any of our specimens, and in particular it must have occupied the curving sides or 'shoulders' of the plinth. Text-fig. 7, A, may perhaps serve as a reconstruction of this early stage.



TEXT-FIG. 7. Diagrams of series of developmental stages (in part hypothetical) of plinth and lagenostome in a *Conostoma*, showing arrangements for entry of pollen into the plinth chamber. The dotted areas represent soft filling tissue. A, young stage; B, cavity of lagenostome cleared of tissue and central patch of plinth tissue ready to separate as the plinth elongates (C); in D the prothallus has encroached on the plinth by means of a 'tent-pole' extension. Pollen present in C and D.

As the solution of the core of the lagenostome advanced the enzyme-action would traverse the base and involve the tissues of the plinth (Text-fig. 7, B).

To account for the next stage one of two assumptions has to be made: either the tapetal membrane (floor of plinth) tended to contract, or else—and we think this the more probable—the summit of the nucellus (including the lagenostome) continued to rise in consequence of a late extension localized in the plinth, whilst the tapetal septum remained stationary or at any rate lagged behind in its growth. Only in one of these two ways does it seem possible to explain the separation of the small central pad which rests on the tapetal septum from the lens suspended above (Text-fig. 7, C).

The state of tension in the plinth tissue, depicted above, would become effective as the enzyme-action, which had already dissolved the core of the lagenostome, advanced basipetally and involved the tissues of the plinth. For the effect of the enzyme would be to soften the central tissue of the cushion or lens (Text-fig. 7 B), thus removing serious resistance to the vertical extension of the plinth cavity. In this way a central pad, adhering to the floor, would be likely to separate from the cushion, which in its turn would remain hanging to the base of the lagenostome. The separation of the flanks of the cushion from the shoulders of the plinth may well have been effected at the same time, whilst in occasional examples (like R. 117, Pl. II, Fig. 16) the whole of the tissue of the cushion may have been liberated from the top of the plinth.

It is of course possible that only the peripheral layers of the core of the lagenostome were softened (and not the whole dissolved), so that when the enzyme-action had reached the base the whole of the central mass of tissue became separated from the lagenostome to form the pad on the floor. This would perhaps account for the unusually large dimensions of the pad in R. 117 (Fig. 15, *pd.*). For a decision on this point, which is not very material, the needful data are wanting. As to this, however, we are satisfied: that a hole was drilled which established communication between the cavities of the lagenostome and plinth, and that the pollen descended through this orifice into the plinth chamber.

The second stage in the development of the mechanism is reconstructed in Text-fig. 7, C.

Our third stage is represented by specimens R. 119 and R. 123 (Figs. 11 and 19), in both of which—unlike any of the others—a prothallus is present. In both we find the lens in contact with the lagenostome above, whilst its lower surface rests on the arched floor. Moreover, in R. 123 a pollen-grain is present in the plinth cavity, whilst one of the bodies in a similar position in R. 119 may possibly be of the same nature, though the preservation is not good enough to say definitely. The problem we are trying to solve is the presence of the grain in this position with the other parts apparently blocking the way (Fig. 19).

Our conclusion as to the course of the pollen has already been stated; it made its way through a temporary orifice.

As the seed continued its development the prothallus made its appearance and a 'tent-pole' prothallial apex pressed on the septum from below, restoring the relations as we see them in specimen R. 123 (Fig. 19). The presence of a 'tent-pole' is indicated in both specimens—especially striking is it in R. 119 (Fig. 11, *t.p.*), where the somewhat oblique plane of section has cut the prothallus twice, once at the projecting shoulder and again at the tip. Williamson's figure[1] appears to represent a seed in the same phase as

[1] Williamson: loc. cit., Pl. XII, Fig. 86.

these two specimens, but we cannot speak critically as the preparation has not passed through our hands.

It still remains to be shown that warrant exists for the assumption that the wall of the plinth underwent a long-continued or intercalary extension which would provide the necessary machinery for 'uncorking' the basal orifice of the lagenostome, and thus letting the pollen through. We have already had occasion to comment on the structure of specimen R. 114 (p. 12), which has all the appearance of being a young seed. It was shown that in this case the plinth had only reached one-half the normal height, so that evidently the main extension of this organ must have been effected at a late period in development.

Again, in the related seed *Lagenostoma Lomaxii*, the plinth was the last part of the seed to develop, as we know from the fact that it had not yet appeared in the small-sized seeds, although the pollen-chamber was already of full size and properly developed (cf. Text-fig. 12, p. 39).[1]

The pollen is not sufficiently well preserved to justify detailed description; suffice it to say that it was multicellular, ellipsoidal in form, and measured $75\,\mu \times 65\,\mu$.

III. Conostoma anglo-germanicum, sp. nov.

1. *Enumeration of Specimens.*

In the spring of 1904 an isolated section passing obliquely through an eight-ribbed seed was obtained from the Shore locality. This specimen remained of doubtful affinity until 1909, when Dr. Kidston put at our disposal a series of four transverse sections from a coal-ball obtained many years before at Langendreer in Westphalia. These emphasized the previous suspicions that the seed was nearly allied to *Conostoma oblongum*. The close relationship was put beyond doubt when, owing to the generosity of Dr. Jongmans, who placed his slides and uncut coal-balls at our disposal, details of the internal structure were obtained.

Owing to the general character of its external form and the almost identical internal structure, we have no hesitation in provisionally referring this seed to the genus *Conostoma*;[2] altogether some twenty sections of this fructification are now available, of which the greater number are from the German material of Dr. Jongmans. Having regard to this double source of origin of our sections we propose the specific name of *anglo-germanicum*. Up to the present time four sections of this seed have occurred from English material, of which one is the oblique section already referred to and the other three are more or less imperfect transverse sections, though of value as supplying our only information as to the histological structure of the testa.

In the following list the sections are enumerated, with their source of

[1] Oliver and Scott: loc. cit., p. 212, and Pl. IV, Fig. 1.

[2] See footnote, p. 38.

origin and approximate plane. Those marked with an asterisk are figured in the plates.

U.C.L., R. 140 *a**, *b**, *c*, *d**	Series of four transverse sections	Langendreer, Westphalia
J. 3*	Oblique through pollen-chamber	Rheinpreussen (nr. Duisburg)
J. 4	Tangential	,, ,,
J. 5	Oblique transverse	,, ,,
J. 6*	Oblique longitudinal	,, ,,
J. 9*	Oblique through pollen-chamber	,, ,,
J. 10	Tangential	,, ,,
J. 11	Oblique through middle	,, ,,
J. 12*	Longitudinal, passing through micropyle	,, ,,
J. 13	Oblique transverse	,, ,,
J. 14	Tangential	,, ,,
J. 15	Oblique transverse	,, ,,
J. 16	Oblique transverse	,, ,,
U.C.L., Q. 18*	Oblique transverse near base	Shore, Littleborough, 1904
U.C.L., R. 141	Very imperfect transverse	Dulesgate
U.C.L., R. 142 *a* and *b*	Imperfect transverse	Shore, Littleborough, 1910

The specimens marked 'J.' are in the collection of Dr. J. W. Jongmans of Leiden. (The numbers of these specimens are provisional.)

The approximate plane of all the more important sections has been plotted on the longitudinal and transverse diagrams in Text-fig. 8.

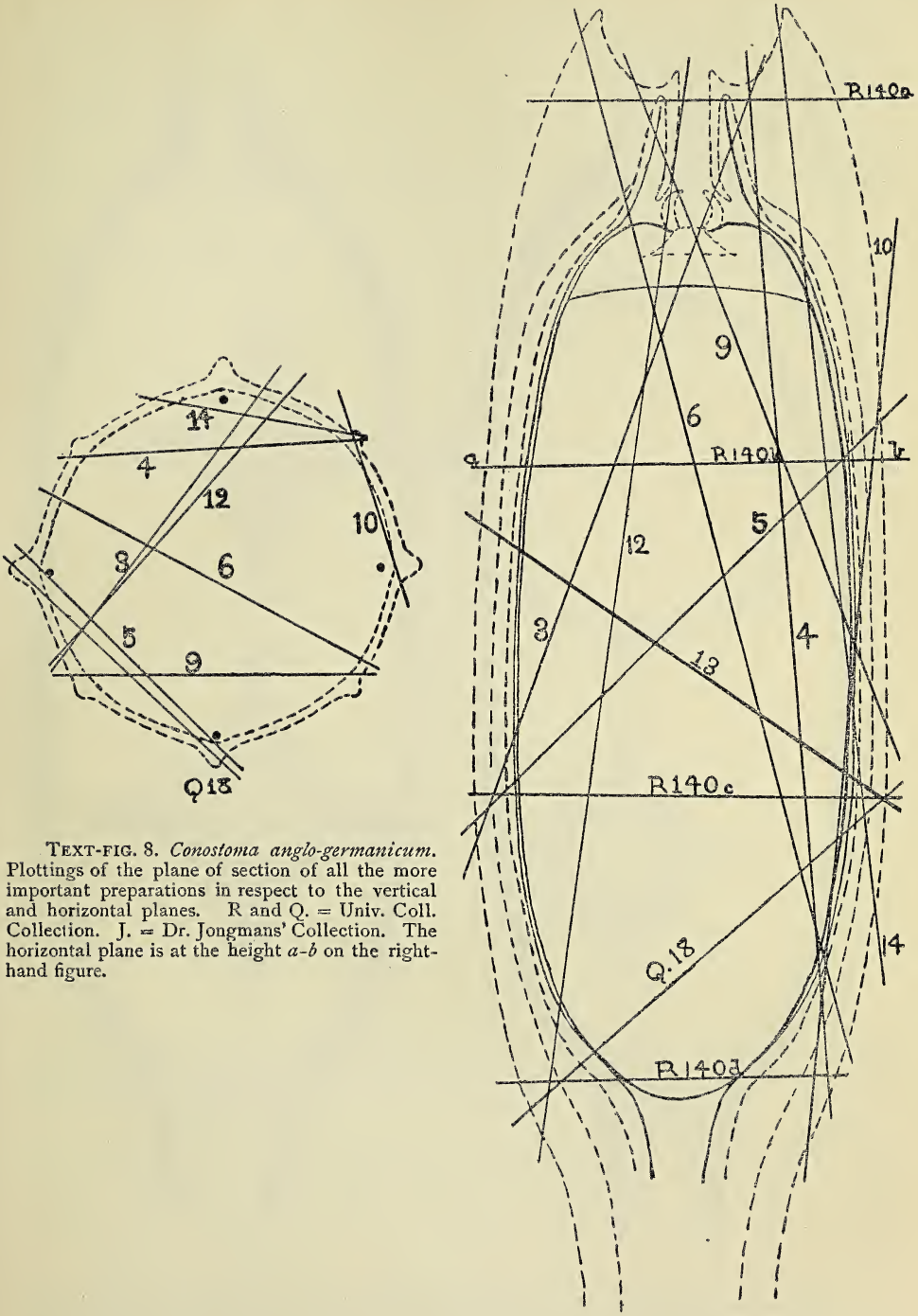
2. *General Features.*

In the fundamental characters both of external form and internal organization the present species agrees closely with *Conostoma oblongum*. Like that species it was a straight, angled seed, roughly bullet-shaped, with a tapering apex and gradual insertion.

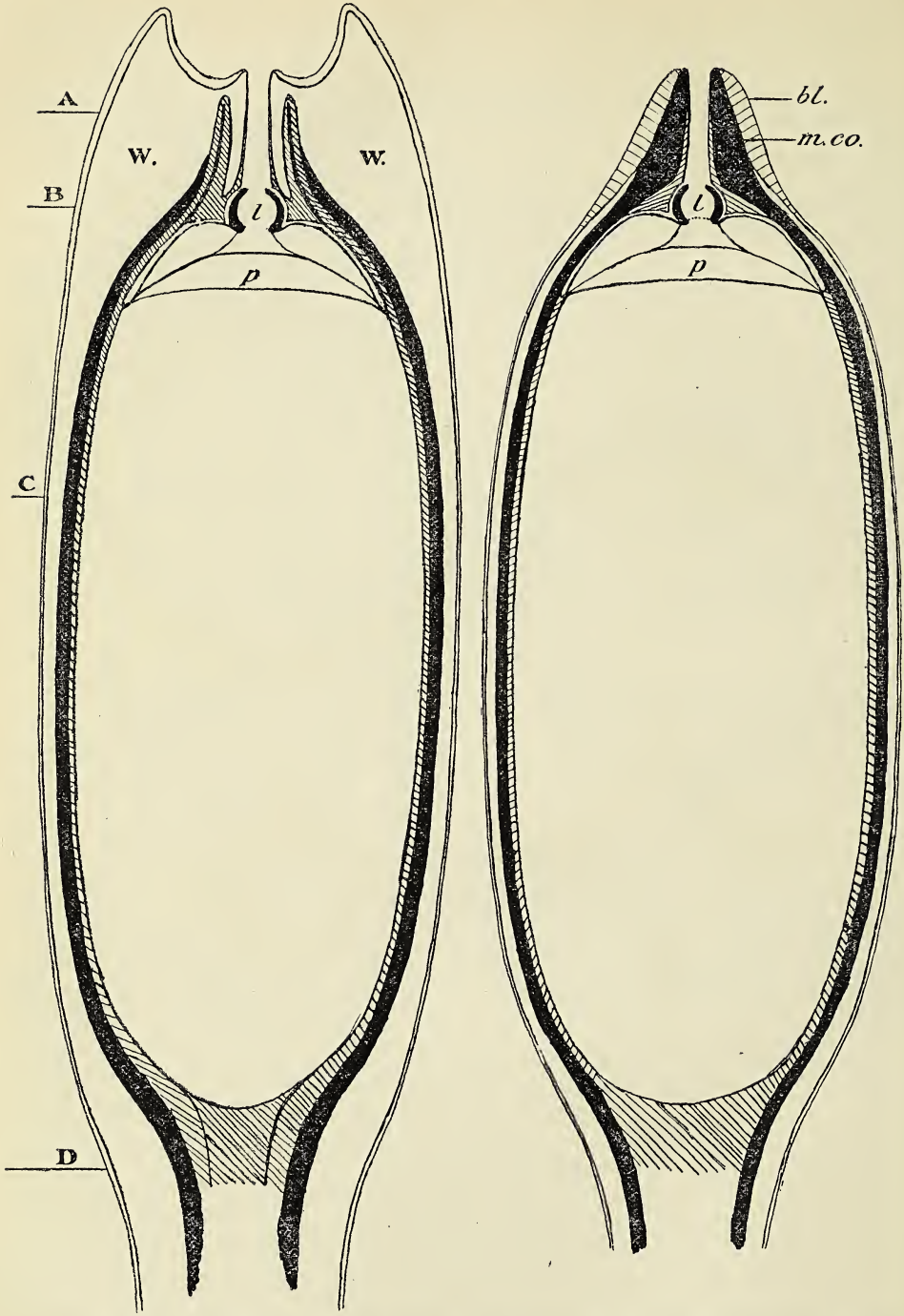
Internally there was an extensive plinth which had a comparatively small globular lagenostome at its apex. But whilst in essentials it was an undoubted *Conostoma* it possessed a very characteristic individuality of its own.

Superficially, the most striking feature was its extreme length as compared with its width ; this latter was the same as in the other species, but it attained a maximum length of 7 mm. The main body of the seed was eight-angled, the angles bearing externally ribs which were alternately large and small ; the former in the middle region being twice as prominent as the latter (0·11 mm. and 0·05 mm.).

The base of the seed tapered gradually to a thick stalk bearing eight wings, the lower extensions of the ribs. At the summit the surface curved inwards almost abruptly, terminating in a short conical apex pierced by the micropyle (Text-fig. 9). At the base of this tube the smaller ribs die out, but the four larger persist as wing-like expansions around the micropylar region, beyond the orifice of which outer margins are produced as free points. At the apex of the seed was an almost hemispherical depression about 0·4 mm. deep, bounded by the internal edges of the wings (Text-



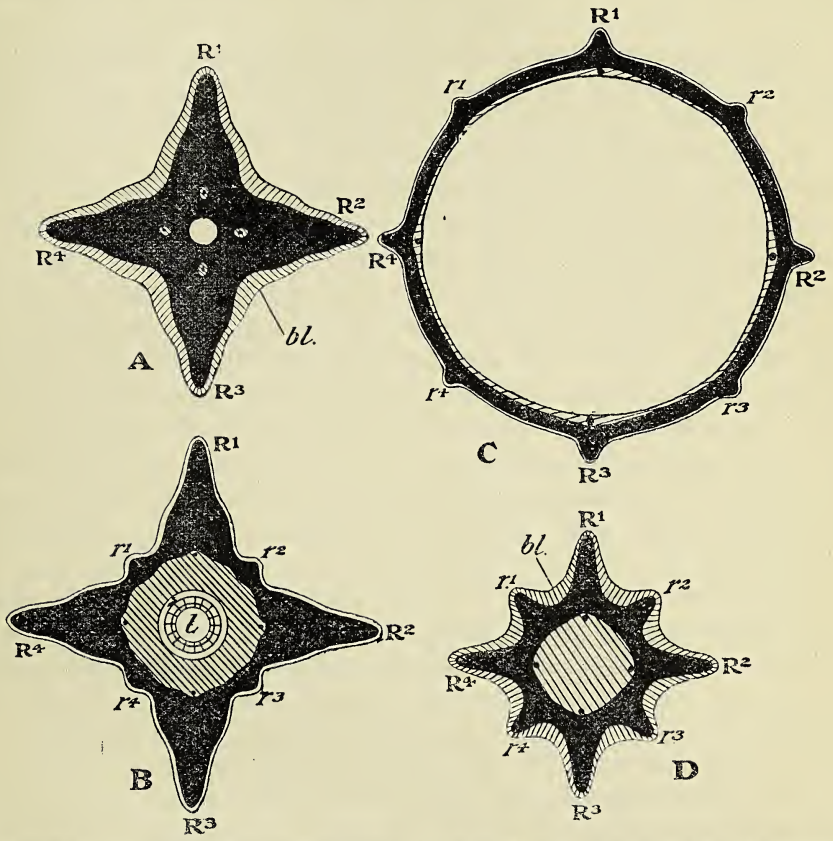
TEXT-FIG. 8. *Conostoma anglo-germanicum.* Plottings of the plane of section of all the more important preparations in respect to the vertical and horizontal planes. R and Q. = Univ. Coll. Collection. J. = Dr. Jongmans' Collection. The horizontal plane is at the height *a-b* on the right-hand figure.



TEXT-FIG. 9. Restorations of *Conostoma anglo-germanicum* passing longitudinally through an opposing pair of major and minor ribs, respectively. The vascular bundle and nucellar wall are represented by a single line. The hard testa and lagenostome are in black, ribs and 'blow-off' white, and parenchymatous tissue shaded. *bl.*, 'blow-off'; *m.co.*, micropylar cone; *l.*, lagenostome; *p.*, plinth; W, W., wings formed by major ribs. × about 25.

fig. 9, w.), and into this depression the summit of the micropylar cone slightly projected (0·01 mm.). The main cavity of the seed was nearly cylindrical, rounded at either end, and passing by a funnel-shaped portion into the tubular micropyle above.

The megaspore cavity was about 4·6 mm. long and 2 mm. wide, surmounted, as in *C. oblongum*, by a dome-shaped plinth which bore the characteristic lagenostome in a slight depression at its apex. Compared with



TEXT-FIG. 10. *Conostoma anglo-germanicum.* Series of transverse sections at the levels marked A, B, C, and D in the vertical reconstructions (Text-fig. 9). The 'blow-off' layer is left white except in A and D. The testa is black and the parenchymatous tissue shaded. R^1, R^2, R^3, R^4, major ribs; r^1, r^2, r^3, r^4, minor ribs; *bl.*, 'blow-off'; *l.*, lagenostome. × about 25.

the internal structure of *C. oblongum*, the nucellus was narrower and much longer, whilst the plinth cavity remained of about the same depth, though slightly less extensive in width. The plinth contents appear to have been as in *Conostoma oblongum*.

The lagenostome was slightly higher and broader than in the other species, perhaps referable to the larger size of the pollen-grains in *Conostoma anglo-germanicum*. From the base of the plinth downwards, the

nucellus and integument were joined, and between these two ran the four vascular strands which corresponded in position to the larger ribs.

The lobes of the canopy internally have almost entirely disappeared, and only four tapering loculi remain, no doubt filled with soft tissue into which the vascular strands passed. The general appearance of the seed may be gathered from Text-figs. 9 and 10, which give restorations in longitudinal and transverse section respectively.

3. *The Testa.*

Although *Conostoma anglo-germanicum* resembled *C. oblongum* in the general features of its testa, yet the angling of the body of the seed, in the latter species obscure, was in the former marked by prominent external ribs, to the number of eight, symmetrically placed around the seed, which interiorly in this region formed a much rounded octagon (Pl. III, Fig. 22). In the middle region, for about half the total length, the sides were nearly parallel, tapering gently below to a thick stalk; whilst above, the surface curved rapidly inwards till the diameter diminished to about a third its maximum, and thence tapered abruptly upwards so as to form a conical tube 0·65 mm. high around the micropyle (Pl. III, Fig. 26, *m.co.*, and Text-fig. 9). The eight ribs traversed the whole body of the seed and formed prominent winged expansions to the stalk (Pl. III, Fig. 21, *R* and *r*, and Text-fig. 10, R^1 R^2 ..., r^1 r^2 ...). The ribs exhibit an interesting differentiation of the alternating members; four were large, and projected in the middle region about 125 μ from the surface, and may be termed the major ribs (Pl. III, Fig. 22, *R.*); the alternating members, which were only about half as prominent, we may term the minor ribs (Pl. III, Fig. 22, r^1, &c.). The relative thinness of the testa between the ribs, which only attains a thickness of 0·11 mm., coupled with the very marked prominence of the ribs, suggests that this latter feature may be of the nature of a mechanical adaptation. The differentiation becomes most marked at the apex; here the minor ribs, which remain of nearly uniform width, follow the outline of the seed, but die out suddenly where the curvature of the surface alters as it passes into the micropylar cone (Text-fig. 9, *m.co.*); the four major ribs continue their former gentle curvature above the shoulders of the seed, so that around the micropylar cone they become extensive wing-like expansions. A section cut at this level has the form of a four-rayed star, with the centre occupied by the micropyle (Pl. III, Fig. 23). Beyond the orifice of the latter the free pointed ends of the ribs projected, and their internal margins bounded the cup-like hollow in which the micropyle stood (Pl. III, Fig. 24, R^1. *e.* and R^3. *e*).

A median longitudinal section passing through two opposed major ribs appears concavely mucronate (Pl. III, Fig. 26), and somewhat resembles a similar section through the apex of *Stephanospermum*, where perhaps the

cup-like expansion might be regarded as a lateral fusion of a number of such major ribs, of which, however, the individuality has been completely lost.[1]

In detail the hard testa was, as in *C. oblongum*, composed of two kinds of elements, viz. an external palisade and an internal fibrous layer; these together attained a width in the middle region of about 0·11 mm. (palisade 75 μ and fibrous 38 μ).

The inner soft portion of the integument is, as in the other species, only represented by a micropylar membrane continuous with the plinth jacket, and was fused with the nucellus from the base of the plinth downwards; the sinus between the plinth and integument is very clearly seen in J. 3 (Pl. III, Fig. 29, *s.*), which forms a close parallel to R. 110 (Pl. II, Fig. 14).

4. *The Vascular System.*

The vascular supply further emphasized the differentiation which existed between the major and the minor ribs. There were four vascular strands, and these occupied the internal angles corresponding to the major ribs (Pl. III, Fig. 27, *v.b.*)—were embedded in the soft tissue between the hard testa and the nucellus. The lowest transverse section (Pl. III, Fig. 21, *v.b.*), which passes through the base of the megaspore cavity, still shows four vascular bundles cut across nearly transversely; so that if they united it was presumably some way down into the stalk. Above, the bundles entered the testa at the base of the micropylar cone and passed into the tapering loculi of the canopy, which reached to just below the apex (Pl. III, Fig. 29, *loc.*); these were no doubt filled, as in the other species, with parenchymatous tissue. We see then that the testa here has reached a much more advanced stage than in *C. oblongum*; the progressive fusion of the unit portions of the canopy has gone further, and, corresponding to the disappearance of the alternate bundles, their loculi have become completely obliterated (Pl. III, Fig. 23, also Text-fig. 10).

This differentiation of alternate members in a whorl, of which *Conostoma anglo-germanicum* is so striking an example, finds a close parallel amongst modern plants in the ribbed seeds of certain Umbelliferae, where, too, we find primary and secondary ridges with vascular bundles situated below alternate members.

There is no evidence for the existence of any soft apical tissue in this seed, but the 'blow-off' layer which extended over the whole seed attained very considerable dimensions between the major ribs at the apex (Pl. III, Figs. 23 and 29, *bl.*); and radial sections through the minor ribs show the cells of this layer with a dimension of as much as 0·19 mm.; it attained, however, its greatest development between the wings at the base (Pl. III, Fig. 21, *bl.*), thus contrasting with the other species in which this layer found its minimal development in this region.

[1] F. W. Oliver: On *Stephanospermum*. Trans. Linn. Soc., 2nd ser., Botany, vol. vi, Pl. XLIV.

The fact that the 'blow-off', in all the sections where preserved, is still *in situ*, seems to point to its either having functioned differently or to the specimens in each case being immature; the presence of pollen in at least one of these preparations seems to render the former alternative the more probable.

Owing to the greater complexity of its outline, especially at the apex, this seed presents very varied appearances when cut in different planes of obliquity; this point is sufficiently well illustrated by specimen J. 6 (Pl. III, Fig. 31), which cuts the seed obliquely, entering near the apex of a major rib and passing out at the base above the insertion of the stalk. The major rib on the left (R^1) and the minor rib on the right at the apex (r^2) are both exaggerated by the obliquity; but owing to the curvature of the proximal end of the seed this does not apply to the corresponding ribs at the base, which are cut nearly transversely. The upper lateral ribs (r^1, R^2) are followed for some way, and consequently appear as obtuse angled appendages of the testa, whilst the lower lateral ribs (r^3, r^4) are rendered cuspidate. The major ribs are respectively R^1, R^2, R^3, R^4, and the minor ribs r^1, r^2, r^3, r^4.

5. *The Nucellus.*

The structure of the nucellus of *Conostoma anglo-germanicum* closely resembled that of *Conostoma oblongum* in all essential points. The dimensions of the various parts differed as between the two seeds, the most notable divergence being in the height of the nucellus, which reached to 5 mm., as compared with 3·7 mm. in *Conostoma oblongum*. As in that seed, the lagenostome rests in a depression of the plinth (specimens J. 3 and 9, Pl. III, Figs. 28 and 30, *lg.*), whilst the plinth itself is characterized by the same peculiar features that have been so fully described in the case of *Conostoma oblongum*. As we find the preservation of *Conostoma anglo-germanicum* to be generally inferior to that of *Conostoma oblongum*, we shall restrict our detailed account of the former to the material points.

6. *The Lagenostome.*

The lagenostome resembled that of *Conostoma oblongum* in form, though its dimensions are slightly larger. Its height is 0·19 mm., and the breadth 0·26 mm., as compared with 0·15 mm. × 0·23 mm. in the allied seed. The cells of the wall appear somewhat more robust than in *C. oblongum* and, as in that seed, the cavity of the lagenostome is destitute of contents. Here also the appearance of a two-layered wall is suggested locally as in specimen J. 9, on the right-hand side low down (Pl. III, Fig. 25, *lg.*); the explanation is doubtless the same as in *C. oblongum* (see p. 18) and depends upon the sectioning of a curved surface combined with post-mortem change. The

state of preservation is as a rule inadequate to show the nature of the cell-walls; however, as at least one cell of the lagenostome bears distinct traces of sculpturing, it may be regarded as probable that in this respect also our seed was in agreement with *Conostoma oblongum*.

7. *The Plinth.*

The wall of the plinth survived as a carbonized crust, much as we find it in the poorer specimens of *Conostoma oblongum*. At its summit a similar depression was present, corresponding with the insertion of the lagenostome (Figs. 25, 29, and 30). The close relations already noted in the case of *Conostoma oblongum* between the plinth and lagenostome on the one hand, and the plinth jacket and micropylar funnel on the other, repeat themselves here in all essentials, even including the well-marked sinus just above the region at which nucellus and integument were confluent (cf. Pl. II, Fig. 14, and Pl. III, Fig. 29, *s.*).

The contents of the plinth are represented by the same structures as in *Conostoma oblongum*. From the base of the lagenostome the 'lens' hung suspended (Pl. III, Fig. 29, *ls.*), and below it in the same figure is found the pad of tissue which adhered to the tapetal septum (*pd.*). In specimen J. 9 the 'lens' is slightly displaced, but its primitive position is still indicated by a connecting shred of membrane (Fig. 25, *ls.*). Pollen-grains are present in the plinth cavity of specimens J. 9 and J. 12 (Figs. 25 and 24, *p.g.*). Their average dimensions are $85\,\mu \times 75\,\mu$—somewhat in excess of those for the companion species.

Very distinct traces of a tapetum enclosing the megaspore chamber are present (Fig. 31), but the character of the preservation hardly warrants detailed description. At the micropylar end the tapetum stretches across the nucellus as a septum, delimiting the base of the plinth (Fig. 30).

IV. Comparison with Related Types.

Comparison with Gnetopsis elliptica.

This famous seed was described and illustrated with some fullness by Renault in 1884 from specimens discovered in one of the Grand'Croix nodules.[1] Apart from the supposed relations with the Gnetales, *Gnetopsis* remained for many years a very isolated type until the reinvestigation of *Lagenostoma* revealed the existence of certain features which these two seeds appeared to have possessed in common.[2]

With *Conostoma oblongum* the points of agreement are numerous and striking, and demand some notice here.

[1] Renault: Cours de Bot. fossile, vol. iv, p. 180, Pl. XX, XXI, XXII.

[2] Oliver and Scott: On *Lagenostoma Lomaxii*. Phil. Trans. B., vol. cxcvii, p. 233.

The lagenostome of *Gnetopsis* has essentially the same form and insertion as in *Conostoma*. It is a small goblet-shaped body resting in a depression of the plinth with which its cavity became continuous.[1] The cells of its wall constitute only a single layer, so far as we can judge from the specimens, but they show evident traces of sculpturing like that of *Conostoma*, though less well preserved.

Our examination of Renault's original type specimens shows that the presence of papillae around the mouth of the pollen-chamber in the preparations B. 230, C. 2 and 11 ('languettes disposées en couronne') is to be interpreted as the result of partial resolution of the wall cells of the lagenostome by means of fissures corresponding in position with some of the vertical lines which, as in *Conostoma*, separated the adjacent rows of sculptured cells. The resulting lobes or finger-like packets, which included as a rule two or three vertical series of these cells, remained attached below to the summit of the plinth.[2]

The dimensions of the lagenostome in the three seeds are as follows:—

	Gnetopsis.	*C. oblongum.*	*C. anglo-germanicum.*
Maximum width	210 μ	230 μ	260 μ
Width of basal orifice	115 μ	180 μ	190 μ
Height	145 μ	150 μ	190 μ

The agreement with *Conostoma* is complete when the nature of the lobing in *Gnetopsis* is apprehended.

The Plinth. The broad low cavity between the prothallial chamber and the lagenostome corresponds with the plinth of *Conostoma*. It was naturally termed the 'pollen-chamber' by Renault as it is here that the pollen is usually found.[3] The central portion of its cavity is occupied by a plug of tissue which rests on the floor, much as in our *C. oblongum*, specimen R. 117 (Pl. II, Fig. 16). Occasionally in *Gnetopsis* (B. 230, C. 12) this somewhat robust lump of tissue is produced towards the lagenostome into an upwardly directed, more delicate continuation. It is hardly possible to say whether the latter alone represents the displaced central core of the lagenostome whilst the lump below is the residual tissue of the plinth, or whether both may not have been derived from the lagenostome—a difficulty also found in the interpretation of our R. 117 (cf. p. 22).

[1] Renault: loc. cit., Pl. XXII, Fig. 4.

[2] In Renault's preparations the lagenostome occurs under two types of preservation, i. e. the fingered or papillose type (which we interpret as macerated) represented in Cours de Bot. foss., vol. iv, Pl. XX, Fig. 3, and Pl. XXI, Fig. 3, *e*, and the intact type represented on his Pl. XXII, Fig. 4, t^1. The information, for which we are indebted to Prof. Bertrand, that the intact type (which differs slightly in other respects) was probably derived from a source other than Grand'Croix (which provided the main series of specimens) explains the differences referred to.

[3] In all the specimens of seeds of the *Conostoma* group that have passed through our hands we have only detected a single pollen-grain in the lagenostome, viz. in *Gnetopsis*, the specimen being the one figured by Renault on his Pl. XXI, Fig. 4.

The pollen-grains found in the plinth have $80\mu \times 65\mu$ as average dimensions, as compared with $75\mu \times 65\mu$ in *C. oblongum*, and $85\mu \times 75\mu$ in *C. anglo-germanicum*.

The Integument. The micropylar tube, funnel, and plinth jacket present in specimens B. 230, C. 2 and 6,[1] were doubtless integumental in origin. The tube reached a considerable length and was named the 'entonnoir' by Renault; in specimen B. 230, C. 6,[2] it contained grains of pollen fossilized *en route* to the lagenostome and plinth cavity.

A conspicuous feature of the integument, especially towards the apex of the seed, was the presence of 'un tissu lacuneux formé de grandes cellules disposées en lames parallèles'.[3] This tissue, conjectured by Renault to function as a float giving buoyancy to the seeds in water, resembles in the closest way the 'blow-off' tissue found in *Conostoma*, and we see no reason to doubt their essential identity.[4]

A great peculiarity of the seeds of *Gnetopsis* was the tuft of apical plumes inserted around the micropyle. Of these structures no trace is shown by *Conostoma*, so that unless they were caducous in the latter, their absence must be regarded as an important point of distinction between the two seeds. In view of the slight flattening detected in the body of *Conostoma oblongum* it is of interest to note that in *Gnetopsis* the symmetry was likewise modified in the direction of platyspermy;[5] whilst in *C. oblongum*, however, the whole of the six vascular bundles are accounted for, in *Gnetopsis* there are only four, those corresponding with the positions we suppose to represent the two major angles being absent. *Gnetopsis* thus appears to combine in itself the peculiarities of both our seeds, in showing the flattening of *C. oblongum*, with the disappearance of some of the bundles as in *C. anglo-germanicum*.

Another point in which *Gnetopsis* perhaps differed from our seeds was in the relatively slight development of a 'tent-pole'. As practically every specimen of *Gnetopsis* contained a prothallus these seeds should be of the right age, as judged by *Conostoma*, to show the 'tent-pole' had it reached any degree of prominence.

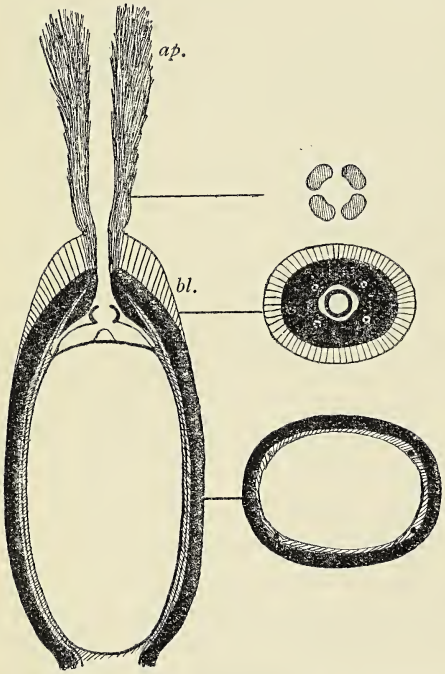
Turning to the cupule which formed the common enclosure of from two to four seeds in *Gnetopsis*, we have, as yet, no parallel in *Conostoma*, where the seeds are only known as detached objects.[6]

Having regard then to the various points cited, viz. the lagenostome, plinth, micropyle and plinth jacket, 'blow-off,' symmetry and distribution of vascular strands, we think the case for the close association of *Gnetopsis* with, or even its inclusion in, the Conostoma group a very strong one.

[1] Renault: loc. cit., Pl. XX, Figs. 2, 3, 4.
[2] Renault: loc. cit., Pl. XXI, Fig. 3, *o*.
[3] Renault: loc. cit., Pl. XX, Figs. 2 and 3, *l*.
[4] Cf. our Pl. III, Fig. 29, and Renault, loc. cit., Pl. XXI, Fig. 3.
[5] Cf. our Text-fig. 11, p. 34, and Renault, loc. cit., Pl. XXI, Fig. 6.
[6] See however pp. 15 and 16.

The only important point in which *Gnetopsis* differs from our seeds is in the presence of plumes at the apex, for which there exists no evidence in *Conostoma*; a minor point is the very slight development of a 'tent-pole'. It is of course possible that even these points of difference may disappear as the seeds of the group become more fully understood.

Having regard to the occurrence of *Gnetopsis*, which reaches from the Middle Coal Measures up to the Stephanian at the top of the Carboniferous Formation, it is a matter of no little interest to find plants with the Conostoma type of seed mechanism persisting from the Lower Coal Measures right on to the close of the Carboniferous.



TEXT-FIG. 11. Longitudinal and transverse restorations of *Gnetopsis elliptica*, largely hypothetical. *ap.*, apical appendage. *bl.*, 'blow-off'.

This long persistence points to the fact that the small lagenostome and large pollen-grain formed a combination at least as perfect as any other of the contemporary seminal arrangements of which we have any knowledge.

Before leaving the subject of *Gnetopsis* it seems worthy of remark that the cupule of that seed presents an interesting point of agreement with that of *Lagenostoma Lomaxii*. After comparing the specimens, we are much struck with the close resemblance in structure between the long tubular hairs with which the cupular lining of *Gnetopsis* was so abundantly provided and those met with in considerable quantity on the cupule of an ovular stage of *Lagenostoma*, and more sparingly on the old effete cupules.[1]

The occurrence of similar hairs in analogous positions on the cupules of the two seeds, though in itself a trivial point, gains in importance when taken in connexion with the other features of organization which these seeds had in common.

Until a detailed knowledge of the structure of the apical region of the testa in *Gnetopsis* is forthcoming, any close comparison between this region and the multilocular canopies of the other seeds is out of the question. The

[1] Oliver and Scott: On *Lagenostoma Lomaxii*. Phil. Trans., B., vol. cxcvii, Pl. X, Fig. 34, *h*, and Pl. VIII, Fig. 8.

presence, however, of a series of apical tufts at the micropyle is at least consistent with a lobed antecedent, and this equally whether these tufts consist of hairy prolongations of the actual ribs of the seed or whether, on the other hand, they are merely the expression of localized proliferations of the trichomes which may, however, have corresponded in position. In order to give precision to the remarks on *Gnetopsis* and to facilitate comparison with the other types, we have embodied our view of the structure of this seed in the convenient form of a text-figure (p. 34). The necessity for a detailed reconstruction happily does not arise, as we understand there is some prospect of *Gnetopsis* undergoing re-investigation at the hands of our friend Professor C. E. Bertrand, to whose good offices we are deeply beholden for the opportunity of consulting the specimens on the present occasion.

2. *Comparison with Physostoma.*

This seed belongs to an interesting generalized type in view of the prominence of the ribbing and the lobing of the testa. Moreover, the presence over the surface of the seed of large hairs probably containing mucilage affords a further variant of the mechanism which is illustrated by the 'blow-off' of *Conostoma* and the mucilage pegs of *Lagenostoma*.

The lagenostome of *Physostoma* with its all but sessile mouth forms a connecting link between those of *Lagenostoma* and *Conostoma*; the former with its tubular prolongation reaching to the surface of the seed, the latter with a true micropylar tube, integumental in origin, which fitted to the rim of the cup-like lagenostome with marvellous nicety.

Within the seed the 'tent-pole' prolongation of the megaspore chamber projected into the floor of the lagenostome, thus outstripping all other known cases. On the other hand the plinth is practically undeveloped. These facts of structure taken in connexion with the large number of ribs and lobes appear to be consistent with the view that *Physostoma* preserves several of the more archaic traits of the unknown precursors from which the various types may be supposed to have sprung. Among the features which on this view would belong to these precursors must be included the lobed, unfused micropyle, the many ribs, the mucilage epidermis, the terminal, indurated, capacious lagenostome, and the 'tent-pole'. The extension of the plinth, on the other hand, is to be regarded as a later intercalation.

3. *Comparison with Lagenostoma.*

The features which unite *Lagenostoma* and *Physostoma* are too well known to need recapitulation here.[1] As compared with *Conostoma* the loculi of the canopy in *Lagenostoma* had a more extensive filling tissue and relatively thinner peripheral and radial layers of sclerized elements, the

[1] Oliver: On *Physostoma*. Ann. of Bot., vol. xxiii, p. 108.

filling tissue in the former being reduced to a mere parenchyma sheath accompanying the vascular strands into the loculi.

A peculiarity of *Conostoma oblongum* was the soft tissue at the apex of the integument, of which no indications have been detected in *Lagenostoma*.

In both genera the integumental units show a high degree of coalescence in the micropylar region, the degree of fusion being considerably greater however in *Lagenostoma* than *Conostoma*, which appears somewhat to approach the condition of *Lagenostoma* (*Physostoma*) *Kidstonii*, Arber.[1] This coalescence of integumental units may be regarded as yet another example of a generally diffused tendency, no doubt correlated with a simplifying of the mechanism of development, viz. the replacement of separate parts borne at the same height by a continuous structure.

The lagenostome of *Lagenostoma* reaches the exterior of the seed by a tubular prolongation, thus contrasting markedly with the etubular condition of *Conostoma*, where a functional micropyle is provided on the lines of most existing Gymnosperms. In view of this difference, it is not possible to regard the two types of seed as very closely related.

Other contrasting features include the nature of the wall sculpturings of the lagenostome—which are not reticulate in *Lagenostoma*; its relatively large size and the persistence of a central core of tissue—not yet detected in *Conostoma*.

The plinth, which was present in full-sized seeds of *Lagenostoma* but not in the small ones (Text-fig. 12), sloped up at a very gentle angle as compared with the corresponding part of *Conostoma*; its presence being correlated rather with a transverse than with a material longitudinal expansion of the nucellus. A 'tent-pole' does not appear to have been produced.

Thus it is plain that whilst both *Conostoma* and *Lagenostoma* have proceeded along similar lines in the coalescence of the integumental units, they show considerable divergence in the details of lagenostome structure, in the plinth, and in the arrangements for the reception of the pollen.

V. Classification and Diagnoses.

In the light of the previous discussion it is convenient to separate the various seeds that have been enumerated into three series or types, all of which possessed in common:—

(1) A nucellus and integument confluent up to the level of the plinth; (2) a free part of the integument consisting either of separate lobes, or of a more or less completely fused series of lobes forming what has been termed a 'canopy'; (3) a vascular system of strands running in the deeper parts of the integument and passing into the lobes or their representatives at the apex; (4) an epidermis to the testa which was mucilaginous, at any rate

[1] E. A. N. Arber: On some New Species of *Lagenostoma*. Proc. Roy. Soc., B., vol. lxxvi.

locally; (5) a terminal, specialized, more or less pear-shaped receptacle for the pollen known as the lagenostome.

These three series of types, with provisional diagnoses, are as follows:—

i. The Physostomeae.

Free parts of the ribbed integument consisting of separate segments surrounding and overlapping the large, globose lagenostome which opened by a small orifice inserted on the apex of a low papilla. Plinth rudimentary. The apex of the megaspore cavity projected into the floor of the lagenostome. Ribbing at base obsolete.

Physostoma elegans, Will.
Physostoma Kidstonii, Arber.

ii. The Conostomeae.

Seeds ribbed or angled, tapering at apex: free part of integument consisting of more or less fused lobes. The lining of the integument formed the passage for the large pollen-grains and was thus a true functional micropyle leading down to the very small, included lagenostome, the wall of which consisted of cells with reticulated or scalariform sculpturings; base of lagenostome communicating with the extensive plinth cavity into which the pollen-grains penetrated. Integument with a mucilaginous epidermis extensively developed at the apex.

Conostoma, Williamson.

Cylindrical or slightly flattened seeds with tapering insertion. Ribbed throughout, or at base with angles passing into ribs. Lobing at apex variable; vascular bundles equalling or fewer than the ribs or angles; loculi of canopy nearly obliterated and equalling the vascular bundles in number; epidermis mucilaginous.

Lagenostome very small, included; cells of wall sculptured.

Plinth conspicuous, dome shaped, with internal tissue; well-marked 'tent-pole' and tapetum present.

1. *Conostoma oblongum*, Will.

Organization of the Fossil Plants of the Coal Measures, Pt. viii. Phil Trans., 1877, p. 243, Figs. 80, 80* (Pl. XI), 80* (Pl. XII), 81 and 86.

Localities. Oldham; Dulesgate; Shore, Littleborough; Halifax; Deighton, Yorks.

Horizon. Lower Coal Measures.

Seed six-angled, winged at base only; six vascular strands; apex ending in six soft, free lobes.

Dimensions: length 6 mm., broadest diameter 2·3 mm.

2. *Conostoma anglo-germanicum*, sp. nov.[1]

Localities. Shore, Littleborough; Dulesgate; Langendreer, Westphalia; Colliery Rheinpreussen, near Duisburg—Seam Finefrau, Nebenbank.

Horizon. Lower Coal Measures.

Seed eight-ribbed, the four major vascular more prominent than the four minor non-vascular, which fall short of apex.

Dimensions: length 7 mm., broadest diameter 2·3 mm.

Gnetopsis elliptica, Ren. and Zeill.

iii. Lagenostomeae.

Free part of integument consisting of more or less completely united segments; very obscurely angled. Lagenostome with tube reaching surface of seed and persistent central cone of tissue; low plinth with gentle gradient; no 'tent-pole'.

Lagenostoma ovoides, Will.
Lagenostoma Lomaxii, Will. MS.
Lagenostoma Sinclairii, Arber, perhaps came here.

An outer envelope or lobed cupule has been described for *L. Lomaxii* and *L. Sinclairii*.

It is probable that Miss Benson's *Sphaerostoma ovale* (Will.) will have to be added as a fourth type to the three enumerated above. Its inclusion here would be premature as the seed is undergoing re-description.

With the exception of *Lagenostoma Lomaxii*, which has been definitely referred to *Lyginodendron Oldhamium*, the parentage of none of the seeds has been determined. In view, however, of the broad agreement in type which they all show, an ultimate reference of these seeds to plants of *Lyginodendron* affinity seems not improbable.

As a convenient collective name for the whole of the seed types or series just enumerated we would suggest, at any rate for provisional use, the name Lagenostomales.

VI. The Pollination Mechanisms of the Lagenostomales.

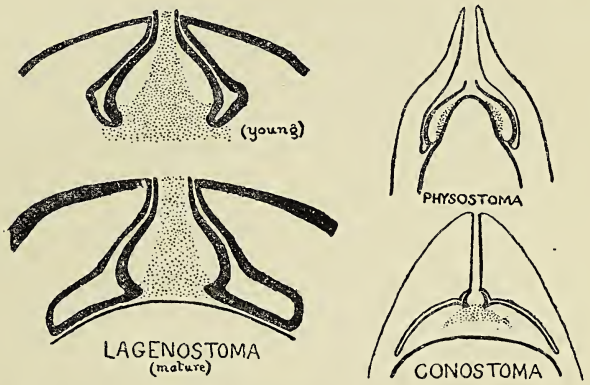
The detailed study of the various seeds grouped under the Lagenostomales has led to the recognition of three distinct types of mechanism

[1] The course followed here of including this seed as a second species of *Conostoma* perhaps demands a word of explanation. The peculiar character of the ribbing, with alternation of major and minor ribs, would go far to justify the creation of a new genus, particularly when regard is had to the relative importance of the testa as a diagnostic character in fossil seeds. Earlier workers in this field have repeatedly founded genera on equally trivial characters, e. g. Brongniart in his Graines silicifiées. Our motive in refraining for the present from raising *C. anglo-germanicum* to generic rank is to emphasize the fact of the essential identity of its internal organization with that of *C. oblongum*.

concerned in the reception and storage of pollen. Briefly stated, these mechanisms were as follows:—

1. The unjoined but approximated lobes of the integument surrounded and overtopped a relatively large lagenostome which bore an orifice seated on a low central papilla. At the time of pollination it is probable that these lobes collectively formed a tube or funnel narrowing towards the mouth of the lagenostome. If so, this type possessed a functional micropyle which played its part in the passage of the pollen (Text-fig. 12, *Physostoma*). This type may be termed a fimbriated micropyle.

2. A relatively massive canopy of united segments was perforated by a long micropyle which led down to a small, included lagenostome, the



TEXT-FIG. 12. Diagrams to show the relations of lagenostome, plinth, and canopy in *Lagenostoma*, *Physostoma*, and *Conostoma*. The regions where soft-filling tissue occurs are dotted, i.e. the central cone of the lagenostome in *Lagenostoma*, a shallow cushion resting on the flanks of the intrusive apex of the megaspore chamber in *Physostoma*, and a lens-shaped cushion below the lagenostome in *Conostoma*. Two stages of *Lagenostoma* are shown.

cavity of which became confluent with that of the plinth below by deliquescence of the filling tissues. The pollen-grains which traversed the micropyle and lagenostome were received into the plinth cavity, where they doubtless underwent maturation. This type is marked by a plinth of considerable vertical extension, an organ not conspicuously developed in type 1 (Text-fig. 12, *Conostoma*).

3. The lobes of the integument were fused into a compact canopy which closely invested the conical lagenostome, the orifice of which reached to the outer surface of the seed. In this type the lagenostome must have been directly pollinated without the intervention of the micropyle. A plinth was present, but had only a trifling vertical extension (Text-fig. 12, *Lagenostoma*).

So far as efficiency in the collection of pollen-grains was concerned, each type appears to have been perfectly satisfactory. The presence of much pollen in the lagenostome of *Lagenostoma*, and especially in the

species *L. ovoides*, has already been a matter of comment,[1] whilst in *Physostoma* no fewer than eighty grains have been counted in a single section that recently passed through our hands—a number that may safely be trebled to get an approximation to the full number. In *Conostoma*, with its long micropyle, tiny lagenostome, and plinth cavity, the evidence shows that an abundance of large pollen-grains found their way into the plinth cavity.

When regard is had to the relations of the parts in these three types, it seems evident that, assuming them to be derived from a common ancestral group, the reception of pollen was originally independent of the integument. If the free lobing of the integument of *Physostoma* is an archaic character, then in so far as these lobes collectively assisted at pollination this pristine method of pollen reception has been lost and an approach made to the entire micropyle as found in *Conostoma* and *Gnetopsis*.

In marked contrast with these types was *Lagenostoma*, where the lagenostome had kept pace with the investing members and, by means of its elongated neck, retained to itself the function of pollen reception. The persistence of this exserted type of lagenostome, a rare condition, may perhaps be regarded as a kind of conservatism which militated against the surrender to the investing structure of the receptive and conductive functions—a conservatism which finds further illustration in *Gnetum*, where the inner integument projects far beyond the envelopes exterior to itself.

This at any rate seems clear, the arrangements which prevailed in the Conostoma type, with its intercalated plinth, had as a result the carriage of the pollen deep into the heart of the seed. The double functions of reception and storage relinquished by the lagenostome were taken over by the micropyle and plinth cavity respectively, the lagenostome persisting as a sort of inner vestibule—a mere piece in an elaborate though doubtless very perfect mechanism. The very smallness of the lagenostome, whose diameter never exceeded 150 μ in the known representatives of this type—a dimension barely equalling the length of two pollen-grains as we know them in the plinth cavitity—fully accords with this vestigial phase upon which the lagenostome would appear to have entered.

Passing on to the seeds of other groups, we come first to members of the Medulloseae, of which *Trigonocarpus*, *Stephanospermum*, and a number of the French Permo-carboniferous seeds afford the best known examples. In these, so far as information is available, there existed a prominent nucellar beak which engaged with the base of the micropyle. The pollen was received by this tubular beak from the micropyle and passed into a deeper lying 'pollen-chamber' below, perhaps comparable to the plinth cavity of *Conostoma*. The flattened seeds usually referred to the Cordaiteae appear to be in substantial agreement, whilst the same remark holds good of the living genera of Cycads and of *Ginkgo*.

[1] Oliver and Scott: loc. cit., p. 214.

In this connexion it is interesting to note that in *Stangeria* and *Ginkgo*, as figured respectively by Lang[1] and Hirasé,[2] the apical papilla of the nucellus which becomes perforated is limited by a prominent epidermis, as to which Lang remarks, 'The superficial cells of the pointed tip seen in Fig. 12 have their walls thickened and form a very definite boundary to the sides of the chamber, suggesting a close comparison with the corresponding region of certain fossil gymnospermous seeds.'[3] The actual place which the pollen reached—the 'pollen-chamber'—is found at a deeper level, a statement also holding good of the fossil seeds to which passing reference has been made. Thus, whilst the facts in so far as they are known are consistent with an elaboration of the nucellus, on lines analogous to *Conostoma*, in the seeds of the Medulloseae, Cordaiteae, recent Cycads, and *Ginkgo*, to say that the beak of the nucellus in these seeds corresponds with a vestigial lagenostome, and the pollen-chamber with a plinth cavity, would be premature if not erroneous. Much fuller details of ovular development than are yet available are required before we can advance further.

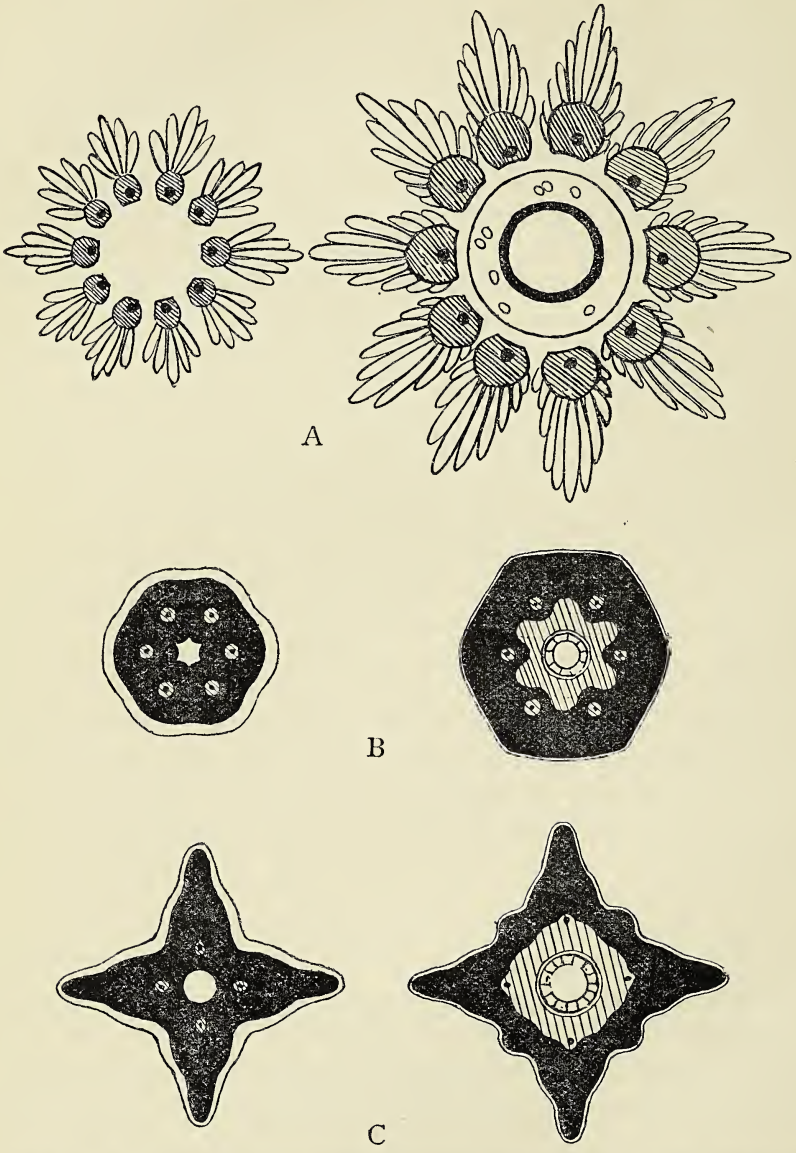
Before leaving this part of the subject reference may be made to the presence in several siphonogamous Gymnosperms of examples of ovules of which the nucellus undergoes spontaneous disintegration at the apex before the arrival of the pollen. Whilst this procedure would appear to be the rule in the three genera of Gnetales, in *Ephedra*[4] and *Gnetum*[5] it was carried so far that definite excavations or 'pollen-chambers' were produced. The functional significance of this peculiarity in plants whose fertilization is accomplished by the agency of pollen-tubes is far from evident, and we must await new light from current or future investigations. We would only remark in this connexion that the past history of the Gnetales and of such Conifers as show analogous arrangements[6] may be the determining factor in the possession of a mechanism which has somewhat the appearance of being an anachronism.

VII. General Discussion on the Testa.

The ribbing, which is so general a character of these and allied seeds, is broadly an indication of a multiple origin of their integuments. Whatever the nature of the members which coalesced to form this organ, it seems reasonable to assume that primitively each of the coalescing members had its own vascular strand, and that the correspondence which usually obtains, both in number and in position, between the ribs and bundles, is an expression of one and the same fact, viz. the multiple origin. In *Physostoma*

[1] W. H. Lang: Ann. of Bot., vol. xiv, Pl. XVII, Fig. 15.
[2] S. Hirasé: Journ. Coll. Sci. Tokyo, vol. xii, Pl. IX, Figs. 31 and 32.
[3] Lang: loc. cit., p. 286.
[4] W. J. G. Land: Bot. Gaz., vol. xxxviii, Pl. V, Fig. 44.
[5] Lotsy: Ann. Jard. Bot. Buitenzorg, vol. xvi, Pl. V, Fig. 35.
[6] e. g. *Sciadopitys verticillata*; see Lawson, Ann. of Bot., vol. xxiv, Pl. XXIX, Fig. 13.

the ribs passed out into free tentacles at the apex, each with its own vascular bundle, and in the young seed the conjunctive tissue between the ribs was



TEXT-FIG. 13. Transverse sections (diagrammatic) passing through the apex and the lagenostome of *Physostoma* (A), *Conostoma oblongum* (B), and *Conostoma anglo-germanicum* (C). Parenchyma shaded : testa in black : 'blow-off' white.

much less developed than in mature specimens, perhaps an ontogenetic recapitulation of phylogeny.[1] This is still further borne out by the gradual

[1] Oliver : Ann. of Bot., vol. xxiii, p. 100, Pl. VII, Fig. 29.

elimination of members as we pass from those seeds in which the lobes are partially free to those in which they are almost completely fused. In *Physostoma*, where the multiple integument is most marked, the number is ten; in *Lagenostoma Lomaxii*, which has a slightly lobed apex, nine; and in *Conostoma oblongum*, where the lobes are internally almost obliterated, six (Text-fig. 13).

The primitive condition of each member was, we think, that seen in *Physostoma*, where the tissue of each tentacle consists of undifferentiated parenchyma. As we pass along the series, we find progressive sclerization proceeding inwards from the whole periphery of each member, thus tending towards the final obliteration of the parenchyma which formed the 'loculi' of the canopy, the sclerization having its inception historically before the fusion of the constituent members. In this way the alternate loculi in *Conostoma anglo-germanicum* have disappeared, though still represented exteriorly by the minor ribs. This gradual decrease in the number of bundles may have culminated in their total suppression, resulting in a condition similar to that in the integument of present-day Phanerogams.

With the more complete fusion of the component members came a gradual loss of vascular tissue. This diminution of the vascular supply of the ovule was restrained in those forms where, as in Cycads, the motile sperms are still retained, but elsewhere, as in most Gymnosperms and the whole of the angiospermic series, where siphonogamy has replaced zoidiogamy, the vascular supply tends to become reduced to a mere basal cup with rare indication of its distal extensions. Thus this view, which has already been put forward, seems to find in these seeds two more links in the chain of evidence.[1]

It seems not improbable that, as already suggested, the prominent ribbing in *C. anglo-germanicum* has a definite mechanical value, for the seed is an exceptionally long one as compared with its width, whilst the testa between the ribs is even thinner than in the much shorter seed of *C. oblongum*. This suggestion seems to find corroboration in *Polylophospermum*, where, too, there is pronounced ribbing associated with a thin sclerotesta and great length.[2] From the integumental standpoint we can then regard *C. anglo-germanicum* as a late stage of the series, in which only four members remain as such, the peripheral portions of all the members still being retained as ribs in relation to their mechanical value; whilst *Gnetopsis*, with four ribs only, probably forms its culmination.

In the medullosean series of forms there is a similar relation existing between ribs and bundles. The latter are, however, situated within the sarcotesta and *exteriorly* to the ribs. In *Trigonocarpus Parkinsonii*, Br.,

[1] Oliver: On the Ovules of the Older Gymnosperms. Ann. of Bot., vol. xvii, 1903, p. 451.

[2] Oliver: Notes on *Trigonocarpus* and *Polylophospermum*. New Phyt., vol. iii, No. 4, 1904, p. 96.

there are three principal ribs, three secondary, and six tertiary. The six bundles subtend the last.

In *Polylophospermum stephanense*, Br., there are six major ribs and six minor, each with a bundle.

If the origin of the integument here was similarly multiple, we must assume that there was complete fusion of the individual members before the inception of sclerization. This latter extended along the inner surface of the fused organs. The ribs may have been purely mechanical and utilitarian in origin, and their relation to the bundles of a similar nature to that which is exemplified in the leaf of a Cordaitean such as *C. angulostriatus*, where sclerization has proceeded at both surfaces and produced prominent ribs at each bundle, and between each pair of bundles a secondary rib and two symmetrically placed tertiary ribs on either side. In modern Cycads, such as *Macrozamia spiralis* and *Encephalartos Altensteinii*, the bundles in the outer flesh overlie the ribs of the sclerotesta.[1] If the integument here be double, as some hold,[2] it could only be homologized on this view by the assumption that sclerization took place, in time, subsequent to the fusion of the outer and inner integuments.

A further point of general interest as regards the testa, and perhaps of some considerable significance, is the flattening observed in the seed of *Conostoma oblongum*; this platyspermy is even further developed in *Gnetopsis elliptica*, where it is associated with a reduction of the number of bundles to four (Text-fig. 11). The appearance of the transverse section of the latter seed could be readily obtained from the former if we suppose the two major ribs to have lost their bundles and the corresponding angles to have been flattened to a gentle curve. These facts, taken together with the general tendency exhibited in the group towards the reduction in number of the vascular strands accompanied by a corresponding reduction in the number of ribs or angles, point to the possibility of this tendency having been carried still further, resulting in the production of a seed with only two vascular strands and a testa exhibiting a bilateral symmetry comparable to that of *Cardiocarpus*. But whether such a seed definitely referable to this chain of affinity be found or not, the facts seem to indicate that, whilst the terms 'radiospermic' and 'platyspermic' have a definite use as morphological distinctions, our attitude towards them as criteria of taxonomic importance may require readjustment.

The presence in *Conostoma* and *Gnetopsis* of the highly specialized layer we have termed the 'blow-off' seems to call for some explanation. Probably to be regarded as homologous with the peg-producing layer of *Lagenostoma* and the epidermis with its mucilage-containing hairs in *Physostoma*, its

[1] M. C. Stopes: Beiträge zur Kenntnis der Fortpflanzungorgane der Cycadeen. Flora, 1904, p. 474.

[2] Coulter and Chamberlain, p. 158, Morphology of Spermophytes, 1901; M. C. Stopes, loc. cit.

secretory nature may be due to the same internal causes that have so frequently rendered vestigial structures secretory among living plants. The 'blow-off' layer and the soft apical tissue of *Conostoma oblongum* may be the remnant of a once much more extensive tissue comparable to the sarcotesta of the medullosean series. The closest analogy which modern plants offer appears to be the megaspore of *Pilularia*. Here the mucilaginous layer which invests the megaspore serves to attract and retain the sperms; above the archegonium the mucilage forms a deep funnel, which becomes filled with spermatozoids.[1] In *Conostoma* the mucilage layer, as in *Pilularia*, reaches its maximum development at the apex. In *Conostoma oblongum* the epidermis split up the flanks of the free apical lobes, as is seen in Pl. I, fig. 6, *bl.*; the expanding mucilage must thus have found its way into the micropyle and in the space between the apical lobes. If the seeds were retained till after pollination this mucilage may well have acted as a drop mechanism comparable to that of the present-day *Taxus*. If, however, as might have been the case, the seeds were first shed, perhaps the mucilage played a part analogous to *Pilularia* in capturing and nourishing the male cells. Our knowledge, however, of the functions of mucilage, even in recent plants, is so incomplete as to render the problem in fossil plants extremely difficult.

In *Conostoma anglo-germanicum* and *Gnetopsis* the 'blow-off' is not exfoliated even in specimens showing pollen-grains. We probably have then in all these seeds to deal with a common physiological cause, and any value the layer may have had in certain cases is to be regarded as a secondary adaptation.

VIII. Conclusion and Summary.

The facts recorded in the foregoing paper go to prove that the seeds of the palaeozoic epoch showed, within certain well-defined limits, a considerable degree of diversity in mechanism.

When regard is had to the dominance which seed-possessing plants afterwards attained, it is hardly surprising that the seeds of Coal Measure times should have shown unmistakable indications of modification and elaboration in a variety of different directions.

This diversity, as it affected the apex of the seed, is fully illustrated in Text-figs. 12 and 13. Whilst the actual parts involved are in fundamental agreement—lagenostome, plinth, and a compound integument—the detailed relations of these parts are altogether different. In *Physostoma* the large lagenostome was enveloped in the lobes of the integument, which collectively formed what may well have been the precursor of the micropyle in this group of seeds. In *Lagenostoma* these arms were united into a chambered 'canopy', which whilst investing the lagenostome, was overtopped by the orifice of the latter, which thus had direct access to the sur-

[1] Campbell: Mosses and Ferns, p. 425, 1905.

face of the seed. The other extreme is afforded by *Conostoma*, where the minute lagenostome lay at the foot of a long and specialized micropyle which traversed a canopy in which the unit parts, though more highly modified than in *Lagenostoma*, were still recognizable.

A peculiar organ, the plinth, claims special attention in *Conostoma*, not merely from its dimensions, but also on account of the part which it played in the reception of pollen. Though this zone or region is represented in all three types, it is only in *Conostoma*, and the probably related *Gnetopsis*, that it attained to any special significance. To what extent the nucellus of existing Gymnosperms—especially Cycads—may have undergone analogous elaboration cannot be stated with any confidence owing to the defective state of our knowledge of the developmental history of the ovules.

The ribbing and angling of these seeds also raises matters of interest dealt with in the body of the paper (p. 41). In these ribs there appear to be presented traces of what may be regarded, in the light of *Physostoma*, as the original segments or lobes of the ancestral integument. As these show considerable variety, even in allied seeds, in the relative prominence and in the presence or absence of accompanying vascular strands, it is evident that no great reliance can be placed on these characters for diagnostic purposes —especially where the larger groups are concerned. Incidentally, it may also be remarked that incipient stages in the passage from radial to bilateral symmetry appear to be illustrated by both *Conostoma oblongum* and *Gnetopsis elliptica*. This shows, if further proof be needed, that the old provisional distinction of palaeozoic seeds into radiospermic and platyspermic types had little or no significance as a guide to affinity.

Though allusion has been made to the modification and elaboration in different directions which the seed underwent, it would be premature hastily to suppose that our different types had necessarily diverged from a common *seed-possessing* ancestor. In these days when the doctrine of polyphylesis is steadily gaining ground, the alternative view that (to take a concrete case) *Physostoma*, *Lagenostoma*, and *Conostoma* had been separately derived from as many related but distinct *cryptogamic* types will certainly have to be considered. On that view, then, the differences between our seeds would depend not only on such divergences as arose after the establishment of the seed habit, but they would be, in part at least, determined by inherited differences already present (or latent) in the several ancestors.

Moreover, the coming of the seed habit must, from the evolutionary point of view, have marked a relatively active period ; for, even if we suppose the qualifications for seed-bearing to have been acquired in cryptogamic days, there must have been a transitional period during which the less immediately serviceable portion of the cryptogamic inheritance was either eliminated or underwent functional change.

These considerations may perhaps serve to indicate some of the diffi-

culties which beset the allocation to their exact place in phylogeny of the various structures and mechanisms which collectively constitute the seed.

In the foregoing paper, the subject-matter of which is set forth in the table of contents (p. 1), we describe in detail two palaeozoic seeds, *Conostoma oblongum*, Will., and *C. anglo-germanicum*, sp. nov.; these, with *Gnetopsis*, are provisionally placed in a separate group, the Conostomeae, ranking with the Physostomeae and Lagenostomeae as subdivisions of the larger class Lagenostomales. The seeds of the Conostoma type are compared with related forms, whilst diagnoses of the species and provisional diagnoses of the groups are given. In the more general parts of the paper especial attention is drawn to the arrangements for the reception and maturation of pollen found in the various seed types and to the peculiarities of the testa.

University College, London,
November, 1910.

IX. Glossary of Terms employed.

'Blow-off.' An epidermal layer of presumed mucilage-containing cells, forming the outermost investment of the testa (p. 14).

Canopy. The apical portion of the hard testa consisting of a varying number of more or less fused members surrounding the free portion of the nucellus.

Cupule. A free sheathing structure arising from the peduncle and investing one or more seeds.

Doubly oblique or **Assymetrically oblique.** Applied to a section which is oblique both to any plane of symmetry and to the axis of the structure cut (p. 12).

Flange. A ring-like projection of the integumental lining of the micropyle (p. 14).

Lagenostome. A differentiated chamber at the apex of the nucellus formed by modification of the epidermis. The lagenostome is either *included* where the integumental micropyle forms an intermediate passage between its orifice and the exterior, as in *Conostoma*, or *exserted* where by upward extension of the lagenostome it communicated with the exterior direct, e. g. *Lagenostoma*.

Lens. The contracted tissue of the plinth which frequently remained attached to the base of the lagenostome (p. 20).

Loculus. A chamber present in the canopy usually represented by a space, but probably filled with parenchymatous tissue continuous with the soft part of the integument lining the seed cavity, and into which the vascular strand passed.

Major rib or **angle.** Applied to the large ribs or angles of a seed irrespective of their vascularity (p. 28).

Minor rib or **angle.** Applied to the lesser ribs or angles of a seed where these latter fall into two categories only; in other cases the terms secondary and tertiary are employed.

Micropyle. The passage to the nucellar apex formed by the integument, which may be of three kinds, viz. a fimbriated micropyle of non-fused members, as in *Physostoma*; an entire micropyle, as in *Conostoma*; or an investing micropyle, as in *Lagenostoma*.

Micropylar funnel. The lower portion of the micropylar tube where it expands to join the seed cavity (lagenostome jacket).

Micropylar tube. The passage formed by the micropyle.

Micropylar membrane. The integumental epidermis lining the micropyle—often found separated.

Oblique. Applied to a section of which the plane is at right angles to a plane of symmetry but oblique to the axis of the structure cut.

Pad. The central portion of the lens (p. 20).

Plinth. The free portion of the nucellus supporting the lagenostome (p. 16).

Plinth jacket. The epidermis of the soft integument surrounding the plinth.

Shoulder. A term applied to that part of a structure where it begins to curve inwards towards the apex.

Sinus. The space between the free portion of the nucellus and the integumental lining or the gaps in a fimbriated micropyle.

Tapetal septum. The septum separating the megaspore cavity from the apex of the nucellus.

Tent pole.[1] A raised central portion of the apex of the prothallus.

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XI. EXPLANATION OF PLATES I–III.

Illustrating Messrs. Oliver and Salisbury's paper on Conostoma group of Palaeozoic Seeds.

U. C. L., R., and Q. = University College London Collection.
J. = Dr. Jongmans' Collection.
W. = Mr. D. M. S. Watson's Collection.

PLATE I.

Figs. 1–11 (Photographs).

Fig. 1. Nearly longitudinal section through seed, showing the boat-shaped outline. The cushion (*ls.*) is in position just beneath the lagenostome (*lg.*), and the micropylar membrane (*m.m.*) has contracted from the hard testa (*t.*). U. C. L., R. 111 (Shore). × 12 (see p. 10).

[1] S. Hirasé: Études sur la fécondation, etc., du *Ginkgo biloba*. Journ. Coll. Sci. Japan, vol. xii, p. 113.

Fig. 2. Apex of young seed cut obliquely, showing the plinth jacket (*pl.j.*) and wall of plinth (*pl.*) in close contact. The cells of the testa (*t.*) show contents, and the plinth tissue is slightly displaced from the base of the lagenostome (*lg.*). U. C. L., R. 114 (Halifax). × 80 (see pp. 12, 14).

Fig. 3. Apex of young seed cut tangentially. The 'blow-off' layer (*bl.*) is in position on the left, but on the right has exfoliated. The cells of the soft apical tissue (*st.*) are well shown and pass gradually into the hard tissue below (*t.*). U. C. L., R. 122 (Shore). × 70 (see pp. 12 and 15).

Fig. 4. Oblique section through winged base of seed, showing basal portions of the cell-walls of the 'blow-off' layer still attached (*bl.w.*) to the prominent wings of the base (r^4, r^5, r^6). U. C. L., R. 110 (Dulesgate). × 36 (see p. 15).

Fig. 5. Transverse section through middle region of seed. The somewhat flattened outline is well shown and the structure of the testa with outer palisade (*pa.*) and inner fibrous layers (*fb.*). *ma.* and *mi.*, major and minor angles. U. C. L., R. 119 (Shore). × 26 (see p. 10).

Fig. 6. Oblique section through apex, showing three lobes of 'blow-off' (*bl.*). The cells of the cushion (*ls.*) are well preserved. *lg.* lagenostome wall; *m.m.*, micropylar membrane; *pl.*, plinth. U. C. L., R. 116 (Shore). × 70 (see p. 18).

Fig. 7. Median longitudinal section through apex of same specimen as Fig. 1, showing the micropylar membrane (*m.m.*) cut obliquely and consisting above of superimposed tiers of cells. The lagenostome (*lg.*) is cut tangentially. *bl.*, 'blow-off' layer; *ls.*, lens or residue of plinth tissue. U. C. L., R. 111 (Shore). × 100 (see p. 14).

Fig. 8. Oblique section through micropyle, showing the soft tissue at the apex (*s.t.*) separating from the testa below (*t.*); the loculus of the canopy on the right (*loc.*) shows the vascular bundle (*v.b.*). *m.m.*, micropylar membrane. W. 267 (Shore). × 64 (see p. 12).

Fig. 9. Transverse section of base of seed, showing seven wings. *bl.*, 'blow-off'; *scl.*, sclerenchymatous cells; *v.b.*, vascular bundles; *c.s.*, central space; *s.p.*, one of the secretory passages. U. C. L., R. 113 (Halifax). × 40 (see p. 15).

Fig. 10. Somewhat oblique longitudinal section through seed passing out above the stalk; seven ribs are present (r^1, r^2, . . .), two of them with their vascular bundles preserved ($v.b.^5$, $v.b.^7$). *s.*, sinus; *lg.*, lagenostome; *ls.*, lens; *pd.*, pad. U. C. L., R. 110 (Dulesgate). × 18 (see p. 12).

Fig. 11. Doubly oblique section through seed, showing contained prothallus (*pr.*) with 'tent-pole' (*t.p.*); the shoulders of the plinth cavity (*pl.c.*) are exaggerated by the direction of the section. *bl.*, 'blow-off'; r^1 and r^2, ribs asymmetrically cut. U. C. L., R. 119 (Shore). × 30 (see p. 12).

PLATE II.

Figs. 12–20 (Photographs).

Fig. 12. Oblique section of seed above the lagenostome, showing the soft apical tissue above (*s.t.*) and with 'blow-off' (*bl.*) exterior to it. The micropyle is in the centre of the figure, its lining membrane (*m.m.*) is seen as a fluted layer. At the top of the figure are two somewhat displaced lobes of the canopy (*l.*, *l.*) with a sinus (*s.*) between. W. 268 (Shore). × 70 (see p. 13).

Fig. 13. Portion of apex of seed given in Pl. I, Fig. 1, showing the outer edge of the testa 'blow-off' raised up by pegs of mucilage beneath (*pe.*); *m.m.*, micropylar membrane. U. C. L., R. 111 (Shore). × 195 (see p. 15).

Fig. 14. Median longitudinal section through lagenostome, plinth, and adjacent parts. *lg.*, lagenostome with apparently two wall layers; *pl.*, wall of plinth; *pl.j.*, plinth jacket; *s.*, foot of sinus between plinth and plinth jacket; *ls.*, remains of plinth tissue ('lens') adhering to lagenostome; *pd.*, pad resting on tapetum; *v.b.*, position of vascular strand. U. C. L., R. 110 (Dulesgate). × 85 (see p. 20).

Fig. 15. Central part of Fig. 16 enlarged. *pd.*, central pad resting on remains of plinth tissue (*pl.t.*); *p.g.*, pollen-grains, the upper right-hand one with internal cells. U. C. L., R. 117 (Shore) × 182 (see p. 20).

Fig. 16. Longitudinal section of upper part of seed, showing testa (*t.*), and base of micropyle (*mc.*), lagenostome (*lg.*), plinth (*pl.*), and remains of tissue of plinth (*pl.t.*) resting on the tapetal septum (*tap.*); part of the tapetum has separated as a blister. Around the pad are several pollen-grains (*p.g.*). *loc.*, loculus of canopy; *s.*, base of sinus between plinth and plinth jacket. Photographed by Mr. W. Tams. U. C. L., R. 117 (Shore). × 38 (see p. 20).

Fig. 17. Longitudinal section of lower end of seed, showing testa (*t.*), and tapetum (*tap.*) several cell layers deep. U. C. L., R. 121 (Shore). × 80.

Fig. 18. Lagenostome enlarged from specimen given in Fig. 16. *lg.o.*, outer layer of wall, *lg.i.*, inner layer of wall of lagenostome; *pl.*, wall of plinth; *pl.j.*, plinth jacket; *u.f.*, upper flange, *l.f.*, lower flange of lagenostome. U. C. L., R. 117 (Shore). × 125 (see p. 7).

Fig. 19. Median section of apex of seed with prothallus (*pr.*); the asymmetry of the internal ribbing of micropyle wall shown. *i.r.*, internal rib; *m.t.*, micropylar tube; *m.f.*, micropylar funnel; *lg.*, lagenostome; *pl.*, plinth cavity; *ls.*, tissue of plinth ('lens'); *p.g.*, pollen-grain; *t.p.*, 'tent pole'; *tap.*, tapetum. U. C. L., R. 125 (Deighton, Yorks.). × 75 (see p. 20).

Fig. 20. Longitudinal section of micropylar tube (*m.t.*) and lagenostome; the wall of the lagenostome is cut tangentially, the cells showing conspicuous reticulated sculpturing. U. C. L., R. 115, 1 (Shore). × 182 (see p. 18).

PLATE III.

Fig. 21. Transverse section through base of the megaspore cavity, showing the basal wings formed by the major ribs (R^1, R^2, R^3, R^4), three of which have corresponding vascular bundles, and the minor ribs (r^1, r^2, r^3, r^4), which have no vascular elements. *tap.*, tapetum; *bl.*, 'blow-off' layer. U. C. L., R. 140 *d* (Langendreer). × 27 (see p. 29).

Fig. 22. Transverse section from the same series as above through the middle region. Major ribs (R^1, R^2, R^3, R^4); minor ribs (r^1, r^2, r^3, r^4). U. C. L., R. 140 *b* (Langendreer). × 27 (see p. 28).

Fig. 23. Section from the same series through the apex; the four minor ribs have died out. The four major (R^1, R^2, R^3, R^4) are pierced by the canopy loculi (*loc.*), in which the black dots, seen in two, are the vascular bundles. *mc.*, micropyle; *bl.*, 'blow-off'. U. C. L., R. 140 *a* (Langendreer) × 27 (see p. 28).

Fig. 24. Longitudinal section through the same seed as in Fig. 26. Micropyle (*mc.*) showing the concave upper edges of the major ribs (R^1 *e*, R^3 *e*). The lagenostome (*lg.*) is cut tangentially. *p.g.*, pollen-grains; *pl.*, plinth; *pl.j.*, plinth jacket; *s.*, sinus; *t.s.*, tapetal septum; *ls.*, tissue of plinth. J. 12 (Duisburg). × 42 (see p. 28).

Fig. 25. Longitudinal section of plinth and lagenostome, enlarged from Fig. 28. *mc.*, micropyle; *m.m.*, micropylar membrane, still adhering to the testa; *lg.*, lagenostome with cells preserved on the left; *pl.*, plinth cavity; *ls.*, lens, slightly displaced; *p.g.*, pollen-grains. J. 9 (Duisburg). × 100 (see p. 30).

Fig. 26. The same section as Fig. 24, through micropyle (*mc.*), showing mucronate apex of seed. R^1, R^3, major ribs; *m.co.*, micropylar cone; *s.*, sinus. J. 12 (Duisburg). × 17 (see p. 28).

Fig. 27. Oblique transverse section near base of seed, showing the four vascular bundles (*v.b.*) corresponding to the major ribs (R^1, R^2, R^3, R^4), minor ribs (r^1, r^2, r^3, r^4). *bl.*, 'blow-off'. Q. 18 (Shore). × 28 (see p. 29).

Fig. 28. Same seed as in Fig. 25, showing the lagenostome (*lg.*), plinth jacket (*pl.j.*), the lens (*ls.*), plinth (*pl.*). J. 9 (Duisburg). × 25.

Fig. 29. Longitudinal section of nucellar apex and testa, showing two loculi (*loc.*) of major ribs, 'blow-off' layer (*bl.*), lagenostome (*lg.*). *pl.j.*, plinth jacket; *pl.*, plinth; *s.*, sinus; *ls.*, lens; *pd.*, pad of tissue on tapetal septum. J. 3 (Duisburg). × 72 (see p. 29).

Fig. 30. Nearly longitudinal section passing out through two major ribs at the apex in which the loculi are cut obliquely (*loc.*); the lens (*ls.*) is seen in position with the descended pad (*pd.*). *bl.*, 'blow-off'; *v.b.*, vascular bundle; *pl.j.*, plinth jacket; *s.*, sinus; *lg.*, lagenostome; *pl.*, plinth. J. 3 (Duisburg). × 17 (see p. 30).

Fig. 31. Section tangential to seed and doubly oblique. The upper lateral ribs (r^1, R^2) are rendered obtuse, the terminal ribs (R^1, r^2) are exaggerated, and the basal laterals (r^3, R^4) have become cuspidate, through the plane of section. *v.b.*^{2}, *v.b.*^{3}, *v.b.*^{4}, vascular bundles; *bl.*, 'blow-off'; *pl.j.*, plinth jacket. J. 6 (Duisburg). × 17 (see p. 30).

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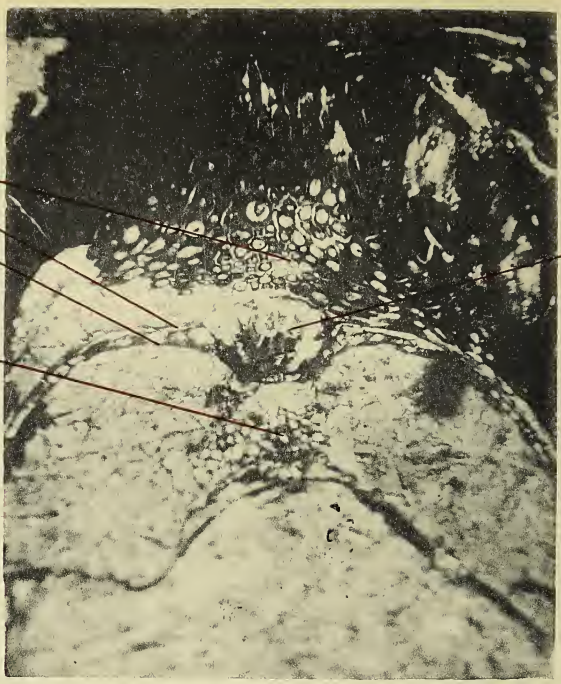

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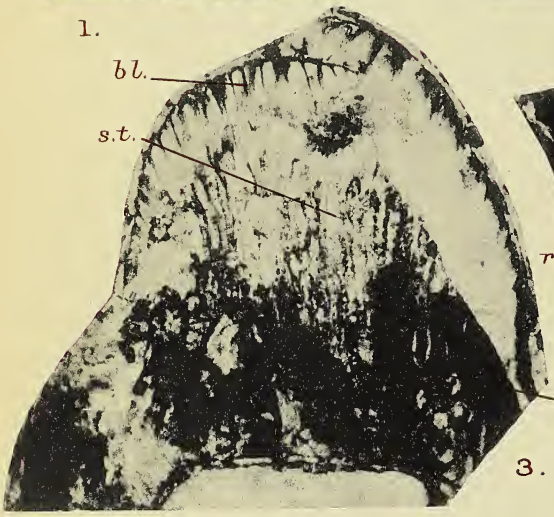

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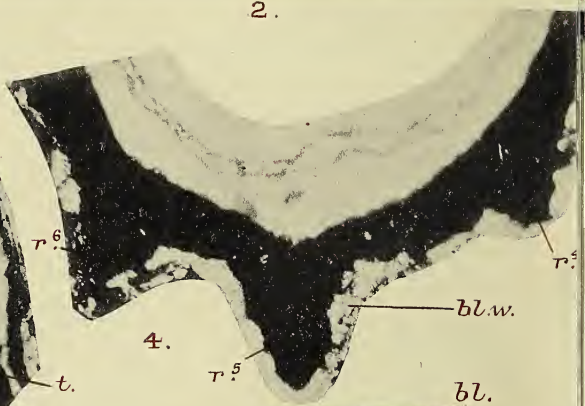

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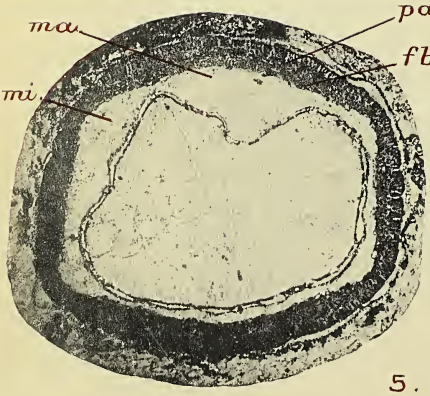

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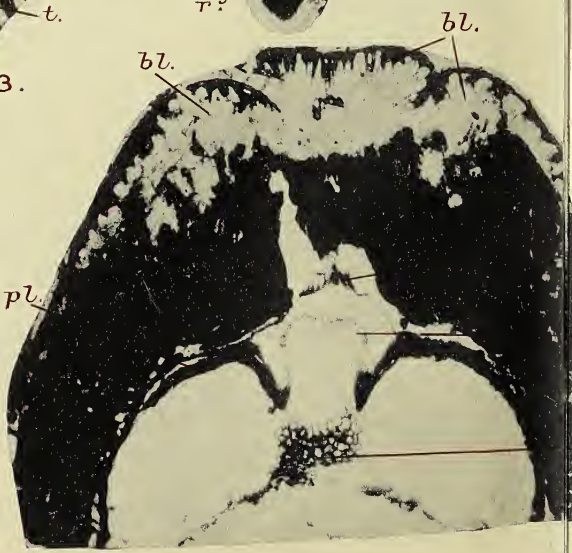

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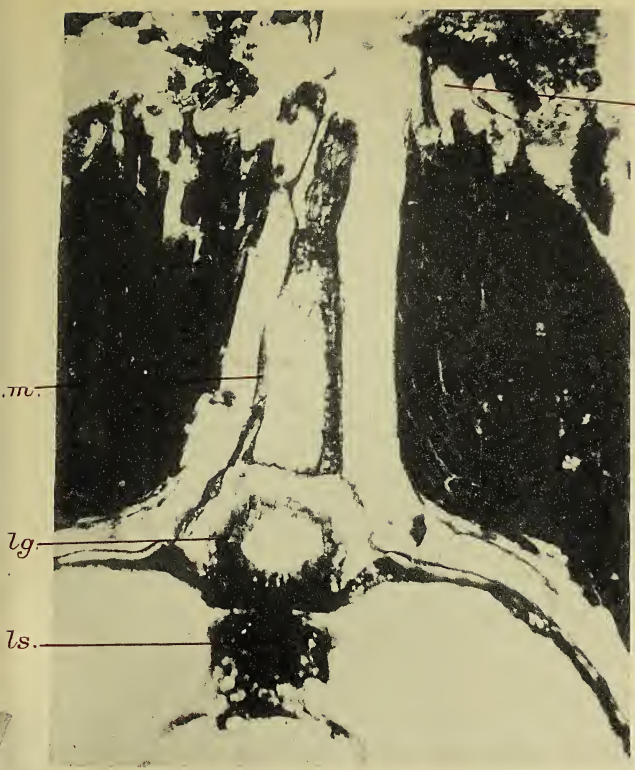

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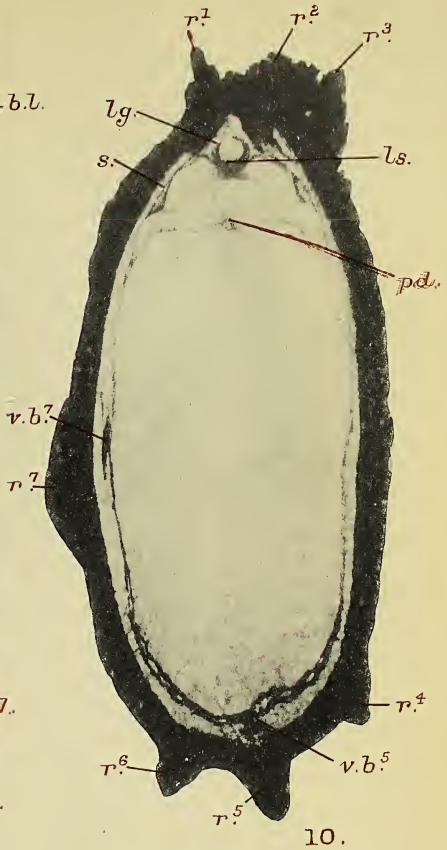

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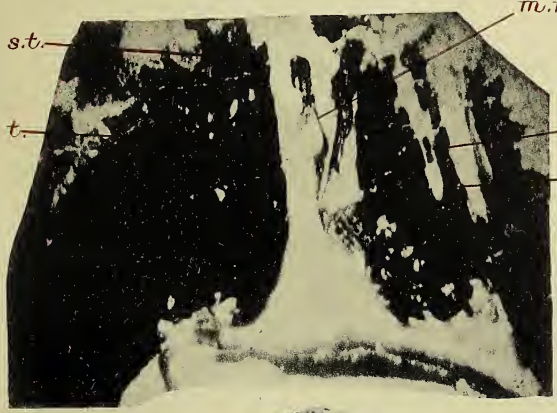

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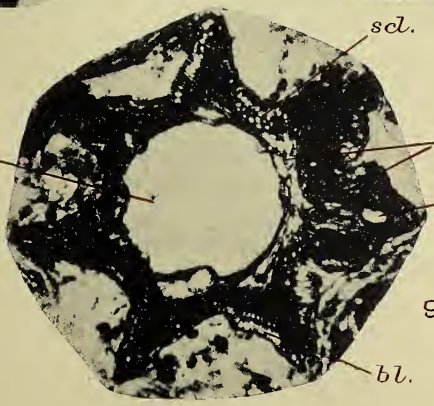

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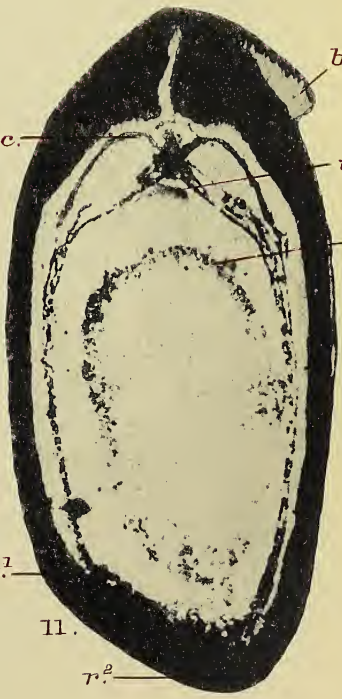

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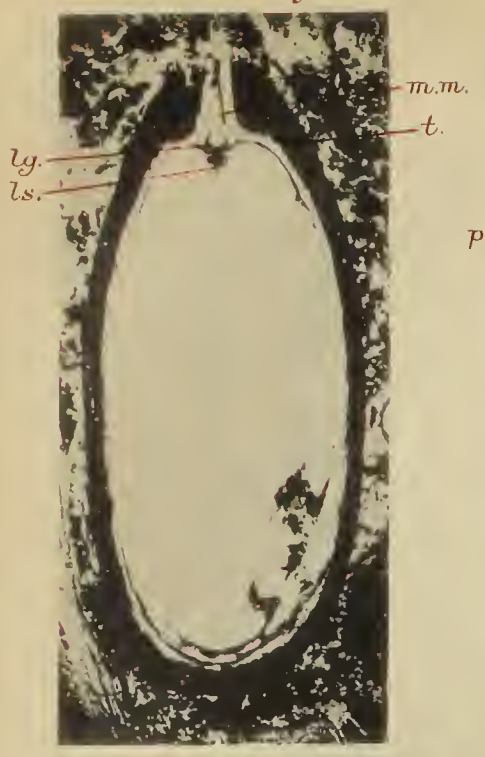

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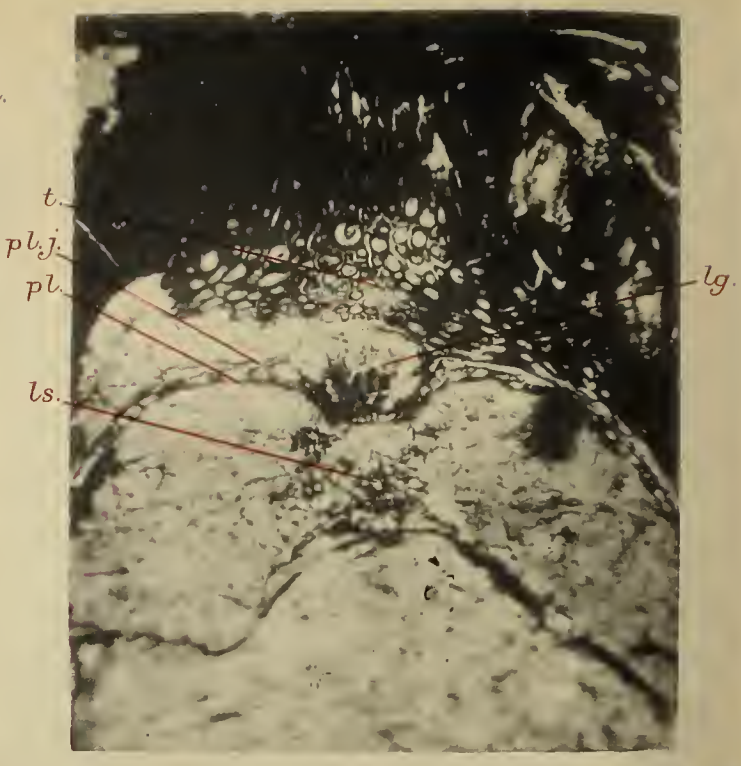

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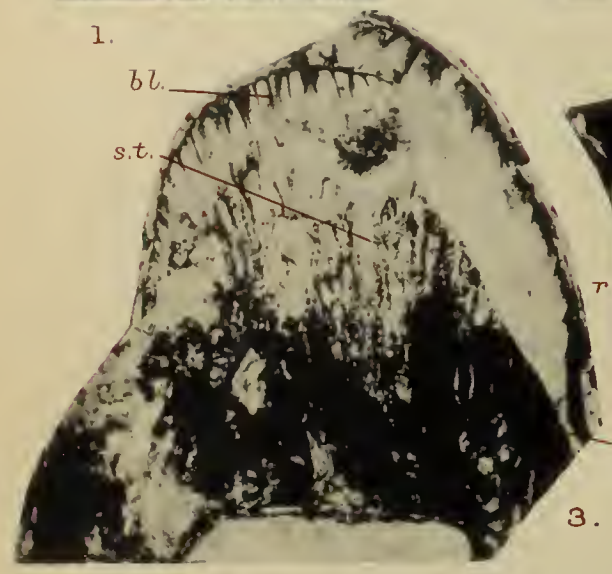

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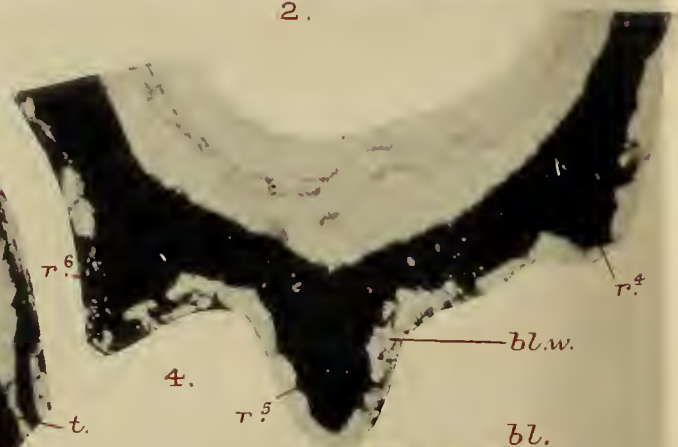

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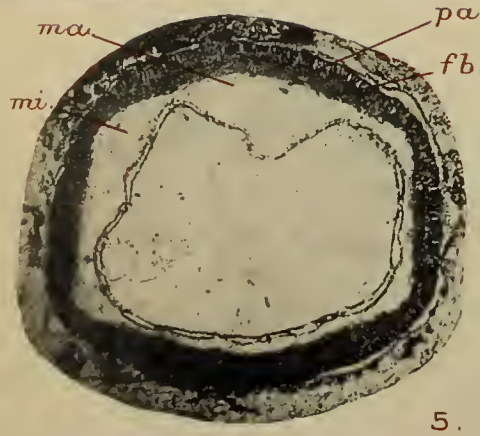

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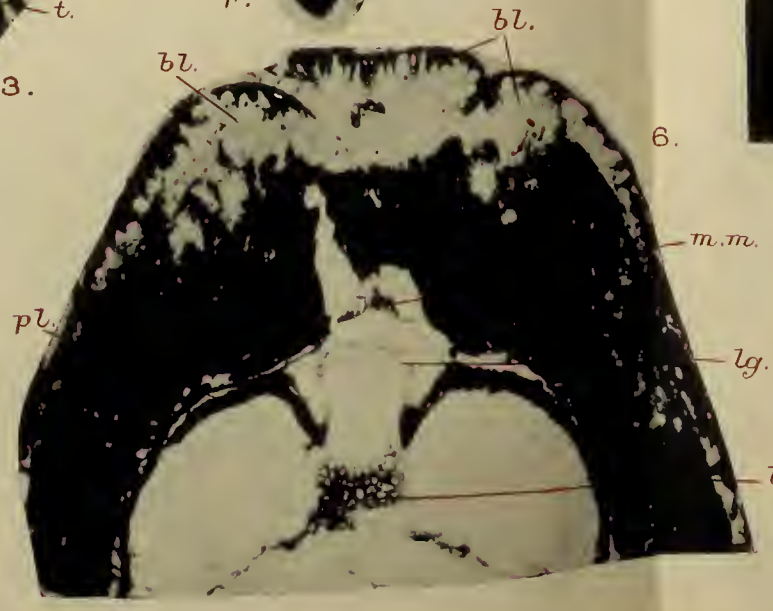

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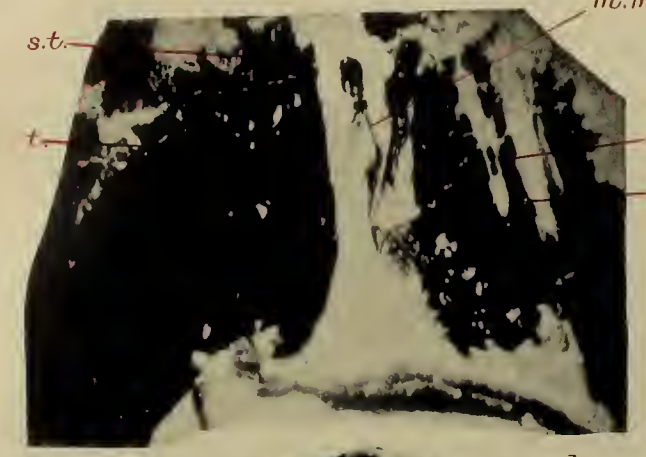

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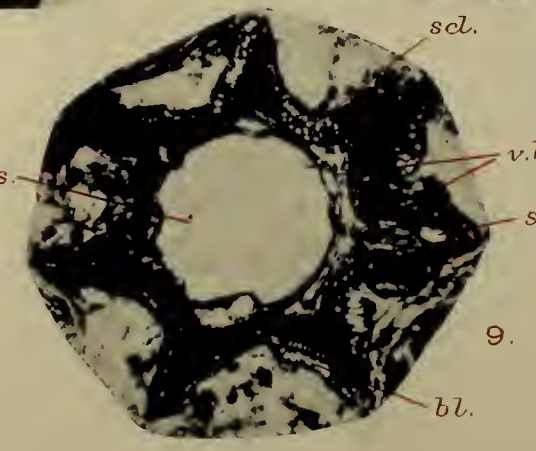

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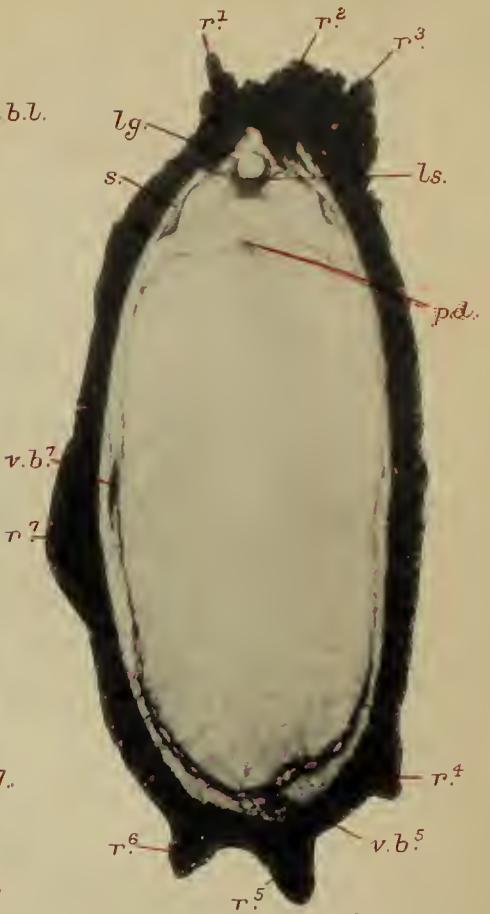

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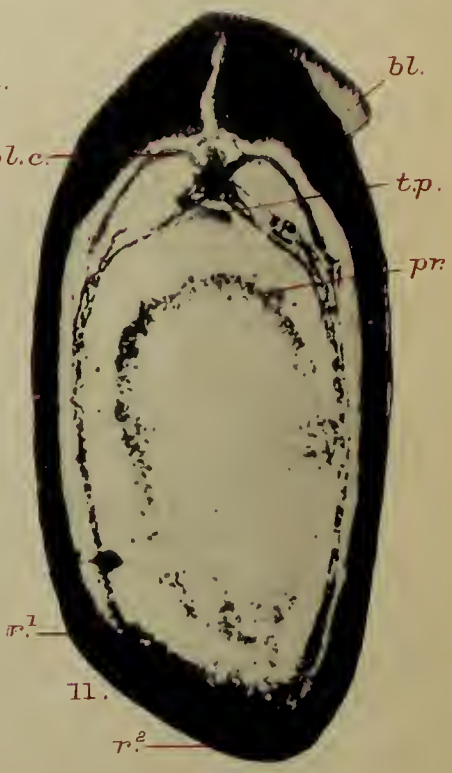

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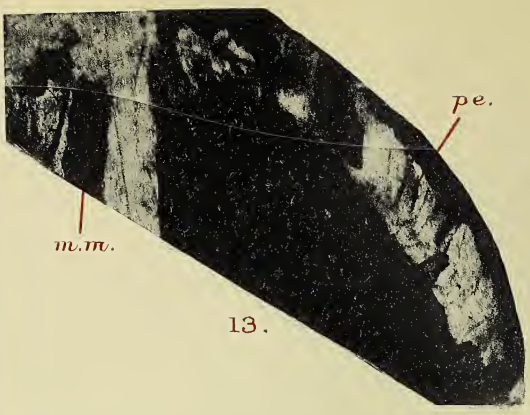

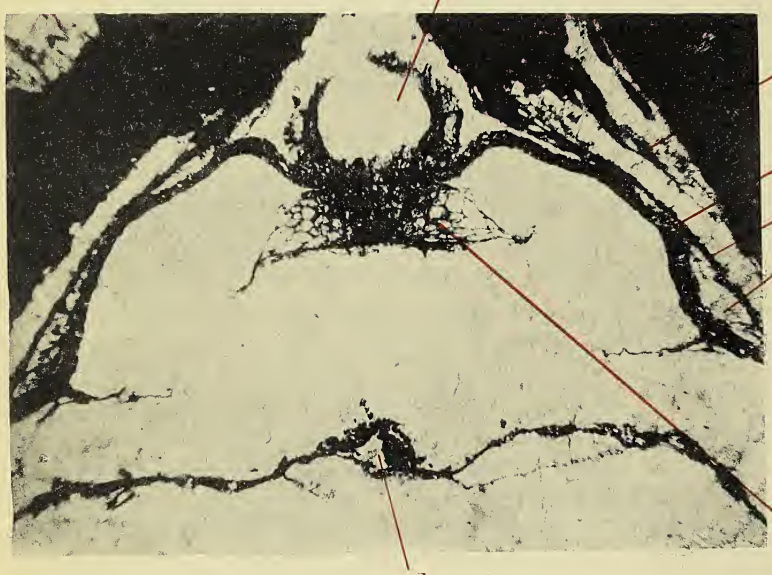

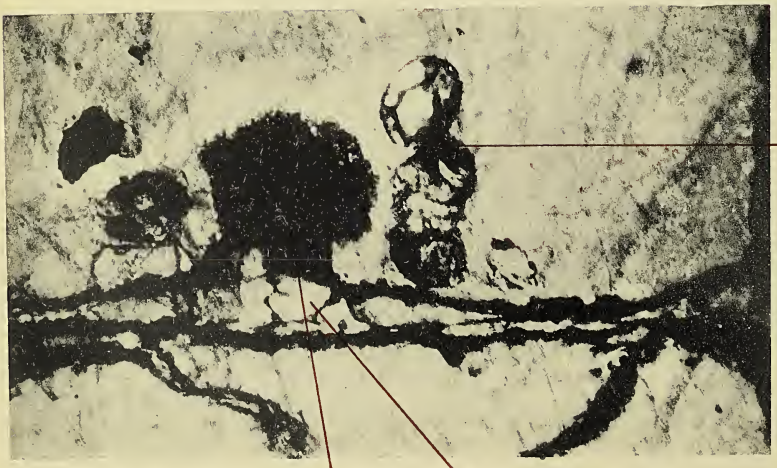

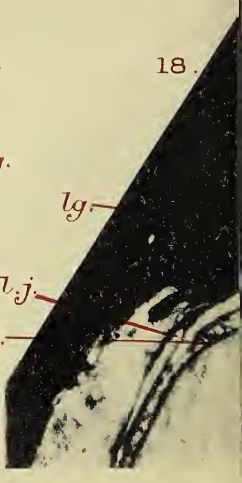

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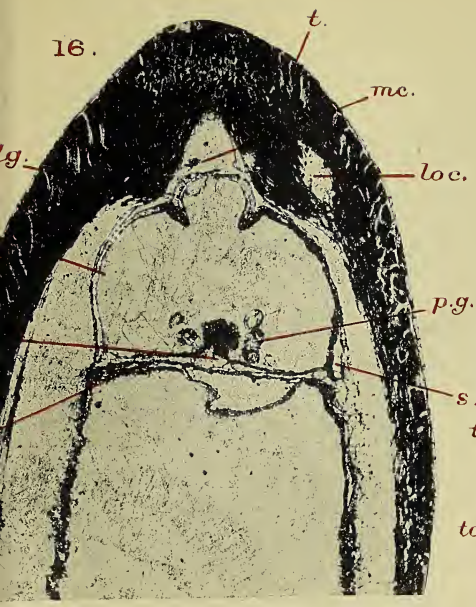

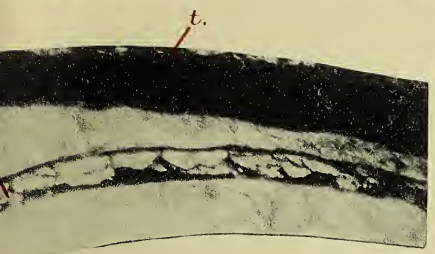

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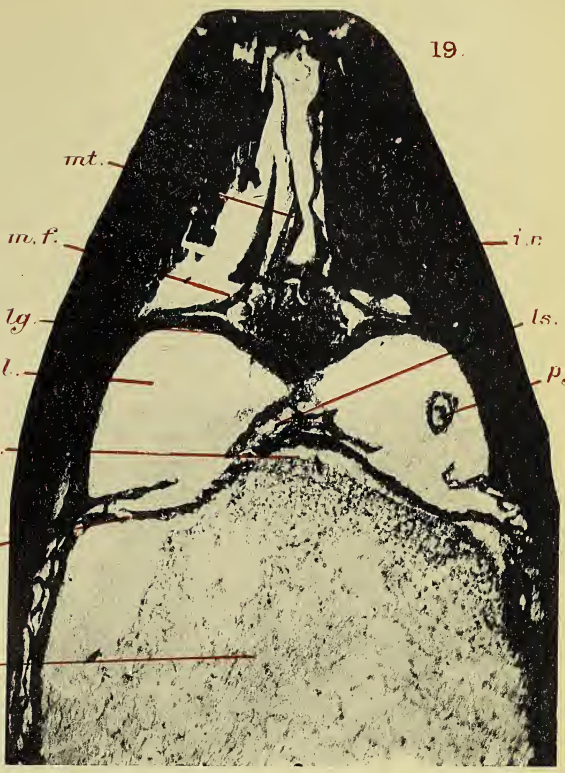

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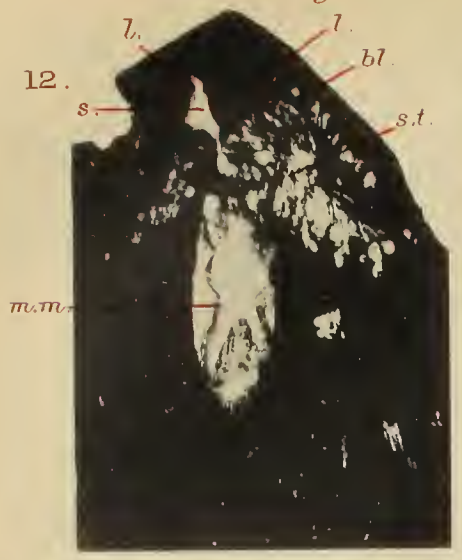

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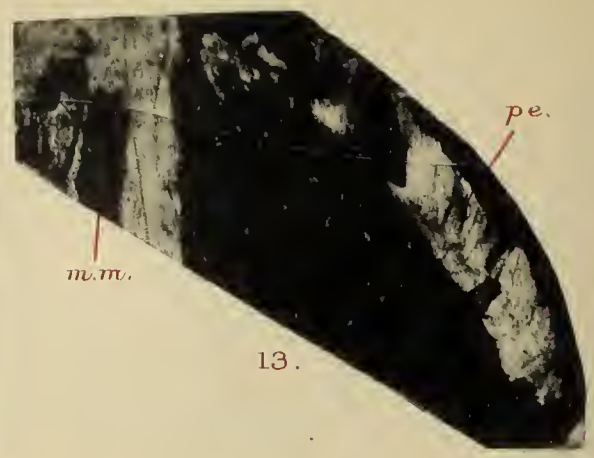

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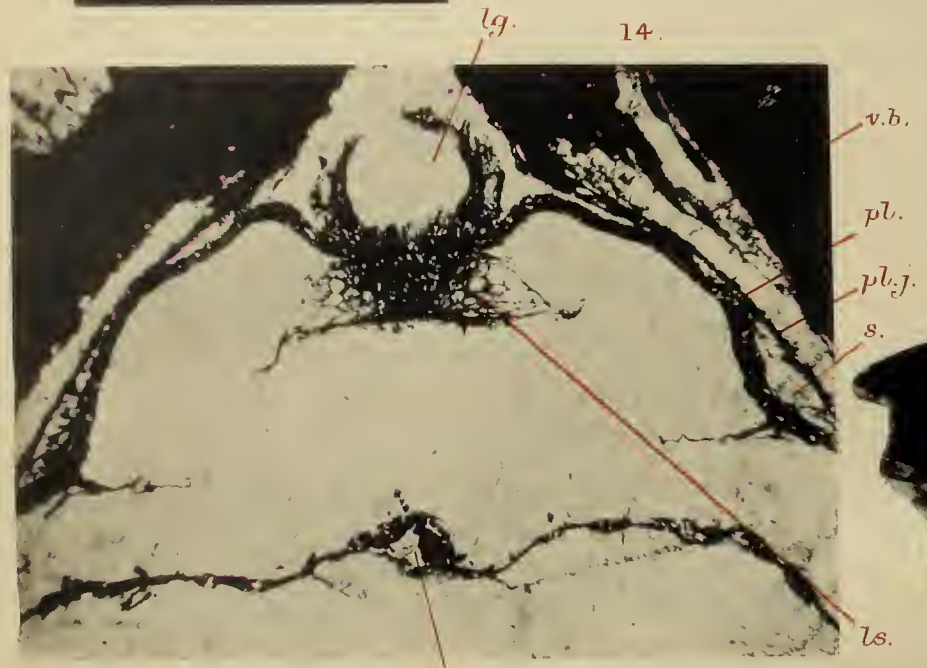

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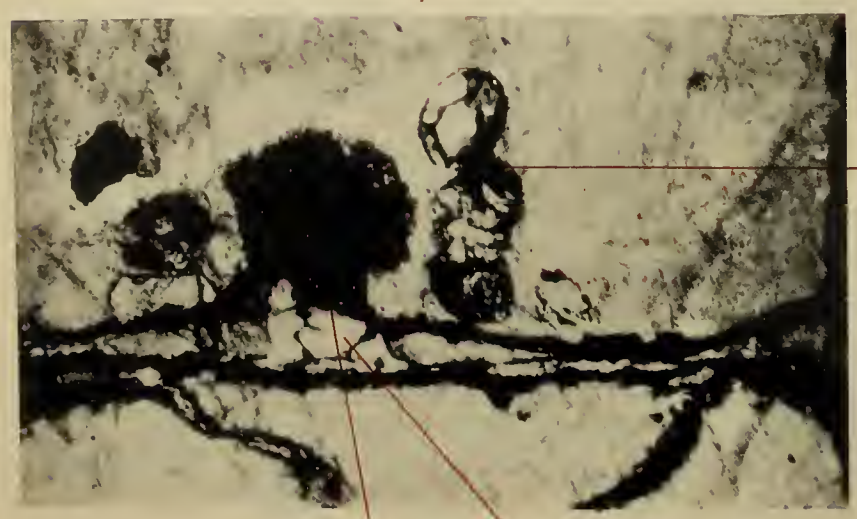

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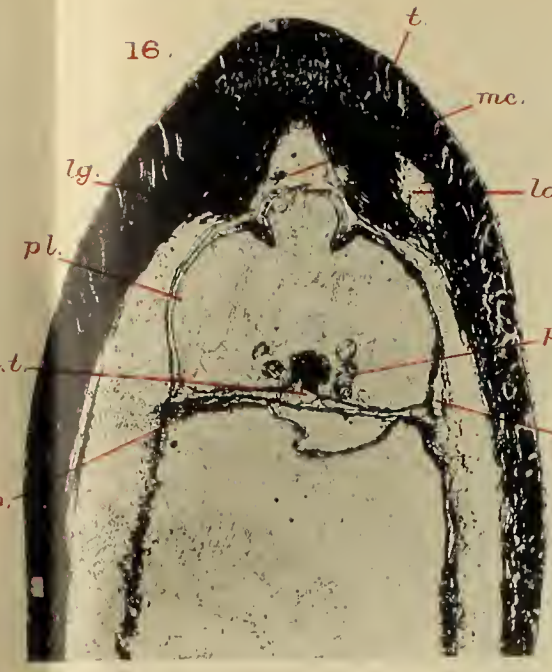

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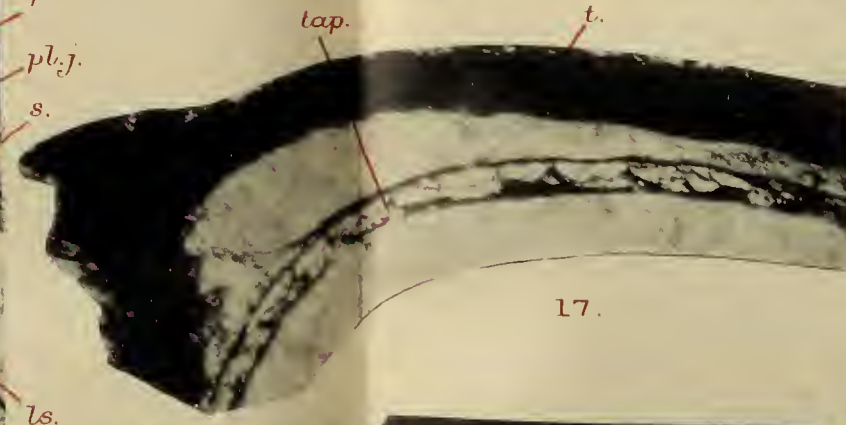

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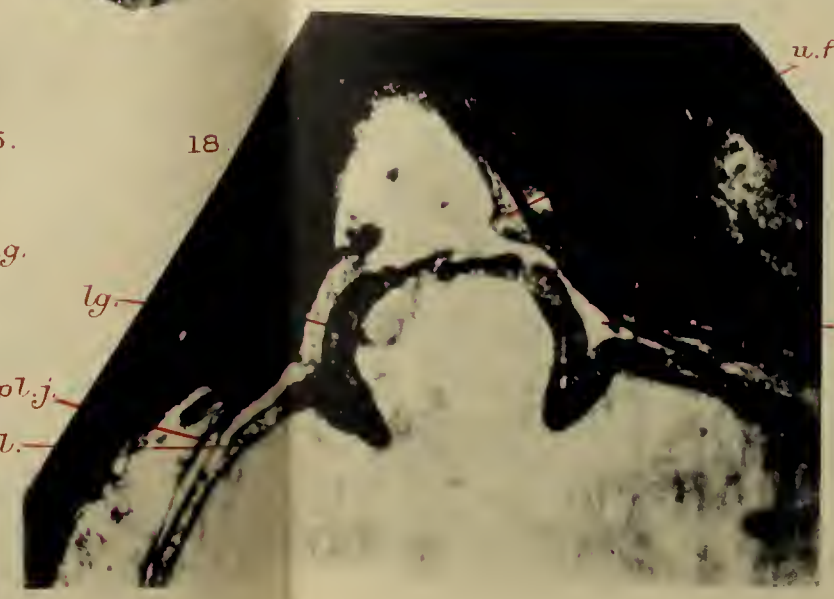

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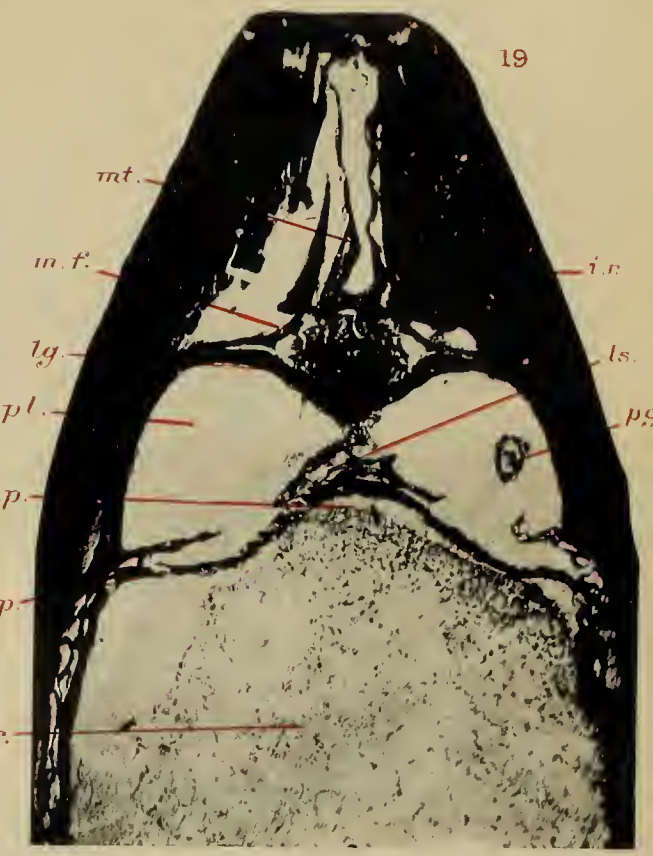

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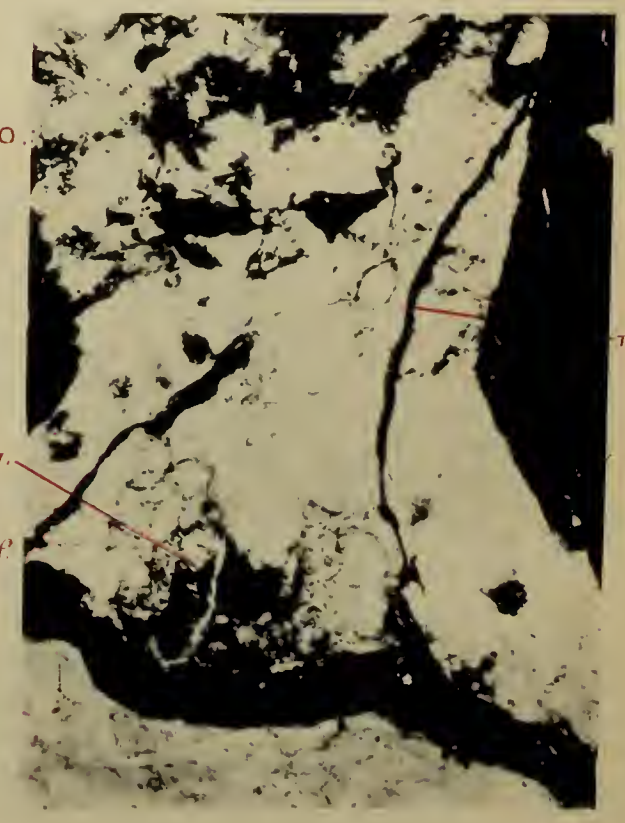

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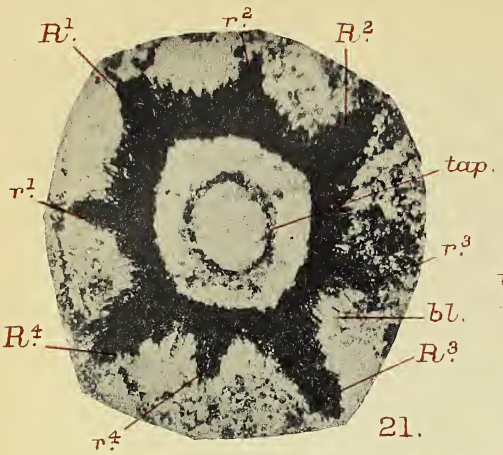
OLIVER & SALISBURY — CONOSTOMA OBLONGUM.



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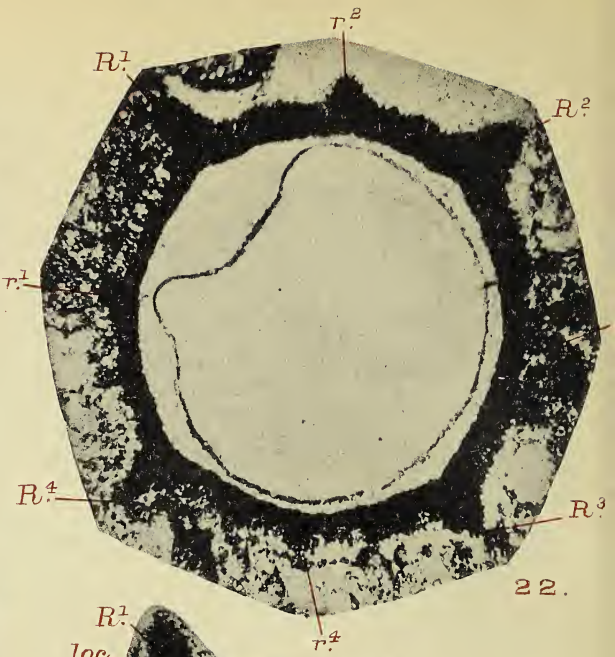

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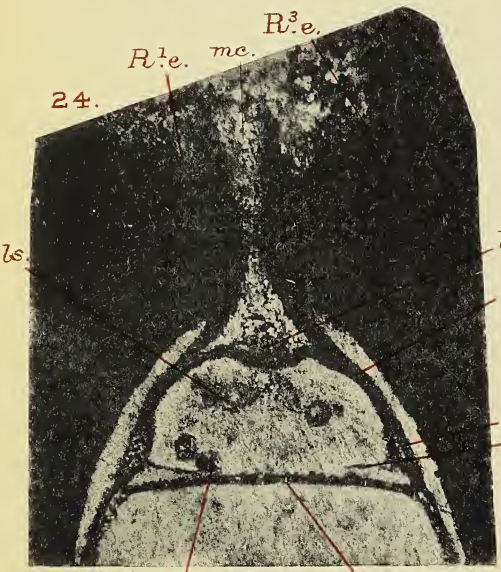

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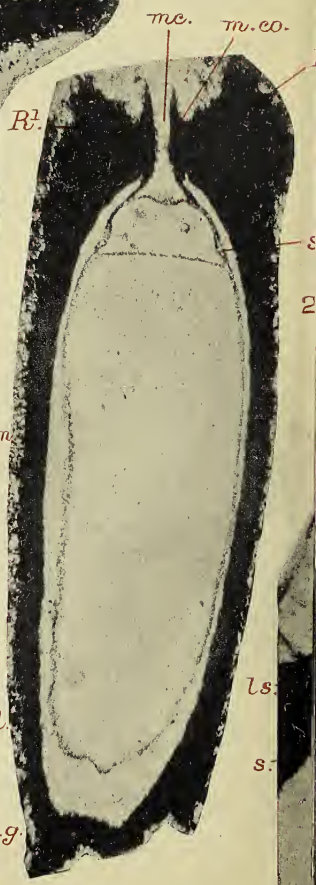

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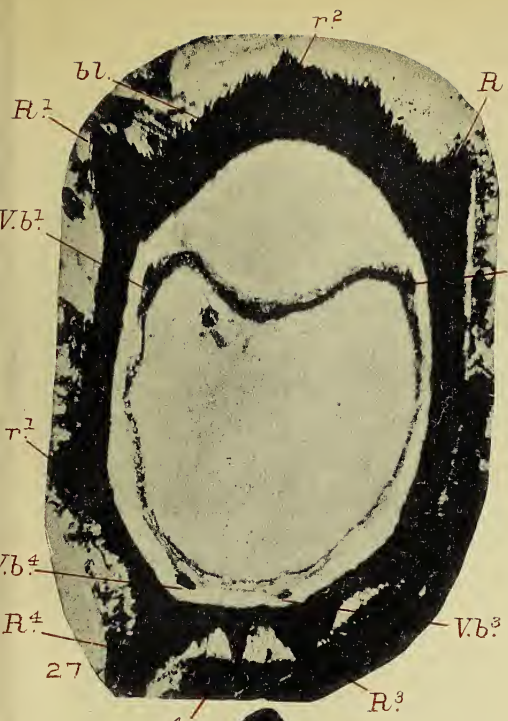
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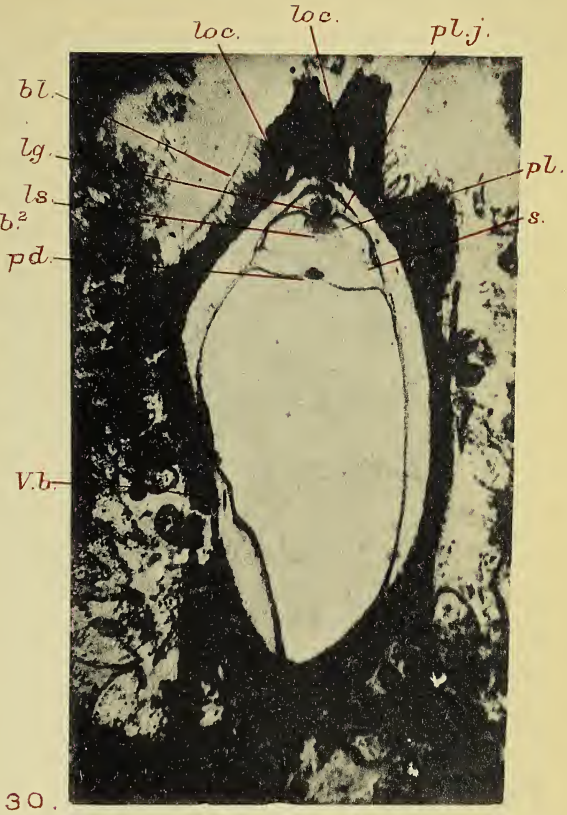
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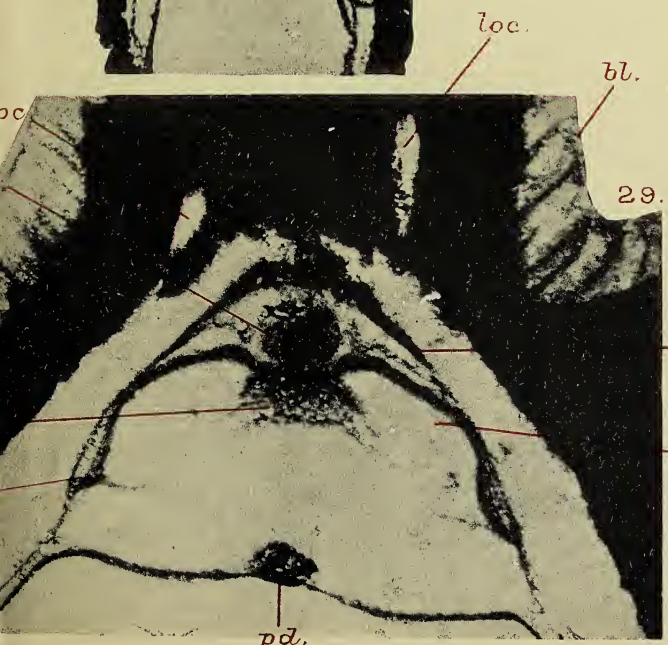
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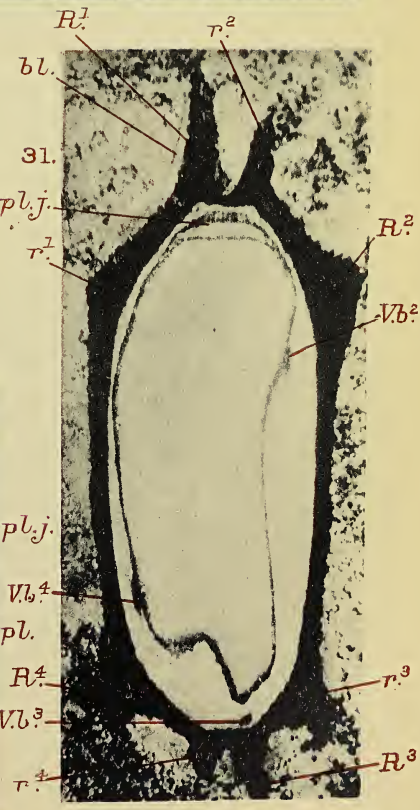
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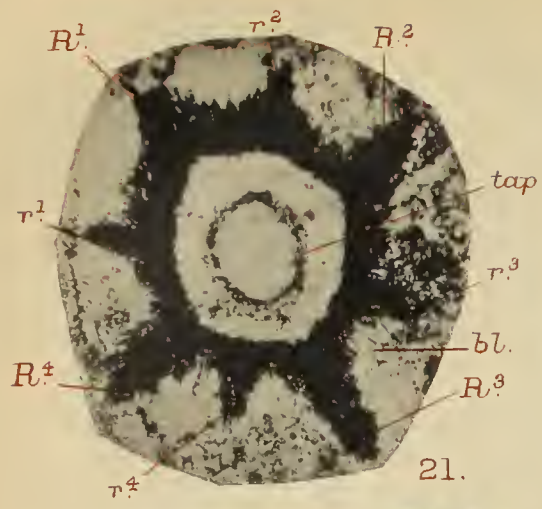

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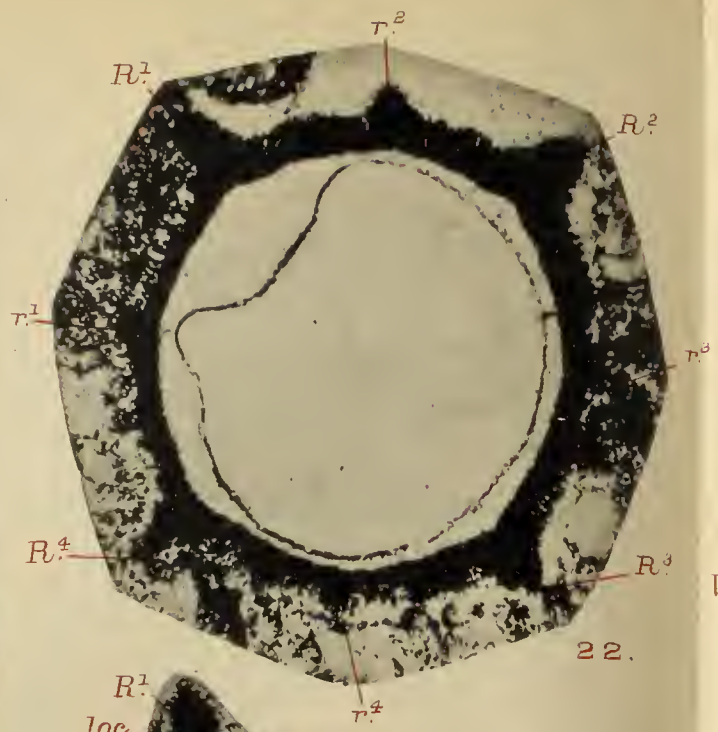

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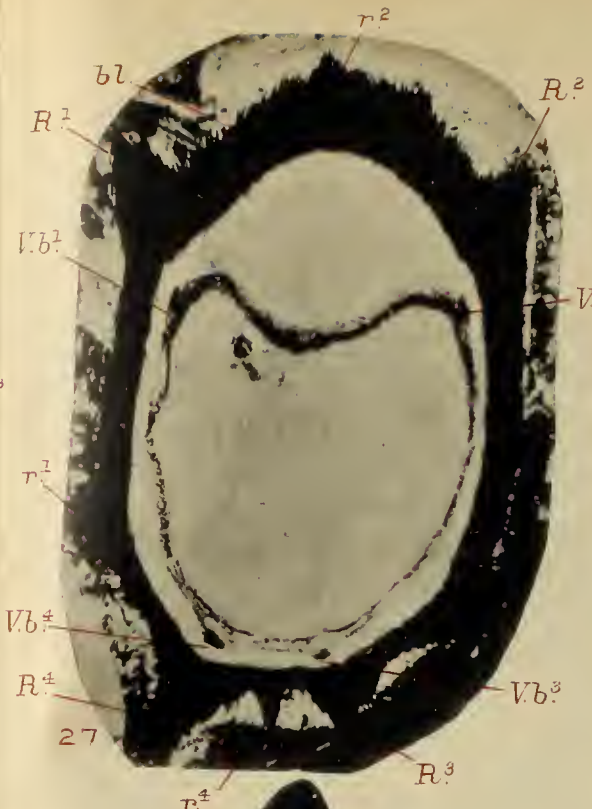

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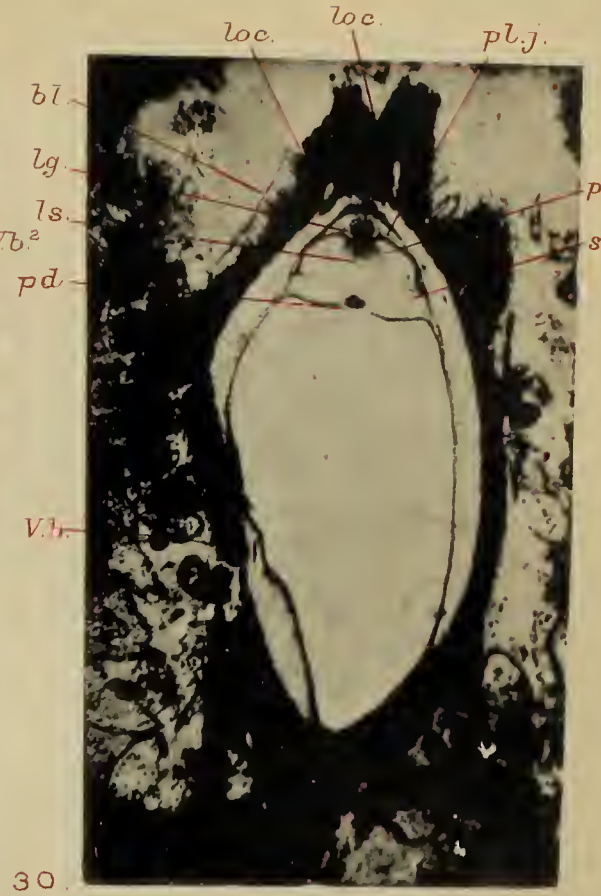

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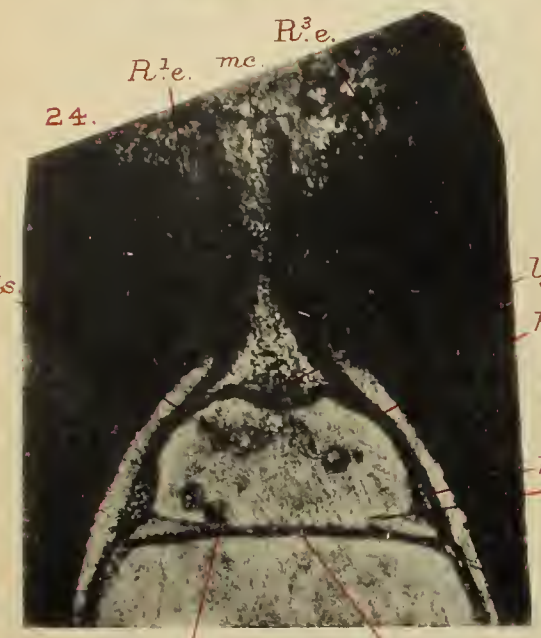

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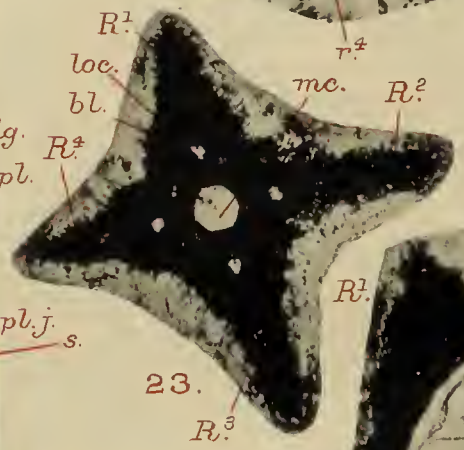

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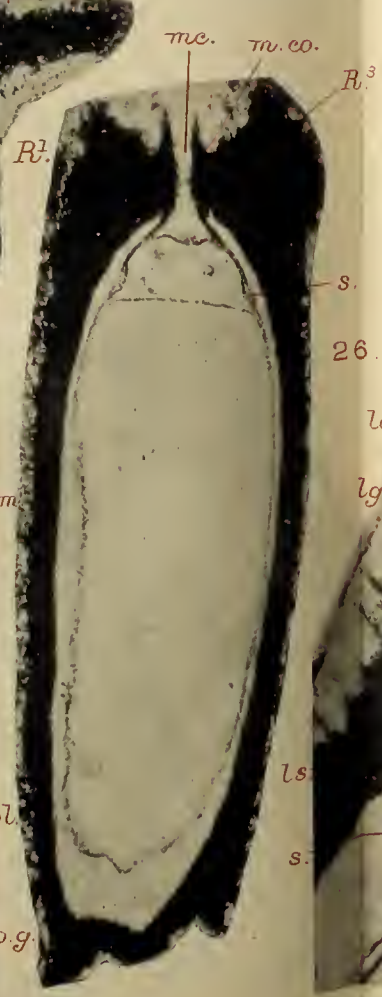

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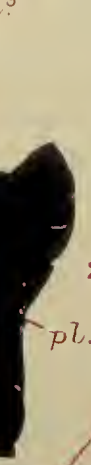

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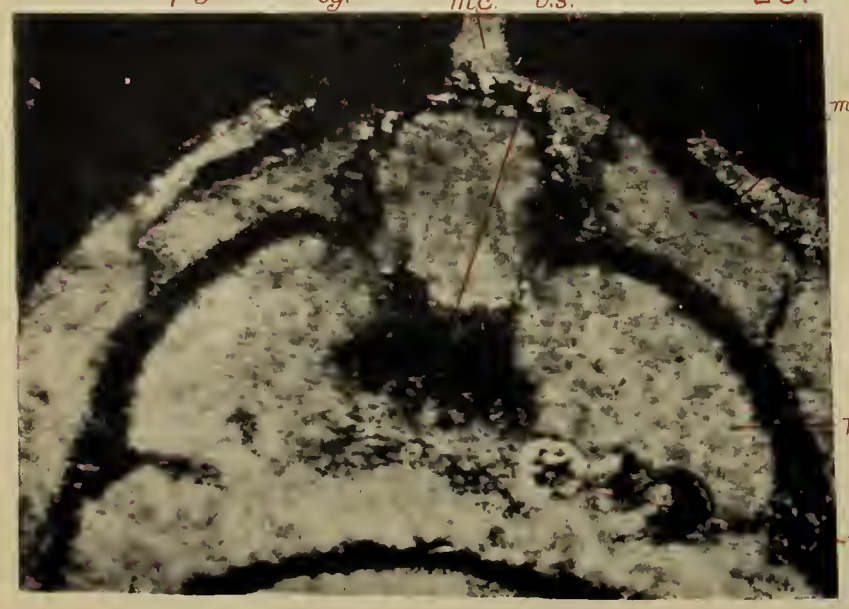

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E.J.S. phot. OLIVER & SALISBURY — CONOSTOMA ANGLO-GERMANICUM. Huth coll.

The Morphology of Leaf-fall.

BY

E. LEE, A.R.C.Sc.

Late Marshal Scholar in the Imperial College of Science and Technology, South Kensington; Assistant Lecturer in Botany, Birkbeck College, London.

With Plates IV–VI and twenty Figures in the Text.

FROM the time of von Mohl's classical paper in 1860 down to the present day, the problem of the physiological and anatomical causes leading to and arising from the natural amputation of the leaf has, with the exception of a short note by Parkin and a passing reference by Woodhead, been exclusively attacked by Continental workers. In the present paper the aspect of the question of the natural amputation of the leaf will be purely anatomical and will relate only to Dicotyledons, and in them exclusively to those species which annually cast off their leaves. A first paper such as this is almost necessarily incomplete, but it is hoped soon to extend the present work to include the examination of other classes of defoliating plants as well as other aspects of this interesting question.

Before passing to a detailed description of the types studied a short summary of the history of the subject will be given, and as the present work is purely anatomical, only a history of observations bearing on that aspect of the subject will now be presented.

As early as the middle of the eighteenth century the phenomenon of leaf-fall had already attracted the serious attention of observers, for in 1758 Du Hamel maintained that—

(1) A layer of cells at the base of the petiole always remained herbaceous, and was therefore incapable of supporting the leaf during the winter; and

(2) After the leaf had ceased to grow in consequence of excessive transpiration, the stem continued to increase in thickness, and this resulted in a tension which ruptured the fibres which unite the leaf to the stem.

Although combated by Mustel (1781), who showed that there is a plentiful supply of cell-sap in the leaf at the time of leaf-fall, the different points in Du Hamel's theory obtained many supporters, among whom were Murray (1785), Link (1812), and Petit-Thouars (1815). In 1796, however, another view was brought forward by Vrolick, who believed that the imme-

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diate cause of leaf-fall lay in the resorption of cells situated between the dead leaf and the living tissue of the leaf-base and belonging to the latter. He was generally supported by Vaucher (1821), Karl Schultz (1823), de Candolle (1827), Christian Tréviranus (1835), and in part by Van Tieghem and Guignard (1882). But the great advance was made in 1860 by von Mohl, who, although foreshadowed to a certain extent by Schacht (1859), showed conclusively that in connexion with leaf-fall two sets of phenomena are brought into operation, viz. those connected with the *separation* of the leaf from the stem, and those which lead to the *protection* of the exposed surface. Since the publication of his classical paper on the subject the labours of a long list of observers have resulted in a general confirmation of his results as well as in a great extension of the problem. The extent of our knowledge of leaf-fall phenomena in ferns was well summarized by Paul Bäsecke in 1908. The chief workers on Monocotyledonous plants have been F. V. Bretfeld (1879) and Fouilloy (1899), while Parkin (1898) and Woodhead (1906) have each added observations on the occurrence of leaf-fall in various geophilous species of Monocotyledons. In Dicotyledons, while many investigators have added to our knowledge of the subject, undoubtedly the most important contribution since the time of von Mohl is a paper by Tison (1900) in which are published observations on upwards of eighty species of plants. Much of Tison's work had been confirmed during the present research before his paper became known to the present writer, but a few points of difference will be noted in the separate descriptions of the species. Relying on various small differences, Tison describes no less than eleven special examples, each of which he makes the type of a separate class. Apart from the fact that the creation of so many classes is undesirable as rendering the subject unwieldy, it will be shown later that such a classification is hardly necessary, but also may result in the separation of species which really belong to the same type.

A description of the phenomena as they occur in the different plants studied will now be given. As far as possible common plants will be taken as types, and these will be fully described and will be followed by concise descriptions of plants which naturally fall into the same class. For the bulk of the material and for much assistance I am indebted to Mr. Hales, Curator of the Physic Garden, Chelsea. The rest of the material was obtained through the kindness of Mr. M. Wilson, B.Sc., Mr. L. A. Boodle, F.L.S., and Mr. A. W. Hill, M.A., to whom I beg to express my sincere thanks.

Class I (*a*). Castanea sativa, Mill.

As an example of the simple type of leaf-fall, the Sweet Chestnut will now be described. It is to be understood, however, that its position in the scheme is not to be taken as an indication that it represents the first stage

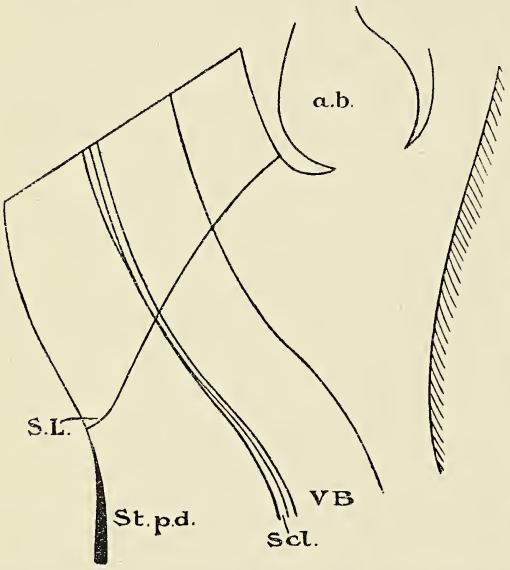
in the evolution of leaf-fall structures. This may or may not be true, but in any case it is fairly certain that many different lines have been separately evolved.

There is no need to describe the appearance of the leaf of *Castanea* Suffice it to say that the petiole is slender and roughly cylindrical, and when mature is delimited from the stem by a light brown ring. It is traversed by a group of vascular bundles which in the upper portion divide previous to entering the leaf-blade. In the stem numerous groups of 'stone' cells surround the vascular ring, and as the bundles are given off to supply the leaves, the respective portions of sclerenchyma accompany them. As the leaf-base is approached the lignification becomes less and less pronounced, and the 'stone' cells finally become quite cellulosic; the groups also decrease in bulk, and at the leaf-base almost disappear (Text-fig. 1), while the vascular elements also decrease in amount at the transition region. In the upper portion of the petiole the vascular bundles assume their former size, while the strengthening tissue outside the bundles increases in amount and again becomes completely lignified. Thus it is seen that the weakest part of the mature petiole is at the very base where later separation will occur.

By cutting longitudinal sections of the leaf-base, the whole course of events is soon apparent. In point of size there is no difference between the cells of the petiole and the cortical cells of the stem, though the junction is marked by an area of smaller cells. Nor is there any difference in the cell contents: in both cortex and petiole there is a scanty supply of starch granules, and numerous cells contain compound crystals of calcium oxalate. In the mature leaf, however, the layer of smaller cells which separates the cortex from the petiole is evidently very active, as indicated by the great increase in the protoplasmic contents as well as by the manufacture and retention of starch granules. The vascular elements in this region also display the results of increased activity by the presence in the vessels of numerous tyloses (Pl. IV, Fig. 2) and a quantity of a gummy substance which, from its property of taking the lignin stains, has been called by Tison 'gummy lignin'. The tyloses and gummy lignin, acting together, more or less completely obstruct the vessels and so stop the flow of nutrient solutions from the stem.

If a leaf that is just about to fall be examined, it will be seen that the cells on the outer side of the active area at the leaf-base are separating from each other, and the reason is not at once apparent. When, however, a sufficient number of leaves are examined, it is found that the walls of the outer cells of the active area first begin to swell, then become gelatinous, and finally the middle lamellae of a layer of cells in that region become converted into mucilage and dissolve, and the cells gradually separate from each other (Pl. IV, Fig. 1). During this period of activity the cells of the *Separation-layer* are distinguished by their abundant protoplasmic contents as well as by a great increase in the number of starch granules present. No

cell-division has occurred, and the activity assumed by the cells in this region cannot possibly have any connexion with the presence of the stem-periderm, which tapers out and stops at some distance below the area indicated (Text-fig. 1, *St. pd.*). The separation itself usually commences near the dorsal surface of the petiole, and extending rapidly across, soon leaves the leaf adhering solely by the vascular elements. The living cells of the vascular bundles split apart as described, and as soon as the non-living elements—vessels, &c.—become ruptured by mechanical means, i. e. the weight of the leaf, wind, &c., the leaf falls to the ground.

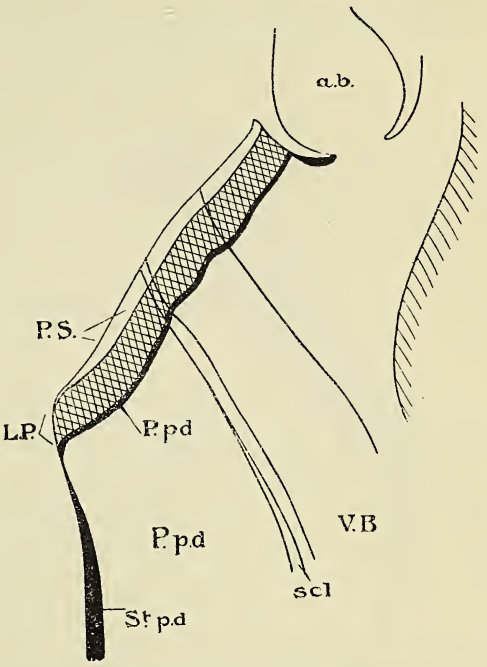


TEXT-FIG. 1. *Castanea sativa.* Longitudinal section of leaf-base at time of leaf-fall.

Beyond the massing of the protoplasm in the cells at the base of the petiole, there has, at this time, been no formation which could be described as protecting the exposed surface. Directly after leaf-fall, however, the activity of the cells below the surface of separation is at once shown by the change in the chemical composition of the cell-walls. Very gradually these become more or less completely lignified. This process commences in regions which vary with the individual specimen, being generally situated near the epidermis, and spreads rapidly until the whole of the Protective-layer has undergone lignification (Pl. IV, Fig. 2). At the same time there is deposited on the inner surface of each cell-wall of the Protective-layer a fine film of suberin, the completion of which is marked by the disappearance of the protoplasm from the cells of the area.

Between the area which undergoes ligno-suberization and the exposed surface, there is a layer consisting of 2–3 rows of cells which for a time remains entirely unaffected by the changes going on around it. For a long time these cells retain their protoplasm and their cellulose character, but gradually the protoplasm in the outer cells disappears, and the latter collapse and form a protective membrane. The change thus initiated spreads to the other cells of this layer until finally all the protoplasm disappears and the walls of the collapsed cells, while still remaining more or less cellulosic, become adpressed to the surface and form a very efficient method of protection in addition to the Protective-layer proper (Text-fig. 2, *P.S.*).

The layer just described is distinguished by Tison as the ' parenchyme sacrifié ', though it would be better perhaps to look upon it as the remains of the Separation-layer. Its premier characteristic—that of retaining for a considerable time its protoplasm and unaltered cell-walls—is possibly due to the production within the separating cells of a small amount of some substance, the effect of which is to overcome the tendency to become ligno-suberized ; while the second characteristic of collapsing is probably a result of the exposed position of the layer. The thickness of this layer varies greatly ; in some cases it consists simply of the remnants of the cells which have separated, while in other cases it is much thicker and consists of several layers of cells. At a later stage there is no obvious distinction between this layer and the Protective-layer, the two having become completely ligno-suberized.

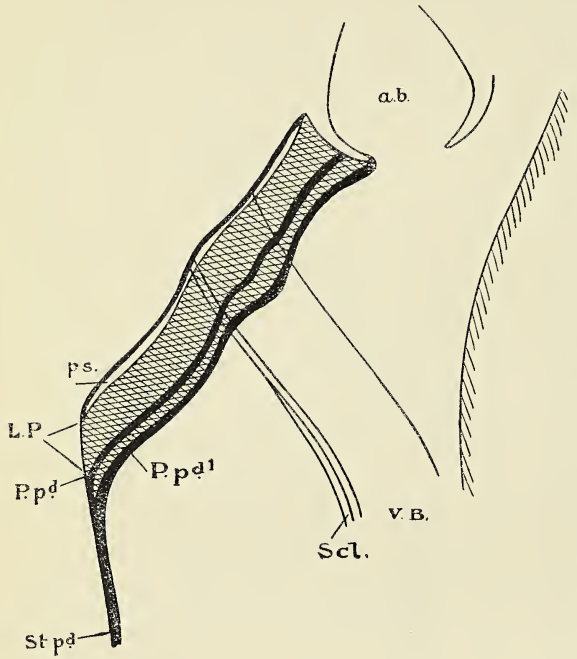


TEXT-FIG. 2. *Castanea sativa*. Longitudinal section of scar in first winter.

In late autumn or winter when the ligno-suberization of the Protective-layer is nearing completion, and when the same change is rapidly occurring in the living elements of the vascular bundle at that level, another process commences which results in the formation of a periderm beneath the Protective-layer. The living cells adjacent to the latter become active, and commence to divide by walls approximately parallel to the surface of the scar (Pl. IV, Fig. 2, *c*). In this way a regular cambium is produced, which, starting from the outer epidermis of the leaf-base at some distance above the upper extremity of the stem-periderm, runs across the leaf-base and traversing the vascular bundles joins the inner epidermis just above the junction of the latter with the axillary bud (Text-fig. 2, *P. pd.*). The new cambium becomes continuous across the vascular bundle by division of the living parenchymatous cells of the latter, and when tyloses are present they also divide in the same manner (Pl. IV, Fig. 2, *ty.*[1]). The generative layer thus produced differs in no particular from the cork cambium of the stem, and like the latter it cuts off the usual regular cells towards the exterior, and occasionally one or two layers of phelloderm cells towards the cortex.

While the latter retain their protoplasm and undergo no change, the former become suberized and slightly lignified, and finally entirely lose their contents. Only 3–4 layers of cork cells are produced in the first year.

In the second year, another cambium is produced beneath the leaf-scar and is situated at some distance below the first, and unlike the latter it immediately becomes continuous with the periderm of the stem (Text-fig. 3, *P.pd.*[1]). It is approximately parallel with that produced in the first year, but on nearing the vascular bundle it dips down and pursues a curved course within the latter. Four to eight layers are produced before the end of the second year, and at this time all the cells above the periderm have become completely ligno-suberized.



TEXT-FIG. 3. *Castanea sativa.* Longitudinal section of scar in second winter.

Before going on to describe other examples the behaviour of the more peculiar elements present within the Protective-layer may be noticed. It is obvious that the lignified elements of the vascular bundle, such as vessels, strengthening tissues, &c., from which the protoplasm has finally disappeared long before the complete maturation of the leaf, cannot participate in the further chemical changes which occur in the formation of the Protective-layer. The sieve tubes, companion cells, and other elements which have retained their living protoplasm undergo the process of ligno-suberization just as do the ordinary parenchymatous cells outside the vascular bundle, and that process is usually not completed until after the formation of the Protective-periderm. Finally there is the behaviour of the crystal cells. When these are included in the Protective-layer the course of events varies with the presence or absence of protoplasm. Sometimes an appreciable amount of protoplasm is present, but more often it is impossible to demonstrate the slightest trace of that substance, and in these cases it appears that the last act of the expiring protoplasm was the secretion of the crystal which now fills its place. It follows then that if protoplasm

is present it is quite likely that the process of ligno-suberization will be complete, but if absent the cell-wall will but participate in the lignin produced in the surrounding cells; and this accords with the facts observed. It is quite easy in some cases to demonstrate the inner film of suberin which may even extend round the exterior of the included crystal; but in most cases no such film is to be observed. And the behaviour of the crystal cells when placed in the path of the developing scar cambium leads to the same conclusion; for beyond the conversion of the cellulose wall into suberin with a little lignin there is no further change. If effective protoplasm is present, why should not the crystal cell divide as do its neighbours? But the crystal cell does not divide, and the chemical changes in its wall are probably the result of the activity of adjacent cells.

Class I (*b*). Ribes sanguineum, Pursh.

In the example about to be described it will be seen that the course of events is not fundamentally different from that which characterizes *Custanea sativa*. The chief difference lies in the fact that here the process of ligno-suberization of the Protective-layer is usually fairly complete *before* leaf-fall. Other differences which have led to the erection of a distinct type by Tison are wholly due to the deep-seated nature of the stem-periderm, and are more apparent than real.

In the mature leaf of *Ribes sanguineum* the upper, green, cylindrical portion of the petiole passes gradually into a sheathing base, the lower limit of which is marked externally by a slight furrow. The axillary bud is large, and its lower portion is completely hidden by the spreading base of the petiole. Both petiole and stem are covered with numerous hairs, which are often glandular and, as is well known, secrete the substance that gives to the plant its characteristic odour.

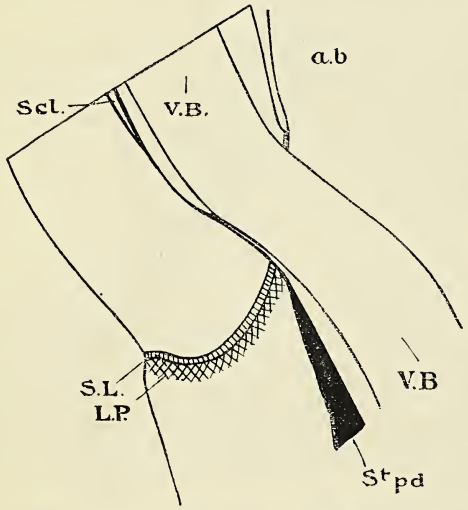
The distribution of starch granules is rather peculiar, the places where these bodies are found being quite localized, and comprising the cork cambium, phellodcrm, wood parenchyma, and the periphery of the pith. At the time of leaf-fall starch granules are entirely absent from the primary cortex and the Separation-layer, two places where in other species they are almost invariably present. Compound crystals of calcium oxalate are numerous in both cortex and petiole.

Apart from the occurrence of fibres in the vascular ring, stereome is entirely absent from the stem, so that here there is no question of the reduction of the strengthening tissue accompanying the vascular bundles as they enter the leaf. Just below each leaf three bundles leave the ring, and as they traverse the cortex and petiole they gradually converge, finally meeting in the upper part of the petiole and forming a large semicircle. Just above the leaf-base thick-walled sclerenchyma appears outside the phloem of each strand and later forms a continuous cylinder enclosing the

three vascular bundles. At the same time the hypoderm of the petiole becomes converted into collenchyma, providing an additional source of strength for that organ. Thus again the weakest part of the petiole is its base, when later separation occurs (Text-fig. 4).

As in other examples which will be described later, the Separation-layer (Pl. IV, Fig. 3, *S.L.*) is well defined long before leaf-fall, its position closely corresponding with the furrow shown externally. It is formed very early by the irregular division of a layer of cells in this region which are distinguished by their dense living and starchy contents, but after once becoming well marked no further change occurs for some time.

As is well known the stem-periderm (Text-fig. 4, *St. pd*) in *Ribes* is very deep seated, appearing in the pericycle or endodermis, and this fact exercises a great influence on the form and appearance of the modifications connected with leaf-fall. Towards the end of the summer the periderm of the stem attains an appreciable thickness, but as the leaf-base is approached a gradual diminution occurs until finally the periderm ceases, usually at a distance of 2–4 cells below the Separation-layer. The cortex thus cut off by the periderm becomes crushed, then dies away, and is finally exfoliated. Its behaviour in many respects is markedly different from that of the layer, 2–5 cells thick, situated just beneath and adjoining the Separation-layer, which, though it undergoes exfoliation along with the general cortex, exhibits modifications which conclusively prove its primarily protective character.

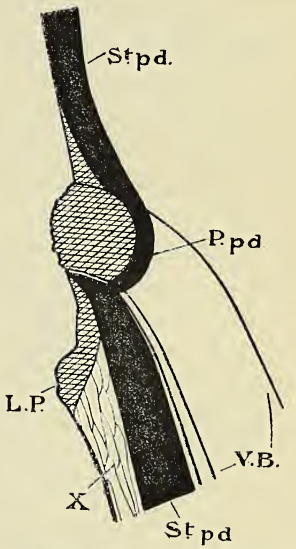


TEXT-FIG. 4. *Ribes sanguineum.* Longitudinal section of leaf-base just previous to leaf-fall.

Just before leaf-fall, the cells of the latter layer begin to undergo lignification. This process, which usually commences in a row about five cells away from the Separation-layer, is very definite and gradually spreads across and through the whole layer, more or less completely delimiting it from the ordinary cortical tissue below (Pl. IV, Fig. 3, *L. P.*). The extent to which lignification occurs before leaf-fall varies greatly, all stages being found from unaltered cells to those completely lignified, and even in very favourable cases partly suberized also. These facts, taken in conjunction with others of a like nature for other examples, point strongly to the conclusion that the degree to which the leaf-fall processes are allowed to occur

before defoliation is largely if not solely dependent upon the external conditions which usually determine the moment of leaf-fall. When external conditions have not interfered a more or less ligno-suberized layer is present before the separation of the leaf from the stem. The process of lignification takes place as above described, and later a thin film of suberin is added to the inner face of each cell-wall. The only other changes which occur before leaf-fall in the elements at this level are the appearance of a few tyloses and the production of a great amount of gummy lignin in the vessels of the vascular bundles.

Defoliation is accomplished by the splitting apart of the cells of the Separation-layer. The middle lamellae between the cells of the lower two rows begin to swell and are gradually converted into pectic mucilage (which is also impregnated with tannin) and later disappear, leaving the neighbouring cells quite free from each other. About the time of separation there is a great increase in size of the walls of the separating cells, and it seems probable that a differential growth of the separating walls occurs which, if true, will greatly facilitate the process of separation.



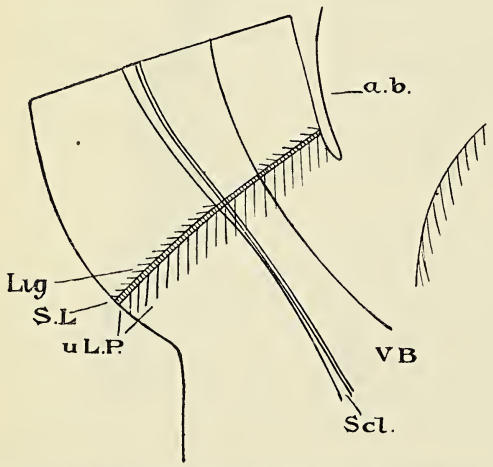
TEXT-FIG. 5. *Ribes sanguineum.* Longitudinal section of leaf scar at end of second year. x = crushed cortex.

In those cases where external conditions have resulted in defoliation before ligno-suberization has been completed, this is rapidly accomplished in the newly-formed scar. By the end of the first year, therefore, the external portion of the scar including the vascular bundle is completely ligno-suberized, but no periderm has been produced which can be said properly to belong to the scar. During the second year, however, divisions occur below the Protective-layer which result in the production of a definite periderm. A fairly thick layer of cork is produced at the expense of the living cells of both vascular bundle and cortex in this region, and this, starting from the outer portion of the periderm of the axillary branch, traverses the vascular bundle in the form of a basin-shaped curve, which finally ends in the stem-periderm at some distance below its upper extremity (Text-fig. 5, *P. pd.*). Subsequently the vascular tissue above undergoes complete suberization in places where lignification had not previously occurred, the change being very striking even in unstained preparations.

Class II (*a*). Tilia europaea, Linn.

We now enter upon a description of a second type of leaf-fall, taking as our first example the common Lime. The sequence of events is in many respects very similar to that of the examples just described for Class I, but there is one essential difference. Here again, taking the class as a whole, ligno-suberization may or may not occur previous to defoliation ; but in every case without exception, new walls running in all directions are produced in the cells of the Protective-layer before ligno-suberization takes place. This is an essential distinction independent of external conditions.

In the present species the petiole is a relatively long, slender, wiry organ which gives place above to the broad lamina, spreading a little towards the base where it curves sharply to join the stem. In the curve is firmly wedged the large asymmetrical axillary bud, which appears slightly to displace the base of the petiole. A slight furrow marks the junction of stem and petiole.



Text-fig. 6. *Tilia europaea.* Longitudinal section of leaf-base just previous to leaf-fall.

In the upper part of the petiole a conspicuous sheath of stereome surrounds the ring of vascular bundles. As the leaf-base is approached the vascular cylinder gives place to three separate bundles ; at the same time there is a gradual reduction in the quantity of sclerenchyma (Text-fig. 6), and the amount of lignification is correspondingly reduced. At the leaf-base itself there is practically no lignification outside the vascular bundles, and the stereome accompanying each has almost disappeared. On joining the vascular ring in the stem the sclerenchymatous patches outside the phloem have again increased to their normal amount and are completely lignified.

There is little or no difference in size between the cells of cortex and petiole, but the junction between the two is marked by a layer of much smaller cells. Early in the season starch granules are present in the cortex, but are entirely absent from the petiole with the exception of the bundle sheath. Simple and compound crystals of calcium oxalate are common to the parenchyma of both organs, and secreting cavities of various size are

also abundant. Periderm is not present in the stem until long after the leaf has fallen.

As is commonly the case, the increased abundance of the protoplasm in the cells of the leaf-base provides the first indication of approaching leaf-fall. The small cells of this area become densely filled with brown protoplasm, and starch granules also appear. Considerable activity is displayed, resulting finally in the production of a few new cell-walls, which are scattered (never more than one in each cell) throughout the leaf-base. Beyond the appearance of a few tyloses and a varying quantity of gummy lignin in the vessels of the leaf-trace at this level, no other change occurs in the Protective-layer previous to defoliation.

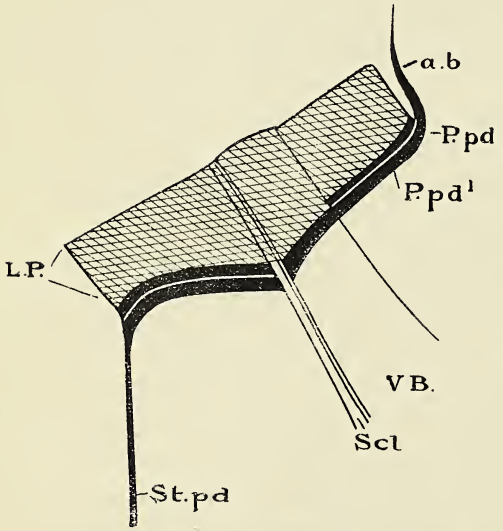
Separation in this species is very simple. A layer of cells (1–3 cells thick) just above the Protective-layer becomes conspicuous by the great increase in the starchy contents. A few irregular cell-walls may appear, or these may be entirely absent. The primary cell-walls begin to swell and take the pectic stains more strongly; the middle lamellae become obviously mucilaginous and finally disappear, and separation, commencing near the dorsal surface of the leaf-base, immediately follows.

In *Tilia europaea*, a further modification is introduced which does not occur in the principal examples previously described. In favourable cases, when the lignin tests are applied to longitudinal sections of the leaf-base taken before the separation of the leaf from the stem, it is seen that the cells above, but adjoining the Separation-layer, are more or less completely lignified. This layer, to which Tison has appropriately applied the term 'Lignified-layer', is usually one cell in thickness near the vascular bundles, but gradually increases to three cells at the periphery. The living contents become reduced in amount and may even disappear. The effect of such lignification is obvious; it gives rigidity to the whole layer, making the adjacent and ever-weakening Separation-layer much weaker by comparison, and thus aiding in the final separation which soon follows (Text-fig. 6, *Lig.*).

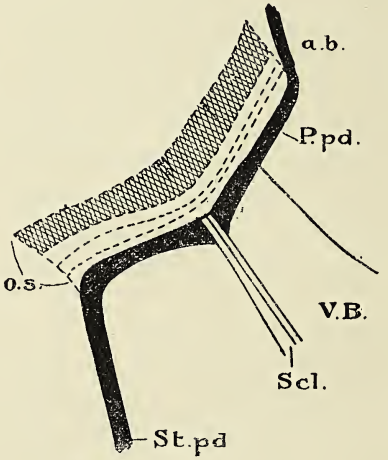
Immediately after the leaf has fallen, the cell-walls of the Protective-layer begin to undergo lignification followed by a slight amount of suberization. The whole process at this stage is very diffuse and not easily recognized until later, when the Protective-layer becomes delimited below by the production of a regular cork cambium. The crystal cells and secreting cavities which occur in the Protective-layer, but which, of course, have not undergone division like their neighbours, now become highly lignified, but do not display any suberization.

During the first winter the unaltered cells just below the Protective-layer undergo division by walls approximately parallel to the surface of the scar, and form a cambium which produces a few layers of regular cells towards the Protective-layer. Almost immediately the cells thus cut off become suberized and lose their living contents (Text-fig. 7, *P. pd.*). A

feature of special interest in this connexion is that cells corresponding to the ordinary phelloderm (Pl. IV, Fig. 4, *ph.*) are also cut off towards the cortex, and this despite the fact that as yet the stem phellogen has not been produced. It is not, however, until the end of the second season that the periderm becomes continuous across the vascular bundle by division of the living cells of the latter, and about the same time it joins on to the recently formed periderm of the stem (Text-fig. 7, *P. pd.*[1]). All the elements above the Protective-periderm have by this time undergone a complete chemical change which, as has been pointed out above, varies with their morphological nature.



TEXT-FIG. 7. *Tilia europaea.* Longitudinal section of leaf-scar in second year.



TEXT-FIG. 8. *Tilia europaea.* Longitudinal section of old scar. *o.s.* = old part of scar which has been cast off; *P.pd.* = Protective-periderm of last year.

In the old scars it is of interest to note that the successive cork layers are thrown off, leaving in each case a perfectly plane surface (Text-fig. 8). It will be noticed later that this is a common phenomenon and corresponds to the exfoliation of the successive cork layers in the stem; and one can scarcely avoid the inference that the throwing off in spring of the dead leaf in such cases as *Quercus* is but the ultimate result of the process, the beginnings of which we see in *Tilia.*

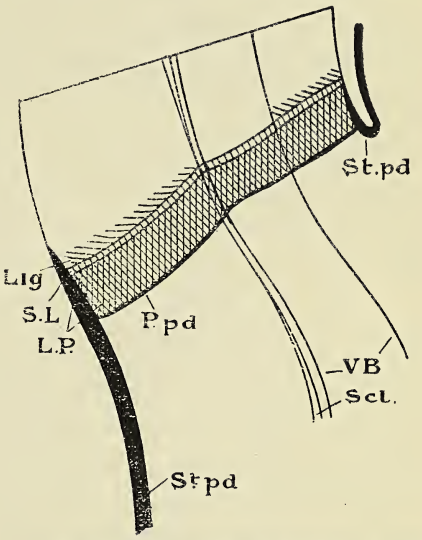
CLASS II (*b*). BETULA VERRUCOSA.

The leaf-fall phenomena in *Betula verrucosa* are identical in most respects with those which occur in the Lime. There is, however, under ordinary conditions, a difference in the sequence of the chief events, for in

the present example ligno-suberization of the Protective-layer occurs previous to the amputation of the leaf.

The external morphology of the leaf of *Betula*, and its relation to the large axillary bud and the stem, are too well known to require description. Internally the arrangements are of the common type. Three vascular bundles leave the ring to supply each leaf, and the patch of sclerenchyma situated outside the phloem of each soon diminishes in amount and finally disappears before the leaf-base is reached. A little way up the petiole it again reappears in its former position, and is assisted in its function by the collenchymatous hypoderm of the petiole.

Starch granules are very numerous in the cortex, but are absent from the mesophyll of the petiole. Compound crystals of calcium oxalate are abundant in both tissues. Periderm is present in the stem long before the commencement of the processes leading to the fall of the leaf.



Text-fig. 9. *Betula verrucosa.* Longitudinal section of leaf-base just previous to leaf-fall.

The first change which occurs in the base of the leaf consists in the increased abundance of the protoplasm of a layer of cells (10–20 cells in thickness) situated above the junction of cortex and petiole. The layer thus affected is very thick (Text-Fig. 9, *L.P.*), and its protoplasmic and starchy contents give it an air of activity which is almost immediately justified by the production of numerous cell-walls. These appear singly in each cell, and are orientated in all directions. Almost immediately the cell-walls of the Protective-layer begin to undergo change in chemical composition, the process of ligno-suberization commencing near the upper limit of the active area, and spreading downwards until the whole layer is completely changed (Pl. IV, Fig. 5, *L.P.*). The whole process is quite similar to what has been described for the other examples; the main mass of the wall becomes lignified, while a thin film of suberin appears on the inner face of each cell-wall, with the possible exception of the crystal cells included in the Protective-layer. The protoplasm gradually degenerates and disappears, leaving the ligno-suberized cells quite empty.

Very early in the formation of the Protective-layer, tyloses appear in the vessels of the leaf-trace bundles at that level. They are usually not very numerous, and are chiefly confined to the secondary wood. Gummy

lignin is produced in great quantity, and fills up the interspaces between the tyloses as well as the vessels from which tyloses are absent.

With the first change in the chemical composition of the Protective-layer, the adjacent cells above begin to organize into a Separation-layer. This layer (Pl. IV, Fig. 5, *S.L.*), which is 2–6 cells thick, is at once distinguished by the abundance of its living and starchy contents, and by the fact that the chemical changes going on in the walls induce the latter to give a reaction different from that of the surrounding cells. A few irregular division walls may appear, but these are never very extensive. The primary walls begin to swell; the middle lamellae between the outer cells become mucilaginous, and later disappear, and the neighbouring cells separate.

Above the Separation-layer the adjacent cells become completely lignified, and finally lose most of their living contents before the leaf separates. The *Lignified-layer* thus formed is generally 2–3 cells thick, and no doubt aids in the separation of the leaf (Text-fig. 9, and Pl. IV, Fig. 5, *Lig.*).

The cells of the Separation-layer which remain on the scar retain for a time their living protoplasm, as well as the cellulose nature of their walls. Later, however, the protoplasm disappears, and the cell-walls collapse and form a thick membrane over the surface of the Protective-layer.

Although most of the changes just described take place quite early, the leaves of the Birch are among the last to fall. Probably this fact is associated with the comparative lightness of the leaf, and the slight resistance it offers to the wind; and this may also explain the degree of completeness attained by the leaf-fall processes in this species before the leaf is finally cast off. Before the latter event occurs, a layer of cells beneath the Protective-layer divides up by regular walls running at right angles to the direction of the petiole (Pl. IV, Fig. 5, *ca.*). A regular cambium is thus produced, which by division gives rise to a layer of cells towards the Protective-layer. As these are cut off suberization immediately takes place, so that a slight layer of cork is produced before leaf-fall. Subsequently the activity of the cambium increases, and before the end of the first season 4–6 rows of cork cells have been produced, which, however, are interrupted by the vascular bundles and accompanying stereome (Text-fig. 9, *P. pd.*).

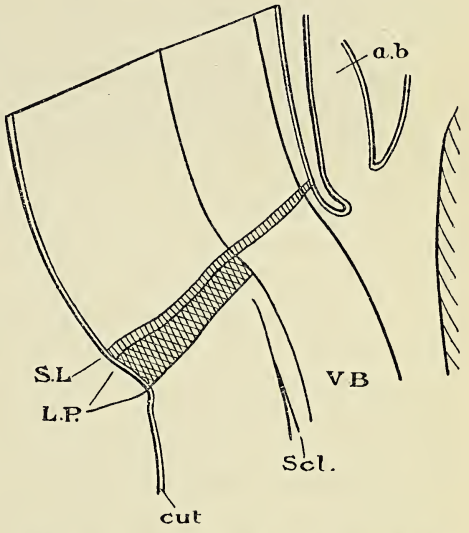
In the second year a new cork cambium is produced below the first, and is at once continuous with the periderm of the stem. It curves downwards and traverses the vascular bundles, and by the end of the second year it gives rise to a thick layer of cork.

The examples which have already been described have only one constant difference, i.e. the presence or absence of irregular division walls in the Protective-layer. With this exception the final result is substantially the same. In all these cases the Protective-layer is formed by the metamorphosis of *existing* cells, and although there is invariably formed beneath

the scar, by the activity of a cambium, a layer of regular cork cells, this is a formation quite distinct in character and usually bears a definite relation to the periderm of the stem. Its function is to reinforce the already existing Protective-layer, and, later, by becoming continuous with the stem-periderm, to assist in removing the now useless scar structures.

Class III. Salix Caprea, Linn.

The next class, of which *Salix Caprea* is the first example, is characterized by the possession of a Protective-layer which is entirely secondary in origin. It is produced by the activity of a single layer of cells which become merismatic, but which for a long time have no possible connexion with the stem-periderm. The whole appearance is quite different from what obtains in other examples even in later stages. But it is upon the morphological distinction that the class is founded.



Text-fig. 10. *Salix Caprea.* Longitudinal section of leaf-base at the time of leaf-fall.

Taking the Willows as a whole, the external morphology is very similar. The petiole is slender and cylindrical, giving place above to the narrow lamina, and below spreading out where it joins the stem. The sheathing leaf-base, which is provided with a pair of stipular wings, encloses the large dorsally-compressed axillary bud, and a distinct furrow marks its junction with the stem.

Internally, there is no obvious difference in size between the cells of the cortex and those of the petiole. In the former starch granules are abundant, but are absent from the latter with the exception of the bundle sheath. Cortex and petiole alike display fine intercellular space systems, and in both tissues compound crystals of oxalate of lime are abundant. No periderm is present in the stem previous to leaf-fall, but a very conspicuous cuticle protects the surface of both stem and petiole.

On leaving the vascular ring to supply the next leaf, each of the three vascular bundles is accompanied by a patch of sclerenchyma, which, however, disappears before the leaf-base is reached (Text-fig. 10, *Scl.*). Sclerenchyma is absent from the petiole, but the thick hypoderm is collenchymatous, and no doubt serves to strengthen the petiole.

The absence of periderm in the stem at the time of leaf-fall rids us of

a complication which in many examples tends to obscure the chief events that precede defoliation. Early in October there is a considerable massing of the protoplasm in the cells of the leaf-base at the level of the furrow shown externally, but, curiously enough, few or no starch granules are to be detected in this area. Very soon regular division walls appear in these active cells, producing a regular cambium which in section has a curved course running across the base of the petiole. By its activity the cambium (Pl. IV, Fig. 6, *ca.*) gives rise to 6–10 rows of cells towards the petiole and 1–2 towards the cortex. The latter (*ph.*) do not increase very much in size, but retain their cellulose walls and living contents, and are certainly homologous with the phelloderm of the stem; the former, however, become highly suberized with the middle lamellae of lignin, and finally lose their living contents. These constitute the Protective-layer (*L. P.*).

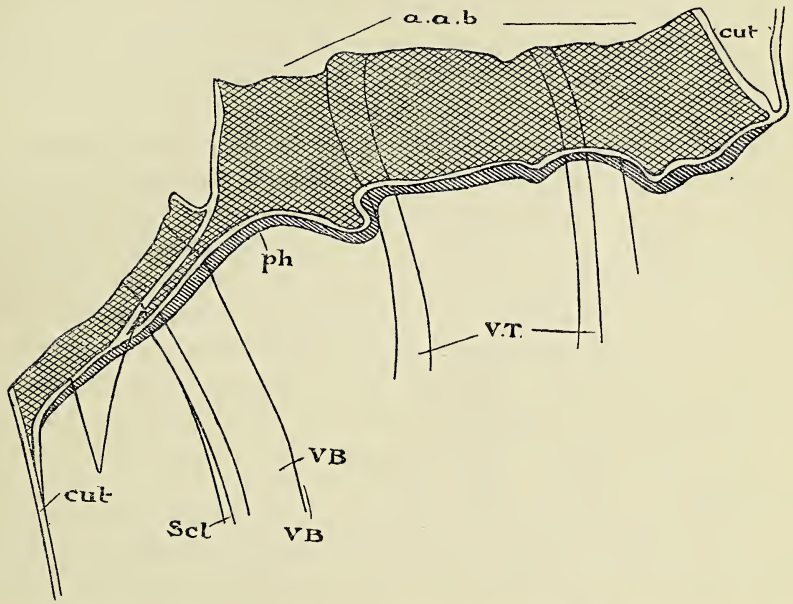
With the first change in the leaf-base tyloses appear in the vessels of the vascular tissue near the active area, and effectively close the vessels of the primary wood, though they are not very numerous in the secondary wood. Gummy lignin is also produced about the same time.

During the formation of the Protective-layer the adjacent layer above has also become active, and has undergone numerous divisions by walls which run in all directions (Pl. IV, Fig. 6, *S. L.*). The protoplasm first increases in amount, and starch granules are produced; this is followed by the appearance of 3–6 irregular walls in each cell of this layer, which is two, sometimes three, cells in thickness. The primary walls begin to swell, the middle lamellae become gelatinous and finally disappear, leaving the leaf free to fall as soon as the rupture of the vascular tissue is accomplished. The separation usually commences near the dorsal surface and spreads rapidly across. A portion of the Separation-layer invariably remains on the surface of the scar; the walls of the cells undergo ligno-suberization, their contents disappear, and the collapsed cells form a ragged outer membrane to the scar tissue. No ligno-suberization occurs in the vascular bundles previous to leaf-fall.

It is singular, in view of the elaborate preparations made to bring about defoliation and subsequently to provide adequate protection for the exposed tissue, that in a remarkably small proportion of the leaves yet examined have these modifications been availing. A well-formed Separation-layer is invariably present at the time of leaf-fall, yet—due possibly to the omission of some internal factor, or perhaps to bad climatic conditions which bring about leaf-fall before, so to speak, the leaf is quite ready for it—it is often ineffective, and separation takes place by the rupture of the thin lateral walls of the Protective-cambium. There is no evidence of a gradual elongation of the lateral walls such as Tison describes for *Aristolochia Sipho*, and it is not likely that such occurs, for when gently cutting longi-

tudinal sections of the leaf-base about the time of leaf-fall, the leaf easily separated along this plane, leaving a clean surface exposed and the underlying tissues quite unprotected.

But whether the Separation-layer is effective or no, the subsequent course of events is the same. In both cases defoliation is followed by the continued activity of the cambium, which produces a few more layers of cells before ceasing for the winter. The ligno-suberization of the new cells does not proceed so quickly or become so complete as in the cells produced previous to leaf-fall, and the last layer formed before the cambium ceases its activity retains its protoplasm and undergoes changes quite different



TEXT-FIG. 11. *Salix Caprea.* Longitudinal section of scar of leaf and axillary bud (*a.a.b.*), showing distribution of internal cuticle (*cut.*). *V.T.* = vascular tissue of aborted axillary bud (*a.a.b.*).

from those previously described. The cells of this layer remain small and have the usual form of periderm cells; their protoplasmic contents are fairly abundant, but no starch granules are present. Soon the outer wall thickens considerably and gives the reactions for cutin. In some cases the increase in thickness is so great that the lumen of the cell becomes almost obliterated. By the end of January, when this internal cuticle is fully formed, it is very conspicuous, and starting from the stem cuticle at the junction of the petiole and axillary bud, it passes beneath the Protective-layer, and curving downwards, runs parallel with the surface for some distance before finally joining the thick outer cuticle of the stem proper, with which it is chemically identical (Text-fig. 11, and Pl. V, Fig. 7, *cut.*, *cut.*[1]).

The living vascular elements above undergo complete chemical change, the wood and bast parenchyma and the companion cells becoming lignosuberized and the sieve tubes submitting to lignification.

During the second year the cambium of the Protective-layer resumes its activity, and usually gives rise to many layers of phelloderm. Sometimes isolated cells in the periderm have been observed to develop a cuticle similar to that just described; and by the end of the second year, a second cuticle, identical with the first and situated a few cells below it, has been produced. This is generally well shown in cases where the axillary bud has become aborted (Text-fig. 11). The conditions of the leaf-scar are then reproduced, and the cuticular layer is continued beneath that of the leaf-scar and joins the internal cuticle of the stem periderm, which has by this time been produced. In all cases the corresponding cells within the leaf-trace also develop cuticular walls. Additional periderm and internal cuticles are formed in subsequent years.

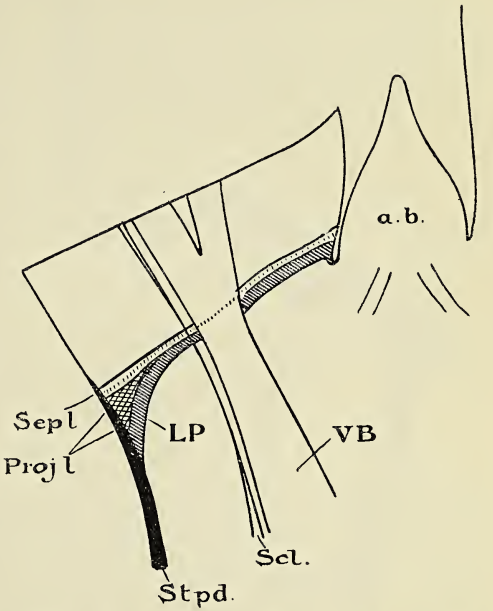
This striking formation of internal cuticle beneath the leaf-scar is not confined to *S. Caprea*, but it is also found in all the species of *Salix* examined, i.e. *S. laurina*, *hippophaefolia*, *purpurea*, and var. *Scharfenburgensis*, *S. alba*, var. *caerulea*, *S. cordata*, *Smithiana*, var. *acuminata*, *S. cuspida*, *undulata*, *rubra*, *daphnoides*, and *Babylonica*, var. *Salmoni*, and *S. incana*. Many variations are apparent in the time of formation and general appearance of the internal cuticle, and without entering into details we may just point out one or two of these. The time of appearance relative to the formation of the secondary cuticle of the stem periderm varies very much; in *S. Smithiana*, var. *acuminata*, and *S. daphnoides*, the time of formation agrees with that described for *S. Caprea*, for here the secondary cuticle affords a protection for the scar before the appearance of the internal cuticle of the stem. This is very different from what as a rule occurs in the remaining species, in which the scar cuticle has a belated appearance and often seems to be formed as a result of the continuation of the internal cuticle of the stem beneath the scar. It is possibly right to conclude that the degree of dissociation which exists in some species between the formation of the secondary cuticle of the scar and that of the stem is due to the difference in the time of formation of the periderm in the stem, for it is only in cases where the formation of the stem periderm is late that the internal scar cuticle has a separate origin.

Another difference to be noted is the different degree of thickness attained by the internal cuticle. The primary cuticle is invariably thick and conspicuous, and in most cases the internal cuticle is very similar. But in *S. incana* it is so thin that it can scarcely be recognized even the second year. Correlated with this, the periderm produced beneath the scar in this species is much more abundant than is usually the case.

Class III (*b*). Populus balsamifera, Linn.

It is not surprising, in view of their undoubted affinity, that the course of events connected with leaf-fall should be almost identical in the two genera, *Salix* and *Populus*. The species of the latter now to be described was examined because the plant happened to be conveniently situated, and also because the somewhat stouter petiole is more convenient to manipulate. Tison's description of the leaf-fall in *P. alba* almost exactly tallies with what has been observed in the present species.

As is commonly the case in this genus, the petiole of *P. balsamifera* is long and slender, and compressed laterally so that a transverse section appears roughly oval in outline. Despite its fragile appearance the petiole is well supplied with sclerenchyma, especially in the upper part, where it is really very rigid. The three vascular bundles which leave the stem to supply the leaf take with them a quantity of the sclerenchymatous sheath which surrounds the vascular cylinder; but the amount accompanying each rapidly diminishes as the leaf-base is approached until in the latter region often only a single strand remains (Text-fig. 12, *Scl.*). The vascular bundles themselves also decrease in bulk as they traverse the leaf-base, but immediately afterwards each divides several times, and after much interesting orientation three groups of bundles emerge and enter the leaf-blade. Just above the leaf-base, the single strand of sclerenchyma gives place to a thick sclerenchymatous sheath, which becomes more pronounced the nearer it gets to the lamina.

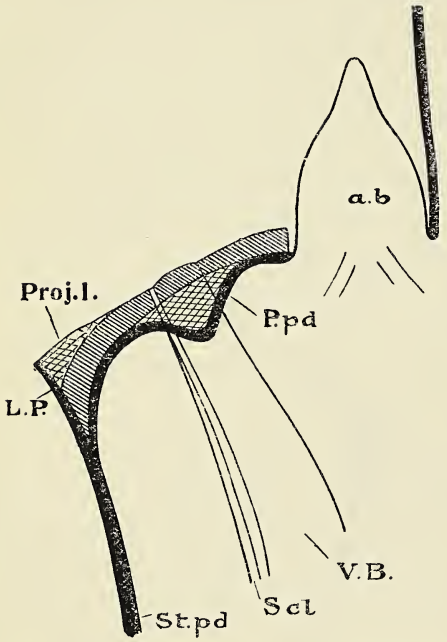


Text-fig. 12. *Populus balsamifera*. Longitudinal section of leaf-base at time of leaf-fall. *Proj. l.* = cells of petiole which occur between Protective-layer (*L.P.*) and Separation-layer (*Sep. l.*).

From the point of view of size, no distinction can be drawn between the cortical cells and those of the petiole. Abundant starch granules are present in the cortex and to a less extent in the petiole, while compound crystals of calcium oxalate are distributed indiscriminately in both. The well-formed hypodermal periderm which is present in the stem extends

well into the leaf-base, and, as will be seen from the diagrams, is distinct from the Protective-layer formed later (Text-figs. 12 and 13).

About the end of August or early in September, the protoplasm becomes very abundant in the cells at the junction of cortex and petiole, and the starch granules increase greatly in number. The layer which shows this increased activity consists of 2–3 rows of cells, and, starting at a point on the inner surface of the petiole just above the junction of the latter with the axillary bud, passes in a slanting direction across the leaf-base, and curving downwards joins the stem-periderm at an appreciable distance below its upper limit. Very soon, division walls appear in the cells of this layer, situated near the dorsal surface, and the process spreads rapidly across, the cells for the most part dividing quite irregularly. The cells of the lower layer, however, divide more regularly and give rise to a cambium which produces 4–8 rows of cells towards the petiole. As soon as they are cut off, the new cells undergo ligno-suberization, losing in turn their starch granules and living contents, until finally nothing but clear, dead cells remain. The cells of the Protective-layer possess the form and general characteristics of periderm cork, and although the middle lamellae and corners of the cell-walls exhibit lignification, the main mass consists of suberin. During its activity, the Protective-cambium has become continuous with the phellogen of the stem-periderm, but in spite of this and the general resemblance of the Protective-layer to the cork in the stem, there is really little connexion between the two, the place of junction invariably remaining well marked.



TEXT-FIG. 13. *Populus balsamifera.* Longitudinal section of two-year-old leaf-scar.

The activity of the cells immediately above the Protective-layer has already been described as having resulted in the formation of more or less irregular walls. These cells still retain their starch and living contents, and division proceeds until walls to the number of 2–5 have appeared in each cell. The Separation-layer thus formed is early marked off, and, except near the dorsal surface, it is in direct contact with the upper limit of the Protective-layer. Occasionally, however, near the dorsal surface a small

triangular mass of cells intervenes, and later becomes ligno-suberized and contributes to the bulk of the Protective-layer (Text-figs. 12 and 13, *Proj. l.*). The cells of the Separation-layer do not increase very much in size, but the walls of the mother-cells begin to swell, the middle lamellae between the cells of the outer layers gelatinize and finally disappear, and the leaf is freed by the rupture of the vascular bundles. Throughout the whole process the formation of tyloses in the vessels is very scanty, only a few small ingrowths being observed in the vessels of the protoxylem. With the first signs of activity in the leaf-base, however, there is an abundant supply of gummy lignin, which lodges in all the vessels in the region of the Protective-layer, and finally results in the complete blocking of these conducting elements.

About the end of the second year, the activity of the Protective-cambium ceases, and a new one is formed by division of the cells in the cortex beneath. At the same time a new phellogen appears in connexion with the stem-periderm, and the two becoming continuous a regular layer of periderm is produced.

Short descriptions of other species examined will now be given, and these will be arranged under their respective types.

Class I.

In the following plants all stages will be found, from the state of events seen in *Castanea sativa* to that described for *Ribes sanguineum*, and in many cases all transitions are to be found within the same species.

Hibiscus syriacus, Linn.

The slender, cylindrical petiole is rather swollen at the point of attachment, and has a row of hairs running up the ventral surface. At the leaf-base there is the usual reduction in the patches of sclerenchyma which accompany the three vascular bundles in their passage to the leaf. Starch granules and compound crystals of calcium oxalate are distributed as usual, and mucilage cavities are also present.

The Separation-layer is formed by the division of 1–3 rows of cells, situated at a little distance above the upper extremity of the stem-periderm, and distinguished by their dense protoplasmic and starchy contents. The walls of the uppermost cells swell and become mucilaginous, and separation occurs by the solution of the middle lamellae. No Lignified-layer is present above the Separation-layer. Tyloses and gummy lignin are abundant in the vessels at the leaf-base before separation occurs. Although easily distinguished by reason of the abundance of its cell contents, the Protective-layer shows no chemical or other change previous to leaf-fall. As soon as the latter has taken place, ligno-suberization, preceded by the disappearance of the starch granules, commences near the exposed surface and spreads rapidly downwards. Lignification is most marked in the lower cells of the

Protective-layer, and is the only process which occurs in the crystal cells and mucilage cavities.

The cambium which appears below the Protective-layer before the end of the first year is not very active, producing only 2–5 layers of cork cells. In the second year, a new cambium arises at a little distance below the first, and becoming continuous across the vascular bundle, produces a thick layer of cork which is generally curved in a curious manner. An appreciable quantity of phelloderm is also produced.

Quercus palustris, Muench.

In this species the cells of the cortex are much smaller than those of the petiole, and are distinguished from the latter by the presence of abundant starch granules. Crystals of calcium oxalate are present in both regions. Groups of peculiar stone cells are found only in the cortex and leaf-base, and the ordinary sclerenchyma which is present outside the phloem of each leaf-trace bundle undergoes great reduction at the leaf-base.

Tyloses appear in the vessels of the leaf-base long before leaf-fall, and are accompanied by the production of a varying amount of gummy lignin. The Protective-layer becomes distinguished by its abundant cell contents, but no other change occurs before leaf-fall. The Separation-layer is produced by division, by approximately parallel walls, of 2–8 rows of cells, situated a little distance above the junction of the petiole with the axillary bud; and separation occurs in the usual way.

Previous to leaf-fall, division of the cells immediately above the Separation-layer is followed by a process of lignification. After the leaf has fallen, ligno-suberization of the Protective-layer commences near the exposed surface, and spreading downwards completely metamorphoses all the cells at that level, including the living cells of the vascular bundle. The crystal cells become lignified, but the lignified elements of the vascular bundle as well as the patches of stone cells included in the Protective-layer undergo no change.

During the first winter the cambium which arises below the Protective-layer produces only 4–8 layers of cork cells, and does not establish any connexion with the stem periderm. In the second year the cambium is much more active and produces a thick layer of periderm.

Ligustrum vulgare, Linn.

The course of events leading to, and resulting from, the fall of the leaf in the Common Privet is very simple. In the cortex and petiole starch granules are very scanty and other cell contents far from abundant. A single vascular bundle supplies the leaf, and the little sclerenchyma which at first accompanies it entirely disappears at the leaf-base. Little or no

change occurs in the cells of the Protective-layer previous to leaf-fall. The latter is brought about by the chemical alteration and subsequent disappearance of the middle lamellae of a layer of cells which is never very obvious, and in which no visible preparations occur. After leaf-fall the cells near the exposed surface undergo ligno-suberization, and the cells beneath the Protective-layer divide to form a cambium, which produces a continuous layer of cork in that region.

A fairly well marked Lignified-layer can often be demonstrated before leaf-fall above the Separation-layer. Tyloses are never very numerous, nor is there an abundant formation of gummy lignin.

Celtis occidentalis, Linn.

In this example there is no essential difference from the method that has already been described for *Castanea*. Reduction of sclerenchyma and distribution of starch granules and crystals of calcium oxalate are as usual; while in addition groups of 'stone' cells are present in the cortex.

The Separation-layer is formed by the slight division of 1–2 rows of cells situated at some distance above the junction of the petiole and axillary bud, and separation occurs by mucilaginization and subsequent disappearance of the middle lamellae. There is no Lignified-layer, and tyloses, though present, are not at all abundant at the time of leaf-fall. Soon after the latter has occurred, ligno-suberization commences in the lower portion of the Protective-layer, and gradually spreads until the whole of the parenchyma in this region—including the living cells of the leaf-trace—has become ligno-suberized. The last cells to undergo this change are those near the surface; but later these collapse and become altered in the usual way.

In the first year, the cambium, which arises by division of the cells beneath the Protective-layer, gives rise to 1–3 layers of cork and a similar quantity of phelloderm (Pl. V, Fig. 8, *co.*, *ph.*), the latter being distinguished by the retention of the living protoplasm and cellulose walls and by the presence in many of the cells of a rhomboidal crystal of calcium oxalate (*c. c.*). The behaviour of these crystal-containing phelloderm cells is very similar to that of many of the stone cells in the cortex which also contain crystals. In both lignification of the cell-wall occurs, and the lignin, instead of being confined to the primary wall, becomes deposited as a film of varying thickness on the surface of the crystal (Pl. V, Fig. 9). This is very noticeable in many of the crystal cells where a connexion exists between the primary cell-wall and the film of lignin which encloses the crystals.

In the second year the activity of the Protective-cambium is continued, and results in the formation of a thick layer of cork and a less quantity of phelloderm.

Pyrus floribunda, Nichols.

Externally the junction of the petiole with the stem is marked by a slight groove, while internally little or no difference is apparent, either in the size of the cells or in the cell contents; in both cortex and petiole, starch grains as well as compound crystals of calcium oxalate are uniformly distributed throughout. There is the usual reduction in the sclerenchyma at the leaf-base. Previous to leaf-fall there is no formation of Protective-layer, the only modification being the formation of the Separation-layer in the petiole at a little distance above the junction of the latter with the stem. This is produced by the fairly regular division of 2–3 rows of cells, distinguished in the usual manner, in each of which 1–2 new walls appear. Little or no growth in size occurs; the primary walls of the outer cells swell considerably, and finally the middle lamellae disappear, and the leaf separates and falls to the ground.

Soon after defoliation, the cells of the Separation-layer which remain on the scar gradually lose their contents and become flattened, forming a well-defined layer which aids in protecting the underlying tissue. The latter also becomes changed and begins to undergo ligno-suberization, the cells finally losing their living contents. At the same time, divisions which take place beneath the Protective-layer give rise to a cambium, which produces a thick layer of cork before the end of the first year.

In the second year the cork cambium continues its activity, and adds considerably to the cork layers. During the whole of the process, few or no tyloses are produced in the vessels of the leaf-trace at the level of the Protective-layer, but the large amount of gummy lignin which appears completely closes these organs.

Ceanothus Gloire-de-Versailles. (Garden origin.)

The leaf-fall phenomena in this species are very similar to what has been described for *Pyrus floribunda*, the chief difference being the absence of stem periderm for a long time after leaf-fall. In *Ceanothus*, also, the position of the Separation-layer is usually fairly high up the petiole, so that an appreciable amount of the latter is left on the stem when the leaf is cast off.

Acer pseudo-platanus, Linn.

In this species the usual characteristics are to be noted, i. e. the presence of starch granules in the cortex, simple and compound crystals of calcium oxalate in both cortex and petiole, the appearance before leaf-fall of a superficial periderm in the stem, and the complete reduction of lignified sclerenchyma at the leaf-base.

In the mature leaf the junction of stem and petiole is marked internally by a layer of smaller cells, in which later the protoplasm increases in amount and starch granules appear. The layer of cells thus distinguished runs rather obliquely from near the upper limit of the stem periderm to the junction of the petiole with the axillary bud, and varies in thickness from 6 to 12 cells. No divisions of any kind occur, but the activity of the cells is soon apparent in the changed character of the cell-walls, which now give slight reactions for lignin and suberin. The process of ligno-suberization, which is really very diffuse, probably commences near the upper limit of the Protective-layer, and from thence spreads in all directions; the protoplasm gradually decreases in amount, and the disappearance of the nucleus marks the complete conversion of the cell-wall.

The amputation of the leaf is effected by the separation of the cells adjacent to the Protective-layer on the petiolar side. This layer (2–3 cells thick) consists of cells which are rich in protoplasm and starch, and which become divided by a single wall in each case. The new walls invariably remain thin and cellulosic, but the mother-cell walls begin to swell and take the haematoxylin and ruthenium-red with great avidity. The swelling continues and the middle lamellae become entirely mucilaginous and finally disappear, leaving the adjacent cells completely separated.

Some time before leaf-fall a phellogen is formed by the regular division of the cells immediately beneath the Protective-layer, which gives rise before leaf-fall to a layer of 2–6 cells. As the cells are cut off they undergo various changes, but are chiefly distinguished by the fact that they become filled with a dense mixture of mucilage and tannin, just as is the case with the cells cut off by the stem phellogen. This stage may continue for a long time, but sooner or later the mucilaginous mass disappears, and the cells assume the regular appearance and composition of periderm cork.

Little attention has so far been paid to the behaviour of the vascular elements near the affected area. Very early in the course of the changes just described, tyloses appear in the vessels of the primary xylem, and are soon followed by the introduction of a great quantity of gummy lignin. The tyloses are usually far from numerous and seldom appear in the later formed vessels of the leaf-trace. Gummy lignin, however, is invariably abundant, and is found in the majority of the vascular elements situated near the leaf-base. The changes which occur in the parenchyma of the Protective-layer are reproduced in the living elements of the vascular bundle, though the process is usually not completed until after the leaf has fallen. Vascular bundles and patches of sclerenchyma alike provide insuperable obstacles to the continuity of the Protective-periderm formed in the first year, but later formed periderm is interrupted only by the sclerenchyma outside the vascular bundles.

Ribes nigrum, Linn.

This species differs from *R. sanguineum* in that: (1) The development of sclerenchyma in the petiole is not so pronounced. In both the rather thick walled cells containing mucilage and tannin which accompany the vascular bundle decrease in number as the latter passes through the Separation-layer. (2) The chief events have a tendency to occur later relatively to the leaf-fall, i. e. ligno-suberization before leaf-fall is much rarer in *R. nigrum* than in *R. sanguineum*.

Diospyrus virginiana, Linn.

The present species, adopted as a type form by Tison, has therefore been fully described by that author, whose observations have been entirely confirmed in the present research. The petiole is short and erect, and supplied with a single vascular bundle. Sclerenchyma is entirely absent from the petiole with the exception of isolated groups of stone cells. Starch granules and calcium oxalate crystals occur in the cells of both cortex and petiole; periderm may or may not be present in the stem previous to leaf-fall.

The Separation-layer, indicated externally by a furrow, is formed very early by the irregular division of 2–3 rows of cells situated a little distance from the base of the petiole, and as no increase in size occurs this layer is a conspicuous feature. Later the Protective-layer is formed by the ligno-suberization, without previous division, of the cells below the Separation-layer. This process commences near the periphery and adjacent to the Separation-layer, and passes like a wave downwards, the protoplasm and starch rapidly disappearing at its approach. About the same time tyloses appear in the larger vessels. No other change occurs until later, when the elements on the periphery of the vascular bundle undergo ligno-suberization, and the protoplasm of the Separation-layer increases in density. The cell-walls of the latter increase a little in thickness, while the cells themselves grow slightly and separate from each other. Apparently there is no production of mucilage, the differential growth of the cells probably accounting for the separation.

At the time of leaf-fall little modification has occurred within the vascular bundle, tyloses and gummy lignin being almost absent. During the second season it is traversed by a cork layer produced by the activity of a cambium; this, however, seldom attains a greater thickness than 3–4 cells.

Halesia tetraptera, Linn.

Quite similar in most respects to what has been very briefly described above for *Diospyrus virginiana* is the course of events in *Halesia tetraptera*. A single vascular bundle invades the petiole. Starch is absent from the

latter but present in the cortex, while single and cluster crystals of calcium oxalate appear in cells of both tissues and of the leaf-base. A rather deep-seated periderm is present in the stem (Pl. V, Fig. 10, *st. pd.*).

The Protective-layer (*L. P.*), at first distinguished by reason of its abundant living and starchy contents, becomes distinctly ligno-suberized long before leaf-fall, the process commencing at localized points, from whence it spreads rapidly, soon extending into the vascular bundle. Near the upper and lower boundaries of the Protective-layer the lignin appears to predominate ; the walls of the crystal cells undergo lignification only. A few tyloses appear in the larger vessels during the process.

The Separation-layer (*S. L.*) is formed by the slight division of 1–3 rows of cells, which as usual are distinguished by possession of much protoplasm and a few starch granules. Separation is effected by the mucilaginization and disappearance of the middle lamellae between the outer layers. No lignification occurs above the Separation-layer.

In the second year a cork cambium arises beneath the Protective-layer, and traversing the vascular bundle, becomes continuous with the stem periderm. As in *Diospyrus*, a thin layer of cork is the result.

Forsythia Fortunei, Hort. (= F. suspensa, Vahl.).

The case of *F. suspensa* has been fully dealt with by Tison, who founds a type on the species. To his general description there is little to add, and only a short summary of the course of events will be given. The petiole is short and spreading towards the base, enclosing in its axil the two unequal buds. Neither externally nor internally is there any clear distinction between the petiole and stem except in presence of starch in the cells of the latter. A single vascular bundle supplies each leaf, and in traversing the cortex it loses the whole of the lignified stereome which at first accompanied it.

The slight massing of the protoplasm in the cells of the leaf-base is followed by the more or less complete ligno-suberization of the cell-walls, the latter process commencing in the upper portion, and gradually spreading across and downwards until a fairly thick wedge-shaped Protective-layer has been produced. No previous divisions have occurred in these cells, and at this time few or no tyloses have appeared in the vessels of the leaf-trace at this level, although the living elements of the bundle have begun to undergo ligno-suberization.

About this time in the average leaf-base the cells below the Protective-layer begin to divide regularly and give rise to a cambium which, without traversing the vascular bundle, soon becomes continuous with the well-marked phellogen of the stem. A few layers of cork may be produced, the number varying with the example and very probably with the position on the branch.

The Separation-layer is formed, without any division, from the cells of the petiole adjacent to the Protective-layer, and is distinguished in the usual way by its abundant protoplasmic and starchy contents. Later the walls swell slightly, and finally the middle lamellae of the outer cells disappear, and separation results. No lignification occurs in the cells above the Separation-layer. In the second year the cork cambium again becomes active, and taking a more horizontal direction traverses the vascular bundle. Eventually all the cells above become ligno-suberized, and abundant gummy lignin and numerous tyloses appear in the vessels near the leaf-base.

In view of Tison's very clear distinctions between the different types, a case which appeared in this example—instances of which are far from rare in this as well as other species—is especially interesting. On cutting sections of quite ordinary leaf-scars it was found that although there had been the ordinary vital separation, and although the Protective-layer was of the usual shape and well formed, there were no signs either of stem periderm or of Protective-periderm. That is to say, in this case the formation on which Tison bases his type was altogether absent, although apparently everything else was quite normal. Such an occurrence cannot be looked upon as exceptional, for in addition to more than one instance being discovered, all transitions from the normal were found in a very small quantity of material.

Baccharis halimifolia, Linn.

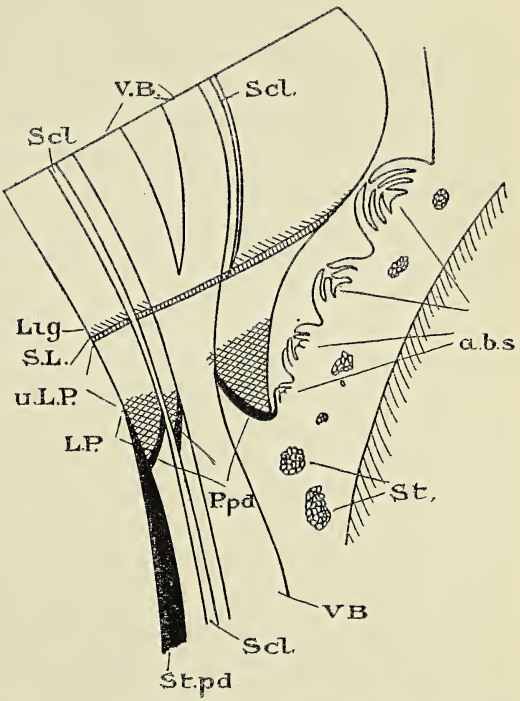
In the mature leaf, the small triangular petiole, which is hollowed out and winged to provide a receptable for the small axillary bud, possesses near its base a deep furrow which marks the position of the Separation-layer. Internally, extensive modifications occur in the three vascular bundles with which the leaf is supplied, for as they traverse the leaf-base, not only are the two patches of sclerenchyma near the xylem and phloem in each reduced to almost nothing, but the bundles themselves decrease considerably in bulk. Another curious feature is the entire absence of starch granules from the cortex and petiole. The junction between the latter is marked by an area of smaller cells in which just before leaf-fall the protoplasm becomes very abundant, and ligno-suberization commences. In the region of the furrow the cells become active, the walls swell and become mucilaginous, and finally separate from each other.

Above the Separation-layer a distinct Lignified-layer is present before leaf-fall. In the fresh scar the exposed cells soon lose their living contents and become flattened out on the surface. Immediately beneath, the process of ligno-suberization, which commenced before defoliation, spreads rapidly (sometimes apparently as two very distinct processes) and extends into the vascular bundles. Later, a cambium arises beneath the Protective-layer and produces from 4 to 8 rows of cork before the end of the second year.

GLEDITSCHIA TRIACANTHOS, Linn.

The elucidation of the phenomena connected with the fall of the leaf in this species is rendered excessively difficult by the presence of various structural peculiarities of the leaf-base. As is well known, the latter organ in *Gleditschia* is much swollen, and hides a cavity which opens to the exterior by a transverse slit on the ventral surface, and in which repose the 3–5 serially-arranged axillary buds (Text-fig. 14, *a.b.s.*). Due to some factor—probably the assumption of the fixed light position—growth on one side of the leaf-base is almost invariably greater than on the other, with the result that this part is usually eccentric. Again, the stem-periderm (*St. pd.*) is rather deep seated and extends up to the narrowest part of the leaf-base ; and the final complication arises from the fact that the quantity of sclerenchyma (*Scl.*) accompanying the three vascular bundles which supply the leaf is large, and scarcely diminishes in any region.

In addition to the sclerenchyma which surrounds the vascular ring of the stem, numerous groups of stone cells (*St.*) are present in the cortex, while the parenchymatous cells of the latter often contain simple and compound crystals of calcium oxalate as well as varying quantities of starch.



TEXT-FIG. 14. *Gleditschia triacanthos.* Longitudinal section of leaf-base at time of leaf-fall.

In the diagram (Text-fig. 14) a median longitudinal section of the leaf-base is shown. There is in this case no conspicuous massing of the protoplasm in the cells at the junction of cortex and petiole. The first indication of the approaching leaf-fall is given by the extension of a branch of the stem periderm in the direction of the vascular bundle, which is interrupted at the phloem of the leaf-trace by the sheath of sclerenchyma (*P. pd.*). A similar branch on the opposite side ends in contact with the xylem, and all the cells thus produced become only very feebly suberized.

About this time a few tyloses are developed in the vessels near the leaf-base, and a quantity of gummy lignin also appears. A little later the cells just above the periderm begin to undergo lignification, a process which at first is most vigorous in the cells adjacent to the vascular bundle. Suberization first commences near the epidermis, and, though spreading in all directions, it is invariably most conspicuous in the region where it first appears. The Protective-layer (*L. P.* and *u. L. P.*) thus formed is thin and indefinite above, and consists of cells which have not previously undergone division. The walls of the included crystal cells become lignified only.

The Separation-layer (*S. L.*) has little or no relation to the Protective-layer. It is situated at some distance above the latter, and is usually composed of 2–3 rows of cells which rarely undergo slight division, and which possess the usual distinction of abundant protoplasmic and starchy contents. Their walls swell and the middle lamellae become mucilaginous and finally disappear, leaving the neighbouring cells quite free. The position of the Separation-layer is noteworthy. It is situated just below the place where the leaf-trace thickens and forks into two, and is so high up as to leave behind enough of the leaf-base to afford efficient protection to all but the largest of the axillary buds.

Lignification occurs in the cells of the petiole immediately above the Separation-layer, and produces a well-marked Lignified-layer (*Lig.*). After leaf-fall the cells beneath the exposed surface, which are still cellulosic, now undergo varying degrees of ligno-suberization, the latter process spreading upwards from the already ligno-suberized portion of the Protective-layer below. In the upper part, however, its further progress is delayed by the desiccation of the cells, which lose their contents and allow their walls to collapse and form a flattened layer on the surface of the scar. Little or no further change occurs in the scar tissue until the beginning of the second season, when a new phellogen arises in the tissue immediately below the old one. Near the epidermis the two phellogens are almost in contact with each other, but towards the centre they diverge considerably, the primary one rising towards the vascular bundle, the secondary cork cambium dipping down a little towards the leaf-trace, which it completely traverses. A thick layer of regular cork cells is produced before the end of the second season, at which time all the tissue above has undergone complete ligno-suberization.

The scar is triangular in shape, and shows on its surface the three cicatrized nodules representing the three vascular bundles. The uppermost and largest axillary bud alone is visible.

Robinia Pseudacacia, Linn.

The leaf-fall and attendant phenomena in *R. Pseudacacia* are quite similar to what has just been described for *Gleditschia triacanthos*; so that here only the differences will be remarked. The Separation-layer is situated relatively higher in the petiole and so produces a more extensive 'lip', which is thus able to completely enclose all the axillary buds. In its formation, too, there is a difference; it is produced by numerous transverse divisions in a single row of cells, though sometimes it seems probable that more than one row takes part.

Clerodendron trichotomum, Thunb.

Externally the petiole is hairy and cylindrical, and passes without any clear distinction into the stem. The internal arrangements are slightly different from what may be called the common type, for in this case only two bundles leave the vascular ring in the stem and pass out to supply the leaf, though as usual the sclerenchyma accompanying each disappears before the leaf-base is reached. Starch in the form of granules is present in the cortex; and in the groups of stone cells which are distributed in both cortex and petiole are seen numerous rhomboidal crystals of calcium oxalate.

The Separation-layer is formed by the regular division of 2–3 rows of cells, and takes a curved course across the leaf-base. It is early defined by the presence of abundant protoplasm and numerous starch granules. Division by 2–4 walls is followed by the thickening of the primary walls, the middle lamellae of which disappear and allow the leaf to separate from the stem. No Lignified-layer is present. Before defoliation occurs the cells below and adjacent to the Separation-layer, without any previous division, begin to undergo ligno-suberization. This process, which commences near the dorsal surface and spreads slowly across and downwards, never becomes extensive before leaf-fall, the layer of tissue altered at that time varying from one cell thick near the ventral surface to three cells near the dorsal surface. After the leaf has been cast off ligno-suberization proceeds, until at length a fairly thick Protective-layer has become differentiated in this way. Finally the adjacent cells beneath divide up regularly, and so give rise to a cambium which, during the first winter, produces a considerable amount of cork, and which continues its activity during the second season.

Tyloses are never very numerous at any stage of the process, but gummy lignin is invariably abundant in the vessels near the Protective-layer. In the second year the Protective-cambium becomes continuous across the leaf-trace, all the living elements above it undergoing complete ligno-suberization. The few crystal ('stone') cells which happen to be included in the Protective-layer become lignified only, and often a slight film of lignin is to be detected surrounding the body of the crystal.

Class II (*a*).

Corylus Colurna, Linn.

Externally the stem and petiole display no special characteristics. Of the internal arrangements it may be noted that the cells of the cortex are smaller than those of the petiole, and are separated by a layer of still smaller cells; starch granules are abundant in cortex and in the transition layer, but are absent, with the exception of the bundle sheath, from the petiole; cluster crystals of calcium oxalate are numerous in both regions, and a superficial periderm is present in the stem before leaf-fall, while at the leaf-base there is an almost complete reduction in the sclerenchyma accompanying the three vascular bundles which supply the leaf.

The irregular division of the cells at the junction of petiole and cortex by walls which appear singly in each cell and which run in all directions, is preceded by a marked increase in the living contents of these cells, so that although no ligno-suberization occurs previous to leaf-fall, the Protective-layer is easily recognized.

The Separation-layer, which now appears, is formed by the division of 1–3 layers of cells situated above the Protective-layer, and is at once distinguished by abundant living and starchy contents, and by its reactions with stains. Separation occurs in the usual way by mucilaginization and subsequent disappearance of the middle lamella. Throughout the whole process a few tyloses are present, and later gummy lignin is very abundant in the vessels at the leaf-base. A slight Lignified-layer is present above the plane of separation.

Ligno-suberization of the Protective-layer occurs after leaf-fall. The double process takes place in the upper portion, the cells below remaining for a time almost wholly lignified. The chemical alteration of the included crystals and vascular elements takes place in the usual way.

Scar periderm arises about the end of the second season. It traverses the vascular bundles (in which it has a curved course) and becomes continuous with the stem periderm on both sides. In later seasons new periderms arise beneath the first one.

Cercis Siliquastrum, Linn.

The petiole is slender, and a slight groove at its junction with the stem indicates the position of the Separation-layer. Three bundles supply each leaf, and the usual reduction in the accompanying sclerenchyma occurs at the leaf-base. The first indication of approaching leaf-fall is the regular division of a layer, 2–4 cells in thickness, which runs in an oblique direction just above the leaf-base. New walls appear in these cells quite suddenly, the only previous indication being the slight massing of the protoplasm in this layer. The plane along which the divisions occur is quite definite and

invariable. It commences near the ventral surface of the petiole just above a small ridge on the inner surface of the petiole, and runs across obliquely to a point very near the upper limit of periderm formation in the stem. The separation which soon follows commences near the ventral surface of the petiole. As the division of the cells (and consequently, their separation) is more and more backward the nearer we approach the dorsal surface, it usually happens that the separation in the latter region is more or less mechanical, and is due to the whole weight of the leaf being thrown on this small area. Although it seems highly probable that the middle lamellae between the separating cells become changed in some way or other to lessen the resistance to separation, no such change could be traced, the tests for mucilage especially giving no positive results. From a comparison of the cell-walls of the Separation-layer before and after separation it seems possible that the disunion is accomplished by differential growth of the respective cell-walls.

At the time of separation there is usually no ligno-suberization of the cells beneath the exposed surface, although a varying number of new cell-walls have appeared. Soon after the cells immediately below begin to undergo change, the process spreading in all directions until a fairly thick layer, including the living tissue within the vascular bundle at that level, has become ligno-suberized. Tyloses appear in some of the larger vessels, and the gummy lignin produced is fairly abundant. Towards the end of the first winter a cambium arises beneath the Protective-layer which in the first year produces a considerable quantity of cork, and which continues its activity in the second year.

Carpinus Betulus, Linn.

The ordinary characters of stem and petiole (both external and internal) are present in this species. In the processes which take place before and after leaf-fall there is little to distinguish it from any of the other examples of this type. Previous to leaf-fall the cells at the base of the petiole undergo irregular division, but no ligno-suberization occurs until after the leaf has fallen. The Separation-layer, which is usually a fair distance above the junction of the stem and petiole, is formed by more or less irregular divisions in a layer of cells 2–3 rows in thickness, and separation occurs between the outer cells of this layer. A well-marked Lignified-layer is almost invariably present above the Separation-layer. After leaf-fall, ligno-suberization commences in the cells of the Protective-layer, but is very diffuse, although apparently aided by the distribution of gummy lignin from the vessels. In late winter divisions in the cells beneath the Protective-layer result in the formation of a cambium, which, however, is not very active.

It is well known that in this species the leaves often remain on the tree during the winter, and it is interesting to see what happens in such

cases. It is found that the usual processes occur, leading to formation of Protective-layer, and that even the Separation-layer may be partially formed. That is to say, an incomplete Separation-layer is produced which possesses the usual characteristics, but not being continuous it is powerless to effect the separation of the leaf, which therefore remains on the tree. In late winter divisions which occur beneath the Protective-layer result in the formation of a second Separation-layer by the agency of which in the following spring the leaf is thrown off. Tison, who has worked out the formation in some detail, states that the divisions beneath the Protective-layer lead to the formation of a cambium which gives rise to many layers of cork towards the scar. The first layer to be formed, however, retains its cellulose character, the side walls elongate and finally rupture, and the leaf separates from the stem. In the cases examined during the present research the complete course of events has not been ascertained due to failure of material. It appears, however, that little or no cork is formed previous to the throwing off of the dead leaf, and that the elongation of the cell-walls of the Separation-layer is not very great.

Prunus Cerasus, Linn.

The external morphology of the petiole and adjacent parts is well known, and calls for no special remarks. It might be noted, however, that a fairly deep furrow marks the transition from stem to petiole, and provides a line of weakness which, quite apart from the fact that the plane of the Separation-layer is later coincident with it, is easily recognized by the ease with which the leaf when submitted to pressure breaks at that place.

We have here again to notice the reduction that occurs in the sclerenchyma accompanying the three leaf-trace bundles. When they first leave the vascular cylinder in the stem each is provided with a conspicuous mass of lignified stereome situated outside the phloem, but this entirely disappears just below the leaf-base, and does not again reappear. Higher up the petiole the strengthening tissue consists of collenchymatous hypoderm, and patches of thick-walled cellulose cells which accompany the phloem; but little or none is present at the leaf-base. In a median longitudinal section the vascular bundle is prominent, and the furrow before mentioned is very well marked on the ventral surface of the petiole. There is practically no difference in size between the cells of the cortex and those of the petiole, but the transition region is marked by an area of smaller cells. Starch is present in the cortex, less abundant in the leaf-base, and entirely absent from the petiole, while compound crystals of calcium oxalate are common in all these regions. A hypodermal periderm is present before leaf-fall.

The first stage in the leaf-fall processes is shown by the increased abundance of the protoplasm in the smaller cells of the leaf-base, which almost immediately begin to divide by irregular walls which appear singly

in each cell. Little growth in size occurs in this Protective-layer, but the cells near the dorsal surface (where the layer is thicker) begin to undergo a chemical change, the process of lignification commencing in this region and extending across the leaf-base to the vascular bundle. Little or no suberization occurs previous to defoliation.

During the processes just described a varying number of tyloses appear in the vessels of the leaf-trace near the active area, and are accompanied by an abundant supply of gummy lignin, together very efficiently closing the lignified conducting elements.

The Separation-layer also arises about the same time. It traverses the petiole in the plane of the external furrow and is produced by the repeated division by (2–6) parallel walls of 2–3 rows of cells above and adjacent to the Protective-layer. There is an abundance of living and starchy contents in these cells; their walls begin to swell, and the middle lamellae between the outer cells degenerate into pectic mucilage and finally disappear, leaving the neighbouring cells quite free.

Before the final separation occurs, the cells immediately above the Separation-layer, after undergoing one or two divisions, become more or less completely lignified. This layer, which always retains a certain amount of protoplasm, is thickest at the sides and gradually decreases as the centre is approached.

As separation takes place between the upper rows of the Separation-layer there is invariably a mass of unaltered cells left attached to the Protective-layer, which for the most part, until properly exposed, retain their contents and cellulose walls. After complete separation, however, these cells soon lose their turgidity, and dying away, they collapse and become firmly pressed to the surface of the Protective-layer.

In *P. Cerasus* little or no suberization occurs in the cells of the Protective-layer before leaf-fall, but subsequently the deposition of suberin on the inner face of each cell goes on rapidly, the living elements of the vascular bundle also undergoing this process. The crystal cells in the Protective-layer become lignified, but no suberin could be detected in their walls.

During the first winter the cork cambium is formed by the regular division of the cells below the Protective-layer, and by its activity gives rise to a few layers of cork. By the end of the second year a thick layer of cork, traversing the vascular bundle and continuous with the stem periderm, has been preserved.

P. VIRGINIANA, Linn.

P. COMMUNIS-DULCIS.

The description of the course of events in *P. Cerasus* applies generally for *P. virginiana* and *P. communis-dulcis*. There is, however, one point of difference. So far as the examination goes, there is never any ligno-

suberization of the Protective-layer previous to leaf-fall. In *P. Cerasus*, as was stated above, lignification of the cells of the Protective-layer almost invariably occurs before the leaf is finally amputated, and in some cases there is a strong tendency towards suberization. But in *P. virginiana* and *P. communis-dulcis* this has not been observed.

CLASS II (*b*).

Platanus orientalis, Linn.

In this well-known plant, the slender cylindrical petiole passes gradually into the swollen base, which externally displays 7–8 prominent ribs corresponding to the leaf-trace bundles beneath. Within the inflated leaf-base there exists a spacious cavity which, while opening to the exterior by a transverse slit on the ventral surface, effectively protects the large axillary bud. Internally, there is the usual distribution of starch granules and crystals (simple and compound) of calcium oxalate, as well as the usual reduction at the leaf-base of the sclerenchyma accompanying the leaf-trace bundles.

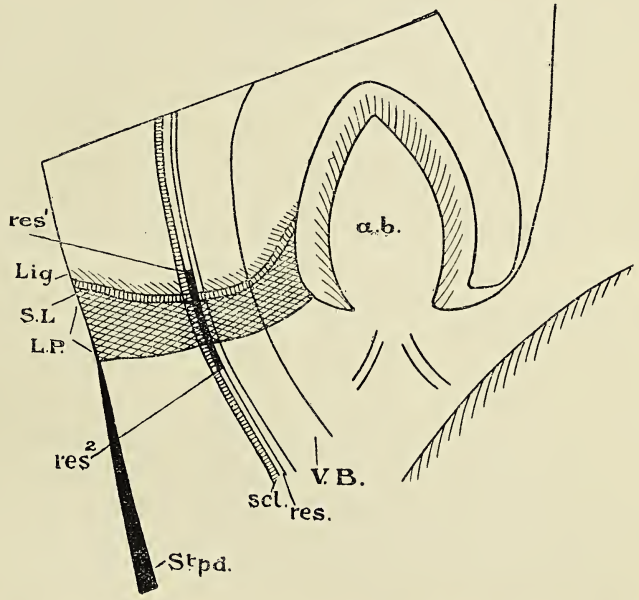
The formation of tyloses in the vessels of the leaf-trace usually gives the first indication of approaching leaf-fall. These appear in small numbers in the vessels of the primary wood near the junction of petiole and stem, and are generally accompanied by a small amount of gummy lignin.

As the tyloses increase in number and the gummy lignin in quantity, there is also a slight massing of the protoplasm in the petiolar cells near the leaf-base, which may at the same time acquire a small amount of starch in the form of granules. Slight division then follows, thin walls appearing singly in a varying number of these cells; rarely do all the cells divide, and it is not uncommon to find that *no* division has occurred at the time of defoliation. Little or no growth takes place in the divided cells, which now begin to undergo ligno-suberization. The cells which first become lignified are usually situated near the epidermis of the basal part of the Protective-layer, and the process spreads rapidly in the cells of the outer part of the leaf-base and to some extent in the cells of the inner portion. In the same way the cell-walls acquire an inner film of suberin, but though the double process is very apparent in ordinary cases before leaf-fall, it is not until long after that the process is complete, the final stage being reached when the whole of the cells at this level become ligno-suberized and lose their living content

In consequence of the conical shape of the leaf-base, and the added strength given by the regular deposition of the numerous leaf-trace bundles (each of which has its own special patch of sclerenchyma), one might expect to find the provision for separation rather exaggerated. As a matter of fact the Separation-layer in the numerous cases examined was found to be a very transitory affair, consisting of cells in which apparently no preparations had

been made. It is separated from the Protective-layer by one or two rows of cells, and although its protoplasm is rather conspicuous, little or no starch is present, and except in rare cases no division walls appear. The Separation-layer first becomes well marked near the dorsal surface ; its walls begin to swell, the middle lamellae become mucilaginous and finally disappear, and the leaf is thrown off.

During the first winter the cells beneath the Protective-layer become active and give rise to a cork cambium which is continuous with that of the stem periderm. The layer of cork produced before the second season is usually not very thick, and is not continuous across the vascular bundles. Towards the end of the second season a new phellogen arises below the first one, and dipping down towards the cortex crosses the vascular bundles and is only interrupted by the thick patch of sclerenchyma outside each leaf-trace.



TEXT-FIG. 15. *Rhus typhina.* Longitudinal section of leaf-base at leaf-fall. *res.* = resin duct ; *res.*¹ and *res.*² = limits of ' tyloses ' in resin duct.

RHUS TYPHINA, Linn.

This species is rather interesting in the fact that in its leaf-fall processes it appears to combine the characters of *R. cotinis* and *R. coriaria* as described by Tison. The whole plant is very hairy. The stout petiole has a swollen base which encloses the large axillary bud. The distribution of starch and calcium oxalate crystals is as usual, and periderm is usually present before leaf-fall. Numerous resin canals surround the vascular ring

in the stem, and each is protected by a layer of sclerenchyma. At least one canal (Text-fig. 15, *res.*) accompanies each of the three bundles which pass into the leaf, and the sclerenchyma (*scl.*) surrounding it does not diminish to any great extent at the leaf-base.

The Separation-layer (Text-fig. 15, *S.L.* and Pl. V, Fig. 11) is formed quite early by division of a layer of cells situated at some distance above the upper extremity of the stem periderm. The protoplasm first becomes conspicuous and starch granules are formed; numerous regular walls appear, usually 2–5 in each cell, and the fully-formed Separation-layer, which often has a thickness of 2–3 cells, may diminish to one cell at the ventral surface of the petiole.

A conspicuous Lignified-layer (*Lig.*) is formed by the lignification of the cells immediately above the Separation-layer. The Protective-layer (Text-fig. 15 and Pl. V, Figs. 11 and 12, *L.P.*) varies greatly in relative time of formation, sometimes appearing with the Separation-layer, at other times commencing to form only after the latter is clearly marked out. Ligno-suberization begins in the upper part near the Separation-layer and vascular bundles and spreads downwards; it is preceded by the irregular division of the cells. The living cells of the vascular bundle at this level may also become ligno-suberized before the leaf finally separates from the stem. The crystal cells included in the Protective-layer undergo lignification, but in other cells the suberization is most marked.

The formation of tyloses in this species is worthy of note. With the appearance of the Separation-layer the production of tyloses in the vessels commences, and the lumen of each of the latter is later completely obliterated (Pl. V, Fig. 12, v^1). The resin canals accompanying the vascular bundles behave in this respect very much as vessels, for although surrounded by thick-walled cells an enormous production of tyloses commences about the time of formation of the Protective-layer; and for a little distance both above and below the latter these structures completely fill the cavity of the resin canals (Pl. V, Figs. 11, 13, 14). Later these ingrowths undergo the changes of any part of the leaf-base in which they are situated, and in this way help to make more efficient the various modifications connected with leaf-fall.

A very curious phenomenon noticed in connexion with this species is the evident individuality of each leaf. In any twig the leaves in which the leaf-fall processes are most advanced are not, as in most cases, the lowest, but in several instances where two adjacent leaves were examined, the one nearer the apex might be on the point of falling, whilst in the other not a trace of the formation of the Separation-layer would be apparent. Quite analogous with this is the difference in degree of completeness of the leaf-fall processes at the time of leaf-fall, for while in some the elements of

the Protective-layer are completely ligno-suberized at this time, in others the process is not completed until long after the leaf has fallen.

Separation occurs in the usual way. The primary walls of the Separation-layer become slightly swollen, the middle lamellae become mucilaginous and disappear, leaving the neighbouring cells quite free.

During the first winter divisions in the cells beneath the Protective-layer result in the formation of a cambium which gives rise to a continuous layer of cork. Later another cambium arises beneath the first one and traversing the vascular bundle adds greatly to the thickness of the layer of cork.

Broussonetia papyrifera, Linn.

Ficus Carica, Linn.

These two plants are being taken together because in their leaf-fall phenomena, as well as in their general affinities, they are very similar. What slight differences there are will be pointed out in the course of the description.

Starch granules and compound crystals of calcium oxalate have the usual distribution, while laticiferous tissue is abundant in both cortex and petiole. Periderm is present in the stem of *Broussonetia* previous to any change taking place in the leaf-base, though it is entirely absent from the stem of *Ficus* for some time after defoliation. Sclerenchyma is absent from stem and petiole in both species.

The usual massing of the protoplasm in the cells of the leaf-base—which are also distinguished by the possession of starch granules and large nuclei—is followed by the production of new walls in the cells of this active area; and although the first walls appear in the cells near the dorsal surface, the process spreads so rapidly that soon there is a complete layer of dividing cells, in each of which 1–4 new walls are produced. As these cells complete their divisions, their activity is transferred to the adjacent cells on the petiolar side, and in these the process of division is continued. The dividing cells always possess abundant protoplasm and starch granules; and the sequence of events is continued until a layer 4–8 cells in thickness have undergone division. The last layer to divide retains its protoplasm, and become the Separation-layer, this statement being especially true of *Broussonetia*; in *Ficus* the Separation-layer may or may not have undergone division. All the new walls are approximately parallel with each other and roughly at right angles to the axis of the petiole.

Almost as soon as the first layer has completed its divisions, ligno-suberization commences and spreads in the direction of the Separation-layer. It is, however, very feeble, and its lower limit indefinite, and the chemical change accomplished before leaf-fall is very variable and usually not very

striking. The crystal cells do not undergo division, and only lignin can be detected in their walls.

The development of tyloses is the important factor in the closure of the vessels at the level of the Protective-layer. At a very early stage they appear in the vessels of the primary, and sometimes in the secondary wood, and later are accompanied by a varying amount of gummy lignin.

As soon as the Separation-layer has become well marked the adjacent petiolar cells, some of which have divided, become highly lignified, and finally lose most of their contents and exhibit great rigidity.

Separation takes place by the disappearance of the middle lamellae of the outer cells of the Separation-layer. In late autumn, after the leaf has fallen, the cortical cells adjacent to the Protective-layer become active and begin to undergo regular division, the result of which, however, is not manifest until the following year, when by their activity a thick layer of cork is added to the Protective-layer. The phellogens of the stem and Protective-layer soon merge into each other, and the layers of cork subsequently produced are identical in form and reactions.

For closure of laticiferous tubes see description of *Morus* and Pl. VI, Fig. 17.

Morus alba, Linn., and M. nigra, Linn.

When mature the stout, cylindrical petiole is distinguished from the brown stem by its green colour. Internally, there is little difference between the cells of the stem and those of the petiole, and while starch is present only in the former, compound crystals and much laticiferous tissues are found throughout. None of the 'stone' cells which accompany the vascular tissue in the stem are found to extend into the petiole. A superficial periderm is present in the stem before leaf-fall.

The massing of the protoplasm in the cells of the leaf-base is followed by the appearance of numerous (2–8 in each cell) new walls in the latter, running in a direction approximately at right angles to the long axis of the petiole (Pl. VI, Fig. 15, *L.P.*). Although the mother-cells increase in length they still retain their distinctive outline; and while it may be said that the whole of the cells of this layer usually divide almost simultaneously it must be noted that there is often a distinct tendency for one row to divide at a time, the activity then passing to the next row of cells on the petiolar side. The final result is that a thick layer of divided cells is produced in which the crystal cells and epidermal cells alone have not undergone division.

As soon as the process of division is well advanced, ligno-suberization commences near the upper limit of the Protective-layer, and spreading rapidly in all directions may even extend to the undivided cells of the cortex. The whole of the Protective-layer becomes uniformly suberized, but the lignification is more marked in the cells near the upper limit. The

last signs of the conspicuous nuclei disappear with the completion, before leaf-fall, of these chemical changes.

Numerous fairly regular divisions which occur in the cells of the petiole adjacent to the Protective-layer result in the formation of a well-marked Separation-layer (*S. L.*), in which starch is present in considerable quantity. After several divisions have occurred, the primary walls begin to swell, at the same time taking the haematoxylin and ruthenium-red more strongly. Later the middle lamellae between the outer cells become mucilaginous and disappear, and with the subsequent rupture of the vascular tissue the leaf falls to the ground. A Lignified-layer (*Lig.*) is also present.

Tyloses and gummy lignin appear very early and become very abundant in the vessels of the primary and secondary wood at the level of the Protective-layer. Tison has already described the manner in which the laticiferous elements behave near the region of separation. Latex tubes in these plants are very numerous, and as those of the cortex communicate with their fellows in the petiole, it follows that a considerable number must traverse the Protective-layer. Those parts which are enclosed within the latter do not become divided as do the ordinary cells of the Protective-layer; their walls, however, become lignified and probably suberized, though the latter process is not at all definite. When separation is taking place there can often be observed a direct squeezing out of the latex tube in the region of the Separation-layer, the process finally culminating in the complete rupture of the tube at this point (Pl. VI, Fig. 15).

But the most interesting point in connexion with the behaviour of the laticiferous tubes appears to relate to the phenomenon mentioned by Parkin[1] for other laticiferous plants, i. e. *Hevea brasiliensis* and *Plumiera acutifolia*. This author found that if a mature leaf is cut off half-way up the petiole, latex exudes copiously from both cut surfaces; nearer the leaf-base the amputation results in copious exudation of latex from one surface only, that of the petiole, while if the leaf is cut off flush with the surface of the stem, the flow of latex is from the stem only. In the species examined by Parkin the only reason he could discover for such behaviour consisted in the appearance near the leaf-base of a number of crystal cells which seemed to cut off the latex tubes. So far as Parkin could discover there was no formation of walls within the tubes themselves.

Now in *M. alba* and *nigra*, as well as in *Ficus Carica* and *Broussonetia papyrifera*, just before leaf-fall definite walls are formed in the laticiferous tubes near the leaf-base, though so far it has been impossible to work out the details of their formation. If longitudinal sections are taken of the bases of leaves which have a fully-formed Protective-layer and the latex is dissolved out by any of the ordinary methods, distinct walls will be found traversing the tubes at a varying distance on either side of the Protective-

[1] Annals of Botany, vol. xiv, 1900, p. 205.

layer (Pl. V and VI, Figs. 15, 16, 17). These walls are invariably very definite; they give the usual cellulose reactions, and are often curved so that the convex side is towards the Protective-layer. This arrangement, although not invariable, is noteworthy, for it often appears to be connected with the presence of abundant latex on the concave side of the walls and with the degeneration (and therefore lower pressure (?)) of the substance enclosed by the walls.

With regard to the formation of these walls nothing very definite has been observed. As Tison has said, they probably arise very rapidly, and the first stages are therefore difficult to obtain. The same author holds that the coagulation of the latex near the Protective-layer often provides an obstruction, and may possibly aid in the formation of the transverse wall. In the present case a coagulated mass of latex, such as Tison describes as being present on the convex side of the transverse wall, has never been found, though masses of various size have been observed dispersed throughout the tubes; and while it is highly probable that there is some relation between the formation of the transverse wall and the nuclei within the tube in that region, it has as yet been impossible to establish any such connexion.

The condition of the Protective-layer at the time of leaf-fall has already been described. The amputation of the leaf is followed by the extension into the leaf-trace of the process of ligno-suberization and by the formation beneath the Protective-layer of a cambium, which gives rise to a few regular rows of cork cells during the first part of the winter. Without traversing the vascular bundle, this cambium soon becomes continuous with the phellogen of the stem; it is not very vigorous during the first year after leaf-fall, rarely more than 4–6 rows of cells being produced. In the second year a new cork cambium arises just beneath the old one, and in turn gives rise to a layer of cork which completely traverses the vascular bundle.

Juglans nigra, Linn.

The course of events is very much as in *Morus* and other examples of this type. The usual reduction occurs in the vascular and strengthening tissue at the leaf-base. A superficial periderm is present in the stem and often extends a little way up the petiole. Aggregation of the protoplasm in the cells of the leaf-base is followed by irregular division, the new walls appearing, 1–3 in each cell. A fairly thick layer is thus produced which has a slightly curved course. Ligno-suberization takes place as usual.

The Separation-layer is formed by the more regular division of a layer of 2–3 rows of cells adjacent to the Protective-layer. Separation follows as in other examples. A slight Lignified-layer is also present.

Protective-periderm does not arise until about the middle of the first

winter. The cork is at first small in amount, but the activity of the cambium in the second year produces a thick layer of periderm.

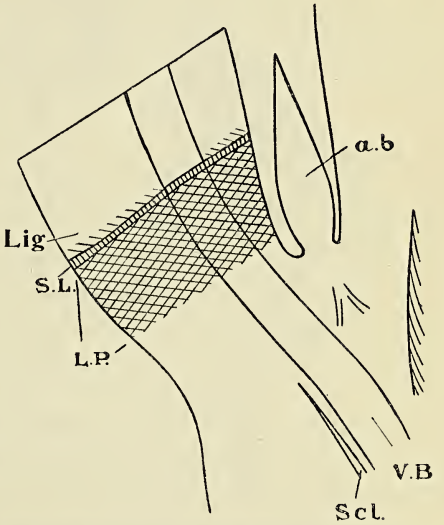
Tyloses are usually very numerous and gummy lignin abundant.

J. REGIA, Linn, and J. RUPESTRIS, Engelm.

So far as these species have been examined, the sequence of events leading to and resulting from defoliation is very similar to what has been briefly described for *J. nigra.*

CORNUS SANGUINEA, Linn.

There is nothing remarkable in the external morphology of the leaf-base and adjacent parts in this well-known species. Each leaf—the junction of which with the stem is marked externally by a slight furrow—is supplied with three vascular bundles, which from the base of the petiole upwards are entirely free from sclerenchyma. Starch granules and compound crystals of calcium oxalate are present in the stem until very late in the first year.



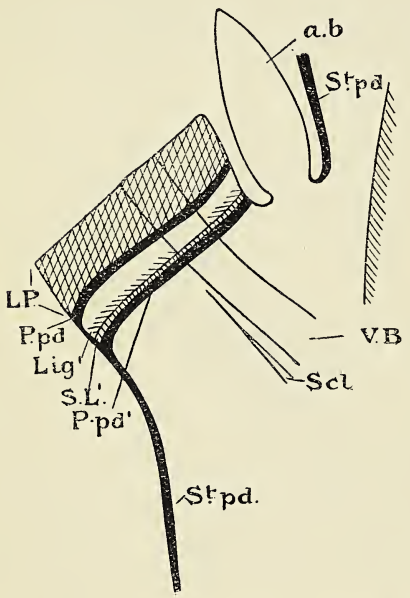
TEXT-FIG. 16. *Cornus sanguinea.* Longitudinal section of leaf-base at time of leaf-fall.

Some time previous to leaf-fall, a large area of cells (*L.P.*, Text-fig. 16) extending an appreciable distance up the petiole become distinguished by their greatly increased living and starchy contents, and begin to divide by walls which appear usually singly in each cell. The new walls, which are orientated in all directions, thicken slightly, and the whole area begins to undergo lignification. The latter process is usually very diffuse, and appears first in the upper part of the Protective-layer, from whence it spreads rapidly downwards until the whole of the cell-walls become more or less completely lignified. At the same time there is also a slight degree of suberization, which finally results in the production of an inner film of suberin on each cell-wall. The whole process is not complete until after leaf-fall, varying in this respect to a remarkable degree in the different examples, the progress made in each case being marked by the amount of protoplasm remaining in the cells.

Divisions which lead to the formation of the Separation-layer take place at any early stage—almost as soon as any marked change has occurred in the cells destined to form the Protective-layer—and affect 2–3 rows

of cells adjacent to the upper margin of the latter (*S. L.*). These cells are at once distinguished by the presence of dense protoplasm and abundant starch ; the new walls, numbering 2–5 in each cell, always remain thin, and run in a direction roughly at right angles to the length of the petiole. The mother-cells begin to swell, and though retaining for a long time their cellulosic character, the middle lamellae of the outer cells disappear, leaving the cells on either side perfectly free.

The continuity of the Separation-layer across the leaf-trace bundle is well shown in this example, though other changes which occur in the vascular elements are not quite so marked as usual. Tyloses are far from numerous in the vessels at the level of the Protective-layer, and gummy lignin, though present, is never abundant. Even after leaf-fall the vascular elements do not become completely closured by these means, but this is accomplished later by the great crushing which takes place when the cells near the surface begin to dry up. A slight Lignified-layer is invariably present above the Separation-layer.



TEXT-FIG. 17. *Cornus sanguinea.* Longitudinal section of leaf-scar in second spring.

It is not until after the leaf has fallen that there is any formation of periderm cork beneath the Protective-layer, and even then it is very variable. During the first winter divisions which take place beneath the Protective-layer lead to the formation of a cambium, by the activity of which a varying thickness of cork is produced before the second season (Text-fig. 17, *P. pd.*).

In the following spring a curious variation from the ordinary course is seen to occur. Most observers will have noticed that in *Cornus* sp. and other examples, the resumption of growth of the axillary bud is the signal for the casting off of the adjacent scar tissue ; and it is not very difficult to ascertain the mechanism by means of which this is effected. About this time the cells of a layer situated at some distance below the Protective-layer become active and give rise to a new Separation-layer in all respects exactly similar to the first. Its action is precisely the same, and results, as was remarked above, in the cutting off of the scar tissue in early spring (Text-fig. 17, *S. L.*). Above the secondary Separation-layer is a well-

marked Lignified-layer, while below it a new periderm is afterwards produced, of which a small proportion is phelloderm (*Lig.* and *P. pd.*[1]).

Cornus Mas, Linn.

In all the essential stages *C. Mas* is very similar to *C. sanguinea*. It was remarked in the description of the latter species that much variation exists in the degree of completeness attained by the ligno-suberization of the Protective-layer before leaf-fall. This is carried to its extreme in *C. Mas*, where at the time mentioned no ligno-suberization has occurred. The later modifications and ultimate result are the same as in *C. sanguinea*.

Nyssa sylvatica, Marsh.

Leaf-fall in this species is very similar to that of *Cornus sanguinea*, and calls for no special description. A small difference which may be noted is the complete absence of the Lignified-layer, a slight formation of which was remarked in *C. sanguinea*.

Ailanthus glandulosa, Desf.

The petiole of *A. glandulosa* is very stout and cylindrical, and its swollen base is delimited from the stem by a well-marked furrow; while in a slight hollow on the upper face rests the small axillary bud. Internally the cells of the petiole are distinguished from those of the cortex by the absence from the former of granules of starch, numerous crystal clusters of calcium oxalate occurring in both tissues.

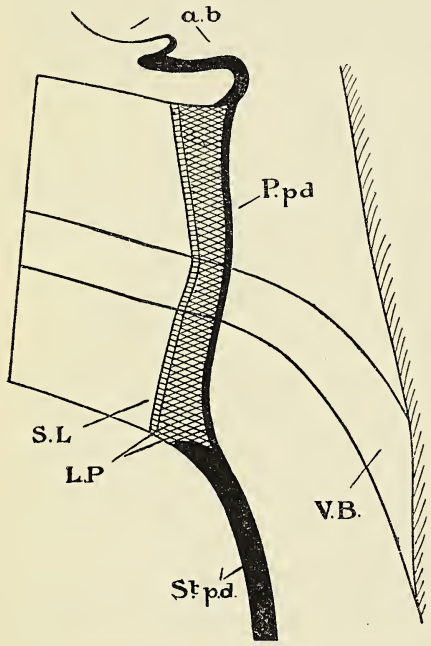
A continuous ring of sclerenchyma surrounds the vascular cylinder in the stem. Five bundles leave the ring to supply each leaf, and as they pass out at different levels the sclerenchyma accompanying them decreases and finally disappears just below the junction of petiole and cortex. In the leaf-base the bundles divide up and later form a continuous ring (with internal groups), which traverses the petiole and which is supplied with an increasing quantity of sclerenchyma situated outside the bast.

In the stem a periderm which arises in the outer layer of the cortex is invariably present before leaf-fall, and in many cases extends well up the petiole.

The Protective-layer is formed by the irregular division of the cells at the base of the petiole, and, compared with the size of the latter, is relatively thin. It appears very early, and curves slightly towards the cortex. After each cell has divided 1–3 times the cell-walls become ligno-suberized, and finally lose their contents. The process of ligno-suberization and its results are invariably complete before leaf-fall, the living elements of the leaf-trace being the last to undergo change. Numerous crystal cells are enclosed within the Protective-layer, and without undergoing division they become

highly lignified. Quite early in the formation of the Protective-layer tyloses appear in the vessels at that level, and in such numbers as to completely close the latter.

The Separation-layer, which appears just before leaf-fall, is formed by the division of 2–3 rows of conspicuous cells situated just above the Protective-layer. These cells possess the usual characteristics and proceed to divide by 1–2 walls. The outer cells increase considerably in length, their walls become quite mucilaginous, and finally the leaf is freed by the solution of the middle lamellae and the rupture of the vascular bundles.



TEXT-FIG. 18. *Celastrus articulatus.* Longitudinal section of leaf-base just previous to separation.

Some time previous to defoliation, the cells below the Protective-layer divide up and form a cambium, which produces 8–10 layers of cork in the first year. Almost immediately it traverses the vascular bundle, and the elements above it soon undergo their final change. In the second year a new periderm is produced below the first one, and this, after traversing the vascular bundle, curves gradually to join the stem periderm, enclosing between it and the first Protective-periderm a small area of unaltered cells.

In the scar tissue the cells of the Separation-layer which remain when the leaf has been removed are the last to lose their contents, and for a time they form a conspicuous layer of unaltered cells. No lignified zone is present above the Separation-layer.

CELASTRUS ARTICULATUS, Thunb.

In the stem the ring of bundles is supplied with an internal layer of sclerenchyma, none being present outside the vascular cylinder. A single vascular bundle leaves the ring and enters the petiole, and during its whole course sclerenchyma is entirely absent. In the cortex, the cells of which are generally smaller than those of the petiole, starch granules are invariably present, while crystals of calcium oxalate are only rarely found throughout the plant.

The formation of the Protective-layer (Text-fig. 18, *L.P.*) takes place in the usual manner of this type. There is a slight increase in the proto-

plasmic and starchy contents of the cells of the leaf-base, followed by the production of numerous irregular walls. Ligno-suberization soon follows, and gradually a layer 6–12 cells in thickness becomes completely metamorphosed, and forms a well-defined Protective-layer, from which protoplasmic and other contents are entirely absent (Pl. VI, Fig. 18, *L.P.*).

More regular divisions which now take place on the petiolar side of the Protective-layer lead to the formation of a well-defined Separation-layer (*S. L.*). Numerous new walls appear in 2–4 rows of cells; the primary walls become mucilaginous, the middle lamellae disappear, and the rupture of the vascular bundle is followed by the fall of the leaf.

Tyloses are introduced into the vessels near the leaf-base at a very early stage, and later there is a copious production of gummy lignin. Before leaf-fall a cambium (*ca.*) is formed by division of the cells beneath the Protective-layer, and soon becomes continuous across the vascular bundle with the phellogen of the stem. In the second year a new cambium arises, and by its activity produces a thick layer of cork.

Phellodendron amurense, Rupr.

The petiole on the whole is slender and cylindrical, but possesses a swollen base which completely encloses the axillary bud. A brown furrow marks the junction of the petiole with the stem. Numerous cells containing clusters of crystals of calcium oxalate, as well as large cavities containing mucilage, are present throughout the cortex and petiole, while starch granules are abundant only in the cortex. In the stem the pericycle possesses scattered patches of sclerenchyma, some of which accompany the three vascular bundles for a short distance before the latter enter the leaf. At the leaf-base, the sclerenchyma having been entirely lost, the bundles form a semicircle of vascular tissue which traverses the petiole and supplies the lamina. Isolated patches of sclerenchyma occur in both cortex and petiole, and a superficial periderm is present in the stem before leaf-fall. Throughout the process tyloses are few in number near the Protective-layer, but the vessels become filled at a later stage with quantities of gummy lignin.

Ligno-suberization takes place in a layer of cells which runs across in a slanting direction from the upper extremity of the stem periderm to the junction of the petiole with the axillary bud. The upper portion of the Protective-layer, having already undergone division by irregular walls, first become ligno-suberized while the lower cells are still dividing, the new walls in the latter being more numerous than in the upper cells. As the divisions are completed, ligno-suberization sets in until finally the whole layer, including the living cells of the vascular bundle, is changed in character. So far as can be seen, the walls surrounding the mucilage cavities and those of the crystal cells undergo complete lignification.

Before the Protective-layer is completely formed, the cells of a layer above, which is separated from the Protective-layer by 1–3 cells, become active, and display abundant protoplasm and numerous starch granules. Numerous division walls appear in this Separation-layer, and the cells increase greatly in size; next their walls begin to swell and become mucilaginous, and finally the complete solution of the middle lamellae paves the way for the full separation of the leaf.

There is no Lignified-layer in this species, but a 'parenchyme sacrifié', consisting of part of the Separation-layer in addition to the cells that intervene between the latter and the Protective-layer, is invariably present. The cells gradually lose their contents, and their walls slowly undergo ligno-suberization.

It is not until late winter that a cork cambium is formed beneath the Protective-layer, and even then it does not become very active, only 1–2 layers resulting previous to the second season. Later, however, a thick layer of cork cells is produced.

Fraxinus excelsior, Linn.

The stout, roughly cylindrical petiole is delimited from the stem by a well-marked furrow, and encloses within its axil a conspicuous spherical bud. The lignified stereome which accompanies the leaf-trace bundles becomes greatly reduced at the leaf-base, and appearances point to the conclusion that there is also a reduction in the vascular elements in that region. A conspicuous periderm is present in the stem previous to the commencement of the leaf-fall processes.

Tyloses and gummy lignin appear in the vessels near the leaf-base at a very early stage, the latter being deposited in considerable quantity. In a layer eight or more cells in thickness situated near the leaf-base, the protoplasm increases in density, and new walls (1–2) appear in each cell. Ligno-suberization, which immediately follows, commences near the epidermis or the vascular bundles, and quickly spreads in all directions, a measure of its advance being gained by noting the diminishing density of the protoplasm, which finally disappears when the chemical change is complete.

Divisions (1–4 in each cell) in the cells of the petiole above the Protective-layer result in the formation of the Separation-layer, which is early distinguished in the usual way, i. e. presence of abundant protoplasm and starch. The swelling of the primary cell-wall and the mucilaginization and disappearance of the middle lamellae are well shown in this example, which altogether furnishes a very easy and diagrammatic type.

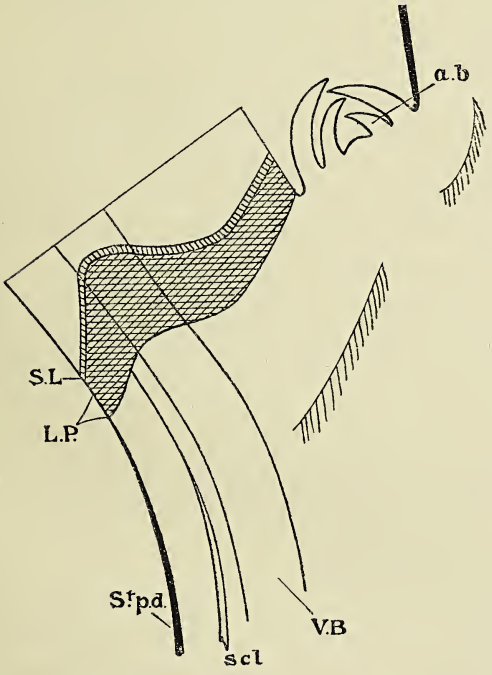
After leaf-fall the vascular elements, which have only partly become ligno-suberized, proceed rapidly to complete that process, which is no doubt

aided by the diffusion from the broken vessels of the abundant gummy lignin which often covers the exposed surface.

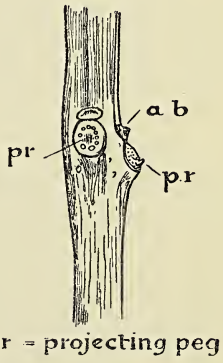
Towards the end of the second season the cells adjacent to the Protective-layer divide up to form a cork cambium, which, becoming continuous with the phellogen of the stem, produces a conspicuous layer of regular cork cells towards the scar, as well as a small amount of phellogen towards the cortex.

Catalpa Kaempferi, Sieb.

The whole sequence of events in this species is quite similar to what has already been described by Tison of *C. bignonioides*. The junction of the stout cylindrical petiole with the stem is marked externally by a dark brown ring, while internally, considerable difference in size exists between the



Text-fig. 19. *Catalpa Kaempferi.* Longitudinal section of leaf-base just before leaf-fall.



Text-fig. 20. *Catalpa Kaempferi.* Portion of stem showing leaf-scars with projecting-peg (*pr.*).

parenchymatous cells of these organs. Starch grains are present in the cortex only, but simple (octahedra) and compound crystals of calcium oxalate are abundant throughout. A single group of bundles supplies the leaf, the stereome accompanying which disappears completely at the leaf-base. A superficial periderm is present in the stem before leaf-fall.

Aggregation of protoplasm in the cells of the leaf-base, division by walls formed singly in each cell, and lignification followed by suberization

(from a number of different points) occur in quick succession. The Protective-layer (Text-fig. 19, *L. P.*) has a peculiar shape, curving upwards from all sides to meet the dorsal leaf-trace, and thus forming a prominent peg which projects above the general surface of the scar (Text-fig. 20, *pr.*). About this time tyloses and gummy lignin become apparent in the vessels of the vascular bundle near the leaf-base.

The Separation-layer (*S. L.*), produced by slight division of the cells adjacent to the Protective-layer, possesses the usual characteristics, and separates in the ordinary way by disappearance of the middle lamellae of the outer cells. No Lignified-layer could be detected, but the remains of the Separation-layer form a slight 'parenchyme sacrifié'.

It is usually not until the second season that divisions in the cells beneath the Protective-layer give rise to a cork cambium. In any case the layer of cork produced by the end of that season is never very extensive.

Liriodendron tulipifera, Linn.

Here the sequence of events is quite similar to that described for *Catalpa*, the only difference being the time of differentiation of the Protective-layer. Numerous leaf-bases were examined, and it was found that in many little or no ligno-suberization had occurred previously to leaf-fall, all transitions being obtained at leaf-fall from the unchanged leaf-base to the one in which a completely ligno-suberized Protective-layer was present.

In addition to this variation the presence of numerous mucilage cavities in the ground tissue must be noted. When these occur in the Protective-layer they undergo no division, though their walls usually become lignified.

Magnolia sp.

An unknown species of *Magnolia* was examined, and, so far as the observations went, was found to agree very closely with *Liriodendron*.

General Discussion.

Having now described the various species on which observations have been made, and arranged them under various types, it remains to examine the available evidence to see whether the scheme of classification advanced is vindicated, and at the same time, if possible, to draw one or two conclusions. The observations here recorded (on species examined by both of us) agree in the main with those of Tison, the variations—usually in time—probably being due to differences of climate and season. The essential modification in connexion with leaf-fall is the occurrence of the Separation-layer, which, therefore, was the first to appear in the course of evolution; other modifications connected with the improvement of the mechanism of the Separation-layer (i. e. the Lignified-layer) and with the protection of the underlying tissues (i. e. the Protective-layer) appeared later, the former in connexion

with the formation of the Separation-layer, the latter after separation had been effected. In many species the relative times of appearance of the Separation-layer and the Protective-layer are reversed, and in these the provision for protection of the exposed tissue is complete before the leaf is cast off. The form of the protective device, so far as the species at present examined are concerned, appears to have followed three distinct lines:—

1. Ligno-suberization without further modification of existing cells.
2. Ligno-suberization of cells after irregular division has occurred.
3. Ligno-suberization of cells produced by the activity of a regular cambium.

So far as the present research goes, the characters here enumerated, and these alone, are invariable; and it is on these that the classification of leaf-fall phenomena has been founded.

The system proposed by Tison is very elaborate, and is based on (1) the mode of formation of the Separation-layer; (2) the mode of formation of the Protective-layer; (3) the degree of ligno-suberization which has been effected at leaf-fall; (4) the amount of cork produced by the cork cambium beneath the scar at the time of defoliation; (5) the origin of the cork cambium of the stem; (6) the exfoliation of scars on dead leaves. Were all the characters just enumerated absolutely invariable, it would still be impossible to form from them classes of co-ordinate value; they are far too dissimilar to be comparable. Again, over and over again in the above descriptions, it has been pointed out how very variable are most of the characters concerned, and one or two variations taken from Tison's paper will be briefly mentioned here. Thus in his second class, which is distinguished by the possession of a Separation-layer formed without division of cells, and by the absence of ligno-suberization at leaf-fall, Tison places *Crataegus monogyna*, in which *the cells of the Separation-layer sometimes present division walls*, and *Euonymus europaeus*, in which *ligno-suberization may occur before leaf-fall.* Similarly in Class IV, which is distinguished by the irregular division of the Protective-layer, absence of ligno-suberization, &c., are placed *Ficus Carica* and *Euonymus latifolius*, in both of which *some ligno-suberization may occur before defoliation.* Other examples might also be given to show that some at least of the characters relied on by Tison are of very doubtful value. In addition the results of Löwi's experiments on the effect of different conditions on the Separation-layer show us that the form and extent of the latter depend largely on external conditions; and any one, by repeating von Mohl's experiment in which he placed a healthy branch in a damp chamber, may prove that the formation of the Separation-layer can be induced without the production of a ligno-suberized layer even in species in which, under ordinary conditions, ligno-suberization is complete before leaf-fall. The form of the Separation-layer, the time of ligno-suberization, and the amount of cork produced by

the cork cambium before leaf-fall are all highly variable, and cannot be relied upon to form the basis of any type.

The species which Tison has classified under Type 10 appear to be more appropriately referrable to other classes, from which indeed they seem to differ only in the deep-seated nature of the periderm; and the species comprised in his last class (11), while they may differ in other ways, agree in that the scar (or dead leaf) is usually thrown off some time during the second year. In the present work the last-named peculiarity has not been regarded as of sufficient weight to justify the erection of a type. This is because the morphological distinction is not regarded as equal in value to the other characters selected, and also because the class as at present constituted affords an easy transition to the ordinary type in which the leaf-scar is exfoliated by formation of periderm. If the class were limited to those cases in which the dead leaves remain on the tree during the winter, the case for a separate class would be much stronger. But it is not so limited, and therefore the conclusion is arrived at that it is better to trust to the morphological distinctions which depend on the cells forming the Protective-layer than to rely on the *time* of exfoliation of the scar.

A consideration of the facts, and especially of the experiments and observations already alluded to, which tend to show that the actual stage which the leaf-fall processes may have reached at the time of defoliation is largely influenced by external conditions, leads to the conclusion that any scheme for the classification of leaf-fall phenomena must be based, not on the features presented at any given time, but rather on the structures which are the ultimate result of leaf-fall. On these lines the simplest and perhaps the most logical scheme appears to be the one now advanced, in which the first class consists of species in which the Protective-layer is formed by the ligno-suberization of cells which have not previously undergone division; the second, of species in which division precedes ligno-suberization; while the third class comprises species in which the Protective-layer is produced by the activity of a regular cambium, and is therefore quite secondary in origin.

In the first two classes there is a gradual transition from species in which the Protective-layer is entirely unchanged at the time of leaf-fall to others in which ligno-suberization is complete at that time; while in the third class, of which only two examples have been described, the Protective-layer is fully formed at the time of defoliation.

The fall of leaflets in compound leaves has not yet been extensively studied, but from various observations which have been made the rule may be laid down that the leaf-fall structures in leaflets are of the same type as those which occur at the base of the parent leaf, but that they are usually much simpler. A Separation-layer is invariably present, a Lignified-layer may be produced, and the Protective-layer may or may not be well marked, but ligno-suberization is seldom well advanced at leaf-fall.

A phenomenon which has been touched on briefly in the separate descriptions is the retention through the winter of dead leaves which are cast off in the following spring. Examples of species which do this are far from rare. In some cases, e. g. species of *Quercus*, *Carpinus Betulus*, &c., only a certain proportion of the leaves are retained through the winter, while in others, such as coppiced beech, the whole of the leaves remain on the tree until the following spring.

Although it is impossible to resist the conclusion that this phenomenon depends in some way on the decreased vitality of the plant, no adequate explanation can at present be suggested, and a full discussion of the phenomenon will be left for a future paper.

It was at first thought that it might be found possible to show some connexion between the degree of evolution of the plant and the type of leaf-fall structure; but no such relation has been traced. A cursory examination of the list of species described shows that the individual classes (especially I and II) comprise plants derived indiscriminately from all the families of Dicotyledons. One advantage of the system of classification here proposed is that all species of the same genus so far examined fall naturally into the same class, and in this respect it appears to present some advantage over the more elaborate system proposed by Tison, where different species of the same genus may have to be referred to quite separate groups.

Summary.

In Dicotyledons the essential modification at the leaf-base in connexion with leaf-fall is the formation of a *Separation-layer* which is produced from existing cells with or without division.

The leaf separates from the stem by the disappearance of the middle lamellae of the cells of the Separation-layer and the subsequent rupture of the sieve tubes and vessels of the leaf-trace at that level.

A *Lignified-layer* may or may not be present, but a *Protective-layer* is invariably produced either before or after leaf-fall.

The mode of formation of the Protective-layer is (1) by ligno-suberization of the cells of the leaf-base with or without irregular division; (2) by ligno-suberization of cells produced by the continued division of a regular cambium.

The following species have been examined:—

Class I.

(*a*) Castanea sativa, *Mill.*
Hibiscus syriacus, *Linn.*
Quercus palustris, *Muench.*
Ligustrum vulgare, *Linn.*
Celtis occidentalis, *Linn.*
Pyrus floribunda, *Nichols.*
Ceanothus Gloire-de-Versailles (Garden origin).

Acer pseudo-platanus, *Linn.*
(*b*) Ribes sanguineum, *Pursh.*
Ribes nigrum, *Linn.*
Diospyrus virginiana, *Linn.*
Halesia tetraptera, *Linn.*
Forsythia Fortunei, *Hort.*
(= F. suspensa, *Vahl*).
Baccharis halimifolia, *Linn.*
Gleditschia triacanthos, *Linn.*
Robinia Pseudacacia, *Linn.*
Clerodendron trichotomum, *Thunb.*

Class II.

(*a*) Tilia europaea, *Linn.*
Corylus Colurna, *Linn.*
Cercis Siliquastrum, *Linn.*
Carpinus Betulus, *Linn.*
Prunus Cerasus, *Linn.*
„ virginiana, *Linn.*
„ communis-dulcis.
(*b*) Betula verrucosa, *Ehr.*
Platanus orientalis, *Linn.*
Rhus typhina, *Linn.*
Broussonetia papyrifera, *Vent.*
Ficus Carica, *Linn.*
Morus alba, *Linn.*
Morus nigra, *Linn.*
Juglans nigra, *Linn.*
„ regia, *Linn.*
„ rupestris, *Engelm.*
Cornus sanguinea, *Linn.*
„ Mas, *Linn.*
Nyssa sylvatica, *Marsh.*
Ailanthus glandulosa, *Desf.*
Celastrus articulatus, *Thunb.*
Phellodendron amurense, *Rupr.*
Fraxinus excelsior, *Linn.*
Catalpa Kaempferi, *Sieb.*
Liriodendron tulipifera, *Linn.*

Class III.

Salix Caprea, *Linn.*
Populus balsamifera, *Linn.*

The protection of the tissues of the stem underlying the scar is aided by the production at a later date of a layer of cork cells which subsequently becomes continuous with the periderm of the stem.

In many species the persistent leaf or leaf-scar is thrown off during the second year.

The above investigation was commenced at the suggestion of Professor J. B. Farmer, F.R.S., to whom the author is sincerely grateful for constant advice and encouragement during the progress of the research.

Appendix.

It has been thought better to give a separate description of the methods employed in this research. The material was collected in July, August, September, October, and November, chiefly in the year 1909, and was preserved in 70 per cent. alcohol. Both longitudinal and transverse sections of the leaf-base were taken, chiefly by hand but also with the microtome, and these were stained with several of the common double stains. For the

detection of mucilage the ordinary methods were adopted, and for cutin the usual tests were confirmed by treatment with a fresh solution of chlorophyll, when a bright green colour was obtained.

To demonstrate the production of lignin and suberin in the Protective-layer and elsewhere, separate tests were employed, i.e. for lignin, phloroglucin, aniline sulphate, &c.; while for suberin, tincture of alkanna and Schultze's macerating fluid were most successful. In favourably stained preparations it is easy to demonstrate the localization of the suberin as an inner film on the lignified middle lamella, but this is better shown by employing the separate tests on consecutive sections. Two other methods are (1) to dissolve away the lignin base by boiling in eau de Javelle, and then to apply the suberin tests; and (2) to dissolve the film of suberin by boiling in macerating fluid, and after careful washing to apply the tests for lignin.

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EXPLANATION OF FIGURES IN PLATES IV–VI.

Illustrating Mr. Lee's paper on Leaf-fall.

The lettering applies also to Text-figures 1–20.

a.b. = axillary bud.
c.c. = crystal cells.
c. = parenchymatous cells dividing to form Protective-cambium.
ca. = Protective-cambium.
cut. = cuticle.
e.s. = exposed surface of scar.
ep. = epidermis.
g. = ground tissue of leaf-base which later will form the Protective-layer.
Lig. = Lignified-layer.
L. P. = ligno-suberized Protective-layer.
P. pd. = first-formed Protective-periderm.
P. pd.^{1} = Protective-periderm formed in subsequent years.
p. = cells of petiole.
ph. = phelloderm of Protective-periderm.
p.s. = cells which for a time retain their cellulose character.
rt. = cortical cells.
scl. = sclerenchyma accompanying leaf-trace.
S. L. = Separation-layer.
st.-pd. = periderm of stem.
ty. = tyloses.
ty^1. = tyloses dividing to form Protective-cambium.
u. L. P. = Protective-layer, not yet ligno-suberized.
v. = vessels containing tyloses.
V. B. = leaf-trace.

Fig. 1. *Castanea sativa.* Longitudinal section of portion of leaf-base showing swelling of cell-walls of Separation-layer (*S. L.*) previous to separation.

Fig. 2. *Castanea sativa.* Part of longitudinal section of scar showing formation of Protective-cambium.

Fig. 3. *Ribes sanguineum.* Part of longitudinal section of leaf-base just previous to leaf-fall.

Fig. 4. *Tilia europaea.* Longitudinal section of periderm beneath old scar, showing well-formed phelloderm (*ph.*)

Fig. 5. *Betula verrucosa.* Part of longitudinal section of leaf-base just before leaf-fall.

Fig. 6. *Salix Caprea.* Part of longitudinal section of leaf-base at time of leaf-fall.

Fig. 7. *Salix Caprea.* Part of longitudinal section of first-year scar showing internal cuticle (*cut.*^{1}). *gum* = deposition of gummy lignin on surface of scar.

Fig. 8. *Celtis occidentalis.* Part of Protective-periderm in longitudinal section. *co.* = cork.

Fig. 9. *Celtis occidentalis.* Isolated lignified crystal cells from Protective-layer. *crys.* = crystal of calcium oxalate. *f.* = film of lignin round crystal.

Fig. 10. *Halesia tetraptera.* Part of longitudinal section of leaf-base just previous to leaf-fall.

Fig. 11. *Rhus typhina.* Portion of longitudinal section of leaf-base showing the continuation of the Separation-layer (*S. L.*) by division of 'tyloses' within the resin duct. *ty*^{1}. = 'tyloses' becoming ligno-suberized. *ty*^{2}. = 'tyloses' dividing to form Separation-layer. *wa.* = wall of resin duct.

Fig. 12. *Rhus typhina.* Part of longitudinal section of leaf-scar showing origin of Protective-cambium (*ca.*) by division of cells, including tyloses. *v*^{1}. = vessels which have become ruptured in consequence of growth and division of tyloses.

Fig. 13. *Rhus typhina.* Transverse section of resin duct. *tyl.* = 'tyloses' in resin duct.

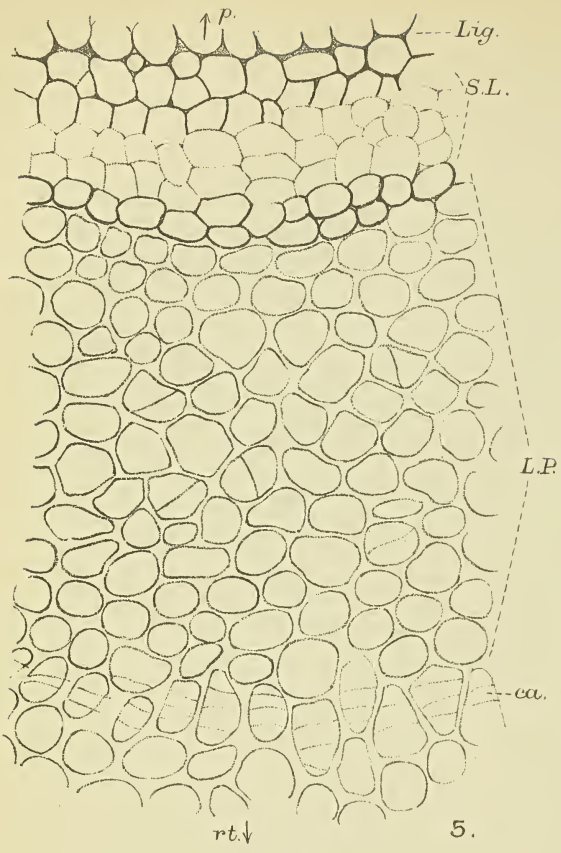
Fig. 14. *Rhus typhina.* Longitudinal section of resin duct.

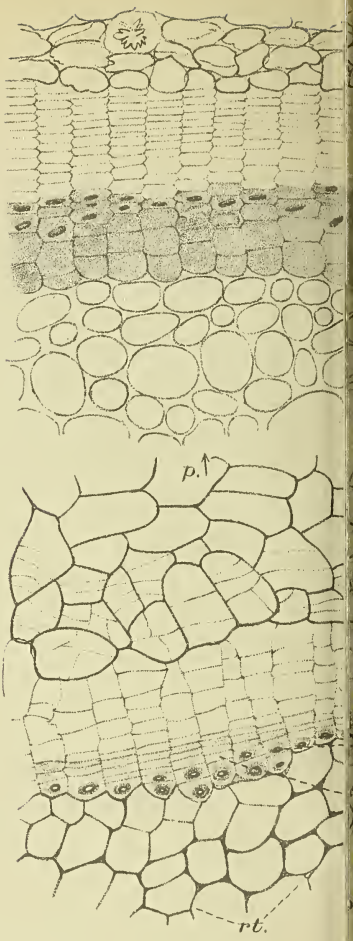
Fig. 15. *Morus alba.* Part of longitudinal section of leaf-base showing latex-tube traversing the Protective-layer (*L. P.*). *wa.* = wall in latex tube above Protective-layer.

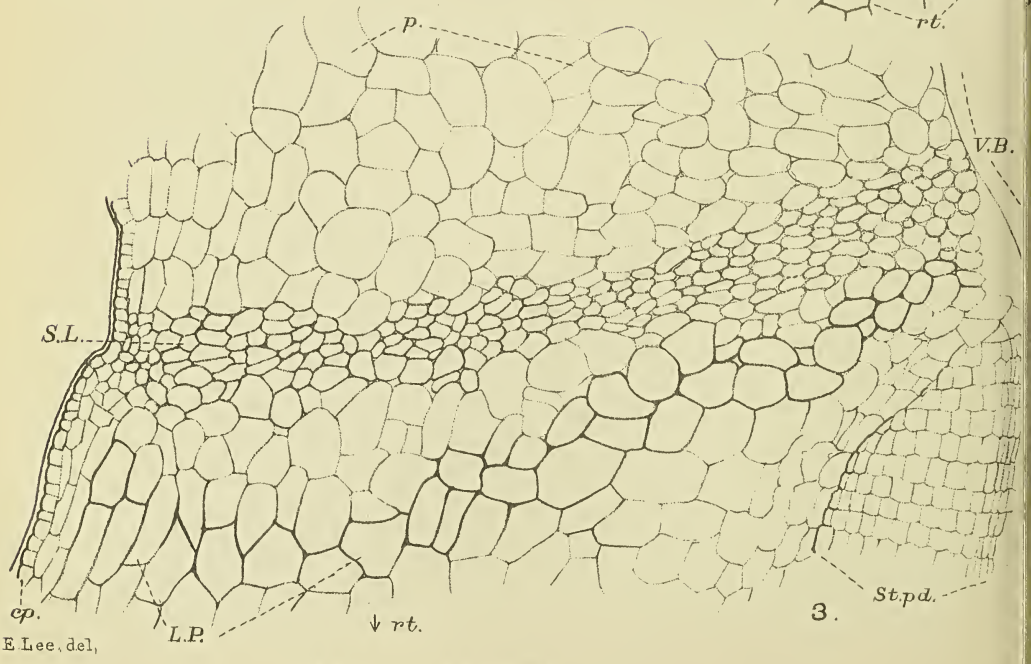
Fig. 16. *Morus alba.* Latex tubes with walls (*wa.*). Arrows show position of Protective-layer.

Fig. 17. *Ficus Carica.* Latex tubes. *a.b.* and *a*^{1}*b*^{1} show position of Protective-layer.

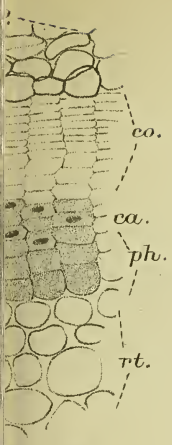
Fig. 18. *Celastrus articulatus.* Part of longitudinal section of leaf-base.







E Lee, del.



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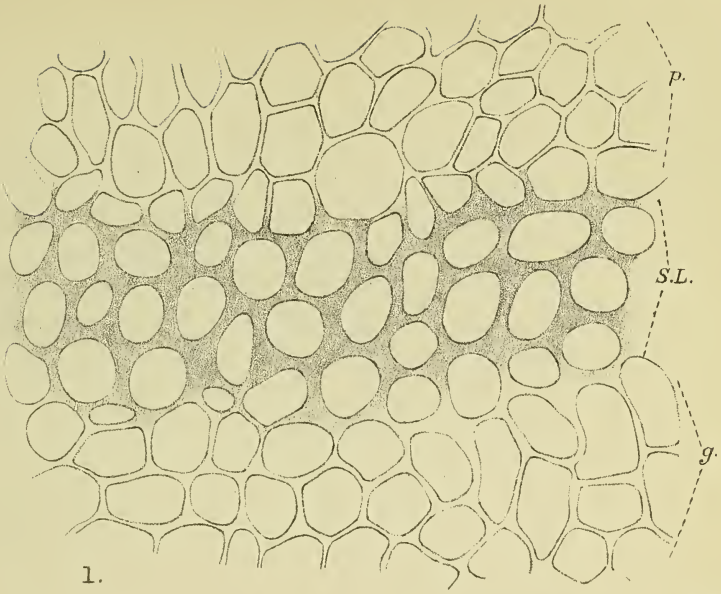


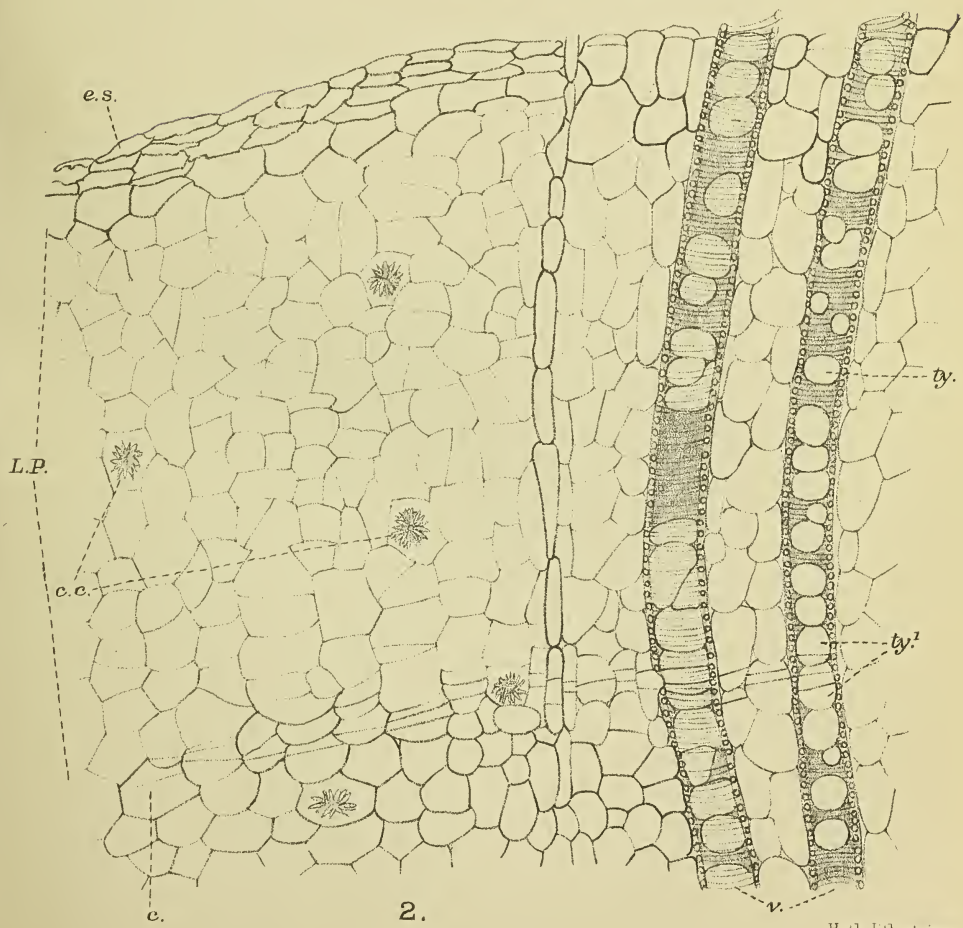
1.

S.L.

L.P.

ph.

ca.



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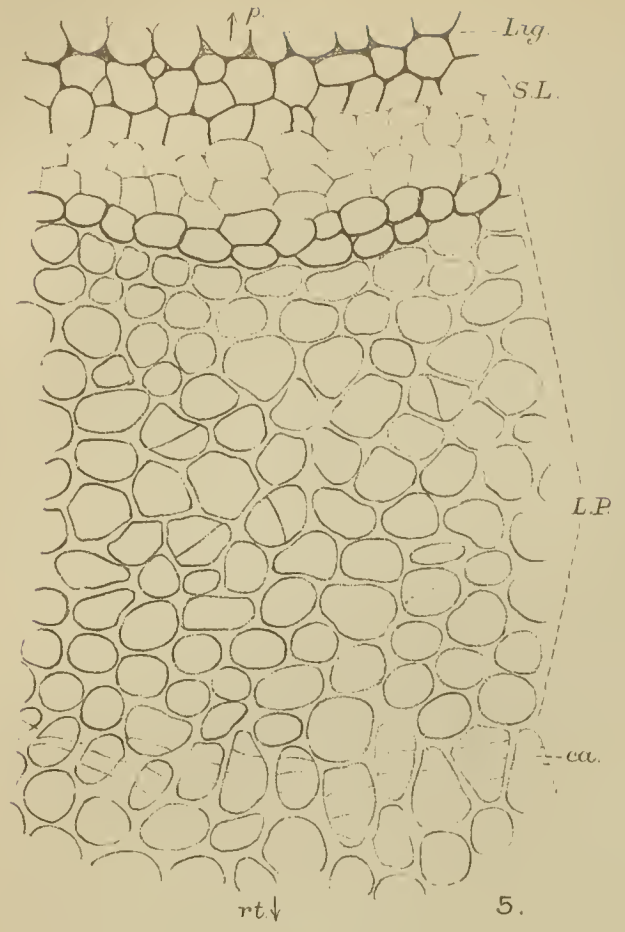

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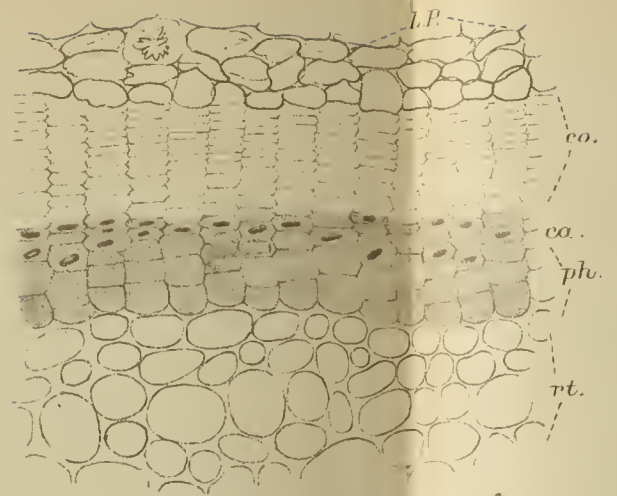

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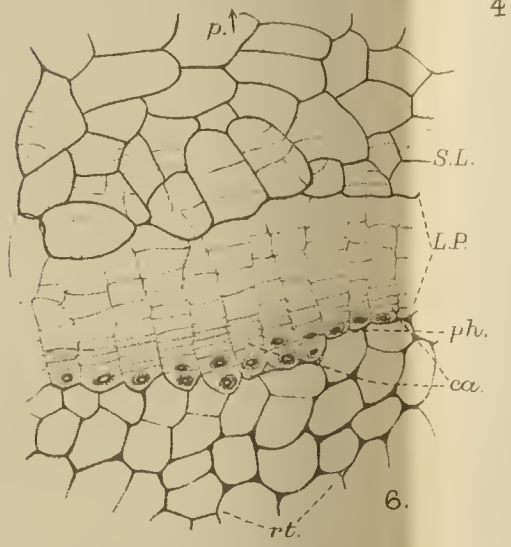

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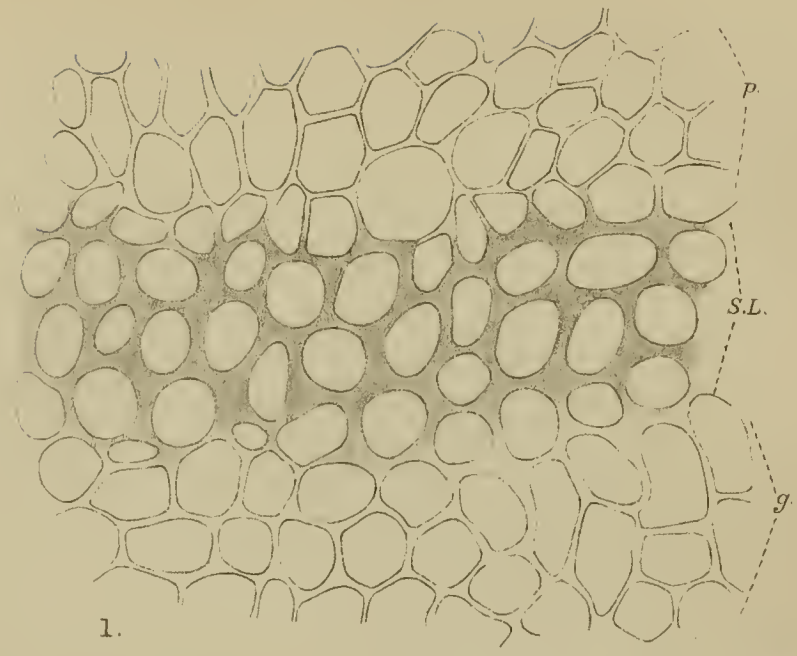

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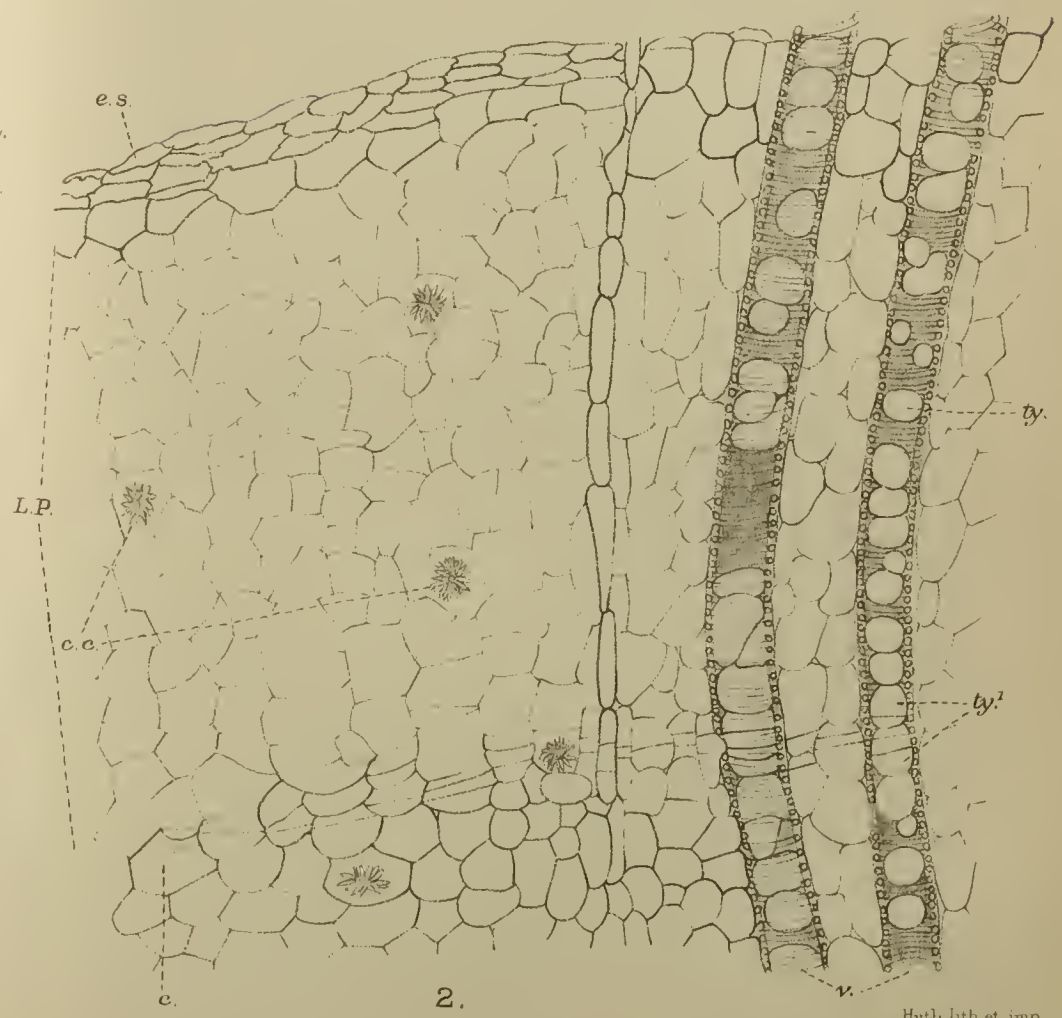

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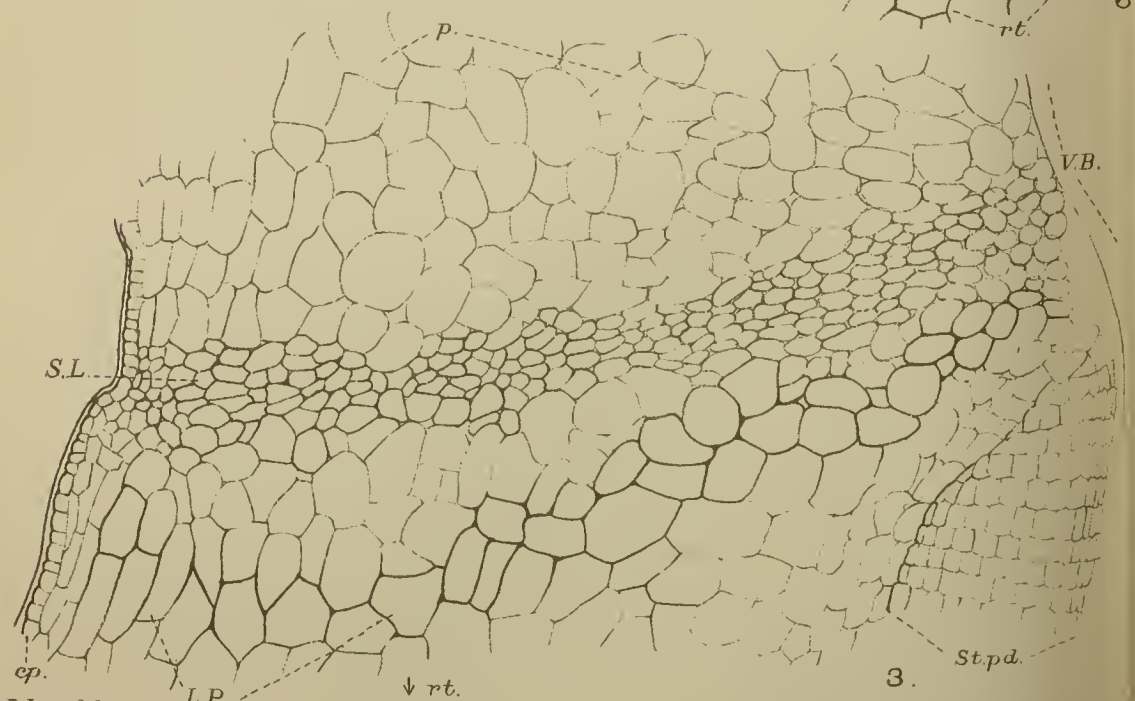

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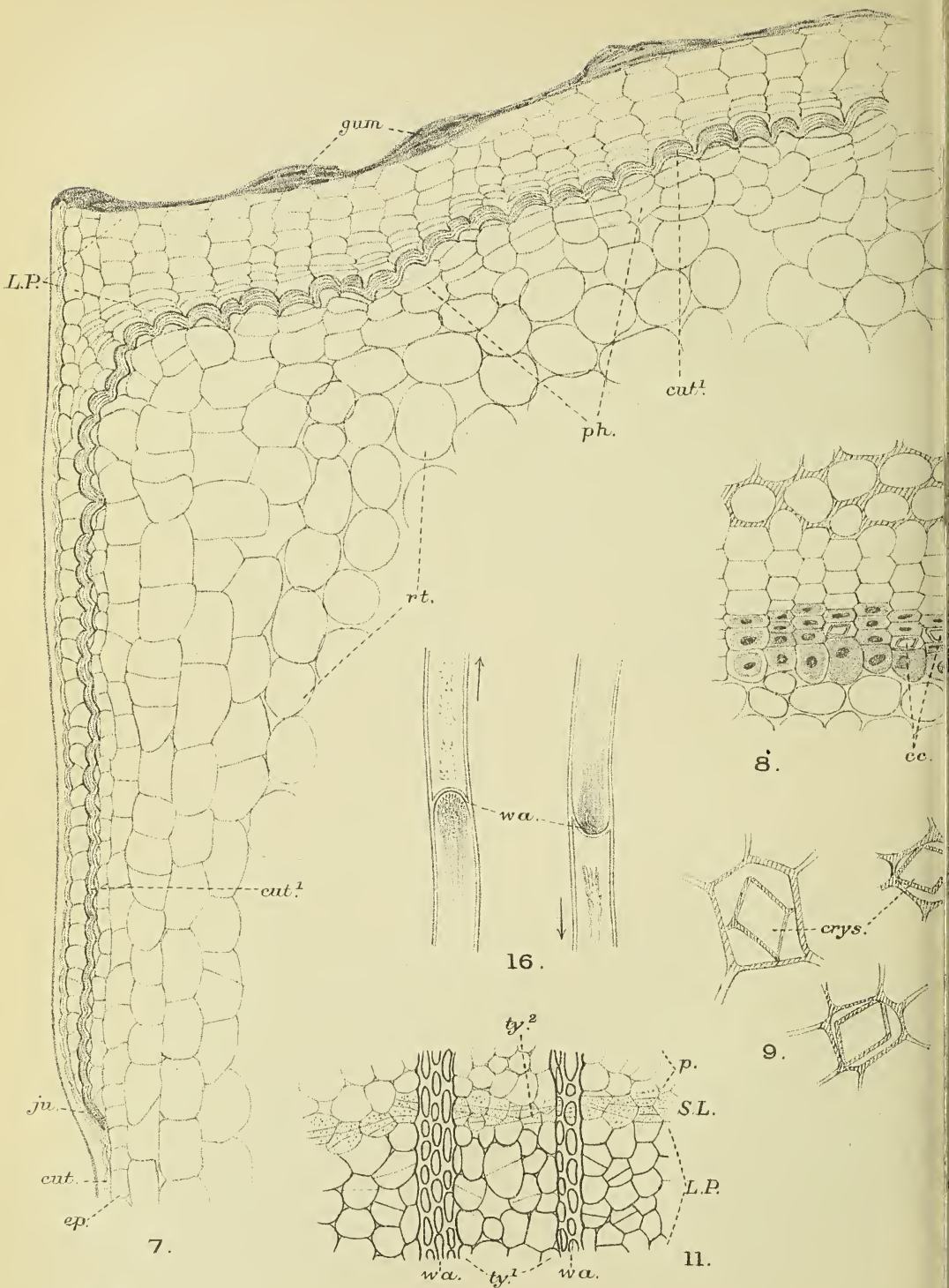

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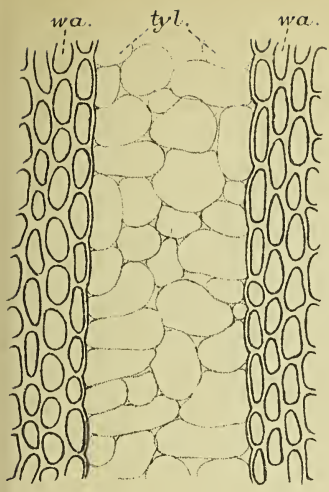
LEE —— LEAF-FALL.



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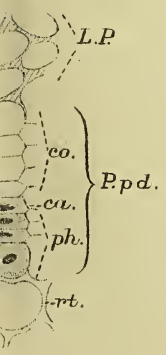

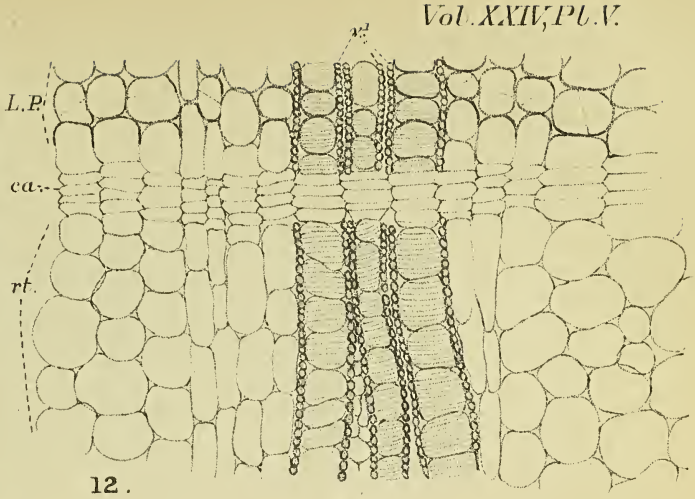

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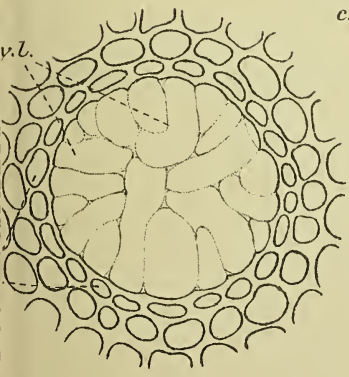

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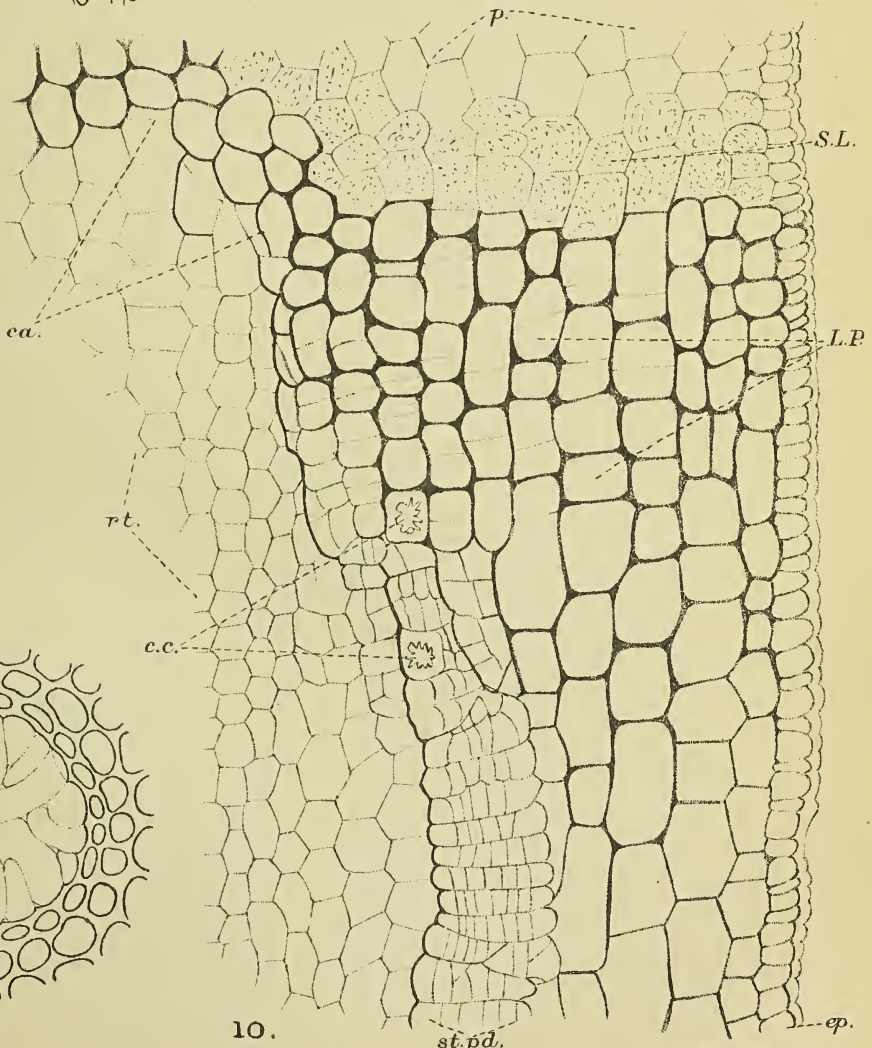

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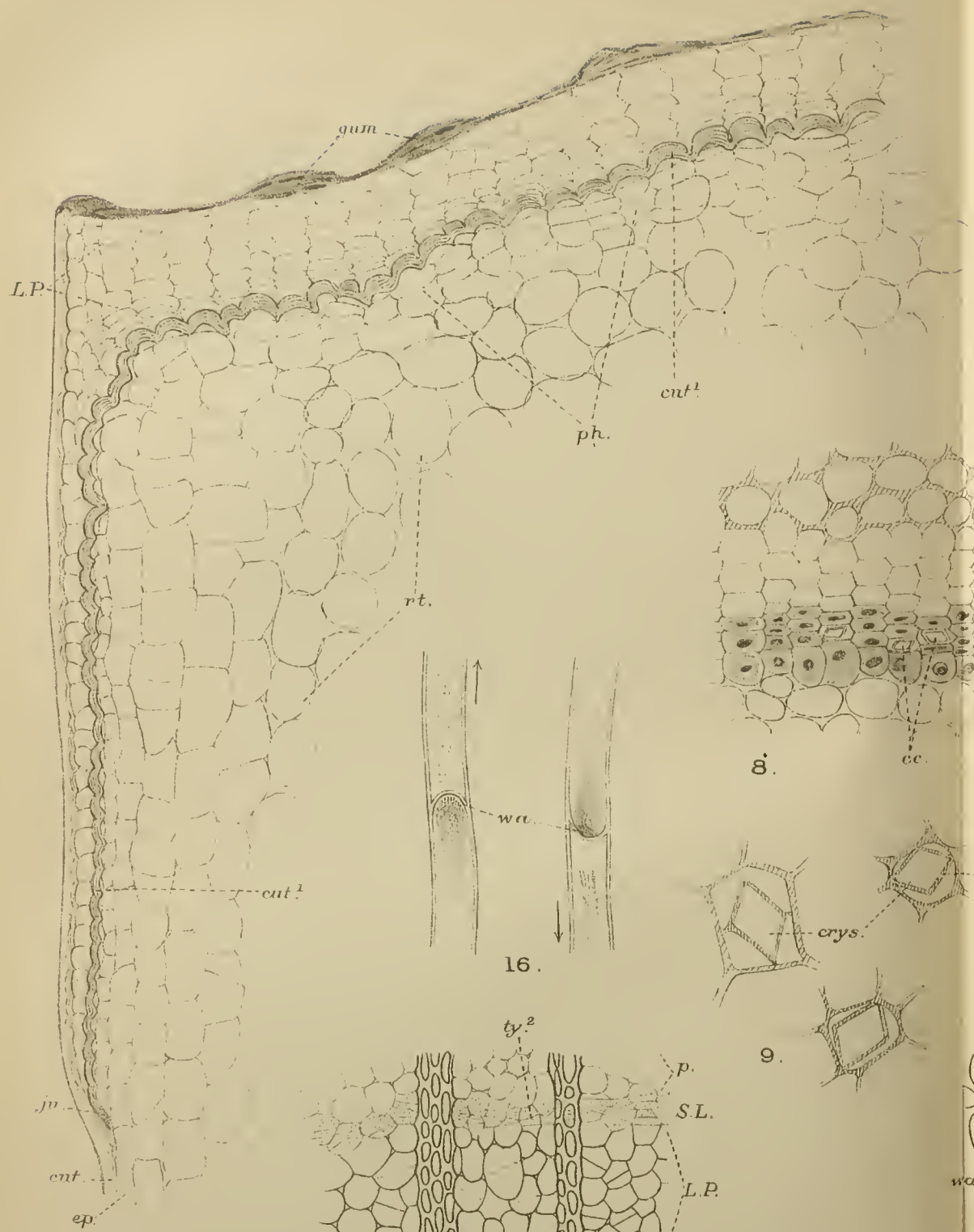

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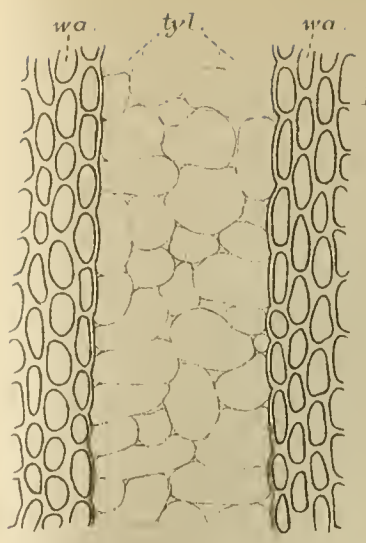

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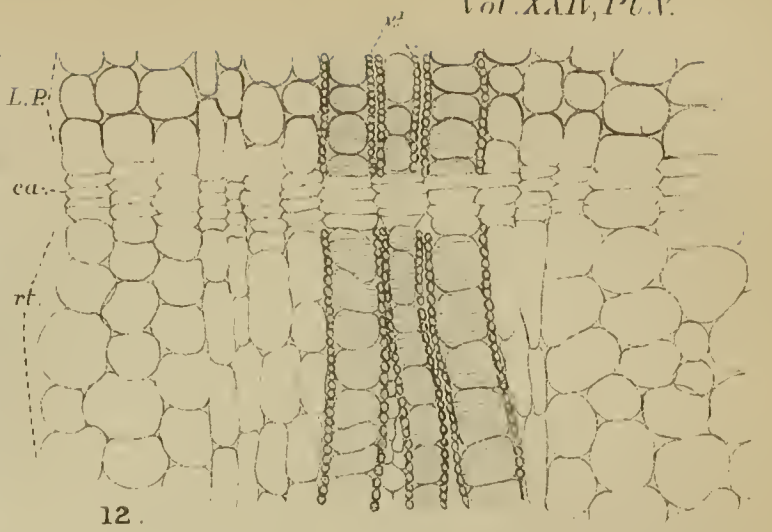

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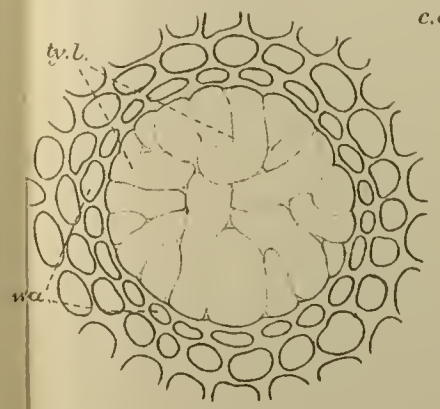

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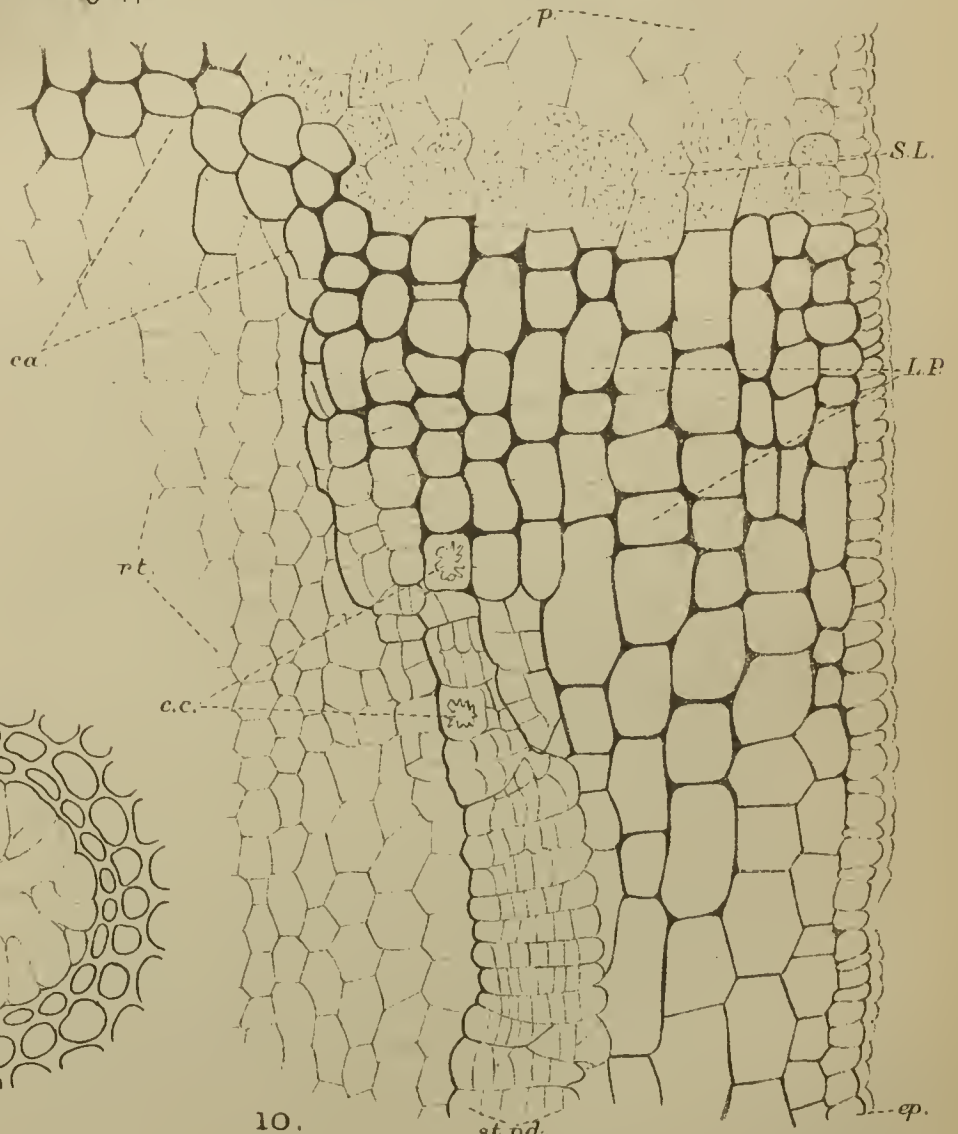
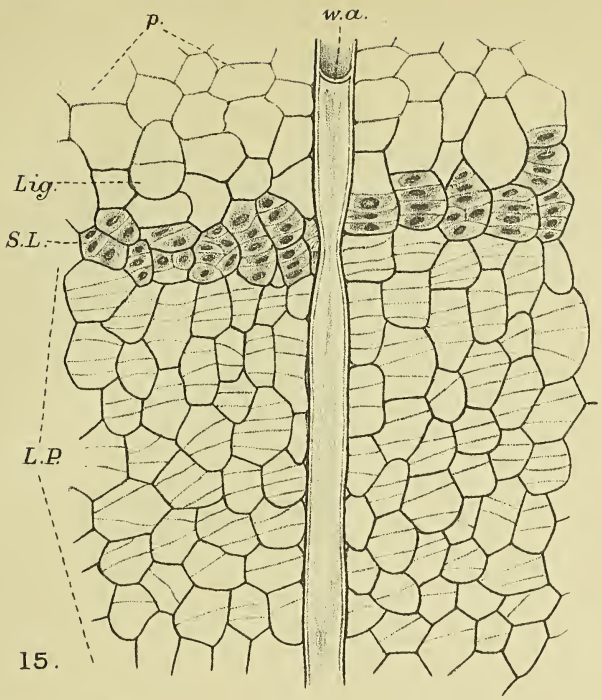


10.

E.Lee, del.

Huth lith. et imp.

LEE —— LEAF-FALL.



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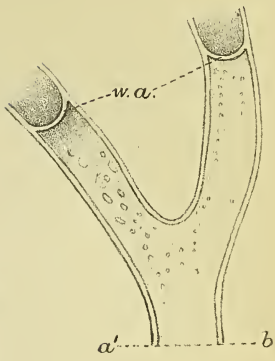

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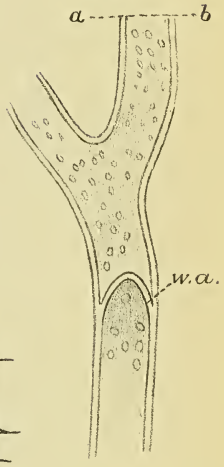
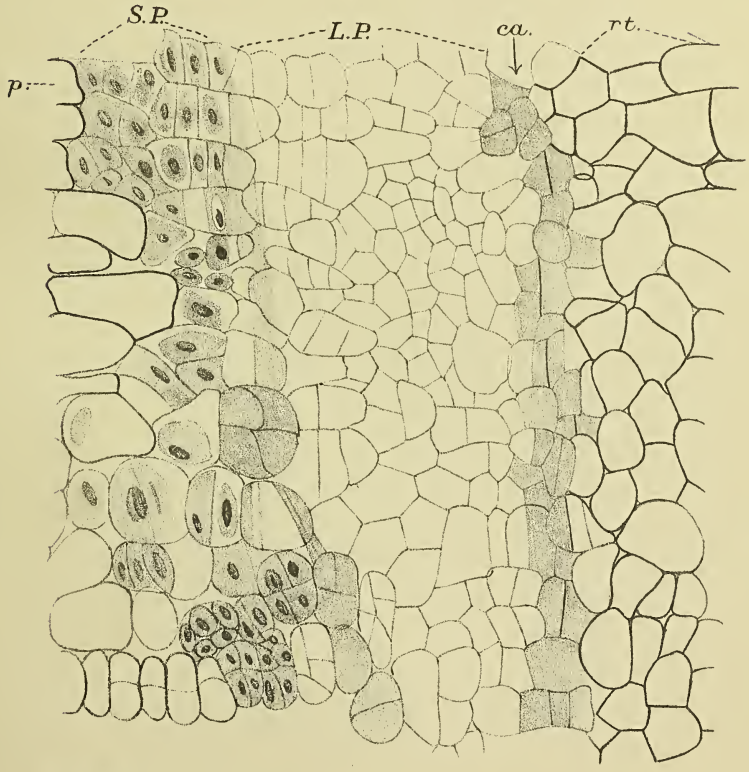




18.

E. Lee, del. Huth lith. et imp.

LEE——LEAF-FALL.

A Study on Gummosis of Prunus and Citrus, with Observations on Squamosis and Exanthema of the Citrus.[1]

BY

ORMOND BUTLER.

With Plates VII–X and three Figures in the Text.

PREFACE.

THE studies which form the groundwork of the present memoir were begun during the autumn of 1907, under the direction of Professor R. E. Smith, at the Pathological Laboratory, Whittier, California, being later continued in Professor B. M. Duggar's laboratory, Cornell University.

The histological studies on gummosis were almost exclusively confined to the *Citrus* during my stay in Southern California. Having satisfied myself that the gum diseases of *Prunus* and *Citrus* were histologically similar maladies—in other words, confirmed the opinion held by Savastano in 1884—I attempted to trace out, using Mikosch's recent memoir as a guide, the elusive first stages of gummosis, but only succeeded in reaching the decided opinion that this author's views would not apply in the case of the *Citrus*. When, therefore, I transferred my work to Cornell University, I decided to reinvestigate gummosis in *Prunus*, as the results arrived at by Mikosch would bear confirmation, while, at the same time, continuing my histological studies on *Citrus*. The present paper is the outcome of this combined study.

Gummosis is a fairly common phenomenon in the vegetable kingdom, but this malady, in other plants, has hardly been sufficiently studied to warrant a comparative review in the present state of our knowledge. I have, however, devoted a few remarks to squamosis and exanthema of the *Citrus*, as these maladies have features common to gummosis, and have not, hitherto, been histologically described.

In closing this preface it is my pleasant duty to acknowledge my indebtedness to Professor B. M. Duggar, and I know not how better to do so than by saying that it is far greater than could be told.

O. B.

June, 1910.

[1] Laboratory of Plant Physiology, Cornell University, Contribution No. 1.

[Annals of Botany, Vol. XXV. No. XCVII. January, 1911.]

I. INTRODUCTION.

Review of Literature.

The identity of the gum disease of *Prunus* and *Citrus*, an identity implied in the title of this paper, has been, if not definitely proved, at least vouchsafed for by Savastano and Delacroix. According to Savastano the histology of gummosis in the *Citrus* is similar to that described by various authors for species of *Prunus* and *Citrus*. He agrees with the histological characters of these diseases as they are given by Beijerinck, Comes, Briosi, Sorauer, Frank, and especially Trécul and Prillieux. Delacroix, on the other hand, observed some differences: starch, always present in the gumogenetic tissues of *Prunus*, is absent from the affected tissues of *Citrus*, and gummosis usually begins at the periphery of the gum parenchyma in the latter, instead of in the centre as in *Prunus*. Otherwise the histogenesis of the disease is the same in both genera. In other works on gummosis of the *Citrus* that I have consulted, the histology of the malady is not studied at sufficient length to warrant any conclusions regarding the validity of Savastano's and Delacroix's conclusions. For this reason it appeared to me that a reinvestigation of the development of gummosis in the *Citrus* could be profitably undertaken.

Gummosis of *Prunus*, on the other hand, has been frequently studied, and it would appear, at first sight, that little remained to be discovered regarding this disease. A review of the most important memoirs that have appeared shows very clearly, however, that our knowledge is still imperfect, and that further studies on this important malady are necessary to elucidate doubtful and obscure points.

Previously to 1860, when Trécul's observations on gummosis of the stone fruits appeared in the Comptes Rendus, no studies of any importance had been published on this malady.

Trécul[1] believed that gummosis was induced by a variety of causes, all of which were instrumental in accumulating in the pathognomonic zone a superabundance of sap. The disease affected either the young wood in formation, or the more differentiated and lignified tissues behind it. In the embryonic wood cells the gum pockets were formed by resorption of the cells, and more especially in the regions where the vessels appeared to be laid down. The lacunae thus formed were filled with fluid containing the dissolved contents of the resorbed cells, the remnants of their cell walls, still in process of dissolution, together with entire cells broken free from the periphery of the gum cavities; these latter cells sometimes enlarged considerably and divided transversely into several cells before being, in turn,

[1] Trécul, A.: Maladie de la gomme chez les cerisiers, les pruniers, les abricotiers, les amandiers. Comptes Rendus, li, 1860, pp. 621-624.

dissolved away. The gum pockets enlarged by continued destruction of the peripheral cells. When occurring within the young wood, the gum pockets were also produced by resorption of the cells, and were even observed originating in the vessels. When gummosis was not severe, and conditions favourable to normal growth recurred, sound wood was formed again by the unaffected cambium, which *de novo* laid down gum-forming tissue when conditions favourable for its production were brought about.

Trécul mentioned one causal condition of gummosis : rain.

In 1863 Wigand [1] published an important memoir on gummosis. He studied gum formation in *Prunus avium*, and, as a result of his investigations, concluded that: (1) the gum found within the vessels was due to a change in the cell-walls, the evidence in favour of this view being derived from the presence and absence, even within the same vessel, of the internal surface sculpturing; (2) except when occurring in the vessels, gum formation in the wood was always preceded by the development of an abnormal wood parenchyma. This abnormal parenchyma was laid down between the medullary rays at any time during the year's growth, and within it the gum pockets were produced lysigenously : one pocket only was usually formed, though at times radial rows of three were produced, each one separated from the other by normal xylem ; (3) the gum pockets could be laid down year after year in the successive annual rings ; (4) gum formation in the cortex was also preceded by an abnormal parenchymatous growth, the extension of individual bast rays ; (5) the formation of gum pockets was due to a centripetal dissolution of the cell-walls, the gum filling the cavities thus formed being derived from the dissolved walls and from the starch within the affected cells which had suffered a synchronous gummous degeneration ; (6) gummosis could begin in the wood and proceed over into the bark, and *e converso* ; (7) cherry and plum gums were a mixture of arabin and of a gum resembling bassorin, i. e. cerasin, which, unlike it, however, was soluble in boiling water.

Wigand did not consider gummosis a malady *per se*, but rather a symptom of weakness of the affected tissues.

In his study on vegetable mucilages, Frank [2] devoted a section to gummosis of the cherry. He found that gum is formed from the secondary membranes in normally formed wood, or through the disorganization of an abnormally formed wood parenchyma and certain cortical tissues. These different areas of gummosis were not necessarily interdependent as regards formation, though whenever the seat of the disease was in the wood all three forms were observed.

[1] Wigand : Ueber die Desorganisation der Pflanzenzelle, insbesondere über die physiol. Bedeutung von Gummi und Harz. Pringsheim's Jahrb., iii, 1863, pp. 115–182.

[2] Frank, A. B. : Ueber die anatomische Bedeutung und die Entstehung der vegetabilischen Schleime. VI : Kirschgummi. Pringsheim's Jahrb., v, 1866-67, pp. 184–198.

In pathognomonic tissues the intercellular substances and primary membranes first changed over into gum, the secondary membranes then became affected, and finally the starch within the cells dissolved away. In certain cases the starch in apparently healthy tissues degenerated with the formation of gum while the cell-walls still remained normal; when this occurred the gum appeared as irregular yellow drops, or in the form of the original granule. The most marked changes in the starch occurred, however, in the cells bordering the gum pockets; the granules lost their form, became irregular masses, and finally gum droplets, the transformation stages being readily followed by their fainter and fainter blue coloration when treated with iodine.

Frank also believed that the plant sap, besides the cell-walls and starch, was an important constituent of gum.

We gather further from Frank's memoir that: (1) cherry gum was insoluble in cold and hot water, in which it did not even swell; (2) when sections of affected tissues were boiled in a solution of caustic potash, the secondary membranes of healthy cells gave the cellulose reaction with iodine and sulphuric acid, while the gum-forming tissues were coloured yellow.

Prillieux [1] in his study on gum formation in fruit trees confirmed in the main the conclusions arrived at by previous workers, notably Wigand, Frank, and Sorauer, but differed from them as regards the formation of the gum. As a result of his investigations, which were confined largely to the apricot, he concluded that: (1) the gum in the vessels was due to infiltration and not to a change in the cell-walls, as the internal spiral and punctiform thickenings remained unaffected even when the lumen was completely filled with gum; (2) the gum filling the lumen of the wood-cells and bast fibre bundles was also due to infiltration or starch; no change in the cell-walls was observed except sometimes a slight swelling in the outermost fibres of the bast fibre bundle; (3) the first stages of gum formation in the abnormal wood parenchyma were a transient rift between the middle lamellae and the cell-walls, followed by the appearance of gum within the suture. Coincident with the appearance of gum, the middle lamellae began to lose their identity, and soon became indistinguishable from the accumulating gum; (4) the starch grains did not gradually change over into gum. With the first appearance of gum within the cells the starch grains became agglomerated. As the production of gum increased the starch became resorbed, but no transitional stages were ever observed; the starch grains at all stages reacted blue to iodine, the gum always yellow.

Hydrochloric acid and chloriodide of zinc were found useful in studying the younger stages of gum formation. The former coloured the wood-cells of plum, apricot, and peach violet, and the gum yellow—recently formed

[1] Prillieux, Ed.: Étude sur la formation de la gomme dans les arbres fruitiers. Annales des Sciences Naturelles (Botanique), sér. 6, vol. i, 1875, pp. 176–200.

gum, however, sometimes coloured violet; the latter coloured the gum yellow at all times.

Prillieux believed that the starch, which is abundant in and around diseased tissues, plays an important rôle in gum formation, the main bulk of the gum being derived indirectly from it.

In his study on *Clasterosporium carpophilum* Aderhold [1] made a number of inoculation experiments on cherry, apricot, peach, and plum trees with the view of determining the rôle played by this parasite in gummosis. He used both pot-grown plants and standard trees, and his inoculation experiments extended over the greater part of the growing season. Numerous inoculations were always made and alternated, with an equal number of witnesses, up the trunk or along the branches, as the case might be. The wounds were made, with one or two exceptions, small and unimportant, but were not protected, as a rule, from the possibilities of contamination. However, this non-protection of the wounds did not affect the experiments, which proved quite decisive. The inoculated wounds always produced gum, which usually pearled more or less upon the surface, sometimes even appearing in three days; whereas the witnesses invariably healed up normally. In some experiments *C. carpophilum* was inoculated, not into the cambium but just beneath the epidermis, or shallowly within the cortex. In these cases no gum appeared in either the inoculated or witness wounds. Aderhold also made one experiment on cherry in which he used *Cladosporium herbarum* in lieu of *C. carpophilum.* No gum appeared in either the inoculated or witness wounds.

Aderhold recorded one or two experiments in which an increased gum flow distinctly followed watering. He made no comment, however.

In the section of his paper devoted to the histology of gummosis, Aderhold described the characteristic appearance of diseased tissues: the production of an abnormal wood parenchyma and its subsequent breaking down to form gum pockets; the reproduction of healthy tissues once more. He also recorded an extensive formation of gum in the mid-rib of a cherry leaf, and the swelling and final dissolution of the pulp-cells of the fruit invaded by the hyphae of *C. carpophilum.* The swelling of the cell-walls and their final dissolution could be brought about either by the secretion of an enzyme, by the hyphae, or from the cells reacting to the stimulus induced by them. Aderhold mentioned both possibilities and accepted the former.

In 1906 Beijerinck and Rant [2] published a memoir dealing very largely with the effect of stimuli on gum formation. They found that the peach

[1] Aderhold, R.: Über *Clasterosporium carpophilum* (Lév.) Aderh. und Beziehungen desselben zum Gummiflusse des Steinobstes. Arbeiten d. biolog. Abt. f. land- und forstwirthschaftl. Gesundheitsamte, ii, 1902, pp. 515, 559.

[2] Beijerinck, M. W., and Rant, A. Sur l'excitation par traumatisme, le parasitisme et l'écoulement gommeux chez les Amygdalées. Archives Néerlandaises des Sciences Exactes et Naturelles, sér. 2, vol. xi, 1906, pp. 184-198.

and almond were very sensitive to wound stimuli, but the cherry, plum, and apricot were less responsive and gummed less readily. When, at the height of the growing season, shoots of peach and almond were wounded into the cambium and secondary wood, at intervals, beginning from the apex, droplets of gum were observed pearling at some of the wounds in less than a week, sometimes even in four days. Dividing the affected shoots into zones they found that gum production was closely related to the position occupied by the wounds on the shoots. At the apices gummosis was slight, then rapidly reached a maximum, decreased again, and finally ceased.

The zone of maximum gum formation occurred just beneath the region of maximum growth in length, and was probably correlated, Beijerinck and Rant believed, with the growth in thickness of the cambium, and also of the procambium.

When shoots were wounded during the course of the summer, but after suberization had taken place, they generally failed to produce gum. In this case the production or non-production of gum appeared to be correlated with seasonal peculiarities.

The gum was formed in the young wood in formation and the diseased tissues, while young, always abutted directly on the cambium. The length and importance of the gum pockets depended largely upon the size and position of the wound upon the shoot; their distal ends were always further removed from the centre of propagation than their basal ends. Wood normally formed never produced gum. The gum pockets buried in the wood were formed by cambial activity at some previous time, and a series of gum pockets in a single season's growth were due to conditions being such that the cambium laid down normal and abnormal embryonic xylem alternately.

Beijerinck and Rant obtained some interesting results with corrosive sublimate and burning as the stimuli of gum formation. When mercuric chloride was introduced into wounds made on young growing shoots of peach, abundant gummosis occurred after the lapse of four to seven days. Burning was also effective, but not as much so as corrosive sublimate, probably owing to the fact that the area of tissue stimulated was less extensive than in the case of the latter, the burns being produced by focusing the sun's rays upon the shoots.

Gummous degeneration of the tissues Beijerinck and Rant believed to be due to a cytolytic enzyme becoming active the moment necrobiosis set in. In necrobiotic cells the protoplasm was killed, but the enzyme remained alive and active, and was able to diffuse out into the surrounding tissues, which it transformed into gum. The greater gum flow when mercuric chloride was used than when the tissues were simply burned or only wounded lent support, they believed, to this view. The gumming of tissue infected by *Coryneum Beyerinckii*, the hyphae of this parasite only showing

gummosis degeneration when growing within living tissues, was regarded as further evidence of the activity of a cytolytic enzyme ; the ferment, passing out from the cells killed by the activity of the fungus, attacked both the living plant cells and the mycelium, transforming them into gum.

The work of Beijerinck and Rant was more fully developed somewhat later in the same year by the junior author.[1]

To the stimuli inducing gummosis mentioned in the joint memoir, Rant made a few additions ; as causal agents he mentioned a number of Fungi, Bacteria, insects, chemical substances, and traumatisms. The histology of the disease was also described at greater length.

Rant did not believe that the gum in the vessels was due to a degeneration of the inner lamellae of the walls, and advanced the view, perhaps somewhat hesitatingly, that it originated either from the decomposition of thylloses, which, however, he admitted never having observed, or from degenerating environing cells. He was of the opinion that the gum could originate both intra- and inter-cellularly. The intracellular gum originated in the manner described by Prillieux. This form of gummosis he considered, however, unimportant, as it did not contribute to the formation of the exudate that appeared on the surface of diseased trees. This latter gum originated exclusively from the degeneration of the cambial and embryonic wood-cells.

The gum cavities arose between the medullary rays. The diseased cells separated from one another and a certain and variable number, depending on the virulence of the disease, became resorbed. The cells bordering the gum cavities thus formed became rounded and grew out into the lacunae ; these ingrowing cells gave rise by division to the cells floating free in the gum. When gummosis was severe the medullary rays were destroyed, and the cambium also sometimes killed. When, however, conditions favourable for growth recurred and the cambium had not been affected, normal wood was laid down over the gum-forming tissues, which gradually lignified.

In 1906 Mikosch [2] also published an extensive article on gummosis of the cherry. He found that branches of plum, peach, apricot, and almond, when cut into 10 cm. lengths and placed in water at room temperature, developed gummosis readily, and his studies were confined very largely to pathognomonic material obtained in this manner. In all the species of *Prunus* experimented with, gum readily formed in eight days, in some cases even earlier, and could be seen with the naked eye pearling between the cortex and the wood. The statement that gummosis developed to a visible extent was generally, though not specifically, correct. To be exact, branches removed in October, November, and June gummed promptly ; branches cut

[1] Rant, A.: De Gummosis der Amygdalaceae. Thesis, Amsterdam, 1906.

[2] Mikosch, K.: Untersuchungen über die Entstehung des Kirschgummi. Sitzungsber. d. k. Akad. d. Wiss. Wien (math.-nat. Klasse), cxv, 1906, pp. 911–61.

in December gummed slowly and less abundantly, and, finally, branches removed in May, when the trees were in leaf, did not show any symptoms of gummosis. There thus appeared to be a certain relation between the time of year at which the branches were cut and their gumogenetic power. Mikosch observed and mentioned this apparent correlation, but offered no explanation for it.

Cherry gum was composed of a water-soluble substance (arabin) and a water-insoluble substance (cerasin); cerasin was, however, soluble in lime-water. These gums occurred together in an intimate mixture. Mikosch was able, however, to separate the arabin almost completely from the cerasin by levigation. When cerasin had been as thoroughly as possible freed from arabin it would, on the addition of alcohol, contract into a homogeneous vitreous mass; pure arabin, on the other hand, gave a white floccose precipitate on the addition of alcohol, or better acid alcohol.

Cherry gum was insoluble in 50 % alcohol. In preparing sections of diseased material for study Mikosch cut from fresh branches, and examined the sections in water, or in 33 % alcohol, if they had to be preserved any length of time. This strength of alcohol was used for the reason that the gum only contracted slowly at this strength. Glycerine and water, equal parts, was also used when the sections were not to be kept for any great length of time.

Mikosch had very little success with staining reagents. He found double staining with neutral red and acid green more reliable than ruthenium red. Chloriodide of zinc was fairly satisfactory; the cell-walls stained blue, as in healthy tissue, and the gum yellow.

The gum was produced in the young wood in formation, and the physiological changes leading to its formation were as follows:—

In the central cells of the groups boxed in, as it were, between the cambium, xylem, and adjacent medullary rays, the protoplasm appeared denser than normally, was granular, and contained starch grains. Following very closely the increased protoplasmic content and appearance of starch, the diseased cells separated schizogenously, and further changes rapidly followed in the cytoplasm. The starch grains became transformed into as many gum droplets, and their remnants, when present, stained reddish brown with iodine solutions. The gum droplets were precipitated *in situ* when sections were first placed in alcohol, but appeared as clear spots when the alcohol was replaced by water, owing to the partial solubility of cherry gum in the latter. Soon after their formation the gum droplets collected together between the protoplast and the cell-wall. If we call, for convenience, the apex of the cell that portion of it which is bounded by the intercellular space, then the gum may be said to have accumulated invariably between the protoplasm and the apex of the cell. Starch does not appear, however, to have been the only source of gum formation,

for Mikosch tells us that owing to the activity of the protoplast gum was continually excreted. This excretion continued until the protoplast was gradually pushed to the base of the cell, where a few remnants of it might be found. The cell-wall was scarcely if at all affected and remained distinct. It may be noted that in some cases, evidently when gum secretion was only active periodically, the protoplast would secrete a layer of gum, then a layer of cellulose, then a layer of gum and a layer of cellulose. Mikosch figured a cell with three distinct walls enclosing two gum layers.

Mikosch did not make it very clear how the gum finally became extracellular. The cell-walls persisted for a long time unaltered, and it was only towards the centre of a gum pocket that they became indistinguishable from the gum matrix. These cell-walls furnished the cerasin component of the gum.

Gum pockets were not necessarily confined to the wood in formation: they also occurred in the cortex.

When the attack of gummosis was not severe, and conditions favourable for its development were soon followed by conditions unfavourable, then normal wood would be laid down on the outside of the gum-forming tissues, and the cells lying within the pathognomonic zone gradually lignified.

Mikosch concluded from his study that gummosis was a pathological phenomenon due to the response of the cambium to wound stimuli.

Ruhland[1] in 1907 advanced the opinion that gum formation was not due *per se* to traumatisms, but to the fact that they allow, when deep enough, air to penetrate to the cambium, or more properly to the young wood in formation. Atmospheric oxygen, he believed, was the active agent in gum formation. This gas acted upon the pectins and pectinates of the cell-walls and upon the carbohydrates within the cells that should have gone to the building of the new septa following cell-division, oxidizing them into gum.

The hypothesis regarding the intracellular formation of gum was arrived at through a study of diseased tissues. Ruhland found that a number of the large swollen cells bordering the gum pockets contained two well-developed nuclei but no cross-walls, and he concluded that intracellular gum was formed through a process of oxydation homologous to that which was effective in producing intercellular gum.

Ruhland performed a few experiments with the view of determining the effect of oxygen exclusion upon gum formation. He used cuttings of peach, plum, and cherry, the bottom ends of which were placed in water, while the distal cut surfaces were capped with a mantle of hydrogen or nitrogen, or some such impervious substance as cocoa-butter, paraffin, &c. The experiments gave very marked results: no gum formed in the cuttings

[1] Ruhland, W.: Zur Physiologie der Gummibildung bei den Amygdalaceen. Per. d. Deutsch. Bot. Ges., xxv, 1907, pp. 302–15.

in which the cut surfaces were protected from the air (oxygen), whereas in the witnesses from 14 % to 100 % of the cuttings showed the characteristic lesions. Taking the most extensive experiment recorded the witnesses showed approximately a mean of 67 % diseased cuttings.

Sorauer[1] in his Handbuch der Pflanzenkrankheiten, art. 'Gummifluss der Kirschen', described the usual appearance of diseased tissues, and ascribed gum formation to a latent capacity possessed by embryonic and full-grown cells to produce gum. He did not lay any stress at all upon the development of gum pockets in the wood in formation, as may be gathered from the summary of his observations, which may be translated thus: (1) gum formation is preceded by a development of parenchymatous tissue within the xylem which presents a nidulose appearance, and lies usually between two medullary rays, and exceptionally includes one or more; (2) the abnormal parenchyma develops independently of wound stimuli; (3) the tissues finally break down into gum and the medullary rays grow out into the pockets thus formed.

Sorauer believed that gum formation was due *pro parte* to a zymogenetic substance present in embryonic and fully developed cells, which became active when certain life functions connected with the growth of the cell-wall were interfered with. But the retardation in the development of the cell-wall thus brought about, as also the transformation of the part already laid down into gum, could also be readily accounted for, he thought, by assuming an increase in the oxygen supply irrespective of the action of enzymes, an opinion also held by Ruhland, as we have seen. This necessary supply of oxygen was obtained directly from the atmosphere through the intermediation of wounds, or from organic or inorganic stimuli.

In order to show that gummosis followed stimulation by an oxydizing agent, Sorauer performed the following experiment: He made two incisions into the limb of a healthy cherry-tree: into one he poured oxalic acid and into the other, which was to serve as witness, distilled water. The wound treated with oxalic acid gummed freely during the course of the summer, the flow only ceasing when the acid had become, in the course of time, neutralized; whereas the witness remained healthy.

II. GUMMOSIS.

Syn.: *France*: Colle, Gommose, Maladie de la gomme; *Germany*: Gummifluss, Gummikrankheit, Gummosis; *Great Britain*: Gumming, gummosis, gum disease, sore shin, foot-rot (erroneously); *Italy*: Mal di gomma, Male della gomma; *Portugal*: Lagrima; *Spain*: Enfermedad.

[1] Sorauer, P.: Handbuch der Pflanzenkrankheiten, i, 1909, pp. 693–701.

1. Historical.

The phenomenon of gummosis has long been known in the genus *Prunus*. The malady has been more frequently noted in the cherry and peach, though, as modern authors have shown, it affects also the apricot, almond, and the plum, both the American and European species. At no time, however, has the disease attained great economical importance, and, unlike other maladies, it has no history. Gummosis has always affected the *Prunus*, now more, now less, but in no sense has it ever been a scourge. In the case of the *Citrus*, on the other hand, gummosis was hardly known, and then not as a malady, before 1834, when, Fouqué [1] informs us, it suddenly appeared in the groves of San Miguel. Watt,[2] however, mentions *Citrus medica*, *C. decumana*, and *C. Aurantium* as yielding an unimportant gum, samples of which were exhibited at Madras in 1855. The orange and citron gums were sent from Masulipatam, but the place of collection of the shaddock gum is not given. Gummosis must, therefore, have been a sufficiently common phenomenon to attract attention, though not so intensive a one as to affect the health of the trees, hence the gum was collected and sent to the Madras Exhibition, as being perhaps of some economic value. Gummosis, we may therefore safely conclude, was no unusual occurrence in the eyes of the natives, and had been for years past a common affection of the trees. The disease was known in India in all probability before it was first observed in the Azores; in fact, there is evidence that the malady, in a very benign form, was in the orange groves of Europe in 1818; it appears even to have been described 'briefly and precisely' as long ago as the middle of the seventeenth century.[3] However this may be, gummosis does not appear to have been a malady of much economic importance prior to its sudden and virulent outbreak first in the Azores, and then in the different orangeries of Europe, towards the middle of the nineteenth century.

The malady was first observed in San Miguel in 1834, where it became, within a few years, enormously destructive. In 1840 the disease was at its height. 'Entire orangeries were destroyed, others partially ruined, and it is estimated that one-quarter of the orange trees on the island had to be dug out.' [4] In 1842 the disease had become less virulent and in 1873, though still extant, was no longer feared.[5] In Portugal gummosis appeared in 1845, affecting first the groves around Lisbon, and was at the height of its destructiveness between 1858 and 1861. In 1851 it was present in the orangeries of Hyères,[6] and in 1855 appeared in the groves at Limone on

[1] Fouqué, F.: Voyages géologiques aux Açores. III: Les oranges de San Miguel, les cultures et le monde organique aux Açores. Revue des Deux Mondes, civ, 1873, p. 836.

[2] Watt, G.: Dictionary of Economic Products of India. ii, pp. 344, 349.

[3] Ferrare, J. B.: Hesperides sive de Malorum aureorum cultura et usu 1646, cited by Savastana, L. Della cura della gommosi e carie degli agrumi. Comizio Agrario, Napoli, iv, 1887.

[4] Fouqué: loc. cit., p. 836.

[5] Ibid., p. 836.

[6] Rendu, V.: Note sur une maladie des orangers d'Hyères. Comptes Rendus, xxxiii.

the Lago di Garda.[1] In Sicily the disease was first observed in the province of Messina in 1862,[2] and was also discovered about this time or somewhat later in Spain, Corsica, and Algeria. The groves of New South Wales were devastated between 1860 and 1870.[3] According to Curtis [4] the disease probably appeared about 1876 in Florida, though few people, he remarked, could remember seeing it prior to 1880; in California it was observed in 1875.[5] At the present day gummosis is known wherever the *Citrus* are extensively grown.

2. Description.

General. Unlike most maladies gummosis appears to be autogenous as well as superinduced by traumatisms of very diverse nature. Fungous, insectile, physical and chemical injuries, when they affect directly the cambium layer, all induce gummosis, provided growth is taking place. Furthermore, gum is not produced in quantities unless the tree affected is well supplied with water. The development of the disease depends, then, upon the rapidity with which new tissues are being laid down at the time of its initiation; and this rapidity, as is well known, is a function, broadly speaking, of the amount of water available to the roots of the affected tree. Growth and water are, therefore, essential to the appearance of gummosis, and both are limiting factors. The relation of these two factors, *growth* and *water*, to gum formation, here only postulated, will be more fully dealt with in subsequent pages of this memoir, and is mentioned at this time solely with the view of rendering more comprehensible the development of gummosis in the orchard, and the cultural and environmental conditions affecting the same.

Gummosis develops sporadically, sub-generally, or generally, depending on the nature of the inducing factor. It is usually only sporadic in the case of insect attack, and the same remark is true for many of the fungi. Some fungus-parasites, notably *Coryneum Beyerinckii* and *Clasterosporium carpophilum*, produce, on the other hand, sub-general or general gummosis, the atmospheric conditions favourable to the development of these parasites being also favourable to the formation of gumogenetic tissues in the host. Autogenous gummosis appears sporadically or sub-generally. In most species of *Prunus* and *Citrus*, the disease develops sporadically, only becoming generalized, as a rule, in *P. Cerasus*, *P. avium*, and *Citrus Limonum*.

[1] Rapporto della Commissione per studiare la malattia degli agrumi in Sicilia, &c. Annali di Agricoltura Siciliana, n.s., i.

[2] Ibid.

[3] Alderton, G. E.: Treatise and Handbook of Orange Culture in Auckland, New Zealand.

[4] Curtis, A. H.: Sore Shin or Gum Disease. Florida Agr. Expt. Sta., Bull. ii.

[5] Mills, J. W.: Citrus Fruit Culture. California Agr. Experiment Sta., Bull. cxxxvi.

The conditions favourable to the development of gummosis (inducive in the autogenous form, conducive in other cases) are: (1) Growing trees in heavy, retentive, poorly drained soils; (2) Growing trees in an otherwise suitable soil, but underlaid by an impermeable subsoil, the situation not being such as to afford ample drainage; (3) Irrigation methods allowing an excessive accumulation of water around the trees for a period of time; (4) High fertility combined with soil and drainage conditions as mentioned under 1, 2, and irrigation methods as mentioned under 3; (5) High fertility and excessive irrigation: (6) Excessive fertilization, especially with nitrogenous manures; (7) Continuous wet weather in spring.

Too much stress, however, should not be laid on any one of the above factors alone. For instance, if we take soil texture in its relation to autogenous gummosis we find that in the *Citrus*, where this form of the malady has been best studied, there is no *ne varietur* relationship whatsoever.

With these few remarks on the development of gummosis in the orchard, I pass to the study of the symptoms of the disease.

Minute. Gummosis begins and attains even a high state of development within the plant before any symptoms appear externally. Only after the gum has accumulated to such an extent that sufficient pressure has been produced to raise the epidermis, or to drive the flux pearling through the crevasses in the same, when it is no longer intact, does the disease become apparent. This is generally considered a young stage of gummosis, though in reality the disease may have developed very considerably by this time. With the further development of the malady the distended epidermis bursts and the gum flows out in greater or less amount, or, if the first external symptom was a pearling through epidermal crevasses, simply shows as an increase in the outflow.

Gummosis may affect a tree locally or generally; the fruit (Saucer Peach, almond, orange, and citron), the twigs and smaller branches, one or more limbs, the trunk, or the entire tree. In *Prunus* the disease appears usually in the larger limbs and trunk (Pl. VIII), and is generally more frequent in the neighbourhood of the crotches. In the *Citrus* gum flow appears more generally, one might almost say exclusively, upon the trunk towards the base, frequently immediately above the union of stock and scion (Plate VII). Gummosis may, however, when extremely intense, affect the entire tree in both *Prunus* and *Citrus*, gum oozing out from both the trunk and the limbs. Such generalized gummosis, though rare in other species of *Prunus*, is not infrequently observed in young cherries; in the *Citrus*, on the other hand, it is quite exceptional and has only been observed on the lemon.

When gummosis progresses sufficiently for gum to appear on the surface of the bark, the cortex in the neighbourhood of the exudate will

be found more or less permeated with gum. The death of this infiltrated bark is but a matter of time; it becomes extremely hard, cracks, curls more or less, and sloughs off.

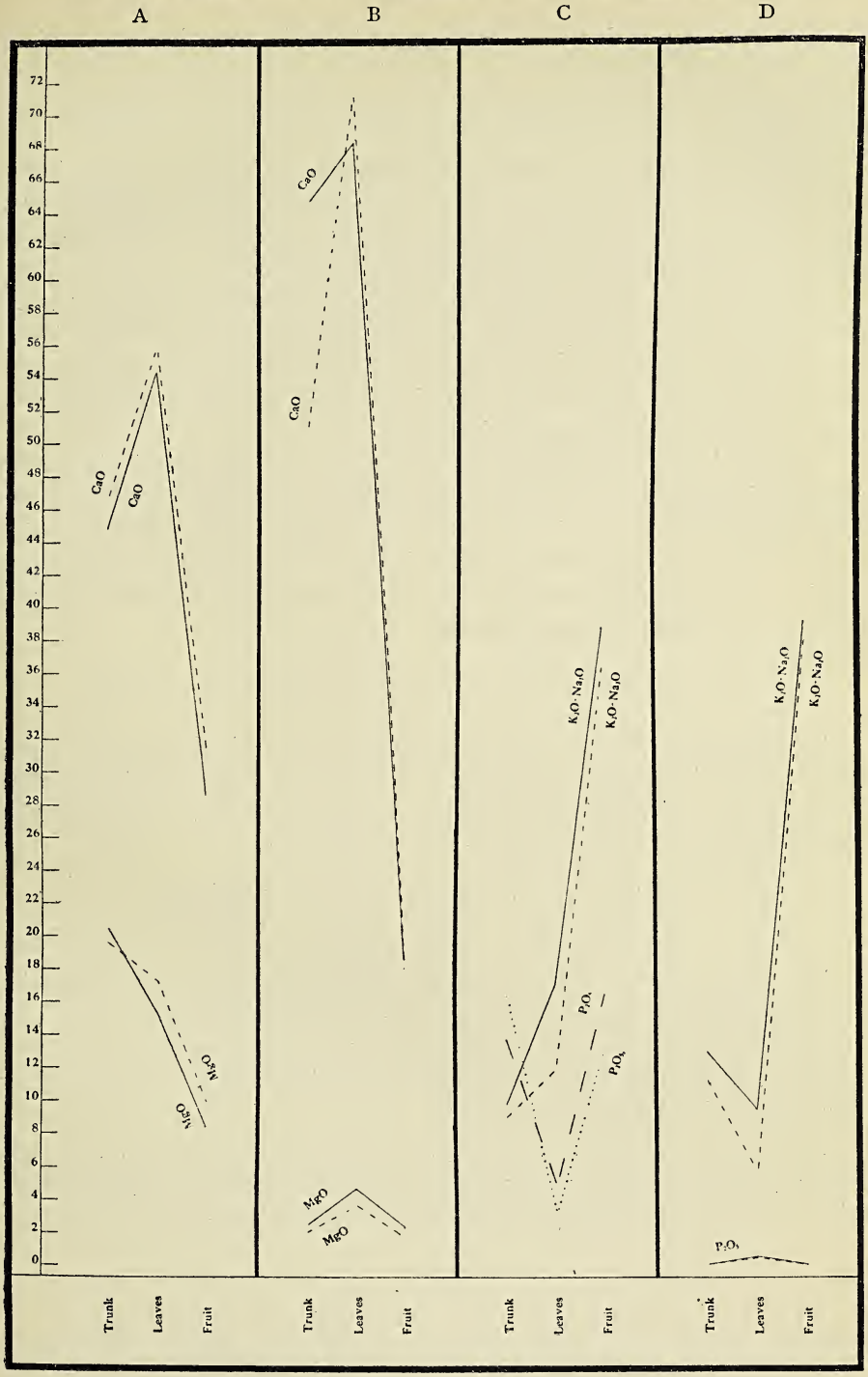
Severe cases of gummosis are always accompanied by chlorosis and, when the malady recurs from year to year, by a marked decrease in vigour. As the disease indirectly, by dehiscing the bark, destroys the avenue of food supply to the roots, death from starvation must ultimately result. With the reduced root development consequent on the failure of the proper supply of elaborated material, there follows a decrease in the absorption of mineral nutriments, and it does not appear to me at all doubtful that it is to this reduced absorption that chlorosis of the diseased trees is due. A number of analyses of healthy and diseased *Citrus* have been made at various times with the idea of throwing some light on the cause of gummosis. Diseased and healthy trees show considerable difference in ash content, but this difference is purely a relative one, and the affected trees show not, as has sometimes been thought, selective absorption, either forced or otherwise, but rather reduced normal absorption. The following curves drawn from the data of Ricciardi and Silvestri clearly show that relative absorption is normal in diseased as well as healthy trees (Text-fig. 1).

Gummosis having been detected by means of the pustulated epidermis or the exudate, the extent of the development of the disease within the tissues may be readily followed by a series of cross-sections taken at intervals above and below the place of gum accumulation or outflow.

The internal characters of the disease are best shown on slightly affected stout branches two or more years old. A cross-section cut through the swollen epidermis or the exudate will show that the pathognomonic tissues are situated in the young wood and young wood in formation, and extend to a greater or less extent around the xylem. The diseased area is fusoid in form from the greater development of the diseased tissues near the point of gum accumulation and their lesser and lesser development as one proceeds further away. The gum exudes *en masse* from the centre of the sickle, but as one proceeds towards its extremities it will appear in droplets of decreasing magnitude, and separated with larger intervening spaces of apparently healthy tissue. The gum in the centre of the sickle is more or less tinted yellow, depending on its age, whereas that pearling from the tissues at its extremities is always colourless (Pl. IX, Fig. 3).

If sections are now cut at various distances above and below the centre of disease, it will be found that gum formation proceeds downward to a much less extent than upward. Furthermore, if we imagine a line drawn through the middle of the pathognomonic tissues, it will be found, as one proceeds upwards and downwards, that the gumming sickle diminishes in size.

GRAPHS SHOWING RELATIVE ABSORPTION OF MINERAL NUTRIMENTS IN PARTS PER 100 BY HEALTHY AND DISEASED LEMONS.



TEXT-FIG. 1.

A.C. from the analyses of Ricciardi.
B.D. from the analyses of Silvestri.
———— CaO, MgO absorbed by healthy lemon.
- - - - - - CaO, MgO absorbed by diseased lemon.

———— $K_2O + Na_2O$ absorbed by healthy lemon.
- - - - - - $K_2O + Na_2O$ absorbed by diseased lemon.
— — — P_2O_5 absorbed by healthy lemon.
· · · · · · P_2O_5 absorbed by diseased lemon.
———— P_2O_5 absorbed by healthy lemon.
- - - - - - P_2O_5 absorbed by diseased lemon.

The cortex remains healthy except where the gum has accumulated sub-epidermally, or broken out on the surface of the bark. In these places, as I have already mentioned, it becomes more or less infiltrated and discoloured.

Within the wood, if the branch examined is old enough, brown maculations and fusoid areas may be observed amidst the healthy tissue; they are due to attacks of gummosis at some previous period, when the now dead tissues were a part of the meristematic zone.

When the attack of gummosis is very severe the branches or trunk, as the case may be, may become completely ringed with gumming tissue, and the fusoid character accompanying mild infection is lost. The wood is more or less deeply infiltrated and discoloured and the disease gives the false impression of working centripetally.

Gummosis is characterized, as we have seen, macroscopically by a more or less copious gummous exudate on the surface of the bark, and by a zone of pathognomonic tissue in the outermost region of the xylem, which, however, is only recognized by the unaided eye when gum flows or pearls from it. The gum is, therefore, an important symptom of the disease, and, as such, is deserving of separate study.

3. Chemistry of the Gums of *Prunus* and *Citrus*.

The gums[1] of *Prunus* are derivatives of the hemicelluloses and represent, to use the illustration of Grüss, the homologous stage in their hydration to that of the dextrins in the hydrolysis of starch. The changes taking place during the hydrolysis may be illustrated thus:—

araban . . .	arabin . . .	arabinose
starch . . .	dextrin . . .	maltose

The gums of the *Citrus* do not appear to have been closely studied up to the present, but, as will be seen from the following table, they behave in essentially the same manner as the gums of *Prunus* towards the various reagents, and like the latter are hemicellulose derivatives. As type of the *Citrus* gums I have taken lemon gum.

[1] The chemistry of the gums is still imperfectly known and consequently not entirely free from inaccuracies. Vide Wiesner, J.: Die Rohstoffe des Pflanzenreiches, vol. i, 2. Bd. Czapek, F.: Biochemie der Pflanzen, vol. i, 1905. The literature is cited in these works.

QUALITATIVE REACTIONS OF *PRUNUS* AND *CITRUS* GUMS.

Reagent.	*Lemon gum.*	*Cherry gum.*	*Plum gum.*
Hydrochloric acid and phloroglucin	bright red, then dark pp.	bright red, then dark pp.	bright red, then dark pp.
Alcohol 95 per cent. excess	white pp.	white curdy pp.	white pp.
Acetic acid, dil.	———	———	———
Glacial acetic acid, excess	white pp.	white pp.	white pp.
Nitric acid	solution coloured yellow	solution coloured yellow	solution coloured yellow
„ „ and ammonia	colour heightened	colour slightly heightened	colour heightened
Millon's reagent	solution deep red	solution yellowish yellow pp.	solution reddened
Tannic and acetic acids	———	———	———
Sodium molybdate	———	———	———
Potassium-mercuric iodide	———	———	———
Phosphotungstic and sulphuric acids	cloudy white pp.	large curdy pp.	liquid cloudy
Sodium phosphomolybdate	———	solution thickened	———
Sodium phosphotungstate	———	white pp.	trace of reaction
Mercuric nitrate and nitric acid	fine pp. remaining in suspension	———	fine pp. remaining in suspension
Adamkiewicz reaction	———	———	———
Biuret test	———	———	———
Lead acetate and sodium hydrate	———	———	———
Hydrochloric acid	bright yellow, then clear amber red gelatinous pp.	bright yellow, then dark brown	bright yellow then dark brown, slight pp.
Ferric chloride	———	———	———
Fehling solution	———	———	reaction doubtful
Lead acetate	milky, slight pp.	———	milky
Ammoniacal lead acetate	abundant gelatinous pp.	abundant gelatinous pp.	abundant gelatinous pp.
Basic lead acetate	fine curdy pp.	———	fine gelatinous pp.
Calcium hydrate	———	———	———
Potassium hydrate	———	sol. slightly thickened	———
Sodium hydrate	sol. thickened	sol. thickened	sol. thickened
Barium hydrate	very slight fine pp. remaining in sol. as a faint cloud.	very slight fine pp. remaining in sol. as a faint cloud	very slight fine pp. remaining in sol. as a faint cloud
Aluminium hydrate	sol. thickened, faint cloud	sol. thickened, slight indefinite pp.	sol. thickened, faint cloud
Sodium borate	sol. thickened	sol. thickened	sol. thickened
Copper sulphate	very slight fine pp. remaining in suspension	———	———

The gums of *Prunus* and *Citrus* I have found to be soluble in water. A 6 per cent. solution of cherry gum is very thick, but the gum dissolves with shaking within twelve hours at this concentration. The lemon and plum gums dissolve much more readily than the cherry gum, and at the same concentration as the latter form a less viscous solution. All the gums contain nitrogenous matter, probably in variable amount.

4. Technique.

In our study of the gums of *Prunus* and *Citrus* we found that they are soluble in water, from which they are precipitated out by strong alcohol, or an excess of glacial acetic acid. In preserving material for examination it was, therefore, quite evident that alcohol and acetic acid mixtures, or

alcohol alone, must be used if the earlier stages of the disease were to be preserved. I early determined that when alcohol and glycerine, equal parts, was used as the fixing and preserving fluid, sections of shoots in which very young gum pockets were to be found always showed a great paucity of gum in the lacunae. I also observed that the glycerine-alcohol solution became more viscous when specimens had stood in it for sufficient time, and this viscosity, upon analysis, proved to be due to dissolved gum. Contrary to Mikosch's observations, then, gum is soluble in 50 % alcohol. As woody tissues are always hardened in alcohol, it became a matter of no small interest to determine what was the minimum strength of alcohol in which the gum remained insoluble. I selected a sample of fresh fluid pellucid cherry gum—in other words, gum recently formed—and placed aliquot parts of it in 50 %, 75 %, 85 % and 95 % alcohol. The gum dissolved in all but the 95 % solution. In alcohol of this strength the gum lost somewhat in volume, and became brittle and perfectly translucent, except at one corner where it was somewhat white opaque.

In acetic acid the gum is quite soluble except in a large excess of the reagent.

Farmer's fixing solution (alcohol absolute 2 pts., glacial acetic acid 1 pt.) may be used in lieu of 95 % alcohol, but the gum, instead of remaining a homogeneous mass, appears as a fine precipitate which is not very suitable for study.

Mikosch found in the course of his study on gummosis of the cherry that staining reagents were of little or no avail, chloriodide of zinc being in all cases the most trustworthy. This opinion is, however, only relatively true. The gum behaves differently towards stains when young than when old, and at its incipiency its reactions cannot be made out at all, for the simple reason that it is too soluble in water; the gum passes into solution and is lost. For this stage a differential alcoholic stain would be necessary, and up to the present I have found none. If we cut our sections, on the other hand, from material showing well-formed gum pockets, and in which, even in alcoholic solution, the gum occupies the lacunae as a homogeneous mass, the gum will be found to stain with Böhmer's haematoxylin [1] usually more rapidly than the cellulose walls (*Citrus* in particular) or colour yellow in chloriodide of zinc (*Prunus* and *Citrus*). If now sections are taken through diseased material in which the gum pockets are well developed, and the gum in the lacunae has assumed centrally a yellow tinge, it will be found that Böhmer's haematoxylin stains the gum peripherally (i. e. where colourless), but has little or no effect where it already shows coloration. Chloriodide of zinc in these cases gives no valuable indications at all; it is

[1] For formula vide Zimmermann, A.: Micro-technique, p. 181. Delafield's haematoxylin (cf. Chamberlain, C. J.: Methods in Plant Histology, p. 249) is a more energetic stain, but does not give as good a differentiation.

difficult to say what effect it has upon the already yellowish gum. Lastly, if we cut a section through a gum pocket already old, and in which gum formation has not taken place for some time, and in fact is no longer capable of taking place, it will be found that the gum no longer stains with Böhmer's haematoxylin or chloriodide of zinc, but, on the other hand, is brought out more or less distinctly by lignocellulose stains—orcein and hydrochloric acid, methylene blue, Bismarck brown, fuchsin.

Other stains useful in the study of the early but not nascent stages are Congo red and aniline blue 2 v. Congo red may be used as a counter-stain with haematoxylin as it does not stain the gum; the cell-walls and protoplasm are coloured red, the gum blue. Aniline blue 2 v. stains the protoplasm only and may be used, therefore, more or less as a check upon the other stains.

Suitable material (fruit, non-ligneous stems) may be embedded in paraffin or celloidin—I think the latter preferable as it hardens less—cut and mounted as usual.

Canada balsam or Venetian turpentine may be used as mounting media, but for unstained sections their indices of refraction are somewhat high, and I am inclined to prefer a mixture composed of castor oil 2 pts. and of oil of thyme 1 pt. When, however, the sections to be mounted will bear passage through water, I use Farrant's medium.

5. Histology.

The anatomy of gummosis has been studied (I refer to *Prunus* in particular) from time to time during the last fifty years, and our knowledge of the various appearances that pathognomonic tissues may present is now very complete as regards the later stages of the disease. Of the initial and post-initial stages, however, practically nothing is at present known,—hence very largely the lack of agreement between authors regarding the nature and origin of gum. In taking up in turn the histology of gummosis, I have, therefore, devoted considerable time to the study of these important stages with the view of answering definitely, if may be, the question: Where and how does the gum originate?

In my description of the gross internal characters of twigs, branches, limbs, or trunks affected by gummosis, I mentioned that, when the disease was not sufficiently intense to completely encircle the stem, the pathognomonic tissues were fusiform in outline. In these cases, it will be remembered, I described gum formation as abundant at the centre, but decreasing in amount and finally becoming unobservable as the apices were approached. The tissues at the centre of the sickle have been longest affected by the disease and may have reached the ultimate stage of decomposition, while those nearer the apices only show young, if not the initial, stages of gummosis. One might, therefore, readily imagine an ideal

sickle of pathognomonic tissues in which all stages of gummosis, from the initial to the large and extensive gum pockets, are to be found. In such an ideal sickle of pathognomonic tissue one would be able to follow, beginning at an apex, the malady through its various developmental stages.

Premonitory to the first symptoms of gummosis or coincident therewith, the cambium frequently lays down centripetally cells rich in granular protoplasm, though this increased protoplasmic content of the embryonic wood cells is not necessary, as Mikosch believed, to gum formation. A dense protoplasmic content is not an invariable accompaniment of gummosis, and essentially it is only an indicator of active metabolism. The importance of starch has also been greatly overestimated. It is invariably absent from the young pathognomonic tissues, and I am unable, therefore, to agree with the view that it plays an important rôle in gum formation. The cell contents take no part in the initial stages of gummosis: it will be shown as we proceed that they remain passive at all times. The cell-wall, on the other hand, is the seat of the malady *ab initio* and throughout its subsequent development.

Material fixed, preserved, and examined in 95% alcohol will show perhaps rather exceptionable activity on the part of the cambium, and frequently an increased protoplasmic content of the embryonic wood cells, giving them a pseudo-nidulose appearance. The wood in formation, as in the case of healthy trees, is more or less distinctly enframed between the xylem, the cambium, and the medullary rays. The sections appear normal, and it would be impossible to say that they were not (Pl. X, Fig. 5). If, however, we replace the 95% alcohol by progressively weaker solutions, finally arriving at water, we will notice that while the protoplasm remains unaffected, a change takes place in the cell-walls. The walls appear to stretch and swell, the swelling taking place in the secondary membrane. This change constitutes the initial stage of gummosis and is quite fugacious, being rapidly followed by further and more marked swelling and disappearance of the primary membrane. By further absorption of water the primary and secondary membranes, henceforth indifferentiable, increase in bulk, become semi-fluid and accumulate, preferably it would appear at the corners of the cells, i. e. at the place where a given pressure would be most likely to force the cells apart from one another (Pl. X, Figs. 6, 7). The gum, however, may also accumulate medianly, pushing the cell-walls apart, forming small elliptical cavities. Gummous degeneration of the cell-walls now continues centripetally, the third lamella rapidly dissolves away, the cell contents become part of the gum matrix, which occupies, as a homogeneous mass, the spot but a short time before filled by the degenerating cells. The cells bordering the gum pockets become markedly convex owing to the release of pressure following the disappearance of the abutting cell or cells, and the real period of the growth of the gum pocket may be

said to begin [1] (Pl. X, Figs. 4, 7). Gummosis now spreads more and more deeply into the circumambient tissues. The cells bordering the pocket are sloughed off from the subjacent cells, which then become, in turn, convex on their free ends, and finally loosened and freed by a process exactly similar to that which brought about the first formation of the gum cavity, and which may continue until all the tissue capable of gummous degeneration has been destroyed. The growth of any given gum pocket is, therefore, only limited by the amount of susceptible tissue (embryonic wood cells) laid down by the cambium, though it may develop to such an extent as to destroy the cambium, the medullary rays, and, in rare instances, the bordering cells of the xylem.

Having described in general terms the method of growth of the gum pocket, I shall now consider at some length the changes that accompany and follow the sloughing off of the cells.

The cells freed during the development of the gum pocket may be either totally destroyed or remain apparently unaffected, depending upon the rapidity with which gummous degeneration is taking place. If gummosis develops slowly or has nearly run its course, the tertiary membrane, now the only envelope of the cell, remains unaffected and life continues ; but, if the disease is progressing rapidly, it gelatinizes centripetally and the cell finally vanishes, completely absorbed by the gum surrounding it.

I shall study at some length the cells sloughed off during the growth of the gum pocket.

If one examines, in a suitable medium, sections cut from material fixed and preserved in 95 % alcohol, and in which there are young and actively growing gum pockets, he will observe that the gum pocket either contains homogeneous gum or is more or less filled with a mesh of thick strands, anastomosing in such a manner as to present an alveolar appearance (Pl. X, Fig. 3), which vanishes immediately when water is run under the cover-glass, being replaced by gum. I at first supposed that this structural pattern was a stage in gummous degeneration of the cells ; but a careful study of a number of sections made it evident that this cellular appearance was due to an alveolation of the gum brought about by the fixative used, i. e. 95 % alcohol.

In young gum pockets, cells will sometimes be observed floating in the

[1] According to Delacroix, gummosis begins towards the centre of the pathognomonic embryonic wood cells, enframed between the cambium and the xylem, on the one hand, and two adjacent medullary rays on the other, in *Prunus*, but rather nearer the cambium in the case of the *Citrus*. I have been unable to confirm this observation. Gummosis begins, I have found, now centrally, now more laterally. When, however, it begins near one of the medullary rays the extension of the disease is necessarily more unilateral (the medullary rays only becoming affected in severe cases), and, when more fully formed, the gum pocket may give the false impression of having originated near the centre. Again, if gummosis began somewhat mildly and gradually grew worse as new tissues were laid down, we might obtain the equally false impression that the disease had worked centripetally when, in reality, it had worked centrifugally.

gum. These cells immediately vanish upon the addition of water, but without any perceptible change taking place in their protoplasm (Pl. X, Fig. 6, section through gum pocket observed in alcohol ; Fig. 2, same section after being placed in water : three cells have vanished).

If now we examine a gum pocket which is no longer developing actively, we will observe at its periphery, or scattered promiscuously within it, a certain number of free cells that no longer vanish upon the addition of water (Pl. X, Fig. 8). In studying these cells we find, as hitherto, that the protoplasm plays no rôle in gummosis. The *Citrus* are best suited for the study of this stage as the gum in the pockets does not dissolve very rapidly, and staining may be resorted to. In sections stained with Böhmer's haematoxylin, the gum colours more rapidly than the cell-walls, and the contents of the cells remain unaffected. Sections stained in Böhmer's haematoxylin may be counterstained with Congo red ; the gum then appears blue, as before, but the protoplasm is always coloured red, as likewise the healthy cell-walls. If on the other hand we stain sections in aniline blue 2 v. the protoplasm only will be coloured. With Böhmer's haematoxylin on the one hand, and aniline blue 2 v. on the other, we are able to determine exactly when we are dealing with gum and when with protoplasm. We, therefore, arrive at the conclusion that the cell contents are free from gum and take no part in its formation from the initial stages of gummosis until they finally vanish upon the gelatinization of the tertiary membrane. With the dissolution of this membrane the protoplasm is added to the gum.

We have seen that when a gum pocket is to be formed the following changes take place : (1) a susceptible tissue, in reality embryonic wood cells, is laid down by the cambium ; (2) at no very determinate position the secondary and primary lamellae of certain of these cells gelatinize and form a nucleus, as it were, for the future gum pocket ; (3) the gelatinization of these lamellae detaches the cells one from the other ; (4) the third lamella gelatinizes and the cells vanish in the gum mass ; (5) the gum is composed of gelatinized cell-walls and of the contents of the dissolved cells (i. e. protoplasm), together forming a homogeneous mass. We have still to describe the changes that take place in this susceptible tissue when the growth of the gum pocket is arrested before it has attained its ultimate development, that is to say, before all the cells predisposed to gummous degeneration have become affected.

Following the arrestation of growth of the gum pocket, and this may occur at any time during its development, the cells of the susceptible tissue lignify. The cells growing into the gum, to use figuratively a phrase that has been erroneously employed in the absolute sense, as well as those bordering the gum pocket, together with the remaining cells of the susceptible tissue, gradually thicken their walls. The lignification of the susceptible tissue cells, however, is never as complete as in normal xylem,

and the extent of this tissue formation can always be traced by this character, as well as by the asymmetry of the cells due to the pressure exerted upon them by the gum, which occupies more space than the cells from which it is derived.

Coincidently with the lignification of the susceptible tissues, starch may appear within the cells ; it will be observed also in the cells within and bordering the gum pocket. I have never observed any quantity of starch in these cells myself, though there is no reason for supposing that it cannot accumulate to a considerable extent in them. The starch is laid down in these tissues when the trees are accumulating food reserves ; its production cannot be considered, therefore, a pathological phenomenon.

Before lignification sets in in the susceptible tissues the gum may undergo changes. I have already pointed out that it becomes yellowish in the more highly developed gum pocket and at the same time less soluble than when colourless ; it will also be remembered that at this stage it no longer stains with Böhmer's haematoxylin. I may now add that this loss of power of fixing haematoxylin is brought about by a gradual increase of affinity for lignocellulose stains, and a further decrease in solubility. Finally, after lignification of the enclosing cells has taken place, the gum becomes apparently quite insoluble, and takes the lignocellulose stains as well as the xylem, or even better.

The changes that take place in the gum parallel very closely those that accompany the lignification of the cell-walls, and we may draw the conclusion that, whether we are dealing with the changes that take place in the cell-walls of the susceptible tissue that do not degenerate into gum, or with the gum that has originated from the cell-walls of certain of them, the ultimate changes are similar in both cases ; the cell-walls lignify and the gum assumes all the characters of lignocellulose.

As soon as the susceptible tissue ceases to be affected by gummosis the conditions favourable for its development no longer exist, and the cambium assumes normal activity. Healthy wood cells are formed once more, and a certain development of xylem may have enclosed the gum pocket and surrounding tissues before lignification has set in in them (Pl. X, Fig. 1).

6. Cause of Gummosis.

Prunus and *Citrus* species, broadly speaking, are susceptible to gummosis whenever conditions are favourable for active growth of the cambium. It will be remembered that the disease may be produced either autogenously or by external agents. The autogenous form of gummosis appears to be confined very largely to the cherry and the lemon, and is induced when vigorous growth is accompanied by an excess of water in the substratum. In the lemon and cherry this form of the disease develops severely under these conditions, and is not induced by injuries of one kind or another, as

one may gather from the fact that the outflow of the gum does not correspond to places of injury, as it always does when the latter are the inciting cause. The autogenous development of gummosis is, however, much less common than that resulting from direct injuries to the cambium.

At one time it was believed that gummosis was due to one or, at most, several forms of injury, but to-day it must be admitted that, provided the plant is in the proper condition when wounded, any traumatism will produce the malady. This will be clearly shown in the following examples.

1. Gummosis is produced by a variety of parasitic Fungi, to wit:— *Coryneum Beyerinckii*, which is capable of producing marked gummosis in the peach ;[1] *Clasterosporium carpophilum*, Lev. Aderh.[2], a common parasite of the cherry, plum, peach, and almond ; *Sclerotinia fructigena*, (Pers.) Shroet. ; *Cladosporium epiphyllum* ;[3] *Valsa leucostoma*, Pers. ; *Tubercularia vulgaris*, Tode ; *Botrytis cinerea*, Pers.;[4] *Plowrightia morbosa*, (Schw.) Sacc. ; *Exoascus deformans*, (Berk.) Fuckel.[5]

2. According to the researches of Aderhold and Ruhland[6] on the one hand, and of Brzezinski[7] on the other, it would appear that Bacteria also produce gummosis ; the former have described a species of Bacillus that induces the malady in the cherry, and the latter has isolated a Bacterium from the peach, plum, and apricot which he believes is the cause of the disease in these trees.

3. Certain insects or their larvae also cause more or less copious gumming : the peach-borer, the curculio on the fruit of plums, the larvae of *Tortrix woeberiana*, Shiff.[8] Gummosis may also be produced by piercing the young shoots near the apex with a needle,[9] tearing the bark, knife-wounds, bruising the bark with a mallet. This latter method I have tried experimentally on orchard trees, but not with very marked success. I hammered the bark of peach, cherry, and plum limbs, being careful not to cause rifts. Gum appeared on the surface in 10 % of the wounded cherries and in 50 % of the wounded peaches and plums.

4. Gummosis may be produced in young shoots by burning with a hot iron, and freezing ; chemical agents are, however, very much more effective. I have successfully used sulphuric (Pl. VIII, upper left-hand figure), phosphoric, nitric, and lactic acids ; acetic acid, on the other hand, has given me

[1] Smith, R. E. : Peach Blight. California Agr. Expt. Sta. Bull.

[2] Aderhold, R. : Über *Clasterosporium carpophilum*, &c., loc. cit., ante.

[3] Massee, G. : A Textbook of Plant Diseases, p. 306.

[4] Rant, A. : De Gummosis der Amygdalaceae.

[5] Pierce, N. B. : Peach-leaf Curl : its Nature and Treatment. U. S. Dept. Agr., Div. Veg. Path. and Phys., Bull. xx.

[6] Aderhold, R., and Ruhland, W. : Der Bakterienbrand der Kirschbäume. Arbeiten kaiserl. biolog. Anstalt, v, 1907, pp. 293–340.

[7] Brzezinski, P. J. : Étiologie du chancre et de la gomme des arbres fruitiers. Comptes Rendus, cxxxiv, 1902, pp. 1170–3.

[8] Rant, A. ; loc. cit.

[9] Beijerinck, M. W., and Rant, A. ; loc. cit. ante.

no results, owing probably to the fact that it volatilizes before penetrating the tissues. Oxalic acid appears to have been successfully used by Sorauer.[1] The disease may also be produced by the alkalies, and I have successfully used potassium hydrate. Corrosive sublimate was found very effective by Beijerinck and Rant.[2] I have tried the hydrocarbon kerosene, but apparently it is not sufficiently penetrating.

7. Nature of Gummosis.

We may now inquire, What is the nature of gummosis? In recent years several hypotheses have been advanced to account for the formation of gum within the plant. Beijerinck and Rant[3] supposed that traumatisms, chemical substances, or fungous parasites acted in an exactly similar manner. The cells of the cambium, according to the view of these authors, contain a cytase, which, while they remain alive, is unable to attack the cell-wall, owing to the semipermeability of the protoplasm. When, however, any cells of the embryonic wood are killed by penetrating hyphae, traumatisms, or a toxic agent such as mercuric bichloride, the contained cytase diffuses out and is able to attack the walls of the circumambient healthy cells, which become gummous, and finally dissolve away. More cytase is thus released, attacks other healthy cell-walls, and in this manner the gum pocket is formed. The authors further point out that mercuric bichloride yields more gum than a traumatism; and this they interpret as strengthening their hypothesis, for mercuric bichloride kills more cells than a simple traumatism. As further proof of the existence of a cytase they point out that the hyphae of *Coryneum Beyerinckii* are affected by gummous degeneration, as well as the cells amidst which they are growing.

Certain objections can be raised against this hypothesis of Beijerinck and Rant. If gummosis is due to the action of a cytolytic enzyme diffusing outwardly from necrobiotic cells it seems to me that all sources initiating gummosis should distinctly tend to cause ultimately an equal development of the disease.

Let us imagine, for simplicity's sake, that the embryonic wood cells occupy a plane figure instead of a solid, i. e. a rectangle instead of a parallelepiped. We will call the base of this rectangle B, and its height H. Now if we kill the tissues somewhere within BH so as to have an area of necrobiotic cells equal to BG, then the cytase formed will diffuse in two directions only. But if we kill the tissues at a point P, the diffusion of the cytase will proceed in all directions and gummosis will develop with increasing rapidity until an area BG' is affected. When this stage in the development of the malady is reached its further progress will be neither more nor less rapid than in the first case considered. The ultimate development of all gum pockets should be, therefore, approximately the same.

[1] Loc. cit., ante. [2] Beijerinck, M. W., and Rant, A.: loc. cit., ante. [3] Loc. cit., ante.

It may also be pointed out that the death of a cell is not necessary, as we have already seen, for the development of gummosis. Nor could we, under the cytase hypothesis, have live cells protected only by the tertiary lamella lying unaffected in the gum. Nor does it appear possible to explain the greater extension of the gum pockets upwards from the centre of initiation if diffusion of cytase from the dead cells is the cause of gummous degeneration, for a solution tends to diffuse equally in all directions. The lesser development of the gum pockets as one proceeds laterally of the inciting centre is also difficult to explain, for the medullary rays are very resistant to the disease, and, therefore, arrest lateral diffusion; but allowing, for argument's sake, the intercommunication of all the gum pockets, the asymmetrical oval development of the tissues affected by gummosis is not explainable on the hypothesis of a cytase diffusion from necrobiotic cells. For these reasons, and for others that will be developed later, I am unable to accept Beijerinck and Rant's explanation.

Ruhland's [1] hypothesis that gum is formed by oxidation of the carbohydrate substances within the cells that go to the making of the cross-septa after division, and of the pectins and pectinates of the cell-walls, is not tenable, as a chemical study of the gums clearly shows that they are not oxidation products of carbohydrates; and for the same reason Sorauer's [2] notion that the increased gum flow induced by oxalic acid was due to oxidation is equally erroneous.

It being, therefore, impossible to accept the views heretofore held regarding the nature of gummosis, it will not be out of place to determine whether or not an explanation may be advanced that does not conflict with any of the observed facts. Our task then will be, first, to correlate the observations recorded in the previous pages, and second, to show that the explanation I would substitute for those of Beijerinck and Rant, Ruhland, and Sorauer satisfactorily explains this correlation.

1. For gummosis to develop two conditions must be simultaneously fulfilled. The cambium must be actively laying down new tissue elements, and a superabundance of water must be present in the soil.

Though neither growth nor a superabundance of water can of themselves induce the disease, their rôle in the development of gummosis may be separately discussed if we are careful to continually bear in mind that neither is operative without the other. I shall, therefore, consider under separate captions the relation between growth and gummosis, and the relation between water and gummosis.

Relation between extent of gummous degeneration and growth. When gummosis is artificially produced by means of sulphuric acid, for instance, we find that the extent to which the disease develops is very closely related to the amount of growth taking place at the time of the wounding. Gum-

[1] Loc. cit., ante.

[2] Loc. cit., ante.

mosis develops freely in actively growing shoots, but less readily and extensively when growth is no longer active.

The relation of growth to gummosis is also brought out by a study of the anatomy of the disease. The gum pockets are formed, it will be remembered, in a sickle of susceptible tissue laid down symmetrically on both sides of the wound. This tissue will be found, upon close examination, to show all the characters, especially noticeable in the medullary rays, of having been rapidly formed. The greatest growth will have taken place in the immediate neighbourhood of the wound, the least at the edge of the sickle. The most extensive gum pockets occur necessarily in the area of greatest growth. If now one studies sections taken from diseased shoots that have shown various degrees of growth activity, he will observe that the size of the sickle of susceptible tissue formed is directly proportional to the rapidity of growth; he will also observe that the gum is the more fluid the more sapid the tissues.

The question will be asked, Why is the growth of the susceptible tissues more marked in the neighbourhood of the wound, and less and less extensive as one proceeds nearer to the apices of the sickle? It is generally supposed that this is due to a response of the plant to traumatic stimuli which are necessarily more marked near the place of origin, but an explanation less vague, I think, can also be offered. It is a matter of common observation that, when a ligneous branch is pruned in the proper position with respect to a bud, a callous tissue forms and the bark becomes slightly raised; again, when the trunk of a vigorously growing tree is slit longitudinally, marked growth occurs, and the two lips of the bark are pushed apart. In these two cases, what, in reality, takes place? It appears to me simply this. The cambium is capable of laying down new tissue elements with extreme rapidity under favourable conditions of growth, but, owing to the pressure exerted by the cortex, the number of cells laid down in a given time are fewer in number than would be the case if the pressure were removed. When, therefore, through any kind of a wound, the pressure exerted by the cortex is more or less greatly reduced the genetic power of the cambium proportionally increases. As the pressure of the ruptured bark from approximately zero will increase gradually as one passes away from the wound, finally reaching the height it had attained before the release was effected, it must necessarily follow that the activity of the cambium will suffer a gradual reduction, and we are prepared to understand the fusoid development of the susceptible tissues. In a similar manner we will be able to explain the greater length attained by the gum pockets upwards from the point of initiation than downwards. The growth of the cambium depends on the amount of elaborated material that the phloem is able to supply, and it must necessarily follow that when it is destroyed the cambium below the lesion must decrease in activity. The amount of

susceptible tissue produced below the wound will be less extensive than that which is laid down above. An elliptical development of xylem tissue round a wound is, therefore, a perfectly normal phenomenon, but whenever the tissues are at the same time gumogenetic, as in *Prunus* and *Citrus*, we must consider this character as an acquired one. Growth and gummosis are nevertheless directly correlated.

Relation between extent of gummous degeneration and water. The relation between gummosis and growth is direct, the relation between gummosis and sapidity of the tissues indirect. Sapidity of the tissues is insufficient in itself for the development of the disease, but as soon as it is combined with the necessary factor, growth, we may say that its rôle changes : it will then govern the extent of gummous degeneration.

The effect of a high water-content of the soil on increasing the susceptibility of *Prunus* and *Citrus* to gummous degeneration has been mentioned by most writers on gummosis, its significance, peculiarly enough, only being overlooked by those who have studied the histology of the disease. Amongst this latter class, however, we find an exception in Trécul,[1] who states that the disease arises from a too abundant accumulation of sap at any point, this accumulation being due to a variety of causes, rain being the only one that he specifically mentions.

In one of Aderhold's[2] infection experiments with *Clasterosporium carpophilum*, we find it mentioned that the gum produced in infected wounds largely increased after watering. The trees experimented on were peach, apricot, and cherry.

In the case of *Prunus* the horticultural writers agree that excessive soil moisture favours the development of the disease. Gummosis occurs in retentive, poorly drained soils; in non-clay soils underlaid by an impermeable subsoil and unfavourably situated as regards drainage; under abnormal climatic conditions, such as a very wet spring, or long sequence of cloudy weather inducing a rupture of equilibrium between transpiration and absorption.

In the case of the *Citrus* the opinions of the writers who have studied the disease in this genus are no less emphatic. Excessive humidity of the soil, they all agree, is very favourable to the development of gummosis. Whether the trees are grown in poorly drained retentive clays, or upon an impermeable subsoil, or are subject to excessive irrigation, the result is the same—the trees succumb to the disease in a comparatively short time. The climatic conditions favourable to the development of gummosis in *Prunus* are also conducive to its outbreak in *Citrus*.

It is, therefore, clear that growth and a high water-content of the tissues are necessary for gummous degeneration. We have now to determine to what degree our studies on the nature and origin of the resulting

[1] Loc. cit., ante.

[2] Loc. cit., ante.

gum are satisfactorily explained on the basis of a growth and water relation.

In our study of the gums it was clearly brought out that they were composed of hydrated carbohydrates and nitrogenous matters. From our histological studies we are able to assign the origin of the hydrated carbohydrates to the cell-walls, the nitrogenous matters to the cell contents. The power of diseased cell-walls to swell upon the addition of water, to contract on dehydration, and of the gum to change from a hemicellulose, when first formed, to a xylose-like substance with the lignification and dehydration of the surrounding tissues, all these facts point to the conclusion that gummosis is due to a hydration of the embryonic wood cells. The importance of the water relation is, therefore, capital. If not enough water is present in the tissues to completely hydrate the affected cell-walls, we will find cells floating free in the gum, or only partly detached from one another, in fact any of the changes described in the section devoted to the histology of the disease.

Growth and high sapidity of the tissues are essential for the development of gummosis. Must we assume also the concomitant action of a cytolytic enzyme? Let us see what the consequences of such an assumption would be.

If we suppose that the gum is formed as a result of enzyme action in conjunction with the essential factors, growth and water, we would find serious difficulties in explaining the unilateral excretion of the cytase, the form that must be assumed if we are not to enter into conflict with the conditions described in our histological study of the disease. Again, the cytase excreted from the protoplasm would necessarily permeate the third lamella, and, while hydrolysing the secondary membranes, would also be acting upon it. But microscopic observation shows that gummous degeneration proceeds centripetally in the cell-walls, and yet we know that the cells, under certain conditions, will vanish instantly when sections are placed in water, without the third lamella showing signs of diffusing into the cell lumen. If an enzyme does induce, in the presence of water, hydrolysis of the cell-walls, for it to act in the manner observation shows that it does it would have to attain the power of action only after having diffused out into the secondary and primary lamellae, thus only being able to attack the third membrane, which it permeates, after having hydrolysed the former, and that in a centripetal manner. Such a mode of action, it must be admitted, would be indeed very peculiar. Again, if the gum originated through enzymatic hydrolysis of the cell-walls, we would have to suppose that, with a change in the water or growth relation, it was acted on by another enzyme excreted from the cells, and transformed gradually into an insoluble xylose-like compound. The cells of the susceptible tissue would first have to excrete a hydrolysing enzyme, and then an enzyme capable of bringing

about recondensation. Such phenomena of solution and recondensation are perfectly well known in the plant kingdom, but I think that to ascribe gummosis to such an analogous process is hardly warrantable. It seems to me, therefore, that gummous degeneration is to be explained in some other way than by assuming the pathological action or excretion of an enzyme acting in the presence of the factors growth and water. But how shall we explain it? I am inclined to believe that were the genesis and development of the cell-wall better known gummosis could be explained on a purely physico-chemical basis, but to venture to do so in the present state of our knowledge would have but a single issue: failure.

8. Preventive and Remedial Measures.

It will be well, before discussing the preventive and remedial measures employed against gummosis, to recapitulate briefly the conditions under which the malady develops.

Gummosis affects trees planted in moist retentive soils and poorly drained soils; excessive irrigation is inducive in any soil, but particularly so in those that, either from their situation, the nature of their subsoil, or their physical composition, are classed as moist soils. Trees planted in rich soils, or highly fertilized, especially with nitrogenous manures, are more subject to the disease, *ceteris paribus*, than those grown in poorer soils and in orchards less intensively cultivated. Plantations in which intercalary cultures requiring a large amount of water and high fertilization, i. e. vegetables, are grown soon become decimated. Various parasitic Fungi, boring and gnawing insects, wounds due to pruning, hailstones, sun-scald, accidental abrasions during cultural operations due to ploughing, cultivating, or fruit-gathering, inordinate pruning during vegetative activity, unequal growth between stock and scion, planting the trees in such a manner that the union between stock and scion occurs below ground—to all these various agencies gummosis has been justly ascribed. In the following remarks on preventive and remedial measures, however, some of these causes of gummosis will not be further considered. When the cause of the disease is traceable to Fungi or insects, sun-scald, or abrasions incidental to cultural operations, the remedies are obvious, and therefore need no particular consideration.

Preventive measures. As gummosis does not occur unless the affected trees are growing rapidly and their tissues are, at the same time, sapid, there will be four possible methods, and they have all been employed, of preventing the development of the disease.

1. Drainage, and, when irrigation is practised, more rational distribution of the water. In draining an orchard, or land intended for an orchard, care should be taken to make the drainage efficient; but whether this shall be accomplished by tile draining, open ditches, by growing the trees on ridges, or by combining two or more of these methods, is a question

which the grower must solve for himself. I may observe, however, that excessive humidity being harmful as regards gummosis only during the vegetative period, it is quite evident that the distribution of the rainfall throughout the year will have to be considered in planning a drainage system.

As regards methods of irrigation, the grower should aim at maintaining in his soil the proper moisture for growth by frequent irrigations. Infrequent and copious irrigations should be avoided, as they produce recurrent periods of saturation favourable to the development of gummosis, which, under such a cultural regimen, if once induced is particularly difficult to cure. In heavy retentive soils flooding ought never to be used and should be replaced, where employed, by a furrow system of irrigation. In light well-drained soils flooding may, of course, be used without danger, which is fortunate, as frequently it is the only satisfactory method of irrigation for such soils.

2. Resistant stocks. It was early observed that, *ceteris paribus*, the various species of *Citrus* differed widely in their resistant power to gummosis, but in *Prunus* we have absolutely no reliable knowledge regarding the susceptibility of the various species. It has, however, been pointed out by M'Intosh that grafting the cherry on a stock that did not develop equally with the scion was more conducive to gummosis than when they were of equal growth, and there is no reason for assuming that the same observations would not apply to the other species of *Prunus* and *Citrus*.

The relative susceptibility of the different species of *Citrus* to gummosis has only been empirically determined, and, consequently, the exact position certain of them should occupy is a matter of some doubt. One may expect, therefore, that careful study will reveal certain minor inaccuracies in the following resistance scale :—

Citrus trifoliata, maximum resistance.
C. amara
Rough Lemon (*C. Limonum* × *C. decumana?*)
C. Limetta
C. bergamia
C. nobilis
C. decumana
C. Aurantium
C. medica
} intermediate resistance.
C. Limonum, resistance zero.

As resistance stocks *C. amara* and Rough Lemon are the most used at the present day. It would seem that *a priori* the stock most resistant to gummosis should be used exclusively, but it is found in practice that another factor is of immense importance. The cultivated *Citrus* frequently form better trees on one stock than on another, hence the necessity of

sacrificing, even at some hazard, a certain amount of resistance power for better affinity. It should also be pointed out that in soils in which *C. Limonum* on their own roots are only slightly affected by gummosis, there is no object in using the very resistant *Citrus* as stocks; the question of affinity should alone be considered.

3. Moderating growth by root-pruning. This method of preventing gummosis is mentioned by M'Intosh and *per se* is unobjectionable. The difficulties of applying it and the complications liable to result from its injudicious use are, however, sufficient to prevent it being ever more than a gardener's palliative.

4. Amendments. The amendment mostly employed in preventing gummosis is lime, and its use is advocated by a number of horticulturists. Its beneficial action is probably due to the increased porosity of the soil following the flocculation of the clay. Salt has also been recommended by Van Hecke, who observed in China a peach orchard which, though covered at flood-tide by salt water, remained quite free from gummosis, whereas an orchard irrigated by river water was badly affected. Van Hecke was particularly struck by the fine appearance of the orchard submerged at flood-tide, and, upon his return to Belgium, tried the following instructive experiment. He selected[1] four peach-trees growing under similar conditions, and treated them as follows immediately before the opening of the buds:—

No. 1. Received 1,500 grm. salt.
No. 2. Received 1,000 grm. salt.
No. 3. Received 500 grm. salt.
No. 4. Received 0 grm. salt (witness).

The result of the experiment was most striking; the witness gummed considerably and even lost several branches, the trees that received one half and one kilo of salt respectively were only very slightly diseased, and the remaining tree was not affected. Van Hecke's observation and subsequent experiment remind one of the remark of Downing's that salt is one of the best fertilizers for the plum.[2] Salt as a preventive of gummosis could be made, it seems to me, the subject of further inquiry.

Besides the major methods of preventing gummosis just described, there are several minor ones that are deserving of mention.

1. In the district of Carcagente,[3] Spain, where the soil is somewhat sandy and very permeable, and the orchards are presumably not over irrigated, as the water has to be drawn from rather deep wells, the oranges, grafted on limes or citrons, are planted sufficiently deeply for the scion to

[1] Hecke, E. van: Le sel de cuisine et les arbres fruitiers à noyau. Journ. des Soc. Agric. Brabant et Hainault, 30 mars 1907.

[2] Downing, A. J.: Fruits and Fruit Trees of America, p. 266, ed. 1850.

[3] Vide Trabut, L.: L'Oranger en Algérie. Direction de l'Agriculture gouv. gén. Algérie, Bull. 44, 68, 1908.

develop adventitious roots. When these roots have become sufficiently stout to support the tree, the soil is removed from around the crown, and the trunk is severed immediately below their insertion. The basin formed by removing the soil from around the crown of the tree is not filled in, and the base of the trunk remains permanently exposed to the air. The irrigation water is, of course, never allowed to flow into this basin, and the rains are never sufficiently abundant to maintain a dangerous humidity round the roots. This method of the Carcagente growers has, horticulturally, its justification ; oranges produce better fruit and grow to best advantage on their own roots, or, which is the same thing, when grafted on orange seedlings. From the physiological point of view the increased resistance to gummosis obtained by growing the oranges with their crowns above ground is probably due to the fact that no change in the pressure of the bark upon the cambium, which is diminished when the trunk comes in contact with soil, occurs. When kept moist the bark becomes more sapid and, therefore, more elastic ; the cambium develops more freely, susceptible tissue may be laid down, and gummosis follow.

2. It was early observed that the resistance of the *Citrus* to gummosis when grafted on appropriate stocks was markedly increased by high budding, and this method of still further reducing susceptibility to the disease has been advocated by the best writers on *Citrus* culture. High budding is successfully and extensively used at the present day. The fact that high budding in itself is capable of ensuring relative immunity to gummosis, even when the stock used is of low resistance, does not appear, however, to have been sufficiently emphasized. For instance the lemon, badly affected by the disease when budded low on the orange, will be quite resistant, *ceteris paribus*, when budded high on the same stock.

Remedial measures. The important preventive method drainage is also remedial, not an absolute remedy in all cases, but unquestionably the only one, under conditions favourable for the development of gummosis, that is really efficient and can effect *per se* a permanent cure. The conditions necessary for the development of gummosis are growth and a superabundance of water in the soil, or in other words very sapid tissues. Without the concurrence of the two essential factors growth and water, the development of gummosis to any marked extent cannot take place. The importance of drainage as a remedial measure is, therefore, capital. That in certain soils and situations, under particular climatic conditions or cultural methods, drainage does not prove an absolute safeguard is to be expected from the nature of the office it has to perform.

Besides drainage a number of remedies have been proposed for gummosis, but they are in reality alleviations only. I may mention root-pruning, which has already been referred to as a preventive, and slitting the bark of affected trees. This latter method is very generally and

successfully employed in cases where trees are only subjected at one season of the year, or irregularly and accidentally, to conditions favourable to the development of gummosis. The bark of the affected trees may be slit cross-wise, longitudinally, or spirally. For cases of generalized gummosis this latter method is the only efficient one.

It is a common practice in the treatment of gummosis, and one that has been advocated in the literature, to cut the bark away on each side of the exudate until clean healthy tissues are reached, removing at the same time all infiltrated and discoloured wood. The wounds thus formed, and they frequently grow to an immense size, are then covered with wax, iron sulphate solution, a mixture of tar and phenol, Saint-Fiacre ungent, and a variety of other substances. This practice has, however, like most empirical remedies, more objectionable than unobjectionable features. The removal of the discoloured infiltrated wood is absolutely without justification, no matter how severely the trees may be affected by gummosis, and cutting away the bark is only advantageous after it has been killed, for we know that as long as the cambium remains alive it is capable, upon the re-establishment of normal conditions, of laying down xylem again immediately over the susceptible tissues or the gum masses resulting from their degeneration. The removal of the cambial layer while it is still alive is, therefore, a serious error. Dead bark, on the other hand, should be cut away as a matter of hygiene, and also that the healing tissues may grow more easily over the area covered by it.

The tissues exposed by the removal of the dead bark may be covered with shellac, or any other suitable substance provided it contains neither free acid nor free alkali.

Summary.

I. Gummosis of *Prunus* and gummosis of *Citrus* are indistinguishable maladies. They are identical in histological development; they are identical in their causal relationships; no species in either genus is entirely immune to the disease, though in both the malady predominates in one (*Citrus*) or several species (*Prunus*); in both genera we find species in which the malady is predominately one of the fruit (citron and Saucer Peach)—in a word, any manifestation of gummosis observed in one genus will be found in replica in the other.

II. Gummosis is due to hydrolysis of the walls of the embryonic wood cells, which develop into a susceptible tissue. The dissolution of the cell-walls begins in the secondary lamella and almost coincidently in the primary membrane; the dissolution of the third lamella proceeds centripetally, and with its final destruction the cell contents become a part of the gum mass.

III. The cell contents are at no time actively concerned in gum

formation. Starch, it should be emphasized, contrary to the prevalent view, plays no rôle whatsoever in gum formation.

IV. The secondary lamella of the wood fibres and that of the vessels, as well as parenchymatous tissue, may show gummous degeneration in severe cases of gummosis when they are near the zone of active development of the malady.

V. Gummosis develops autogenously and is induced by all manner of traumatisms, provided they act directly or indirectly as growth stimulants to the cambium. Once incited the simultaneous concurrence of two conditions, one physiological, the other environmental, is necessary for the development of the disease; the cambium must be actively growing and an abundant supply of water must be available to the roots; either factor is inoperative alone.

III. SQUAMOSIS.[1]

Syn.: Scaly bark.

1. Historical.

Squamosis is at present only known in the *Citrus* groves of Southern California and Florida. The malady was probably first observed in California prior to 1880, but it has always remained so inactive that little or no attention has been paid to it. In Florida, on the other hand, squamosis has recently excited some interest, though its general behaviour in that State must be very much the same as in California, since it was first observed there about 1860.

2. Description.

General. Squamosis is primarily a disease of the orange-tree; in fact, the malady does not appear to have been observed on the other *Citrus*. The disease always develops sporadically, and the conditions that favour its development appear only to occur at infrequent and long intervals of time. I have a note regarding an orange grove in which, during a period of twenty years, no new trees became affected.

Besides the infrequency of its appearance in *Citrus* groves, squamosis has another marked peculiarity: the disease develops extremely slowly on the affected trees. The scaling of the bark, which is the striking and characteristic symptom of squamosis, first appears as a single scale or group of scales upon the trunk or limbs, the area of affected tissues gradually growing from this small beginning until the trunk or the limbs are completely girdled. An affected tree may live for fifteen to twenty years, but

[1] Bibliography.

Smith, R. E., and O. Butler: Gum Disease of *Citrus* Trees in California. California Agr. Expt. Sta., Bull. cc, 1908, pp. 256–63.

Fawcett, H. S.: Report of Assistant Plant Pathologist. Florida Agr. Expt. Sta. Report, 1907.

————: Scaly Bark of *Citrus*. Florida Agr. Expt. Sta., Bull. xcviii, 1909, pp. 75–80.

this probably only occurs when the disease begins in the trunk, for if it starts in the smaller limbs these latter are soon girdled and chlorosis sets in, followed later by progressive death from the apex downwards (Pl. VII, lower figure. Squamosis on limbs has practically destroyed one half the tree). When the disease begins in the trunk or large limbs chlorosis appears very much later and is much less intense; in these cases the foliar appearance of diseased trees is not very indicative. In fact one might say that squamosis under these conditions, may be present in a grove without the affected trees showing, to the casual observer, any pathognomonic symptoms, a scaling of the bark, though abnormal in the *Citrus*, being sufficiently common in trees in general not to excite any particular fears, especially since the disease is not correlated with definite environmental conditions. Squamosis may be observed in light soils and heavy soils, in dry soils and moist soils, but apparently never when the conditions are favourable for the development of gummosis; it is, however, a form of this latter malady, and from this fact we are necessarily led to the conclusion that its development must also depend on a growth and water relation.

Minute. The characteristic of squamosis, i. e. the scaling of the bark, has already been briefly mentioned. I shall now describe in greater detail the symptoms of the disease.

Squamosis first appears, as has already been mentioned, as a rounded or an irregular exfoliation of the bark, about an inch or somewhat less in diameter, which is pushed out by the growth of the subjacent tissues upon which it stands in slight *alto rilievo*. The detached bark soon dies from the periphery inwards, and curls more or less. The curling of the dying bark reveals the subjacent cortical tissues, which appear rugose, white, or white with a yellow tinge, and somewhat mealy. When the bark finally falls a more or less highly developed pustule will be found occupying the centre of the exfoliated area. The centre of the pustule is navicular, and gum infrequently oozes from it in small amount. Not infrequently the pustular outgrowth, especially after the disease has attained a certain development, becomes less prominent and is replaced by a general swelling of the sub-epidermal cortical layers which causes the bark to flake off in large strips (Pl. VIII, upper right-hand figure). In Florida the shoots are also affected by squamosis, but in California this form of the disease is confined to water sprouts coming from the neighbourhood of diseased tissues. The shoots are discoloured subepidermally into rather well-defined shield-like maculations somewhat raised above the surface. With age these discoloured maculations become indurated, darker in colour, and further raised above the normal surface of the bark. The epidermis ruptures around the diseased areas, which then present, even more markedly than before, a scuteliform appearance. When the shoots are more seriously affected the maculations lose their definiteness of outline, become larger, and the

epidermis apparently less brittle, for it rifts within the diseased areas and not at their boundary. The rifts are parallel to the axis of the shoot, more or less numerous, and inclined to be labiate. In these cases the pathognomonic tissues form marked swellings on the shoots.

When diseased limbs are observed in cross-section they will appear more or less zoned with brown, and rings of growth will be quite prominent. The dark zones indicate the beginning of a new growth annelid. In some cases, however, the zonation may be somewhat masked by a more general discoloration of the wood. The innermost zone of brown indicates when squamosis first affected the tree, but the innermost extent of wood discoloration should be considered as indicating a certain amount of centripetal infiltration. In the youngest wood it will be observed that the pustules are composed of an outer layer of normal xylem enclosing a thin-walled xylem parenchyma ; the whole structure would remind a mycologist of a young sorus. When the shoots are examined in cross-section they may show a similar zonation to the older branches, but instead of many dark circles or partial circles, there will be generally only one, sometimes two, rarely more ; the bark shows infiltrated yellowish brown areas which appear to be cut off by suber, and, when the shoots are old enough, rings of growth are plainly discernible.

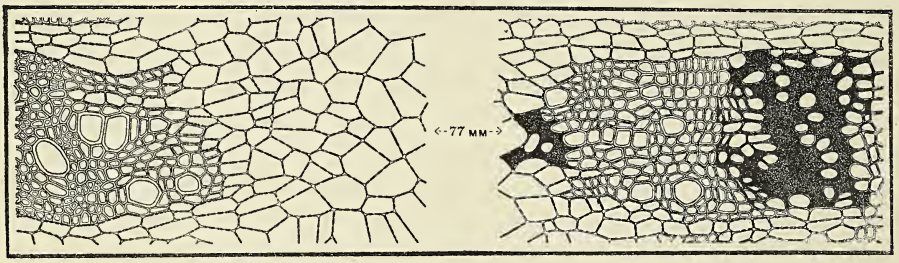
3. Histology.

I have mentioned that squamosis has characters in common with gummosis; we have also seen that it has characters particular to itself. In the following histological sketch, I shall consider squamosis in the former rather than in the latter aspect.

Gum pockets are the histological characteristic of squamosis. As in gummosis, they are formed in a susceptible tissue, but do not develop to the same extent except in transitional stages. In squamosis the gum pockets are typically inextensive, and the medullary rays are not, as a rule, affected; nor do the cells become totally destroyed to the same extent as in gummosis. The secondary and primary membranes are affected by gummous degeneration, but only a few cells suffer complete dissolution. The failure of the gum pockets to develop as in gummosis is undoubtedly due to the fact that conditions favourable to gummous degeneration are transient, as is shown by the gum in young pockets being largely insoluble and soon tinged with yellow. Furthermore, lignification soon begins in the susceptible tissue, and no sharp distinction between it and the xylem can well be drawn ; one might readily be deceived by the appearance into believing that it was xylem parenchyma that suffered gummous degeneration and not embryonic wood cells.

Circular rows of the gum cavities are produced with considerable regularity at the beginning of every new annelid of growth, and it is, therefore, to them that the zonation of the xylem is due.

In the trunk and limbs, besides the gum pockets just described, we also find, corresponding to the pustules, large almond-shaped areas of susceptible tissue being laid down, degenerating somewhat and then lignifying. Over this tissue normal xylem may be formed, or the cells, while regularly laid down, may remain comparatively thin-walled and show rows of gum pockets wherever an increase in the rapidity of growth takes place. It should be noted, *en passant*, that localized areas of rapid growth occurring quite close together may be frequently observed in the xylem without the accompaniment of gummous degeneration, thus proving that the diseased trees function most irregularly.



TEXT-FIG. 2. Cross-section through edges of an almond-shaped area, showing development of ligneous parenchyma and one of a row of gum pockets beyond which lies the cortex (not shown).

When the xylem appears discoloured to macroscopic vision this is due largely to infiltration, though it appears that, in some cases, the middle lamellae of the vessels show gummous degeneration, as likewise those of the wood fibres.

In the bark the tissues die from the periphery inward, the cells being largely occluded by a homogeneous yellowish brown mass, and are cut off by suber or a proliferation of the subjacent cortical cells.

4. Cause of Squamosis.

Gummosis, as we have seen, may be brought about by various agencies: in the case of squamosis, however, we are still ignorant as to the cause of the malady. It may be an autogenous disease, though it would appear from the manner in which it develops, especially on the trunk and limbs, that it is due rather to bark-binding, the particular symptoms of the malady being induced by the manner in which the pressure on the cambium is released, the release occurring at a time when the affected tree was growing rapidly and the tissues were quite sapid. This explanation may perhaps also apply in the case of the shoots, seeing that the irregularities of growth distinctly shown in the older parts of diseased trees may also be seen in them. Added the death of the bark from fungous or other injury introducing changes of pressure on the cambium and we can conceive how the

pathognomonic structural characters of the xylem arise. Squamosis, however, must be further studied before its cause or causes can be definitely ascertained. We are only certain of one thing: the disease is a form of gummosis, and consequently growth and water are factors in its development.

5. Preventive and Remedial Measures.

Little is known regarding the remedial and preventive methods for squamosis. The only preventive measure that appears, at present, to offer any guarantee of success is growth regulation. This may be accomplished by cultural methods. As for remedies, there are at present none. Slitting the bark may be considered a palliative, nothing more.

IV. EXANTHEMA.[1]

1. Historical.

Exanthema is at present only known in the United States. In Florida it is found throughout the orange-growing region, and has probably been a malady of the *Citrus* in that State since the introduction of their culture. In California exanthema is only known in the orange groves of Los Angeles, San Bernardino, and San Diego Counties, Southern California, and, as in the case of Florida, the date of the first appearance of the malady does not appear to have been recorded.

2. Description.

General. According to Hume, exanthema affects all varieties of the *Citrus*. Young trees—the malady has even been observed in nursery stock—and old trees are equally susceptible. The malady develops in California either on very permeable granitic soils or in shallow clay soils underlaid by an impermeable subsoil, in other words, in dry soils. In Florida the soils are typically sandy and belong, therefore, to the class of permeable dry soils. In that state the disease is worst when the soils are poorly drained or underlaid by an impermeable ferruginous sandstone, but it is also found in groves growing on the best 'hammock' soils.

Minute.[2] Exanthema is characteristically a disease of the small branches and shoots, though the fruit shows symptoms of diagnostic value.

[1] Bibliography.

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[2] Largely after Swingle and Webber, loc. cit., p. 16 et seq.

A luxuriant growth, a deep green colour of the foliage, and thick-skinned fruit, though not in themselves specific, precede the first symptoms of the disease sufficiently frequently to possess the value of an indication. Exanthema, however, cannot be diagnosed until the shoots become more or less stained sub-epidermally by a yellowish brown material and begin to die back; or the fruit becomes similarly stained and the epidermis so indurated that it cracks and splits, due to the pressure of the developing pulp-cells. Either one or the other of these symptoms must be observed before exanthema can be accurately diagnosed.

When conditions are not very favourable for the development of exanthema, an affected tree may live for years without developing any further pathognomonic symptoms; when conditions are favourable for its development, however, further changes take place, especially in the shoots and branches. When young the shoots swell at the nodes (Pl. VIII, Fig. A), infrequently on the internodes—the homologues of these swellings are sometimes observed on the fruit, but as they become more mature linear erumpent pustules break out on the internodes (Pl. VIII, Fig. C); these pustules may even alternate with nodal swellings. On the older branches the nodal swellings are not observed, but the pustules may become exceedingly numerous, and a small amount of gum may be observed in them. Gum may also be observed exuding through the bark in small amount. On shoots and branches that are not covered with swellings and pustules, there frequently occurs a marked proliferation of young buds (Pl. VIII, Fig. D), which may develop into short branches with chlorotic foliage, thus producing a pseudo witch's-broom effect (Pl. VIII, Fig. B).

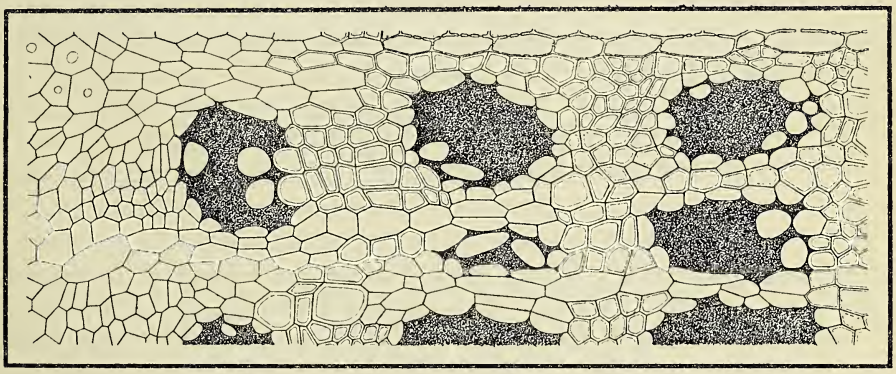
When diseased shoots are examined in cross-section it will be observed: (1) that the swellings are due to an accumulation of gum and are entirely similar to those sometimes met with in gummosis; (2) that the pustules are produced by a proliferation of the cortical tissues, accompanied by the formation of a sickle of susceptible tissue containing gum pockets. It will also be observed that rings of growth are extremely marked, much more so than in squamosis, and altogether too numerous to be considered variations in growth due to seasonal changes.

3. Histology.

A cursory histological study of the swellings and erumpent pustules—in other words, the most striking and typical characters of exanthema—is sufficient to show that this malady is very closely related to gummosis; in fact, were it not for the erumpent pustules, it would be impossible to point out any essential anatomical differences between the two maladies. The swellings are nothing more nor less than well-developed gum pockets in which the gum has accumulated to a considerable extent, it not having been able to break through the epidermis. These gum pockets are not only

histologically identical with those formed in gummosis, but are even found, to my knowledge, on the shoots of young trees affected by this malady. This form of exanthema need not, therefore, be further studied.

In the development of the erumpent pustule we meet with a character not observed in gummosis. Owing to the pressure from beneath, the epidermis ruptures and the cortical cells begin to proliferate, the proliferating cells, at first sub-epidermal, gradually extending deeper into the cortex until they finally make up all the tissue exterior to the last formed row of pericyclic fibre bundles. With the beginning of the proliferation or coincident therewith, a sickle of susceptible tissue is laid down by the cambium and gum pockets are formed, in the manner described in our histological study of gummosis, between the medullary rays, which themselves are rarely involved in gummous degeneration. When large pustules are pro-



TEXT-FIG. 3. Cross-section through sickle of pathognomonic tissue showing development of gum pockets in rows, and separated from one another by more or less perfectly formed ligneous cells.

duced the sickle of susceptible tissue laid down may become relatively thick at its middle point, and contain several rows of gum pockets more or less perfectly separated by a fillet of tissue not affected by gummosis (Pl. IX, Fig. 4; Text-fig. 3). The growth in thickness of the sickle of susceptible tissue is thus clearly due to a period of normal or subnormal growth being rapidly followed by the further laying down of gummogenetic tissues. Such a peculiar development as this, and one in every respect homologous, may be observed infrequently in gummosis.

When the shoots affected by exanthema show typical gummosis characters (Pl. IX, Fig. 1), normal wood may re-form over the gum pockets, but I have not observed it laid down *de novo* over the pathognomonic sickles. Lignification of the cells of the susceptible tissue (sickle formation) begins with the cessation of the conditions favourable to gummous degeneration, but is less perfect than in squamosis or gummosis.

From the brief sketch of the histology of exanthema that I have just given it appears evident that were it not for the erumpent pustules this malady would be undifferentiable from gummosis. I am inclined to think that the proliferation of the cortex may not be a differentiating character of very great importance. May it not be simply due to the epidermis becoming inelastic and preventing for a time normal cellular division, which is able to proceed with vigour the moment a rupture occurs? At the time the rupture in the epidermis takes place there is every reason to believe that metabolism is unusually active, and the tissues at the same time very sapid —gummous degeneration which only occurs, as we know, when the cambium is active, and when the plant has an abundant supply of water at its command, is an evidence of this—and that owing to this concurrence the cells of the cortex become meristematic the moment pressure is released. With the release of pressure due to the rupture of the epidermis the growth stimulus should be transmitted centripetally and only to a slight extent tangentially if my hypothesis were correct, and we have seen that this is exactly what happens.

4. Cause of Exanthema.

The conditions favourable for the development of exanthema, as well as an histological study of the tissues of diseased trees, indicate that the malady is induced, like gummosis, by the concurrence of active growth and sapid tissues. In the case of the latter malady these conditions prevail for a period of time, but in the case of the former they are extremely transient.

The soils in which exanthema occur are typically dry soils, which, when saturated by irrigation water or rains, promptly become dry once more when the weather clears or irrigation is discontinued. The rings of growth, which, as we have seen, are very marked in diseased shoots and branches of trees affected by exanthema, could not be caused except by a more or less rapid succession of maxima and minima of growth, and such an alteration could only be accounted for by synchronous changes in the amount of available water present in the soil, or a like succession of favourable and unfavourable climatic conditions. Obviously climatic changes cannot be considered as in any way favouring or inhibiting the development of the disease, and we have no alternative but to assign the unusual and marked development of rings of growth to changes in the water relation.

Exanthema has another character in common with gummosis: trees are *ceteris paribus* much more severely affected in rich soils than in poor soils. In Florida it is even considered that heavy fertilization with organic manures, such as cotton-seed meal and dried blood, is alone sufficient to cause the appearance of the disease in healthy trees. This opinion is in a certain sense well founded, for nitrogen is a growth stimulant, and would, therefore, tend to widen the range between the maxima and minima of

growth in the various rings; and it is quite conceivable that the widening of this range could be sufficient to produce exanthema. The inorganic manures are reputed to be less favourable to the development of exanthema than organic manures, and it is altogether probable that this is due to the solubility of the former. Nitrates are soon carried into the drainage waters and lost, whereas the organic nitrogen must first be nitrified, and this only occurs under suitable conditions of soil moisture, conditions which would be realized when the available water in the soil became sufficient to promote vigorous growth in the trees. Hence the bad effects following the use of organic manures.

Swingle and Webber[1] observe that cultivation increases the susceptibility of the *Citrus* to exanthema, and even causes a more virulent outbreak of the disease in affected trees. They explain this effect of cultivation on the ground that the surface roots are destroyed and the trees forced to grow down into an unfavourable subsoil, but this supposition is not well founded: cultivation simply increases nitrification and, when deep enough, prevents excessive evaporation of water from the soil. We must, therefore, consider in humid climates nitrification due to cultivation as a possible source of danger and one not to be neglected if no cultivation is as effective a prophylactic measure as it appears to be.

5. Preventive and Remedial Measures.

All the preventive measures used in combating exanthema are also remedial; similarly, the remedial measures are all prophylactic. It is unnecessary, therefore, to distinguish between the two.

To successfully combat exanthema one must prevent the irregularity of growth which is such a marked feature of the disease. The methods employed for attaining this end are quite various, and sometimes even apparently conflicting. I shall not attempt to exhaust the list.

When exanthema is traceable to the excessive use of organic manures we know that for these manures to be inducive sufficient available water must be present in the soil, and we have two cases to consider: (1) exanthema does not appear when the groves are not fertilized, and (2) the malady is present in a mild form when manures are not employed. In the first case drainage would be beneficial though not absolutely essential; in the second drainage would be indispensable, and in both the amount of nitrogen furnished the trees should not exceed their immediate requirements. The nitrogen would probably be most advantageously obtained from green manure crops, though the various other sources are not precluded. Heavy fertilization with potassic and phosphatic manures, which salts act somewhat as growth restrainers, is, in many cases, desirable, and has proved effective.

[1] Loc. cit., p. 20.

In the arid regions where the trees depend on irrigation for their supply of water, and the appearance of the disease is traceable to this source, there will be again two methods of combating the malady: (1) in very permeable soils the periods between irrigation should be shortened and the humus content of the soils increased by green manure crops; (2) in shallow clay soils underlaid by an impermeable subsoil the periods between irrigation should be shortened and the amount of water given at any one time reduced as much as possible; in these soils drainage would undoubtedly be beneficial.

I have already mentioned that exanthema develops more readily in highly cultivated groves than in those which are less perfectly tilled. Swingle and Webber[1] observe that groves planted in soils underlaid by an impermeable subsoil, and which succumb to the disease when cultivated, may be successfully maintained under a régime of no cultivation, the soil around the tree being simply mulched with fine straw, oak leaves, or other suitable material.

To conclude: Any method of cultivation which tends to promote regular instead of fluctuating growth may be considered a preventive or a remedial measure for exanthema.

[1] Loc. cit., p. 20.

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DESCRIPTION OF PLATES VII–X.

Illustrating Dr. O. Butler's paper on *Gummosis*.

PLATE VII.

Upper figure. *Citrus Limonum*. Cultivated variety affected by gummosis, environs of Whittier, Los Angeles County, California.

Lower figure. Same tree as in upper right-hand figure, Plate VIII, showing marked loss in vigour and development of chlorosis due to squamosis on limbs.

PLATE VIII.

Upper left-hand figure. *Prunus avium*. Cultivated variety. Limb on right washed with sulphuric acid diluted one-half with water over a small oblong area near origin, and showing gummous exudation twenty-two days after treatment.

Upper right-hand figure. *Citrus Aurantium.* Cultivated variety affected by squamosis, environs of Whittier, Los Angeles County, California.

Lower figures. *Citrus Aurantium.* Cultivated variety. Shoots from tree affected by exanthema. A, nodal gum postules; B, pseudo witch's-broom growth following bud proliferation; C, erumpent pustules; D, bud proliferation (enlarged). Fig. B from California Agr. Expt. Sta., Bull. cc.

PLATE IX.

Fig. 1. *Citrus Aurantium.* Cultivated variety. Cross-section of shoot affected by exanthema and showing development of gum pockets as in gummosis.

Fig. 2. *Prunus avium.* Cultivated variety. Cross-section of a shoot showing various stages in the development of gum pockets. Gummosis was induced by *Plowrightia morbosa.*

Fig. 3. *Prunus* sp. Cross-section of shoot of plum showing first stage of disease (fusoid development of pathognomonic tissues). Shaded area shows part of bark killed by burning with sulphuric acid diluted one-third with water.

Fig. 4. *Citrus Aurantium.* Cultivated variety. Cross-section of shoot affected by exanthema showing typical fusoid development of gumogenetic tissues and cortical proliferation.

Fig. 5. *Prunus avium.* Cultivated variety. Cross-section through a shoot burned with a drop of sulphuric acid diluted one-half with water, and showing development of gum pockets and re-formation (in progress) of normal xylem after the lapse of fourteen days. The shoot was sectioned somewhat above the spot burned.

B, bark; C, cambium; E, epidermis; G, gum pockets; M, medulla; P, pericycle bundles; S, suber; X, xylem; X′, imperfect xylem.

PLATE X.

Fig. 1. *Prunus avium.* Cultivated variety. Cross-section through part of a gum pocket in branch affected by *Plowrightia morbosa* some little distance above the 'black knot'. Normal xylem is being laid down over the gum pocket.

Fig. 2. *Citrus Aurantium* var. Valencia. Cross-section through gum pocket showing disappearance of cells upon the addition of water. Compare with Fig. 6, which represents the same section in alcohol before the addition of water.

Fig. 3. *Prunus domestica* var. Robe de sargent. Cross-section through gum pocket showing pseudo-cellular structure of gum.

Fig. 4. *Prunus domestica* var. Robe de sargent. Cross-section through young gum pocket.

Fig. 5. *Citrus Limonum.* Cultivated variety. Cross-section through portion of a fibro-vascular bundle in rind of fruit showing in the dense granular protoplasmic area the first stage in gummosis, degeneration of cell-walls. Change in cell-walls only becomes manifest when sections are placed in water.

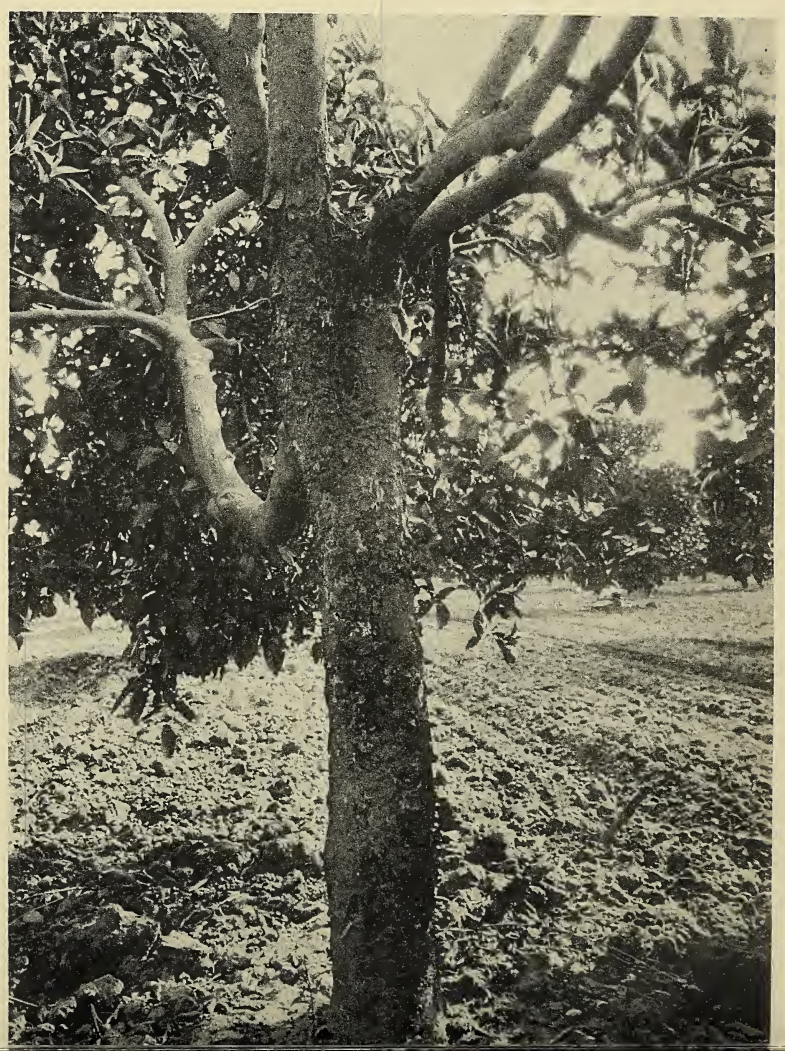
Fig. 6. *Citrus Aurantium* var. Valencia. Cross-section of a gum pocket. Section in alcohol to compare with same section (Fig. 2) in water. Figs. 2 and 6 illustrate the disappearance of cells upon the addition of water.

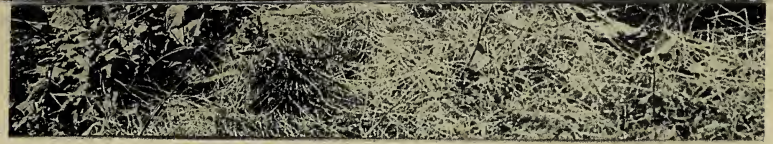
Fig. 7. *Citrus Limonum.* Cultivated variety. Cross-section through young gum pockets in various stages of development.

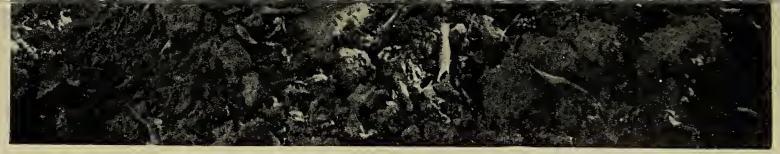
Fig. 8. *Citrus Aurantium.* Cultivated variety. Cross-section through a gum pocket showing a number of cells floating free in the gum that do not vanish when alcohol is replaced by water.

BUTLER —— GUMMOSIS.

Huth London.







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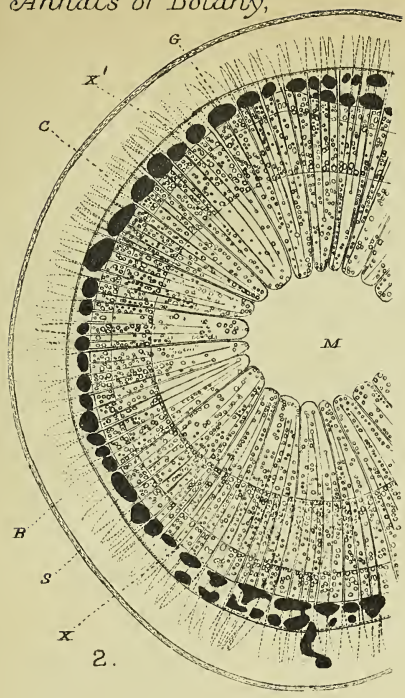

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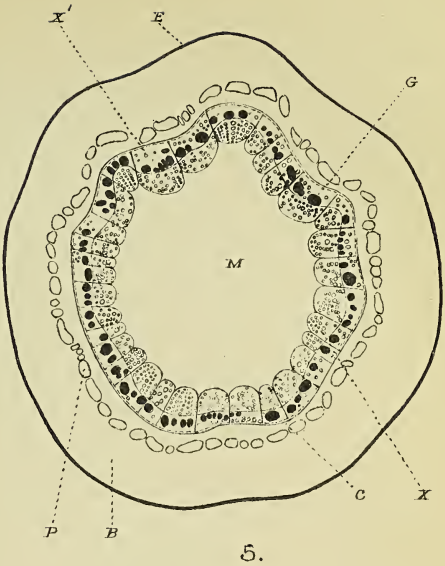

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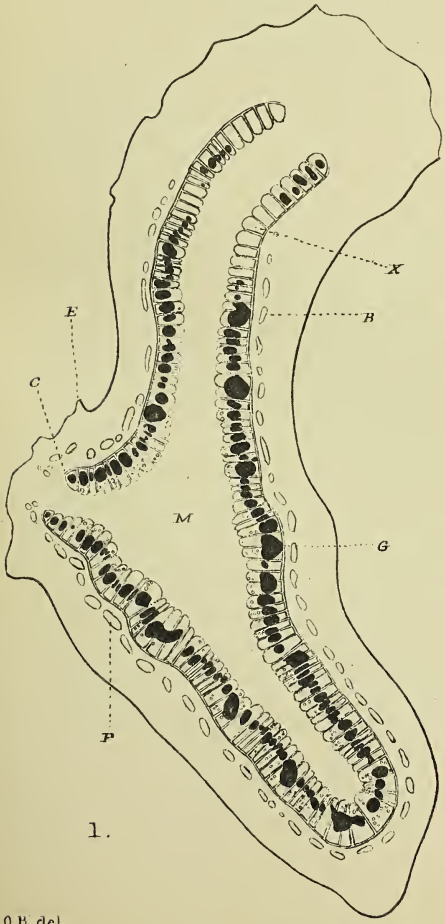

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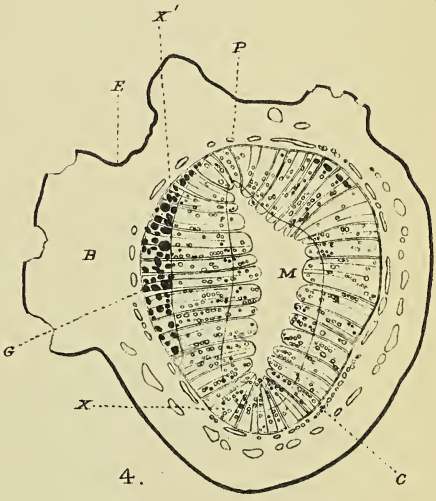

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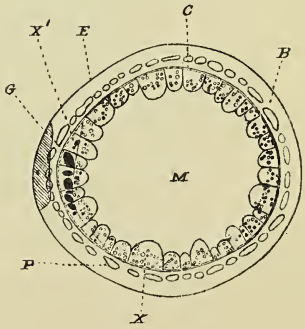

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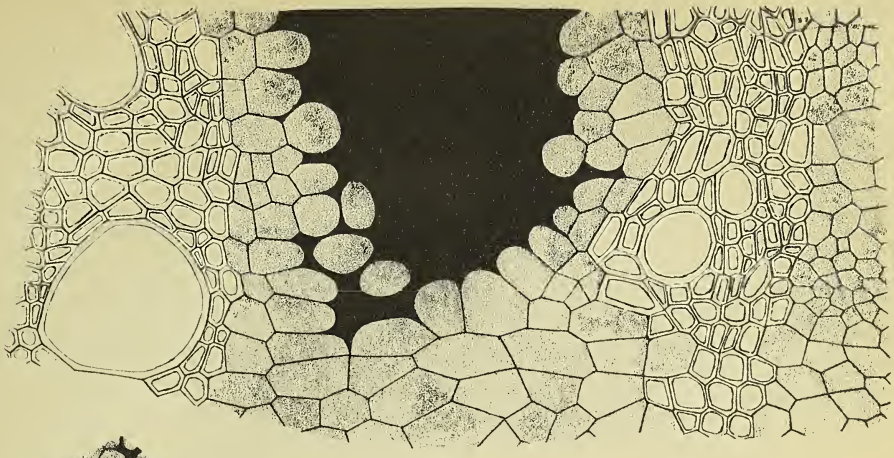
Huth, lith et imp

BUTLER —— GUMMOSIS.



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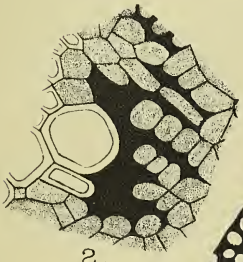

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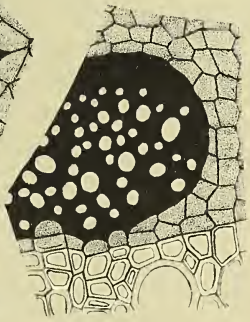

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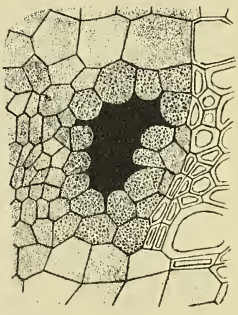

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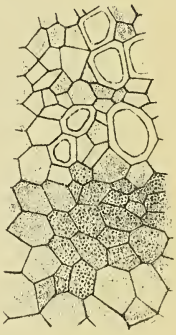

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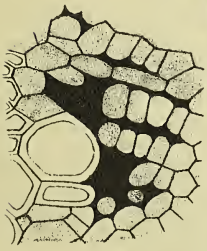

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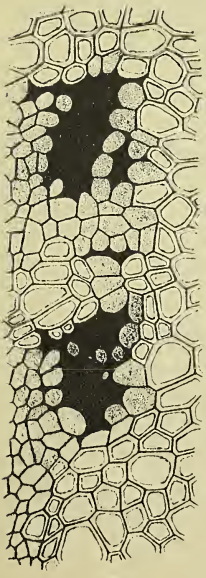

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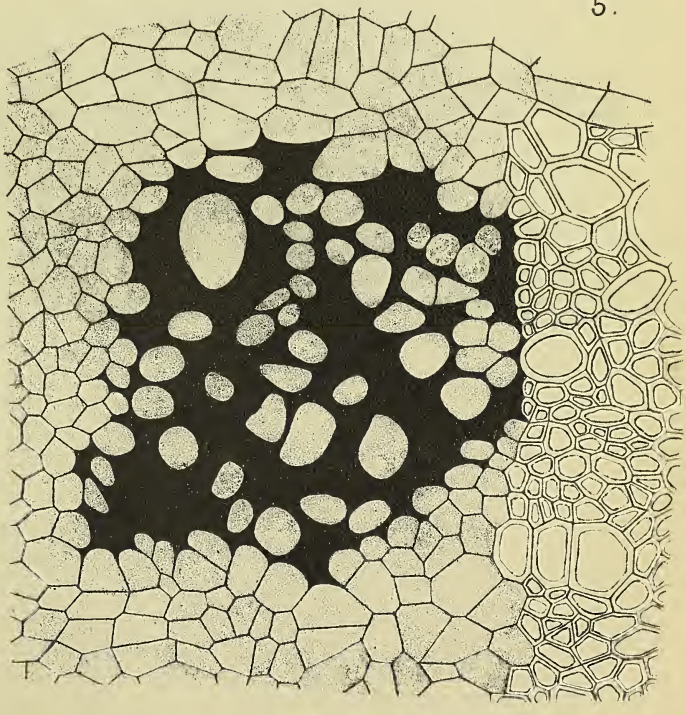

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BUTLER — GUMMOSIS

The Weeds of Arable Land in relation to the Soils on which they grow.

BY

W. E. BRENCHLEY, B.Sc., F.L.S.

Lawes Agricultural Trust, Rothamsted.

DURING the last fifty years a considerable amount of work has been done on the various species of plants which are to be found associated in grass-land. The relations existing between the plants and the soil on which they grow have been well investigated, while the effects of different manures on the growth of the different species have been exhaustively tested at Rothamsted, the permanent experiments to this end extending over a period of fifty-four years.

While so much is known about grass-land vegetation, our knowledge of the relations existing between the weeds of arable crops and the soil on which they grow is more or less indefinite and vague. Many observers have recorded their impressions and observations,[1] but these have never been so tabulated and reduced as to give the information required in a systematic form.

This being the case, an attempt was made in the spring and summer of last year (1910) to devise some means of finding out how far the weeds of arable land are characteristically connected with particular soils and crops, and how far they are independent of either soil or crop.

As a beginning it was decided to limit the work chiefly to the soils overlying the well-defined geological series of rocks between Harpenden and Bedford, where within about twenty miles the Chalk, Gault, Lower Greensand, and Oxford Clay appear in well-marked succession. The bulk of the work ultimately centred in the Bedfordshire districts round Dunstable (Chalk), Harlington (Gault), Flitwick (Lower Greensand and Oxford Clay), and Woburn (Lower Greensand and Oxford Clay). Some investigations were also carried on near Hertford and Hitchin (Hertfordshire).

[1] 'On the Indications which are Practical Guides in Judging of the Fertility or Barrenness of the Soil.' By John Bravender. Journ. Roy. Agric. Soc., vol. v, 1845, pp. 567–78.

'On Agricultural Weeds.' By Professor Buckman. Journ. Roy. Agric. Soc., vol. xvi, 1855, pp. 359–81.

[Annals of Botany, Vol. XXV. No. XCVII. January, 1911.]

The working plan adopted was that of visiting as many farms as possible during the season (extending from May to August), and detailing the nature of the soil, the crop grown, and every weed found in each individual field. So far as was practicable the relative prevalence of the weeds was noted in each case, falling under five headings:—

(1) Dominant.
(2) Sub-dominant.
(3) Very generally distributed.
(4) Occasional.
(5) Scarce or rare.

Much information was frequently obtained from the landowners or farmers as to the soil, rotation of crops, manuring, and troublesome weeds.

The data obtained in the field have been tabulated and analysed. It must be fully realized at the outset that what is set forth in this paper is essentially preliminary and to a large extent tentative in nature. Of necessity the year's work was confined to a very limited area, and before any definite conclusions as to the relations between the weed, soil, and crop can be arrived at, the work will need to be extended to embrace more numerous geological formations, and also to include the same formations in different parts of the country, in order that true comparisons can be drawn. It must be borne in mind that this paper simply sets forth the results of a careful study of a limited and localized area in one particular season.

During the season's survey about 150 fields were visited, yielding 107 species of weeds, representing 74 genera. Of these, 30 species, representing 28 genera, were each seen once only. In noting the weeds those plants only growing actually among the crops were taken into consideration, those occurring on the banks and hedgerows being ignored.

The classification of the soil was necessarily somewhat haphazard, as no mechanical analyses were undertaken during this preliminary survey, an arbitrary opinion on the nature of the soil in each case being formed on the field, and frequently modified or corroborated by the verdict of the farmer. In the absence of mechanical analyses it is very difficult to distinguish between a true clay and a clayey or heavy loam, a sand and a very light loam, &c., but experience enables one to gauge the texture of the soil sufficiently for the practical purpose of the investigation.

The more important species of weeds, with their habitats and relative dominance, were as follows:—

Ranunculaceae. *Ranunculus acris.* Most frequent on chalk and light loam; no record for gravel. Never dominant, and rarely found on very sandy soil.

Ranunculus arvensis. Chiefly found on clay land, but it occurred on

all other types of soil except sand. One record of dominance, on heavy loam.

Papaveraceae. *Papaver sp.* Usually associated with light sandy soils, where they were occasionally dominant. Sometimes found on chalk.

Fumariaceae. *Fumaria officinalis.* Occurs chiefly on chalk, where it is often dominant. Frequently found on sand (with one record of dominance); absent from clay lands.

Cruciferae. *Brassica alba.* Characteristic on chalk, where it is very often dominant; frequently occurs on sandy soils, but is *very* rare on heavy land.

Brassica Sinapis. Found on all types of soil, but most frequently on chalk and clay, where it is often dominant. Where it occurs on light soils it is usually scarce, though instances of dominance are recorded.

Capsella Bursa-pastoris. Occurs on all types of soil, but probably more frequent on light and sandy land. Rarely dominant.

Violaceae. *Viola tricolor.* Practically confined to light and sandy soils and chalk. Very rare on clay. Never dominant.

Caryophyllaceae. *Cerastium vulgatum.* Apparently distributed on all types of soil, but never very prevalent.

Spergula arvensis. Confined to sand and very light sandy soil. Frequently dominant.

Stellaria media. Chiefly found on sandy and light soils. Recorded from clay, chalk, and black land. When dominant it was usually on light and sandy soils.

Geraniaceae. *Geranium sp.* (It was difficult to absolutely fix the species in some cases.)

G. pusillum and *G. molle.* Only occurred on chalk and sand. Rarely dominant.

G. dissectum. Found on light loam.

This genus is practically confined to temporary leys of clover or 'seeds'.

Rubiaceae. *Sherardia arvensis.* Practically confined to chalk.

Galium Aparine. Of general occurrence, but possibly more frequent on light lands than on heavy soils. It rarely occurs in any quantity, but is usually occasional or scarce.

Compositae. *Anthemis arvensis* and *A. Cotula.* (Species were not clearly separated in the field.) Recorded from both light and heavy soils, but infrequent in occurrence in this area.

Chrysanthemum segetum. Only found on sand; very local, but characteristic.

Gnaphalium uliginosum. Usually associated with light sandy soils; never seen on chalk, and only once on clay. It seldom occurred in any quantity. (There is just a possibility that this represents a mistaken diagnosis of a certain small form of *Filago gallica,* which much resembles

Gnaphalium uliginosum. The habitats hardly correspond to those usually associated with the latter species.)

Matricaria Chamomilla. Confined to light sandy soils, with one solitary notice on clay. No record from chalk. Occasionally dominant on sand.

Matricaria inodora. Most frequently occurred on sandy soils, but was also found on clay. Seldom recorded from chalk. Dominant at times both on clay and sand.

Senecio vulgaris. Universally distributed, and of very frequent occurrence. Rarely dominant, and then on heavy soil.

Sonchus arvensis. Universal in distribution, both as to soil and dominance.

Thistles (usually *Carduus arvensis*). Universally distributed as to soil. When dominant it was usually on the lighter types of soil, not on clay.

Tussilago Farfara. 'Coltsfoot.' Universal in distribution, found on all types of soil. Frequently in association with *Equisetum arvense.* Rarely dominant.

Convolvulaceae. *Convolvulus arvensis.* Universally distributed, both as regards soil and dominance.

Scrophulariaceae. *Bartsia Odontites.* Recorded from heavy and light soils. Characteristic of heavy soils—clay; scarce on light lands.

Veronica agrestis. Of universal distribution, both as to soil and dominance.

Veronica hederaefolia. Recorded from all soils except gravel, though rarely seen on chalk. Dominant on all types of soil.

Labiatae. *Mentha arvensis.* Recorded from all soils but chalk. Frequently dominant on clay; usually occasional or scarce on light land.

Chenopodiaceae. *Chenopodium album.* Characteristic of light sandy soils, though frequently found on clay. No record on chalk, and very scarce on gravel. Rarely dominant, and then on sandy soil.

Polygonaceae. *Polygonum aviculare.* Very frequently found on sandy soils, and also occurred on clay. Very rare on chalk. Equally dominant on sand and clay.

Polygonum Convolvulus. Most usual on sandy soils, but frequently found on clay. Absent from chalk and gravel. Only dominant on sandy soil.

Polygonum Persicaria. Found on all soils except chalk. Only dominant on boggy land.

Rumex Acetosella. Characteristic of acid sandy soils.

Rumex crispus. Universally distributed. Probably more characteristic of clay and chalk than of light sandy soil. Dominant on clay, chalk, and gravel.

Gramineae. *Agrostis stolonifera.* Frequently dominant when found on gravel and chalk. Only occasional on clay and sand.

Alopecurus agrestis. Distributed over different types of soil, but probably more typical of heavy clay; scarce on chalk. May be dominant on either light or heavy soils.

Poa annua. Distributed over all types of soil except chalk. Only dominant on heavy clay.

Poa trivialis. Chiefly found on clay and chalk, but occasionally on rather sandy soil. Dominant both on chalk and sandy loam.

Triticum repens. Chiefly found on the lighter types of soil, though recorded from clay. Sometimes dominant on sand, once on chalk.

Equisetaceae. *Equisetum arvense.* Universally distributed and frequently associated with Coltsfoot. Occasionally dominant.

An examination of the tabulated and classified lists of results reveals the fact that while some weeds are universal in distribution, occurring on all types of soil indiscriminately, other plants are definitely symptomatic, only occurring in certain habitats, being absent from all others. (N.B.—While a weed may be said to be absent from any particular soil, still it is possible for it to occur in that very habitat as a stray, though very rare or occasional.)

A. Clay and Heavy Loam.

These soils originate from different geological formations.

(1) Clay with flints (e.g. Hitchin).

(2) Gault (e.g. Harlington, Tingrith).

(3) Oxford Clay (e.g. Flitwick, Houghton Conquest, Woburn Sands, and Ridgmount).

Of these, very little data was obtained with regard to the clay with flints, and most of the results apply to the latter two.

The weeds on the heavy lands were relatively few as regards the number of species, especially in comparison with those on light and sandy soils, though the quantity of weed was equally abundant. It was found that with the solitary exception of *Mentha arvensis*, all the common clay weeds occurred on soils derived from both the Gault and Oxford Clay. *Mentha arvensis* was only found on the Gault and is very local, but it is quite possible that further search might discover it on the Oxford Clay as well.

The weeds most characteristic of clay soils were:—

Bartsia Odontites
Brassica Sinapis
Chenopodium album
Matricaria inodora
Mentha arvensis
Plantago major
Poa trivialis
Polygonum aviculare
„ Convolvulus
Ranunculus arvensis
Senebiera Cononopus
Veronica hederaefolia

Of these *Bartsia Odontites* and *Mentha arvensis* may be regarded as practically confined to clay, while *Polygonum Convolvulus* finds its home on clay or sandy soils only.

B. Chalk.

This includes both the chalk proper and the heavy chalk marl underlying it. The flora on these soils is somewhat sharply marked out, in that several plants which are otherwise universal in distribution are totally absent, because they are calcifuges.

Bromus sp.
Geranium pusillum
Scabiosa arvensis
Sherardia arvensis
Silene Cucubalus
} are symptomatic of chalk.

Brassica alba
Fumaria officinalis
Geranium molle
} while very characteristic of chalk are also frequently found on sandy soils.

Agrostis stolonifera
Alchemilla arvensis
Brassica Sinapis
Lychnis vespertina
Poa trivialis
Viola tricolor
} are of very frequent occurrence.

C. Light and Sandy Loam, including Sand.

These soils are of very mixed origin, as all the light lands on the different geological formations (excluding chalk) fall under this heading. Thus the loams may either be derived from—

(1) Alluvium,

(2) Lower Greensand;

or they may be loams originating from drift overlying—

(1) Gault,

(2) Oxford Clay;

while the sands proper are either—

(1) Alluvial sands, or

(2) Lower Greensand.

However, it is evident that the derivation of the soil has little or no effect upon the distribution of the weeds, the texture being the determining feature.

Chrysanthemum segetum
Rumex Acetosella
Spergula arvensis
} are confined to sand and are very symptomatic, being probably associated with acid soil conditions.

Thlaspi arvense, though found in but one locality, was also on sand only.

Geranium dissectum
Gnaphalium uliginosum
Lamium purpureum
Matricaria Chamomilla
Papaver sp.
Rumex obtusifolius

are characteristic of light and sandy loams.

Alchemilla arvensis
Brassica alba
Chenopodium album
Fumaria officinalis
Geranium molle
„ pusillum
Lamium amplexicaule
Lychnis vespertina
Matricaria inodora
Polygonum aviculare
„ Convolvulus
Triticum repens
Veronica hederaefolia
Viola tricolor

are all chiefly associated with these light soils, though occurring on other types as well.

Besides the weeds detailed in the foregoing lists, there are several which are quite universal in distribution, occurring on all types of soil, and which may be dominant on any soil. Such plants are the following—those which are of universal occurrence, with the exception of chalk, being given separately :—

Weeds of universal occurrence.

Anthemis sp.
Capsella Bursa-pastoris
Cerastium vulgatum
Convolvulus arvensis
Equisetum arvense
Galium Aparine
Myosotis arvensis
Phleum pratense
Plantago lanceolata
Ranunculus acris
Rumex crispus
Senecio vulgaris
Sonchus arvensis
Stellaria media
Taraxacum Dens-leonis
Thistles
Tussilago Farfara
Veronica agrestis

Scandix Pecten—not on sand.

Weeds universally distributed, but absent or very rare on chalk.
(*Calcifuges.*)

Alopecurus agrestis	Polygonum Persicaria
Anagallis arvensis	Veronica arvensis
Euphorbia sp.	„ hederaefolia
Poa annua	

Besides the plants noted in the foregoing lists, a number of species occurred which were observed very seldom or once only during the season. The habitats are appended for purposes of reference.

Ranunculaceae	Ranunculus bulbosus	light and sandy loam
Cruciferae	Arabis Thaliana	„ „
„	Barbarea vulgaris	bog peat
„	Brassica campestris	sandy loam
„	„ oleracea	sandy loam and chalk
„	Raphanus Raphanistrum	sandy soil
„	Sisymbrium officinale	sand
Resedaceae	Reseda lutea	chalky loam
Caryophyllaceae	Arenaria serpyllifolia	chalk
„	Lychnis diurna	sand
Geraniaceae	Geranium columbinum	chalk marl
Leguminoseae	Vicia hirsuta	sandy loam
„	„ tetrasperma	„ „
Rosaceae	Potentilla anserina	light loam
Umbelliferae	Aethusa Cynapium	various
„	Carum Petroselinum	heavy clay
„	Conopodium denudatum	chalk
„	Daucus Carota	„
Valerianaceae	Valerianella olitoria	chalk
Compositae	Centaurea nigra	clay
„	„ Scabiosa	chalk
„	Filago Germanicum	sand
Campanulaceae	Campanula hybrida	sand and clay
Boraginaceae	Borago officinalis	sand
Plantaginaceae	Plantago media	various
Scrophulariaceae	Linaria spuria	heavy clay
„	„ vulgaris	chalk and clay
„	Veronica Buxbaumii	light loam
„	„ serpyllifolia	sandy loam
Labiatae	Glechoma hederacea	clay
„	Prunella vulgaris	„
Illecebraceae	Scleranthus annuus	sand

Polygonaceae	Rumex Acetosa	sand
Euphorbiaceae	Euphorbia exigua	various
„	„ helioscopia	sand
„	„ Peplus	various
Urticaceae	Urtica dioica	sand
Dioscoreaceae	Tamus communis	chalk
Liliaceae	Allium vineale	heavy clay
Gramineae	Bromus arvensis	chalk
„	„ asper	„
„	Holcus lanatus	sand and clay
„	Hordeum pratense	heavy clay
„	Lolium perenne	various
„	Phleum pratense	„
„	Poa pratensis	gravelly loam

Points of Interest.

1. While the nature of the soil plays such an important part in determining the local weed flora, the character of the crop, generally speaking, is a matter of indifference. The one exception to this rule is in the case of seed crops—clover, lucerne, sainfoin, trefoil. Many weeds, though they are to be found with every other crop, are entirely absent or very rare with seeds. This may be due to the difference in habit, as the Leguminous plants cover the ground so closely with such a dense mass of herbage that probably many weeds are choked out. One or two species, especially *Geranium* sp., *Cerastium vulgatum*, and *Sherardia arvensis*, are almost entirely confined to such crops, and it is possible that these weeds are not indigenous, but are introduced with the seeds of the crop.

A very few species are practically *confined to cereal crops*:—

Anthemis Cotula
Brassica alba
„ Sinapis
Centaurea nigra
Poa annua

Weeds absent or very rare in seed crops.

Agrostis stolonifera
Anagallis arvensis
Chenopodium album
Equisetum arvense
Galium Aparine
Gnaphalium uliginosum
Mentha arvensis
Plantago major
Poa annua
Polygonum aviculare
„ Convolvulus
„ Persicaria
Ranunculus arvensis
Scandix Pecten
Spergula arvensis
Stellaria media

Triticum repens	Veronica arvensis
Tussilago Farfara	„ hederaefolia
Veronica agrestis	

It should be noted that while *Poa annua*, *Plantago major*, and *Ranunculus arvensis* are absent from seed crops, other species of the same genera, e. g. *Poa trivialis*, *Plantago lanceolata*, and *Ranunculus acris*, are very frequent in such surroundings. While the variety of crop does not greatly influence the weed flora, still the cultivation frequently affects the prevalence of certain weeds the following year.

2. Care was taken to discover the various species of plants designated as 'Twitch' in the different localities. Any grass which runs along the surface of the soil or covers it to any great extent is apparently so called, the following species being included under this heading :—

Agrostis stolonifera	Phleum pratense (var. stolonifera)
Alopecurus agrestis	Poa annua
Lolium perenne	Triticum repens

3. Special investigations were made to determine whether Coltsfoot and *Equisetum*, which so often occur in company, are in any way characteristic of some particular quality of soil. Samples of the soil were tested with litmus for acidity and hydrochloric acid for alkalinity, with the result that both species were found to occur indiscriminately, and so could not be regarded as indicative or symptomatic.

Spergula arvensis, 'spurry,' on the other hand, is very symptomatic of acid soils, and it disappears entirely where lime is applied, reducing the acidity. Lime also kills out Mayweed in many cases.

4. 'Mayweed' (*Matricaria* sp. and *Anthemis* sp.) seems to be very impatient of competition. If the crop is in any way heavy little or no Mayweed is to be found among it, though it occurs in enormous quantities round the edge of the fields, along lines of drainage, and wherever there may be a clearing in the crop.

Fumaria officinalis does not generally occur in any great abundance, but occasionally it is so prevalent on chalk that it is necessary to hand cultivate to get rid of it in order to save the crop. This is notably the case in the Maiden's Bower, an old Roman encampment near Dunstable.

Allium vineale was only met with in one locality on the very heaviest of clay land. This weed reproduces itself by offsets, bulbils, and seeds, and its vitality is so great that no known method of cultivation seems competent to eradicate it.

Summary.

1. A definite association exists between the species of weeds of arable lands and the soil on which they grow. The determining factor is the actual texture of the soil, and not so much the geological formation from which it is derived, except with soils overlying chalk.

2. The crop has very little influence on the weeds occurring except in the case of seed crops, which probably smother out a number of species which would normally occur.

3. Certain weeds are definitely symptomatic of particular types of soil, though the majority of species are not so strictly circumscribed in distribution. Some species, also, are to be found indiscriminately on any soil.

In conclusion I have to express my great obligation to Mr. Spencer Pickering, F.R.S., who obtained for me numerous introductions, without which the work would have been impossible. Also I desire to thank all those who so greatly facilitated the work by permitting me to visit them and by giving me much information and practical help.

The Evolution of the Filicinean Leaf-trace.[1]

BY

EDMUND W. SINNOTT.

With Plate XI and eleven Figures in the Text.

THE anatomy of the leaf in the Filicales has recently attracted much attention among botanists, for the reason that about it have centred some of the most fundamental problems of plant morphology, such as those concerning the retention of primitive characters by the leaf, the inter-relations of the foliar vascular supply and that of the axis, and the general questions of stelar theory and the origin and differentiation of plant tissues. Although the structure of the petiole and blade of the mature leaf has been worked out in considerable detail in the various fern families by several authors, notably Bertrand and Cornaille (1), Thomae (24), &c., and although the general relation of its vascular system to that of the stele is well known from the investigations of Prof. Jeffrey and others, the more minute anatomy of the base of the leaf-trace, as it leaves the central cylinder and while it is still in the cortex or the lower part of the petiole, has apparently not received the attention it deserves, for it is in this portion of the course of the leaf-bundle that the effects of changing external conditions, working upward from the axis and downward from the frond, must be felt last and least. It is therefore here, if anywhere, that we should expect to find indications of what was the structure of the primitive foliar bundle in the ferns. A brief comparative account of the anatomy of this region in the main groups of the Filicales, and of the relation of structures here to those in the petiole, as well as a discussion of the evolutionary history of the order in the light of the results obtained, is the purpose of the present paper.

A considerable number of species were examined by the writer,[2] but

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 33.

[2] These include the following: *Osmunda cinnamomea*, L., *O. Claytoniana*, L., and *O. regalis*, L.; *Todea barbara*, *T. superba*, and *T. hymenophylloides*; *Helminthostachys zeylanica*, *Botrychium virginianum*, (L.) Sw., and *Ophioglossum vulgatum*, L.; *Marattia alata* and *Danaea alata*; *Lygodium dichotomum*, *Aneimia fraxinifolia*, and *Schizaea pusilla*, Pursh; *Gleichenia Speluncae*, *G. circinata* v. *macrophylla*, *G. dicarpa* v. *longipinnata*, *G. rupestris*, and *G. dichotoma*; *Cyathea Macarthuri* (young plant); *Dennstaedtia* (*Dicksonia*) *punctiloba*, Moore; *Onoclea sensibilis*, L.; *Pteris*

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for much information, especially on the rarer groups of ferns, he is indebted to the researches of others, especially of Mr. L. A. Boodle and Professor D. T. Gwynne-Vaughan. 'The Origin of a Land Flora' by Professor Bower (6) also presents in a condensed form much information regarding the morphology of the group, and was found very useful.

The base of the leaf-trace in living ferns presents three main types of differentiation, which may be characterized as the primitively monarch, the primitively diarch, and the primitively triarch, possessing one, two, or three clusters of protoxylem, respectively. The first type is found in the Osmundaceae and Ophioglossaceae, the second in the Marattiaceae, and the third in all other families of the Filicales.

In the Osmundaceae, the first family in the monarch group, three species of each of the two genera *Osmunda* and *Todea* were examined. The structure of the very base of the leaf-trace in all of them is similar. It consists of a solid monarch strand of xylem, surrounded by phloem and elliptical in cross-section with its long axis at right angles to the stem radius, though in *T. barbara* the bundle tends to be slightly constricted in the middle and to be arched from the very first. As the trace passes through the cortex it grows larger and becomes steadily more and more concave towards the axis till the typical arch-shaped undivided petiolar bundle is produced. The protoxylem group, which is single at the base but soon begins to divide, shows a very strong tendency to be mesarch,[1] and numerous instances were observed in every species examined where there were one or more centripetal tracheides on the adaxial side of the protoxylem (Pl. XI, Fig. 12). By the time the trace is well out into the cortex, however, it has become clearly endarch, which condition is maintained throughout the leaf. Mesarchy in the leaf-trace of the modern Osmundaceae is interesting in connexion with the recent discovery by Kidston and Gwynne-Vaughan (19) of various fossil members of the family which show the base of the leaf-trace to be strikingly mesarch (Text-fig. 1).

In the Ophioglossaceae, the other family in the primitively monarch group, the trace at its base seems to be characteristically single and provided with one protoxylem group. This is the case in *Helminthostachys*, *Botrychium*, and nearly all the species of *Ophioglossum*, though in the sections

aquilina, L., *Adiantum cuneatum*, and *Pellaea atropurpurea*, (L.) Link.; *Cystopteris bulbifera*, (L.) Bernh., *C. fragilis*, (L.) Bernh., *Polypodium vulgare*, L., *Phegopteris hexagonopteris*, (Michx.) Fee, *P. Dryopteris*, (L.) Fee, *P. polypodioides*, Fee, *Woodwardia virginica*, (L.) Sm., *W. areolata*, (L.) Moore, *Asplenium Filix-foemina*, (L.) Bernh., *A. platyneuron*, (L.) Oakes, *Aspidium Thelypteris*, (L.) Sw., *A. spinulosum*, (O. F. Müller) Sw., *A. cristatum*, (L.) Sw., *A. marginale*, (L.) Sw., *A. Boottii*, Tuckerm., *Polystichum acrostichoides*, (Michx.) Schott, and *Marsilea quadrifolia*, L.

[1] The term 'mesarch', as used in the present paper, is descriptive of any protoxylem group, whether situated at the axis of the stem or not, which is surrounded by metaxylem. 'Endarch' describes any condition where the protoxylem is not at the axis and where there is no metaxylem (centripetal wood) formed on its inner face. 'Exarch' is used in the accepted sense, as the opposite of endarch.

Ophioderma and possibly *Cheiroglossa* of the last genus, the foliar supply consists of several bundles even at its attachment to the cylinder. This condition is quite generally agreed to be a specialized and not a primitive one. Unlike that of the Osmundaceae, however, the leaf-bundle in this family does not long remain unbroken, but is soon divided into a series of strands which become variously disposed. The single protoxylem cluster at the base of the trace, which tends to be stretched out parallel to the long axis of the bundle, is at first in a clearly mesarch position (Fig. 13) in *Helminthostachys* (in other characters the most primitive member of the family), but soon becomes entirely endarch, a condition which is apparently the only one ever found in the other two genera. Both the leaf-trace and the petiolar bundle seem to be usually collateral, though they are concentric in certain cases.

In this first group of the ferns, therefore, a single and probably concentric strand, with one mesarch protoxylem group, seems to have been the primitive state of the leaf-trace, and so, very probably, of the whole petiolar system (Text-fig. 1).

In the second main subdivision of the Filicales, comprising those ferns with primitively diarch traces, there is but one family, the Marattiaceae, which is represented by five genera : *Marattia, Danaea, Kaulfussia, Archangiopteris*, and *Angiopteris*. In the first four of these, the leaf-trace departs from the cortex in much the same way, and consists at its base of two concentric strands, from circular to elliptical in outline, which arise from the sides of the gap, and each of which possesses a single cluster of protoxylem. As these traces pass through the cortex they begin to divide, and in the base of the petiole there are many bundles arranged in an arch. In *Angiopteris*, however, the whole stele is very intricate and the foliar supply is inserted on it in a complex manner. The trace consists at its base of a large number of bundles which are given off in the form of a rough arch, and each of which is concentric and possesses one to three protoxylem clusters. It is noteworthy, however, that in young plants of *Angiopteris*, Farmer and Hill (13) found at the base of the leaf the typical double bundle of the other genera.

The position of the protoxylem in the leaf is variable. In *Archangiopteris*, Gwynne-Vaughan found the trace, through its whole extent, to be endarch (16). In *Angiopteris*, there is always a protoxylem group on the adaxial side of every bundle while it is in the cortex, and there are often others in a mesarch position, but in the petiole all the groups are apparently endarch. *Marattia* shows a uniformly endarch condition throughout the whole vascular system of the leaf. In *Danaea*, however, distinct centripetal elements were found in the petiole by Brebner (9). This observation was confirmed by the writer, and, in addition, the protoxylem in the two bundles at the base of the trace was found to be mesarch in more than half the cases

investigated. Fig. 14, Plate XI, shows a typical double bundle, in both parts of which the protoxylem occupies a central position.

It would seem reasonable to conclude, then, that the primitive condition of the leaf-trace in this group of ferns consisted of two concentric bundles, each possessing a single protoxylem cluster which was possibly, and as we shall later see, probably, in a mesarch position (Text-fig. 2).

The third main group of the Filicales, which has been characterized as primitively triarch, comprises all the remaining families of the order. Many of its members have reached a high degree of development, and in them the structure of the base of the leaf-bundle is very complex. In the opinion of the writer, however, the primitive condition from which they have all been derived was one where the leaf-trace consisted of a single roughly triangular

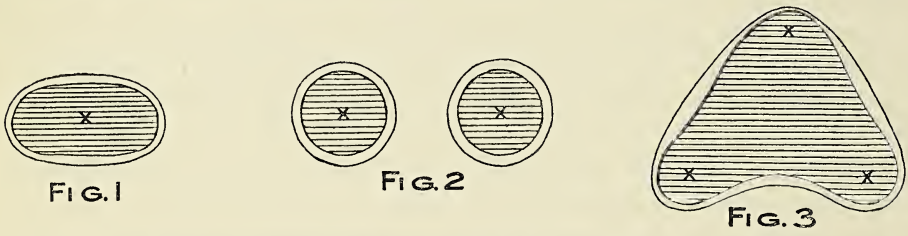


FIG. 1. The primitive monarch type, as represented by the base of the leaf-trace of *Thamnopteris*.
FIG. 2. The primitive diarch type, as represented by the base of the leaf-trace of *Danaea*.
FIG. 3. The primitive triarch type, as represented by the base of the leaf-trace of *Gleichenia Speluncae*.

and concentric strand, near each corner of which was embedded a mesarch protoxylem group (Text-fig. 3). In support of this view, evidence derived from a study of the leaf-trace in the various fern families will be brought forward.

The Filicales have recently been divided by Bower (7) into three main groups, the Simplices, the Gradatae, and the Mixtae, according to the order of development of the sporangia in the sorus. This classification has received considerable support along anatomical and other lines, and is now accepted by most botanists as a rather close approach to a natural arrangement, the three groups representing an ascending series in evolutionary history, with the Simplices as unquestionably the most primitive. Aside from the ferns which we have already discussed, this group includes the simplest of the triarch Filicales, the Schizaeaceae, Gleicheniaceae, and Matonineae.

The Schizaeaceae include four genera: *Lygodium*, *Aneimia*, *Mohria*, and *Schizaea*. *Lygodium* is protostelic, and for this reason, as well as on other evidence connected with its reproduction, is well considered the most primitive member of the family. *Aneimia* and *Mohria* are more advanced in their stelar anatomy, spore output, and gametophytic characters, while *Schizaea* is in all probability a reduced form.

The leaf-trace in all four genera is a single bundle. In *Lygodium* it is concentric and, while in the cortex, roughly circular to oval in cross-section. In the base of the petiole, four prominences appear on the abaxial face of the xylem, of which two are close together and median and two are lateral. On these prominences appear the scalariform elements which higher up become typical exarch protoxylem. The petiolar strand of *L. palmatum*, as described by Boodle (4), forms the only exception to this general description. Here the bundle is in the shape of an equilateral triangle and there is only one median protoxylem group, which instead of being peripheral is distinctly embedded in the xylem. The leaf-bundle of *Lygodium* differs from that of *all* the other Filicales in the fact that it is 'protostelic' or approximately isodiametric throughout its whole course, and that its protoxylem is *exarch*, on the abaxial side of the bundle. The protoxylem in the petiole of every other known fern [1] is endarch in its position. Taking all the evidence together, *Lygodium* probably approaches more closely to the conditions in the ancient Filicales than does any other living fern, and it is itself found from the Cretaceous period.

In *Aneimia* the trace leaves the stele as a flattish arch surrounded by phloem and possessing one median protoxylem group. This is exarch or mesarch at the very first, but the centripetal wood is almost immediately broken through and the subsequent endarch condition originates. On passing through the cortex, the arch becomes more concave and an endarch protoxylem group appears at each of its ends, which become hooked. The phloem in the middle of the adaxial side gradually disappears and the arch becomes distinctly flat-topped. This general condition persists throughout the petiole.

The structure of the trace in *Mohria* is much the same as in *Aneimia*, according to Boodle. It is a flat-topped arch, at first entirely concentric, but later only partially so. The protoxylem is endarch, and there may sometimes be five groups instead of three.

The leaf-trace and petiolar bundle of *Schizaea* are peculiar and seem to point towards reduction. Phloem occurs only on the abaxial side of the bundle, which is usually roughly elliptical in cross-section, with its long axis tangential to the stele. In the lower part of its course in *S. digitata*, as described by Boodle (4), there is no true protoxylem, but the earliest formed elements appear to be at each end of the ellipse. Higher up one protoxylem group appears in the middle of the adaxial side. In the leaf-bundle of *S. pennula*, according to Prantl (20), there are two lateral or terminal groups instead of one median one. *S. elegans*, as observed by the same writer, shows a much more complex leaf-bundle. In the middle of the xylem band is a large, abaxial projection, giving the whole bundle a roughly anchor-shaped appearance, with a protoxylem cluster at the end of each lateral

[1] With occasional exceptions in *Danaea*.

arm. The resemblance of this bundle to that of certain species of *Lygodium* is increased by the fact that the most outward elements, those on the end of the projection, are the first of the metaxylem tracheides to lignify, a condition which Prantl considers the beginning of the third protoxylem cluster found in the other members of the genus, but which instead seems rather to be its persistence in a reduced form. The only other type of bundle observed was in *S. fistulosa*, by Boodle, where certain of the traces in the cortex showed an arched structure somewhat resembling that found in *Aneimia*. *S. pusilla* is a very delicate form, and never seems to possess more than one small protoxylem cluster.

To summarize conditions in the family, then, *Lygodium palmatum* approaches most closely the roughly triangular mesarch trace which we have considered primitive, and has departed from it only in the position of its two lateral protoxylem groups, which are exarch instead of mesarch. The other species of the genus have progressed still further, the median group having bifurcated and become exarch also. The leaf-trace in *Schizaea* seems clearly to be reduced from the *Lygodium* condition by the loss of the adaxial phloem and the disappearance of one or more of the protoxylem clusters. *Aneimia* and *Mohria*, which are rather far advanced along other lines, show a leaf-bundle much modified from the hypothetical prototype. At the very base, however, the only difference is the band-shaped lengthening of the trace. The first-formed elements are still peripheral and the phloem surrounds the xylem, though these characters are soon lost at a higher level.

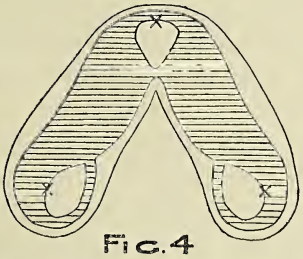
Another important family of the Simplices is the Gleicheniaceae, comprising the genera *Gleichenia* and *Platyzoma*. These are all ferns of simple structure, possessing for the most part a protostelic central cylinder, near the periphery of which occur a number of mesarch protoxylem groups. *Platyzoma* and several species of *Gleichenia* have clearly attained a siphonostelic structure.

The leaf-trace is always a single strand. The simplest method of its departure from the stele was observed in *G. Speluncae*, where a concentric triangular mass of xylem, with three mesarch protoxylem groups at its corners, is constricted off from the central cylinder (Text-fig. 3 and Pl. XI, Fig. 15). Before this is fairly started on its way through the cortex, however, a wedge of phloem and parenchyma enters it from the adaxial face. This soon comes in contact with the median protoxylem, and though the two lateral groups long retain their mesarch position, they, too, soon lie next the parenchyma and the typical endarch, arched petiolar bundle of the family is formed. The nodal structure of this species rather closely resembles that described for *G. dicarpa* by Boodle (5).

In *G. circinata* v. *macrophylla*, and *G. dicarpa* v. *longipinnata*, a nodal island of parenchyma forms in the cylinder before the departure of the

trace, which at its exit, though triangular in outline, never shows the solid character of that of *G. Speluncae*. Its three protoxylem groups retain their mesarch position for some time, and the median one especially seems to tend to be shut off from the bay of parenchyma by the coming together of the two large lateral masses of metaxylem (Text-fig. 4 and Pl. XI, Fig. 16). This seems much more pronounced in the former of the two species than in the latter.

The base of the leaf-trace in the sub-genus *Mertensia* displays a more advanced condition than it does in *Eugleichenia*, to which all the species above described belong. In *G. dichotoma*, a large nodal island is formed, dividing the stele from a smoothly rounded arch of xylem, not triangular in outline, which departs with its three protoxylems as the leaf-trace. The ends of the arch soon unite, completing a continuous ring of xylem. The protoxylem is endarch from the very first.



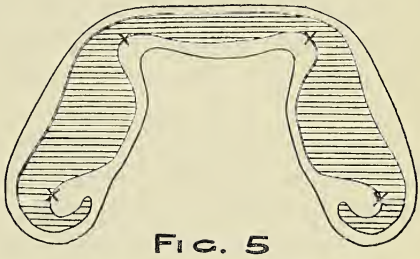


Fig. 4. The base of the leaf-trace of *Gleichenia circinata* v. *macrophylla*. The three protoxylem groups are still mesarch.

Fig. 5. Leaf-trace and petiolar bundle of the *Loxsoma* or *Dennstaedtia* type.

In the siphonostelic species there is of course no nodal island, but a smooth, rounded and rather flattish arch of xylem departs to the leaf with apparently three protoxylem groups, all of which are endarch.

In all but the simplest species, the number of protoxylem groups increases in the petiole, usually to six; but in *Mertensia*, with its larger leaf-bundle, there tend to be more, reaching an extreme in *G. longissima*, where Boodle found twenty distinct clusters.

Platyzoma is a highly specialized and reduced form. Its leaf-trace is very small and possesses but two protoxylems. In the cortex it is endarch and collateral, but in the petiole it becomes concentric and in the rachis apparently mesarch.

The structure of the vascular supply of the leaf in the Gleicheniaceae represents an advancing series. In *G. Speluncae*, the base of the trace approaches our hypothetically primitive condition even more closely than does *Lygodium*. Unlike the case in the latter genus, however, this structure soon changes to a much modified one in the petiole. In the other species of *Eugleichenia*, which is recognized as being the more primitive of the

two sub-genera, this type of trace is not seriously modified. In *Mertensia*, however, more complex conditions appear, due to the approach towards siphonostely. *Platyzoma* represents the extreme of modification.

The last family included under the Simplices is the Matonineae, represented by the single genus *Matonia*, which includes two species, *M. sarmentosa* and *M. pectinata*. The former is very simple in structure, its central cylinder, as observed by Compton (12), being an endarch amphiphloic siphonostele, surrounding one large medullary bundle. The leaf-trace departs from the stele as a single concentric strand in the shape of a triangular arch, and with three endarch protoxylems, which persist undivided throughout the petiole. *M. pectinata*, on the other hand, is much more complex, possessing a central cylinder which contains often as many as three concentric siphonosteles. The leaf-trace goes off as a single wide arch, which contains at its very base five endarch protoxylem groups. This number becomes greatly increased in the petiole, where the bundle is much larger and more complex. Young plants of this species show but a single siphonostele and a much smaller leaf-trace. This would apparently be triarch, if typical protoxylem, which is very late in appearing, could be made out.

M. sarmentosa seems to be clearly the more primitive of the two species. In its fronds as well as in its vascular anatomy it resembles young plants of *M. pectinata*, and it is also very much nearer to the Gleicheniaceae, the family which the Matonineae most closely approach in sporangial and soral characters, and to which they are doubtless nearly related by descent.

In the Gradatae, that group characterized by Bower as possessing sori in which there is a definite succession in time and space in the development of the sporangia, the first family to be considered is the Hymenophyllaceae, the anatomy of which has been carefully investigated by Boodle (3). These small, filmy-leaved ferns exhibit considerable diversity of structure, the central cylinder ranging from a collateral or nearly collateral bundle in the very simplest forms, to a much larger protostelic mass in which the first-formed elements of the xylem may be indefinitely scattered, may be massed with parenchyma in a pith-like island in the centre, or may be distributed in an exarch position along the periphery. The leaf-trace is a single, usually collateral, strand, which in the simpler forms is much like the central cylinder and possesses one protoxylem group. In the slightly more complicated species, such as *Hymenophyllum dilatatum* v. *Forsterianum* and *Trichomanes reniforme*, where the stele consists of a ring of metaxylem surrounding a group of parenchyma and protoxylem, the leaf-trace at its base is very similar to the central cylinder, but higher up the xylem changes from a ring to an arch, and the protoxylem splits into two endarch groups. In certain species of *Trichomanes*, such as *T. Prieurii*

and *T. radicans*, which possess much stouter steles than does *T. reniforme* or any of the Hymenophyllums, but which resemble them in having central protoxylem, the leaf-trace and the petiolar bundle are distinctly triangular in shape and always have more than two protoxylem groups. In *T. Prieurii* there are three of them in the trace as it passes through the cortex, but from the division of the median group there are four in the petiole. In *T. radicans* the protoxylems are more numerous, but tend to be restricted to the three angles of the trace, at least while it is in the cortex.

In the stoutest members of the family, represented by *T. scandens* and *T. apiifolium*, the cylinder is a solid exarch protostele, much more comparable than have been any of the others to the condition found in *Lygodium* and the Gleicheniaceae. This similarity is also clearly apparent in the petiolar bundle, which is arched and possesses three protoxylems. The incurving of the ends of the arch in *T. apiifolium* suggests *Aneimia*. The leaf-trace in the cortex was observed by Boodle only in *T. scandens*, where he notes the important fact that here and at the node it possesses considerable peripheral or exarch protoxylem which is continuous with the exarch protoxylem of the stele.

There would thus seem to be a rather complete series, both in the anatomy of the stele and of the leaf-trace, between the simplest and the most complex species of the family. As to their inter-relationships, the view which has the widest acceptance, and which is supported by Boodle, Bower, and Tansley, considers the type represented by *H. dilatatum* and *T. reniforme* to be the most primitive, and the smaller forms to be derived from this by reduction, the larger by amplification. The fact that such species as *T. scandens* approach so closely the protostelic Simplices in the structure of the stele, and more especially of the leaf-trace, as well as in certain sporangial characters, makes it seem very probable that there must be a phylogenetic relationship between the two groups. If this close affinity is acknowledged, however, the Hymenophyllaceae are apparently either very primitive ferns, from which the triarch Simplices have been derived, or else they represent a reduction series from them, beginning with *T. scandens* as the least modified and closest to the ancestral type, and ending with the small collateral species of *Hymenophyllum*. The latter view seems to the writer much more worthy of acceptance, both on account of the filmy-leaved character of the family, which is clearly a reduced condition, and from the fact that the sorus shows a basipetal development, putting these ferns among the Gradatae, instead of among the Simplices, where they should certainly be placed were they an extremely primitive group. The resemblance of the family to the Botryopterideae seems to be of little significance.

The bulk of the remaining ferns in the group Gradatae are comprised in the *Loxsoma-Dennstaedtia-Dicksonia* alliance, the anatomy of many

members of which has been investigated by Gwynne-Vaughan (14 and 15). These are typically siphonostelic ferns, which both in their soral characters and in their anatomy are intermediate between the Schizaeaceae and Gleicheniaceae on the one hand, and the Polypodiaceae on the other.

Loxsoma is clearly the least specialized and most primitive of the three genera. In its sporangial characters it clearly shows an affiliation with certain of the Simplices and possibly with the Hymenophyllaceae, but in the form of the indusium and in its general habit, as well as in anatomical structure, it is closely allied to the Dennstaedtineae. It possesses a well-developed exarch siphonostele from which the leaf-trace departs as a single arched concentric strand, concave towards the axis and with incurved ends. This has four endarch protoxylem groups, one in each lateral hook and one in each of the two angles of the arch (Text-fig. 5). In the upper part of the petiole these two median groups approach each other, and they finally fuse in the rachis. This type of trace is also characteristic of the Dennstaedtineae and of many of the lower Polypodiaceae. It has probably arisen from our hypothetically primitive condition much as has the leaf-bundle of *Aneimia* or *Mohria*.

Dennstaedtia and its allies show a higher development of the sorus, which often displays a marked tendency towards the 'mixed' condition of the Polypodiaceae. Anatomically the family is almost identical with *Loxsoma*, though the siphonostele may be mesarch as well as exarch. In *D. punctiloba*, as examined by the writer, the endarch protoxylems of the leaf seemed to be continuous with the peripheral scalariform elements of the stele. It would perhaps seem from this that the two distinct kinds of protoxylem—cauline and foliar—noted by Gwynne-Vaughan in *Loxsoma* (14) might, in closely related forms at least, be really the same.

The Dicksonieae[1] are much larger ferns than *Loxsoma* or the Dennstaedtineae, and show a close approach to a dictyostelic condition. The leaf-trace arises as a long and narrow band of xylem with incurved ends, and either in the cortex or the base of the petiole usually breaks up into a large arch of separate strands. Though little information is available as to the ontogeny and more minute histology of the trace, it has most probably been derived through the amplification of such a bundle as is found in *Loxsoma*, for Chandler (10) found the young trace of *D. antarctica* to be single and later double, a condition which we shall observe to be characteristic of the higher Gradatae and of the Mixtae.

The genus *Onoclea*, though possessing a gradate sorus and in some ways showing a resemblance to the Cyatheaceae, is identical in its anatomical structure with certain of the simpler Mixtae, and represents the next step in development above the *Dennstaedtia* condition. The cylinder is dictyostelic and the leaf-bundle is a double one, each of its diarch members representing

[1] The name *Dicksonia* is here used for the genus exclusive of section *Patania* (*Dennstaedtia*).

one-half of the *Dennstaedtia* or *Loxsoma* bundle, and composed of a mass of metaxylem with an adaxial incurving or hook at each end, in the concavity of which occurs an endarch protoxylem group. This double trace, the '*Onoclea* type' of Bertrand and Cornaille, is characteristic of the great mass of smaller and simpler Mixtae as well as of *Onoclea* (Text-fig. 8). In all ferns examined where this trace was present the important fact was noticed that the adaxial (lateral) protoxylem group of each bundle, whenever it was distinguishable reasonably early on the course of the trace through the cortex, was always mesarch and near the outside of the bundle (Text-fig. 7

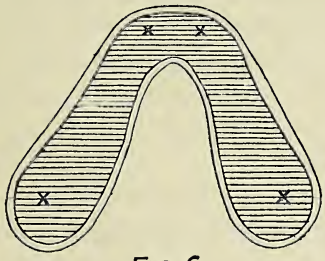


FIG. 6

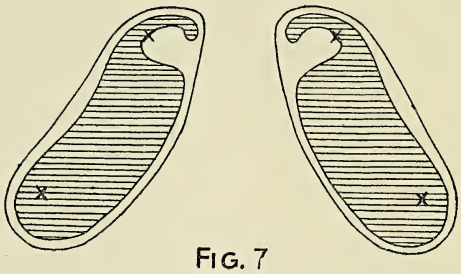


FIG. 7

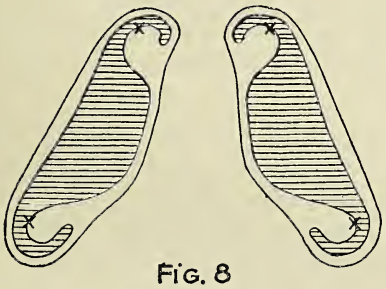


FIG. 8

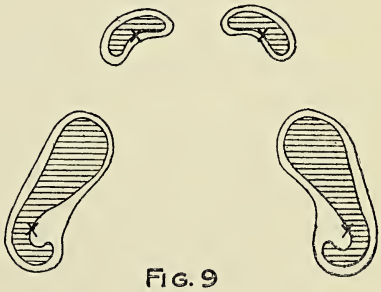


FIG. 9

FIG. 6. The base of the leaf-trace of *Plagiogyria*.
FIG. 7. The base of a leaf-trace of the *Onoclea* type.
FIG. 8. The petiolar bundle of the *Onoclea* type.
FIG. 9. The base of the leaf-trace of *Cyathea Macarthuri*, showing the method of formation of a many-bundled arch.

and Pl. XI, Fig. 17), becoming endarch only by the gradual disappearance of the metaxylem between it and the adaxial parenchyma. Several instances were observed where the other protoxylem (median or abaxial) was also mesarch, but never in such a striking way. This condition of mesarchy was apparent even in such delicate ferns as the three species of *Phegopteris* which were examined. It is important in connexion with our hypothetically primitive leaf-trace, for two such Onoclean bundles as shown in Fig. 17, if closely approximated by their adaxial faces, would produce a triangular mesarch bundle very similar to that which we have considered ancestral for the group.

The only remaining members of the Gradatae to be mentioned are the Cyatheaceae. These are tree-ferns with large stems, and display considerable complexity in their stelar system and in their leaf-bundles. *Alsophila pruinata*, as described by Karsten (18), shows the simplest conditions in the family—a siphonostele with the leaf-trace departing as a single strand. A study of the development of young plants of *Alsophila excelsa* by Gwynne-Vaughan (15) makes it clear that the advanced adult state has come through the protostelic and siphonostelic stages, as in all ferns, and that the leaf-trace was originally a single bundle. This latter conclusion was borne out by the writer's examination of some young plants of *Cyathea Macarthuri*, in which the base of the leaf-trace is a single triarch strand in the shape of a triangular arch and much resembles the leaf-bundle of *Eugleichenia*. This trace soon becomes separated into two diarch bundles of the *Onoclea* type, each of which later divides into two again (Text-fig. 9). By subsequent division the complex adult leaf-trace is formed.

In the position of the sorus and the structure of the sporangium, as well as in the above-mentioned anatomical resemblance,the Cyatheaceae show considerable similarity with the Gleicheniaceae, among the Simplices, and have probably arisen from somewhere in their vicinity. The family constitutes an independent line of advance, and seems to have led to no higher forms.

The Mixtae are usually all included in the Polypodiaceae. This great family comprises the vast majority of living ferns, and is usually divided into two groups: those with marginal sori, or the Pterideae, and those with superficial sori.

The Pterideae seem to have had a somewhat different origin from the rest of the Mixtae. The recent researches of Professor Bower on the genus *Plagiogyria* (8) prove that though this fern possesses sori which are marginal and clearly of the mixed type, they show no signs of having passed through a basipetal or gradate condition. In its habit, in its anatomy, and in the structure of its sporangium, this genus presents close resemblances to several families of the Simplices. It probably is a primitive form and near the ancestral type of the Pterideae, which thus seem to have developed a mixed sorus directly from a simple one.

Plagiogyria possesses a simple mesarch dictyostele from which the leaf-trace departs as a single, concentric, arched strand in the form of a rather broad inverted V. In this are three protoxylem groups, one median and two lateral. The median one, and from the figures apparently the other two as well, is at first distinctly mesarch (Text-fig. 6), but as the trace passes through the cortex it gradually becomes endarch. The median group soon divides into two, and this arch, very similar to that of the Dennstaedtineae, continues through the leaf. The early condition of the trace, therefore, shows a very close approach to our primitive bundle, being triangular in shape with three mesarch protoxylem groups.

In *Pellaea atropurpurea*, which was examined by the writer, a somewhat similar state of affairs was observed. The leaf-trace here is a single strand throughout its course, triarch at its base, and with the two lateral protoxylems sometimes mesarch ; but tetrarch and endarch higher up.

In *Adiantum*, however, although the trace is a single arch at its base, it soon becomes double, and assumes the *Onoclea* condition in the petiole.

These simpler Pterideae show a less highly developed structure than do the simplest forms of the other Polypodiaceae, but the more advanced members of the group may display extreme complication. The genus *Pteris* itself is an example of this. In *P. aquilina*, as is well known, the leaf-bundle is very intricate and in the shape of a much involuted arch. Its insertion upon the stele is complex, for it is derived from the medullary bundles as well as from the outer ring. It is interesting to note that at the base of the petiole the arch of bundles is much smaller and simpler than it is higher up, and that each strand is mesarch, as are the stem bundles, and not endarch, as is ultimately the case.

Young plants of this species were investigated to discover what was the probable primitive condition of the leaf-trace. Professor Jeffrey (17) has described the early siphonostelic condition of the central cylinder in this species. In the youngest stage observed by the writer, the leaf-trace departed as a single roughly triangular strand with but one protoxylem group as yet developed, which was median and mesarch (Fig. 18). A root-trace was inserted with it. As it passed through the cortex, a protoxylem group appeared at each lateral corner and the trace divided into two, each of which was a small but complete bundle of the *Onoclea* type. This condition continued through perhaps half an inch of the base of the petiole, but higher up the bundles increased in size, became irregular in shape, and merging and separating several times through the length of the petiole, formed an arch somewhat comparable to that in the mature leaf.

In a slightly older condition, the first indication of the departure of the leaf-trace was the appearance of a large island of parenchyma near the outside of the stele, which caused a 'swelling' in the xylem. On either side of this was a protoxylem cluster, each representing half of the single median group of the earlier stage. These protoxylems with the surrounding segment of the stele passed off as the leaf-trace and the peripheral swelling broke through, forming two xylem hooks, in the concavity of each of which was a cluster of protoxylem (Fig. 19). At each adaxial corner was a group of small cells which eventually became protoxylem also. The whole trace at this stage resembles two *Onoclea* bundles fused by their median masses of metaxylem. This is really the state of affairs, for the strand soon divides into two diarch traces. It would thus seem that in *Pteris*, at any rate, the very complicated petiolar condition is a derived one and has come through

the *Onoclea* stage from the simple triarch stage which we have considered primitive.

There are also certain members of the Pterideae which show a reduced condition, such as *Lindsaya* and most of the sections *Odontolema* and *Stenolema* of *Davallia*. Here the siphonostele is very much modified, the 'pith' consisting merely of an eccentric pocket of phloem. The leaf-trace at its exit is a small elliptical or slightly curved strand with a protoxylem group at each end. In the petiole, however, the typical triarch bundle is present although the adaxial hooks are lacking. This condition of the stele, known as the '*Lindsaya* type', has been much dwelt on by various writers and considered of importance as showing the origin of the siphonostele from the protostele. However, aside from its occurrence in a family of ferns admitted from their soral characters to be highly specialized, the fact that the nodal conditions are so widely at variance with what we have seen to be the universal rule in families of this series in lacking the triangular triarch bundle leads almost inevitably to the conclusion that we are dealing with a reduced rather than with a primitive structure.

In the Pterideae, therefore, we see the development of the leaf-trace from a single triarch and mesarch strand at the node of *Plagiogyria* to the endarch and tetrarch condition by the disappearance of the centripetal wood; the further modification of this to the *Onoclea* type by division into two; and finally the elaboration of this simple double bundle into the complex petiolar system of *Pteris* and its allies.

Most of the remaining Polypodiaceae probably originated from somewhere in the vicinity of the Dennstaedtineae and have in general progressed further than have the Pterideae. Their simpler forms show the *Onoclea* condition, and in *Asplenium Filix-foemina*, where the petiole displays this type of bundle, numerous instances were observed of the occurrence at the base of the leaf-trace of a single strand, of which the two median protoxylem groups, the only ones developed at this level, showed a strong tendency to be surrounded by metaxylem, and several cases of complete mesarchy were noted. One of these is shown in the left-hand group in Fig. 20. Such a structure as this seems undoubtedly the primitive one from which the typical double and diarch bundle has been developed.

Another fact noticed in these two-bundled forms was that the traces were always close together and sometimes, as we have seen, completely fused just as they departed from the stele, but that the sides of the gap seemed to pull away from them, and that although the traces themselves slowly spread apart, it was not until the base of the petiole was reached that they were as widely separated as were the two limbs of the gap from which they sprung. This constriction at the base of the leaf-trace seems to be present in all ferns.

In the more advanced Mixtae the petiolar bundle may take on every

variety of complication, but it seems reasonably clear that all of these have been derived, as was that of *Pteris aquilina*, from the simple double strand which we have called the *Onoclea* condition.

Two genera, *Woodwardia* and *Aspidium*, in which large and rather complex petiolar bundles occur, were investigated as to the history of the foliar strand. In *W. virginica* the trace is an arch of from six to ten bundles, of which the terminal or adaxial ones are by far the largest. It is interesting to note that in this case, as in *Pteris*, almost all the bundles are mesarch at their base. No young plants of this species were available, but as the other native species, *W. areolata*, which is much smaller, showed on investigation the presence of the typical *Onoclea* leaf-bundles, it seems reasonable to suppose that the more complicated condition has in this case been derived from the simpler one. Perhaps the two large adaxial strands in *W. virginica* correspond roughly to the two bundles which it originally possessed.

In the case of *Aspidium* and the closely related genus *Polystichum*, the larger native species, such as *A. spinulosum*, showed a petiolar bundle very similar to that of *Woodwardia virginica*, namely an arch of bundles, concave towards the axis and with two large terminal members (Fig. 21). In the smaller species, however, such as *A. Thelypteris*, the typical *Onoclea* condition was again found to be present. The young state of some of the larger species, such as *A. cristatum* and *A. spinulosum*, were examined and found to possess at first a single bundle, but later a double one throughout the leaf (Fig. 22). Each strand was very small and had no clearly defined protoxylem, but smaller cells were seen to be clustered at either end. Even at a very young stage small bundles began to be cut off from the abaxial ends of the traces and made the beginning of the later arch. In every case, however, both in young and in mature plants, the simplest condition of the vascular supply and the smallest number of bundles were always found at the very base of the trace. Each of the two large strands in the mature petiole possessed a hook and a protoxylem group only at its adaxial end, the other one being very obtuse. It seems a fair inference, especially from what we have seen as to the origin of the mature petiolar system of *Cyathea* (Text-fig. 9), that the abaxial protoxylem group of each strand, with its adjacent tracheides, has gone to form half of the band of small bundles which connects the two large ones. It thus appears that in *Aspidium*, as well as in *Woodwardia*, the primitive condition of the foliar supply is the double *Onoclea* bundle.

Chandler (10) describes the vascular system in many of the seedlings of these Polypodiaceae, and finds the leaf-trace to be at first a single strand which later divides into two, a condition which is usually retained for a long time. In *Nephrolepis cordifolia*, the only species in which the protoxylem is figured, the leaf-trace in its early state is a single strand

which is clearly triarch and apparently exarch, as we should expect. The structure of the leaf in young plants of the Polypodiaceae is a subject worthy of much further investigation.

The only family of the Mixtae which has apparently not arisen through the general line of ascent which we have outlined above is the Dipterineae. These ferns in the adult condition display a very close resemblance to *Matonia* in stelar structure, save that the siphonosteles are mesarch instead of endarch. The petiolar bundle is a single arch with much involuted ends and very many endarch protoxylems. The number of these, however, is much smaller as the trace leaves the stele, though it apparently is never as low as three. Young plants were examined by Seward and Dale (22), but are not described in sufficient detail to make clear the histology of the leaf-trace. From several lines of evidence, however, the family seems to have been derived from *Matonia*, and is therefore without much question primitively triarch.

The heterosporous genus *Marsilea* was also investigated and seems clearly to have come from among the triarch ferns. The leaf-trace, as it departs from the stele, is an arch with three endarch protoxylems. The median one of these soon bifurcates, and the trace then divides into two bundles, each of which has at either end a cluster of protoxylem, though no definite hook. This condition, which resembles that among such delicate ferns as *Asplenium Platyneuron*, is doubtless a reduction from the *Onoclea* type.

From the rapid survey of the Filicales which has just been completed, it would seem that our original classification of the order into three groups possessing primitively monarch, diarch, or triarch leaf-traces respectively, is one which holds for living families of ferns. The conclusions reached, though in accord with several writers who consider a relatively simple leaf-trace as primitive, do not agree with the opinions of Bertrand and Cornaille, who contend that a wide polyarch arch is the ancestral condition and that the *Onoclea* type of bundle represents a reduction from this. The evidence which we have cited from conditions in primitive families and from ontogeny do not support such a conclusion however. The results derived from our comparative study of the leaf-trace in living ferns strengthen most of the recent conclusions as to their relationships that have been based on other evidence, and it remains in conclusion to see what light is thrown on the subject by the structure of the fossil Filicales.

Anatomical knowledge of Mesozoic and Tertiary ferns is unfortunately very slight, and it is the beautifully preserved fossils of the Coal Measures which give us our best idea of what primitive members of the order were like. These plant remains are comprised in two main groups: the first, of large tree-ferns included under the comprehensive genus *Psaronius*; and the second of much smaller and simpler plants, the Botryopterideae.

Psaronius shows the most complex vascular system of any fern which we know. Its stems are large and contain many vascular strands arranged in a series of concentric circles, the relation of which to each other and to the leaf-trace has only recently been worked out. The trace itself departs from the outermost circle as a single broad arch, though this sometimes appears to be divided into two separate bands. The whole structure of the genus seems clearly not a primitive one, but the result of long development.

The Botryopterideae, or *Inversicatenales* of Professor C. E. Bertrand, on the other hand, are protostelic and probably much nearer to the original condition of affairs in the ferns. Three main families of this order, the Zygopterideae, the Anachoropterideae, and the Botryopterideae, are recognized by Dr. Paul Bertrand (2) in his masterly work on the anatomy of the Zygopteridean leaf. In all but the most primitive members of the first family the leaf-bundle is of the well-known 'H' type, more or less modified, with four mesarch protoxylem groups; in the second, it has the shape of a C with its convexity towards the stem axis and with two groups of endarch

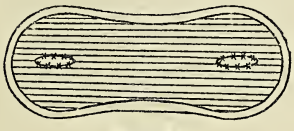


FIG. 10. The petiolar bundle of *Clepsydropsis*.

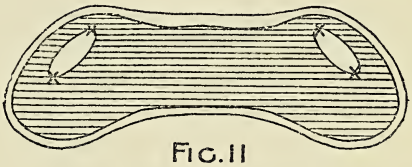


FIG. 11. The petiolar bundle of *Asterochlaena*.

protoxylem; and in the last, it is elongated radially, with one or sometimes two groups, which are also endarch.

In the genus *Clepsydropsis* of Unger, Dr. Bertrand finds a type of leaf-bundle which seems very primitive and which is probably ancestral for the whole family. It consists of a more or less elliptical body of xylem, at either pole of which is completely immersed an island of parenchyma with protoxylem at its margin (Text-fig. 10). Dr. Bertrand believes that by the disappearance of its centripetal wood and by the lateral and abaxial extension of its centrifugal wood, this bundle has given rise to such arched types of leaf-stele as are found in *Anachoropteris* and *Tubicaulis*, and that by further reduction these have produced the condition found in *Botryopteris*. By its increased constriction and the splitting into two of each protoxylem cluster, the 'H' type of bundle characteristic of *Zygopteris*, *Etapteris*, *Ankyropteris*, and *Stauropteris* has been derived. A rather clear series of connecting forms has been established by Dr. Bertrand in all these cases and his hypothesis seems well founded.

It is noteworthy in the light of these researches that a very primitive condition of the leaf-bundle was a clearly mesarch one with two protoxylems.

The monarch state was probably still earlier, for it occurs at the attachment of the leaf-trace to the stele in *Clepsydropsis* and *Asterochlaena*, as shown by the observations of Dr. Paul Bertrand. The single bundle with one mesarch protoxylem also occurs at the base of the leaf-trace, and very often in the foliar bundle among all the main groups of the Lycopsida, *Equisetum*, *Lycopodium*, *Phylloglossum*, *Selaginella*, *Psilotum*, and *Tmesipteris*, as well as in the fossil forms *Lepidodendron* and *Sigillaria*. It is thus probably the most primitive type of foliar strand.

The xylem of the trace in fossil ferns seems to have always been surrounded by phloem. This concentric structure, though absent in several living families, is clearly the primitive condition of the leaf-bundle in the Filicales.

In the Osmundaceae and Ophioglossaceae, families which stand apart from the rest of the ferns on many counts and which have become much modified from the ancient type, the base of the leaf-trace shows the primitive monarch and mesarch structure. Though this may possibly be a reduction from a diarch or triarch state, it seems reasonably clear that in the Osmundaceae, at any rate, it is the original condition, for in ancient members of the family from the Jurassic, as described by Kidston and Gwynne-Vaughan (19), the base of the leaf-trace is an elliptical mesarch bundle with one protoxylem group (Text-fig. 1). It is worthy of note that Chrysler (11), who has made a careful study of the leaf in the Ophioglossaceae, comes to the conclusion that this family approaches the Osmundaceae more closely than it does any other group of ferns. It seems reasonable to suppose, therefore, that these two families might have left the primitive fern stock before the leaf-bundle had become complicated, and that in them its later modifications, as well as those of the stele, proceeded independently of the rest of the ferns.

The occurrence of two bundles at the base of the trace in the Marattiaceae, a family where the leaf-bundle and the stele are so highly complicated, is logically explained only by assuming that we have here to deal with a persistence of a much simpler condition, which prevailed throughout the petiole and rachis of the ancestral leaf. This double bundle, which is so clearly mesarch in *Danaea*, has very possibly arisen by a splitting into two of the diarch and mesarch strand of the primitive Zygopterideae, the constriction of which is very evident in *Clepsydropsis* and *Metaclepsydropsis*. The presence of scattered centripetal tracheides in the petiole of *Danaea*, which is almost certainly an ancestral character, seems to indicate that this genus is more primitive than the others, in which case the occurrence of mesarchy in the base of its trace is all the more significant. That *Psaronius* is near the direct line of descent of the simple living Marattiaceae seems very doubtful, and it is perhaps nearest the truth to consider the whole family, which, like the Osmundaceae and Ophioglossaceae, stands apart from other ferns in many of its characters, to be a very primitive one which

arose from the early diarch Zygopterideae and perhaps gave off as a side branch the highly developed tree-fern flora of the Carboniferous, but which has persisted to the present day in a relatively simple state.

The question now arises as to the origin of the leaf-trace in that great body of living ferns which we have called the primitively triarch group. There is no clear indication in any of the Botryopterideae of a bundle with three protoxylems. *Asterochlaena*, a genus closely related to *Clepsydropsis*, presents a suggestive structure, however, for here the two terminal immersed parenchyma islands, each possessing two protoxylem groups, become elongated and oblique in the upper part of the petiole, as shown in Text-fig. 11, though the bundle resembles that of *Clepsydropsis* in the lower part of its course. This general flattening of the bundle may possibly be an adaptation to the recently assumed dorsiventrality of the leaf. If such a trace as this should become somewhat reduced, and if the two abaxial or median groups of protoxylem should tend to approach each other and eventually to fuse, a triarch mesarch trace rather triangular in shape would result. Some such process as this seems the most probable one by which the leaf-strand of *Lygodium* or *Gleichenia* might have arisen. This explanation is further strengthened by the fact that in living ferns it is always the median group of protoxylem which is the first to divide in the course of the higher development of the trace. This is perhaps shown most clearly by the series from *Plagiogyria* to the *Onoclea* type. Indeed, from the great readiness of the median protoxylem to bifurcate, it may perhaps be inferred that complete fusion has been attained only in certain cases, and that the condition of two closely approximated median groups may be the ancestral one. Against this should be placed the evidence from *Lygodium palmatum*, the only species of the genus with but a single median protoxylem. Here it is mesarch instead of exarch, and thus in a distinctly primitive position.

The evidence from the leaf-trace, then, as far as it goes, seems to point to the triarch Filicales as originally perhaps a somewhat reduced branch from the primitive Zygopterideae, that group which apparently stands so close to the common ancestry of all the ferns. *Lygodium* is the most ancient of all, for its solid bundle, with no sign of endarchy, runs through the whole leaf. In all the rest centripetal xylem is present only at the base of the trace, which becomes successively a double bundle and finally a complex series of separate bundles. The fact that *Lygodium*, which has held ancestral characters so well, is a climbing fern, and that the Botryopterideae may have clambered among their larger neighbours, leads to the suggestion that the ancestors of our living ferns may once have possessed the climbing habit.

It is worthy of note that in *Lyginodendron*, one of the most ancient and primitive of the Cycadofilices, the bundle at its very base is a monarch and mesarch strand, but that this soon becomes diarch, and finally divides into

two diarch and mesarch bundles which enter the base of the leaf. Here they approach one another and fuse by their abaxial or outer ends into a tetrarch arch (Fig. 23) which is very similar to the leaf-bundle of certain modern ferns, except that it is mesarch instead of endarch. Its first-formed tracheides lie near the outer edge, and the whole structure is very similar to the bundle of *Asplenium Filix-foemina* shown in Fig. 20. The number of protoxylems increases considerably, and the strand may become divided into several in the upper portion of the petiole, but these all unite into such a triarch and mesarch bundle as is described and figured by Scott (21). The gross structure of the vascular system of the leaf in *Lyginodendron* is very similar to that in many of the simpler triarch ferns, and besides showing the close similarity in development between the Lyginodendreae and the modern Filicales, it throws considerable light on the origin of the triarch type itself from the monarch and diarch conditions.

In conclusion, a word may be said as to the bearing of this whole matter on the important question of whether the stem in the Filicales is the leader in evolutionary development and the leaf lags behind it, retaining primitive characters as it does in the Lycopsida and Cycadaceae, or whether the reverse is the case and the leaf leads with the stem following. Important arguments in favour of the latter alternative have been made by Tansley and by Gwynne-Vaughan, both of whom consider the siphonostele to have been produced by the influence of an arched leaf-trace on a protostele. The former also cites certain cases of medullary bundles as probably intrusions into the stele of the complications of the petiolar vascular system (23).

The opposite view, however, seems to the writer much more convincing. The stem and the leaf, though doubtless interacting more or less on one another, serve very different purposes and seem to have developed independently, but of the two the leaf shows the greater conservatism and fixity of structure. This is evident in the first place from the position of its protoxylem. Tansley and Bower have emphasized the extreme irregularity and consequent unimportance of the position of these early tracheides in the Filicales as compared to its fixity in other vascular plants, and it might seem from this that the emphasis laid in the present paper upon the position of the first-formed elements of the wood was much too great. If we look carefully, however, we shall see that, no matter how it varies in the stele—for there are many cases of endarchy, of exarchy, and of mesarchy among the vascular cylinders of the Filicales—in every fern of which we know the anatomy except *Lygodium*, the protoxylem of the petiolar bundle is always endarch. This striking constancy, together with the fact that the leaf-bundles are almost without exception concentric, although internal phloem may be lacking in the stele, emphasizes the conservatism of foliar anatomy in the order and the apparent uniformity of conditions under which its

modifications have been developed. Perhaps more important even than this evidence is the fact that in every case the simplest and most primitive structures and relations of the vascular system occur at the node or in the base of the leaf-trace. It is a noteworthy fact that in very many ferns the leaf becomes slender at its attachment, and that in almost all of them the vascular supply, besides being much less complicated at this point than higher up in the petiole, is also composed of a much smaller number of cells. This is strikingly shown in *Osmunda* and in the Marattiaceae, but is very evident in almost all non-degenerate ferns. As the bundle ascends the increasingly wide petiole, it shows a strong tendency to broaden laterally, with the result that a single compact trace may become much stretched and sometimes divided, or that two bundles, at first close together, may be widely separated and broken up. It has often been pointed out by Gwynne-Vaughan, Tansley, and others that as all the water going to a leaf must pass through the base of its petiole, it is at this point that the greatest modifications for efficiency in water-conduction will appear. That increased size and complexity are evident in the lower portion of the leaf-stalk is quite clear, but the fact that this condition does not continue to the stele itself, but that the structure becomes continually more simple towards the very base of the leaf, makes it extremely doubtful if the size of the transpiration current has had much influence on the development of the vascular supply, for the stream is as well accommodated by the small double trace of *Danaea*, for example, as by its large petiolar bundle. From this fact, the argument for the derivation of a siphonostele from a protostele by the influence of a continually enlarging leaf-trace—a theory in which the shape of the trace and the appearance of the pith are accounted for by changes in the size and course of the transpiration current—loses much of its weight. This is further emphasized by conditions in many ferns with the *Onoclea* type of leaf-strand, where the two traces as they leave the stele are united or close together, but where they, as well as the two bundles from which they departed, gradually draw far apart from one another. The influence of the wide petiolar system can surely not have caused the widening of the dictyostele, for both systems are constricted when they meet.

Against the argument that medullary bundles in ferns are caused by the influence on the stele of the complication of the petiolar bundle, it may be pointed out that in the Marattiaceae the intricate vascular systems of stem and leaf communicate with one another by only a double trace, and that in young plants of *Pteris* a medullary bundle appears in the stele, while the leaf-trace is still a very simple strand.

It appears much more probable that the stele and the leaf-bundle have increased in size and complexity independently, as suggested above, and that the base of the leaf-trace, occupying an intermediate position and subjected to no great mechanical stress, has preserved most fully a primitive

state of affairs. In any fern the structure of the base of the leaf-trace represents a condition which probably once obtained throughout the entire petiole.

But why, if the smaller bundle is sufficient for the conduction of the necessary water, should it be later increased in size and pulled apart? In answer to this it may be pointed out that the lower and slender portions of the petiole are almost always either subterranean and thus supported by the soil or are immersed in a packing of old leaf-bases. The widest part of the leaf-stalk is where it becomes free, for at this point the strain of supporting the leaf is greatest, and there is therefore the most need of strength. The increase in size of the whole petiole here is probably a response to this need, and the amplification of the vascular bundle is either for increased rigidity or is simply complementary to the enlargement of the whole and serves to distribute the vascular elements more uniformly throughout the leaf-stalk, and also, perhaps, to facilitate the departure of the pinna-trace.

The simplified conditions found in the upper part of the rachis may show a retention there of ancestral characters, as suggested by Gwynne-Vaughan, but as the lamina is subject to change in external influences more than any other part of the plant, it would not be safe to conclude that the simplicity found in its upper portion was always a primitive simplicity.

In several cases observed there seemed to be an influence exerted by the stele upon the histology of the base of the leaf-trace. Its mesarchy in the mature leaves of *Pteris aquilina* and *Woodwardia virginica*, as noted above, is probably due to the carrying up for a little way of the mesarch condition of the stelar bundles. This influence is also felt in such cases of reduction as *Platyzoma* and the *Lindsaya* type. In these forms the degenerate state of the stele has influenced the base of the leaf-trace, and here it is the petiolar bundle which is least affected and therefore nearest the original condition.

From the conservatism of their general foliar anatomy, therefore, and from the fact that at the very base of the leaf-trace are found the simplest and most primitive structures and relations of the plant body in this order, it is clear that the Filicales form no exception to the general principle that the leaf of vascular plants is the seat of ancestral characters.

Summary.

1. The base of the leaf-trace in living ferns presents three main types of structure: the primitively monarch, with one group of protoxylem; the primitively diarch, with two; and the primitively triarch, with three.

2. The first type is characteristic of the Osmundaceae and the Ophioglossaceae, where the base of the leaf-trace is a single monarch strand, which is often mesarch as well. It is more strikingly so in the fossil

ancestors of the former family. This single strand becomes in the petiole a broad arch, which is continuous in the Osmundaceae and broken up in the Ophioglossaceae. The primitive condition of this type of trace is an elliptical concentric strand with one mesarch protoxylem (Text-fig. 1).

3. The second type is characteristic of the Marattiaceae. In all the members of this family save *Angiopteris*, and in young plants of this genus, the base of the leaf-trace consists of two bundles, each of which has a single protoxylem. This is endarch in all except *Danaea*, where it is often mesarch. A complicated arch of bundles in the petiole develops from these two early ones. The primitive condition of this type of trace is two circular concentric bundles, each with one mesarch protoxylem (Text-fig. 2).

4. The third type is characteristic of all remaining ferns, and its primitive condition is a single, roughly triangular, concentric bundle, with its base towards the stem-axis and with three mesarch protoxylems, one near each corner (Text-fig. 3). In the Schizaeaceae, Gleicheniaceae, and primitive Matonineae among the Simplices, the strand is single throughout, and at its base is always triarch and often mesarch. *Lygodium* alone among the Filicales shows a petiolar structure which is neither endarch nor arched, and which is doubtless very primitive. In the simpler Gradatae, the trace becomes broadened into a tetrarch, flat-topped arch, which becomes separated into many strands in the Dicksonieae and Cyathaceae. The Hymenophyllaceae form a reduction series from the Simplices. In the lower Mixtae, the undivided tetrarch trace persists, but in the bulk of the smaller and simpler forms it becomes divided into two equal diarch bundles. The complicated petiolar system of the higher Polypodiaceae is always referable to this simpler type.

5. The monarch trace may be considered as the persistence of a very primitive condition. The diarch type has perhaps been derived from the constriction and separation into two of such a primitive diarch bundle as that of *Clepsydropsis* among the Zygopterideae, while the triarch condition may have arisen by the amplification of a similar bundle into a tetrarch strand, a slight reduction of this, and the fusion of the two median protoxylem groups.

6. The constant endarch and concentric structure of the leaf-bundle, as opposed to the varying conditions in the stele, point to the former as a conservative organ.

7. The petiole at its attachment is in many cases very slender, and the base of the leaf-trace presents always its smallest and most compact condition. Structures are often retained here which are undoubtedly very primitive. The petiole is largest at the point where it first becomes free, probably for mechanical reasons, and the bundle here is in its most complex form.

8. Since the transpiration current seems to be accommodated equally by

the slender trace and by the large petiolar bundle of the same leaf, it is improbable that it has had much influence in producing the complications of the foliar vascular system. The stele and the leaf-trace have developed almost independently and the latter has had little influence in moulding the former.

9. The condition found in the base of the trace in any fern was probably once present throughout the whole leaf-bundle.

10. The simple condition in the upper part of the leaf may possibly be regarded as primitive.

11. The histological influence of the stele is sometimes carried up into the base of the petiole, especially in cases of reduction, where the petiolar bundle, better than the lower part of the leaf-trace, retains conditions which may be regarded as primitive.

12. In the Filicales, as in all other vascular plants, the leaf-trace is the seat of ancestral characters.

The writer is much indebted to Professor E. C. Jeffrey, Professor M. A. Chrysler, Mr. A. J. Eames, and the authorities of the Botanic Garden of Harvard University for aid in securing material, and to Professor Jeffrey for helpful advice during the course of the work.

This investigation was carried on in the Phanerogamic Laboratories of Harvard University.

Harvard University.
June, 1910.

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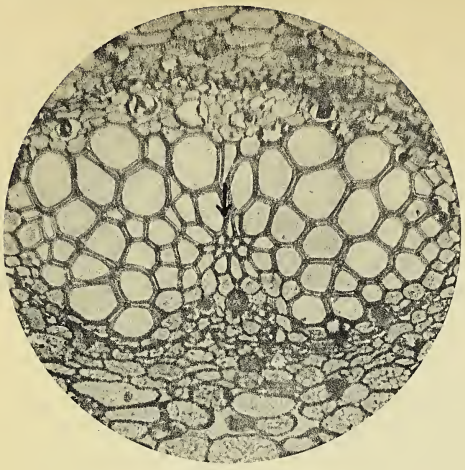
DESCRIPTION OF FIGURES IN PLATE XI.

Illustrating Mr. Sinnott's paper on the Filicinean Leaf-trace.

A cross indicates the position of the protoxylem in the text-figures; an arrow, in the photographs.

Fig. 12. The base of a leaf-trace of *Todea hymenophylloides*. × 120.
Fig. 13. The base of a leaf-trace of *Helminthostachys*. × 80.
Fig. 14. The base of a leaf-trace of *Danaea*. × 40.
Fig. 15. The attachment of a leaf-trace of *Gleichenia Speluncae*. × 150.
Fig. 16. The attachment of a leaf-trace of *Gleichenia circinata* v. *macrophylla*. × 70.
Fig. 17. The base of one of the two leaf-traces of *Onoclea sensibilis*. × 100.
Fig. 18. The attachment of the leaf-trace in a very young plant of *Pteris aquilina*. A root-trace is inserted with it. × 80.
Fig. 19. The attachment of a leaf-trace in an older individual of the same species. A root-trace is present here as well. × 150.
Fig. 20. The base of a leaf-trace of *Asplenium Filix-foemina*. × 80.
Fig. 21. Petiolar bundle of *Aspidium spinulosum*. × 15.
Fig. 22. Petiolar bundle of a very young leaf of *Aspidium cristatum*. × 40.
Fig. 23. Bundle in the base of the petiole of *Lyginodendron Oldhamium*. × 40.



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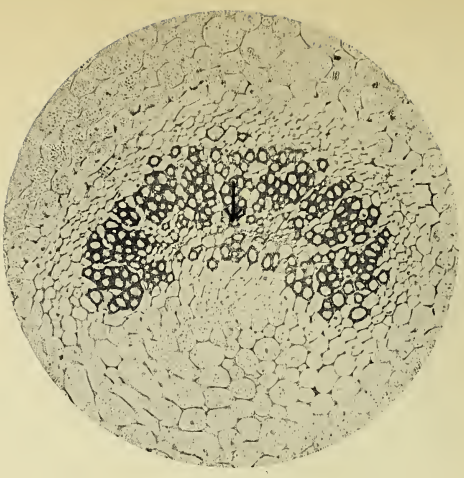

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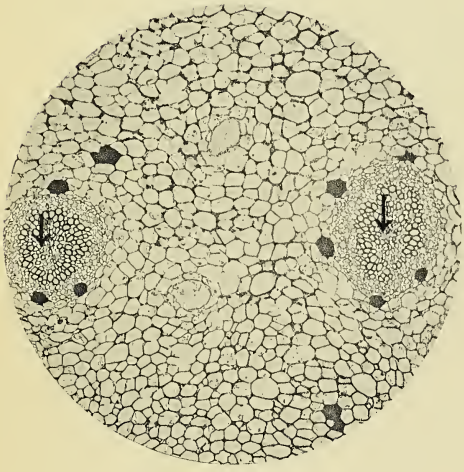

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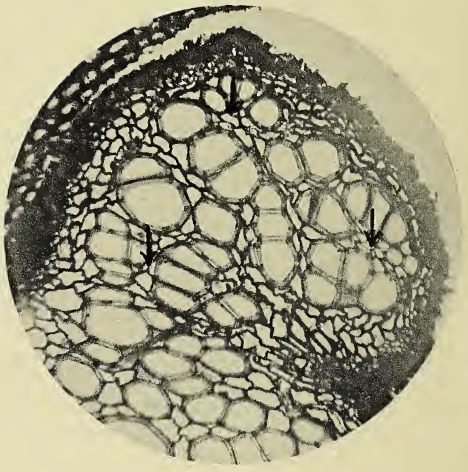

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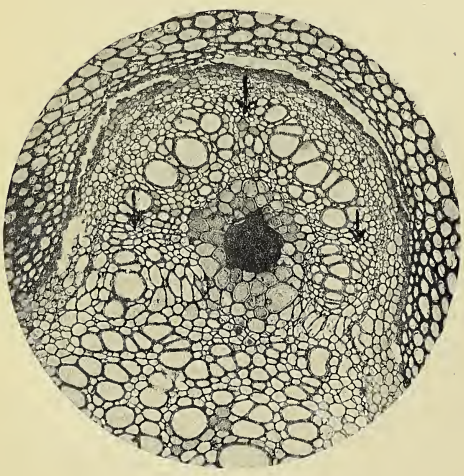

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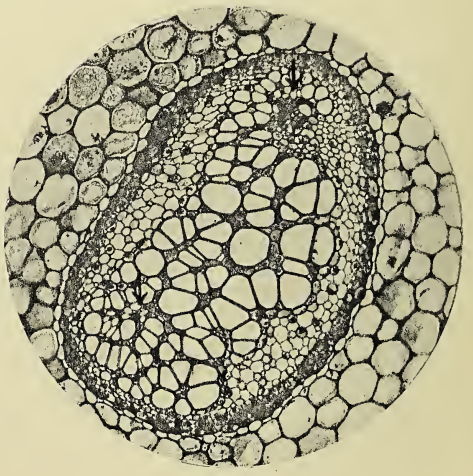

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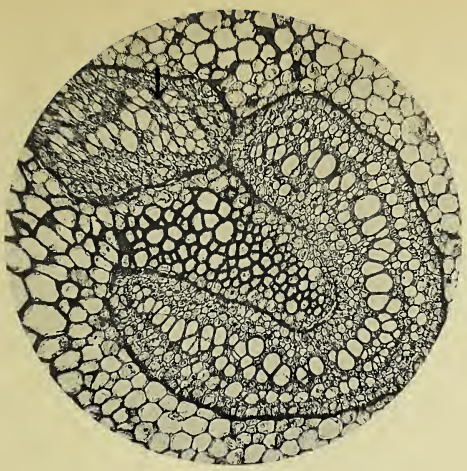
E.W.S. photo.

SINNOTT — FILICINEAN LEAF-TRACE.



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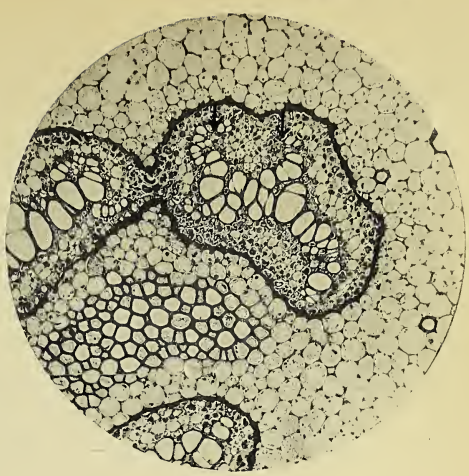

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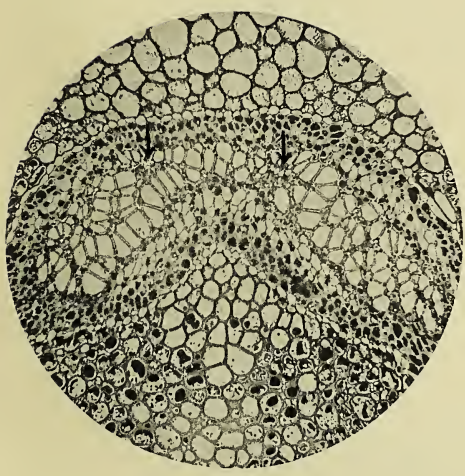

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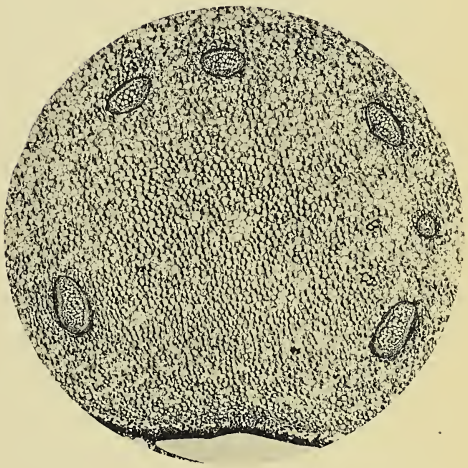

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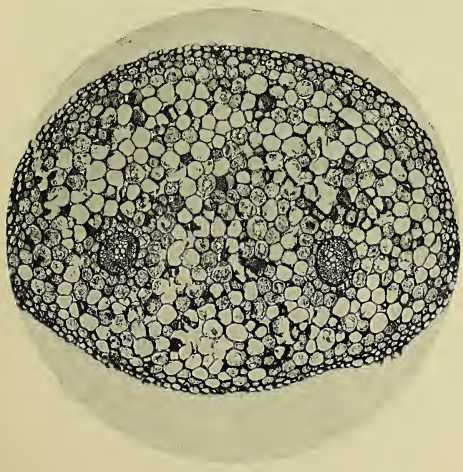

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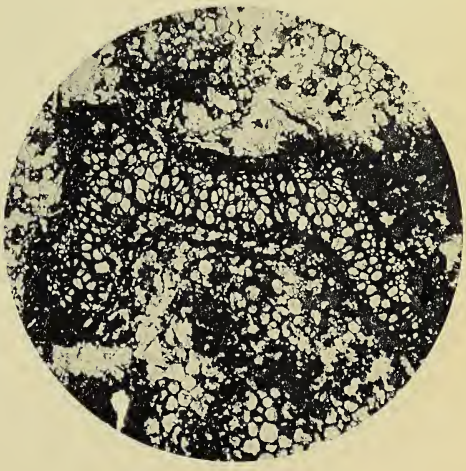


23.

Huth, coll.

A Lower Cretaceous Species of Schizaeaceae from Eastern North America.

BY

EDWARD W. BERRY.

Associate in Palaeobotany, Johns Hopkins University.

With Plate XII and one Figure in the Text.

THE present brief communication is published in order to demonstrate that certain leaf impressions from the lowermost Cretaceous of the Atlantic coastal plain, which have been referred to the Ginkgoales, are to be referred to the Filicales, and considerable evidence will be brought forward for the reference of these remains to the family Schizaeaceae or to the Mesozoic representative of the modern family.

When Professor Fontaine undertook the study of the flora of the Potomac group, which culminated in Monographs XV and XLVIII of the U.S. Geological Survey, a variety of *Baiera*-like forms were figured and described. These were made the basis of the genus *Baieropsis* referred to the order Ginkgoales, in which ten species were described, and the genus *Acrostichopteris* referred to the Filicales, in which five species were described. In a re-study of the original, as well as a large amount of additional material, it became obvious that the specific differentiations which had been put forward were absent in nature, and that the remains of both *Baieropsis* and *Acrostichopteris* were for the most part congeneric in cases where they were not specifically identical, and that all were indubitably ferns, as shown by the habit of the fronds and their manner of subdivision and fructification. These fructification characters are obscure in all but two of the forms, and hence in a recent revision[1] the writer has dropped the name *Baieropsis* altogether and referred all of the forms except these two to the genus *Acrostichopteris*, whose exact position among the Filicales remains unknown.

The two forms above referred to as showing fructification characters of value, although described by Professor Fontaine as *Baieropsis macrophylla* and *Baieropsis expansa*, prove to be identical. They have furnished certain rather definite criteria for their reference to the family Schizaeaceae, using

[1] Berry, Proceedings U.S. Natural Museum, vol. xxxviii, pp. 625-632, 1910.

that term in a somewhat general sense, and since the term *Baieropsis* is not available as well as grossly inappropriate, a new generic name is demanded, and *Schizaeopsis* is here proposed. The single known species with its synonymy becomes as follows :—

Schizaeopsis expansa (Font.).

Baieropsis expansa, Fontaine, Potomac Flora, 1890, p. 207, Pl. LXXXIX, Figs. 1, 3 ; Pl. XC, Fig. 1 ; Pl. XCI, Fig. 2 ; Pl. XCII, Fig. 5.
Baieropsis macrophylla, Fontaine, ibid., p. 212, Pl. XC, Fig. 6.

Fronds relatively large, about 11 cm. in length by 6 cm. in width, apparently short stalked, divided almost to the base into two principal ribbon-like divisions, which in turn are almost immediately subdivided dichotomously into two similar subordinate divisions, which are dichotomously forked in a like manner at varying heights. In the nearly complete specimen figured, from which the restoration has been made, the outer main division of the frond is somewhat less developed and less cut up than the inner main division. The texture is coriaceous. The veins are thin but strong, in some specimens suggesting a double vascular strand ; they fork dichotomously near the region where the frond forks, and then repeatedly at varying intervals, but they are for the greater part of their course unbranched and approximately parallel. They are somewhat more numerous than in the comparable modern species of *Schizaea*. The fructifications as preserved are brownish spindle-shaped bodies about 4 mm. in length and 1 mm. in diameter. They were observed and figured by Fontaine in the specimens named by him *Baieropsis macrophylla*, and were considered to be of a pathological nature, i.e. fungal, but were not noticed on the specimens which he described as *Baieropsis expansa*, although they are readily seen in the figure here reproduced, which is from a photograph of the specimen from which Fontaine drew his Fig. 1 on Pl. LXXXIX of the Potomac Flora. These fructifications are borne at the distal ends of certain of the veins at varying heights, usually along the margins, but occasionally on the face of the laminae. Ordinarily they are massed towards the distal ends of the ultimate divisions of the frond, as in the modern *Schizaea elegans*, the ultimate ones appearing as continuations of the ultimate teeth which terminate the distal lacinae. Numbers of these fructifications are in organic connexion with the fronds, so that there is no room for any mistake in observation. These objects are found upon microscopic examination to be made up of masses of closely packed, relatively large spores, in the ground mass of which there are traces of other tissues which cannot be made out, but which evidently represent peduncles and synangial walls. These spores are nearly spherical in form, a feature common to the genera *Aneimia* and *Lygodium*, but apparently not to

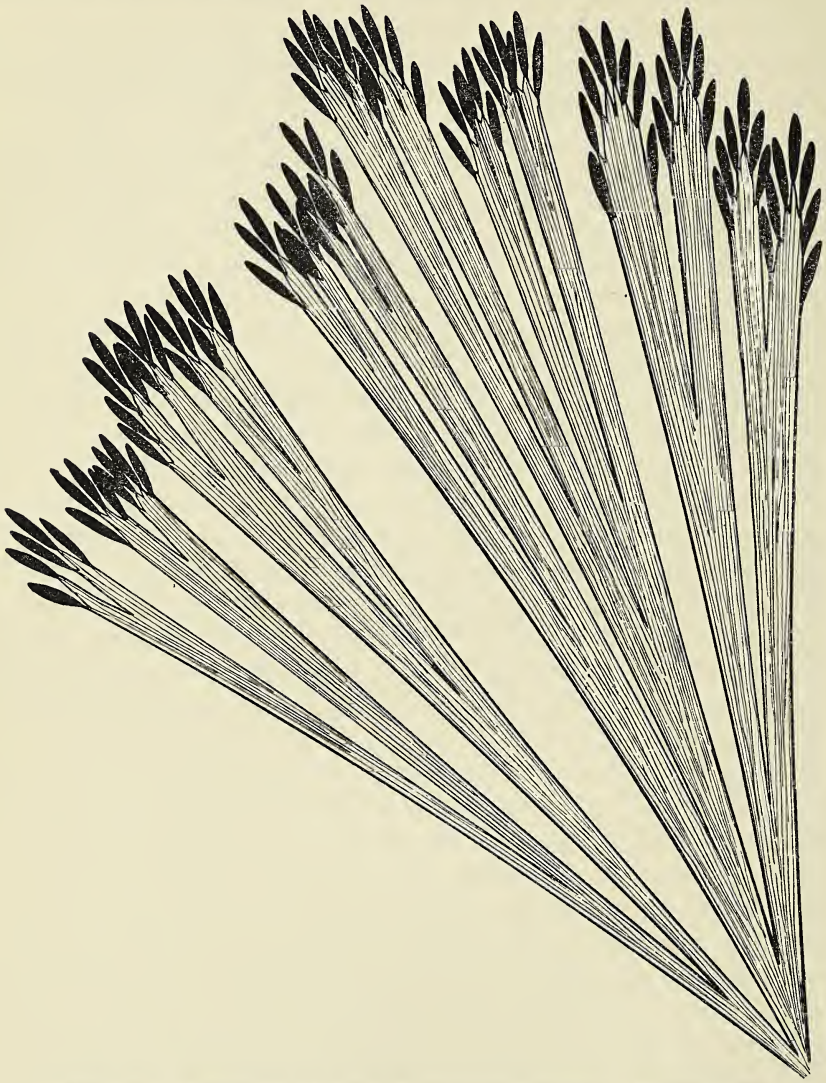
Schizaea. They are of large size, averaging about $\frac{1}{10}$ mm. in diameter. The tetrad scars are small but well marked, but show no protuberances or ornaments at the outer angles. The walls are thick and strongly striated, another feature of the modern Schizaeaceae, especially of the genus *Aneimia*, and well shown in the camera lucida drawings of the fossil spores reproduced on Pl. XII, Fig. 2. The spore contents are for the most part dissipated, only the yellowish exine remaining, and the walls are frequently collapsed, so that with a low power they appear cross-lined because of their juxtaposition.

Since only the spores are preserved, the morphology of these fructifications is conjectural. They have the appearance of simple fusiform sporangia of gigantic size, but it is believed that they represent a large number of pairs of more or less confluent, or at least close packed, sporangia.

The specimens are found in a partially lithified sandy clay, almost an argillaceous sand, but the sand is fine-grained, so that the fossils are well preserved, as indicated by the specimen photographed. In this specimen the spores were evidently nearly mature, as indicated by their size and configuration. None seen are in tetrads, and yet the sporangia could hardly have dehisced before fossilization, since each tiny rock cavity which represents these fructifications is packed with the spores. In some of the impressions there are faint transverse lines on the matrix, as if they marked the line of demarcation between successive pairs of sporangia, and in one case the vein upon which the fructification was borne can be traced the entire length of the fructification, clearly indicating that it is not a gigantic simple sporangium, but an aggregate of sporangia comparable to that of the modern genus *Schizaea.*

With regard to the botanical affinity of this species, the writer's convictions are indicated in the generic name. No modern group of ferns fulfils the conditions as does the family Schizaeaceae. The fossil fern is identical with various modern tropical members of this family in vegetative habit, no other modern ferns known to the writer resembling it in the character of the fronds except the genus *Rhipidopteris* of the Polypodiaceae, which has a quite different habit and type of fructification. The venation is closely similar to *Schizaea.* The fructifications are similarly borne and the spores are similar in form and markings to the closely allied modern species of *Lygodium* and *Aneimia.* It is believed that the combination of close agreement in vegetative characters with a similar close agreement in fructification characters, in so far as they are determinable from the nature of the material, justifies the reference of these ferns to the family Schizaeaceae, a family which on theoretical grounds we would expect to find represented in the lower Cretaceous. Whether the detailed organization of the fructifications conforms to that which obtains in the modern members

of the family cannot be determined; presumably there were differences, but these were probably not greater than those between the existing genera referred to this family. A restoration of the fossil drawn from the specimen figured in Pl. XII is shown in the Text-Fig., enlarged $1\frac{2}{3}$ times.



TEXT-FIG. Restoration of a frond of *Schizaeopsis expansa*. $1\frac{2}{3}$ natural size.

The species occurs in the oldest formation of the Potomac group, the Patuxent formation, which in age corresponds in a general way with the upper Wealden of Europe. The figured specimens come from Fredericksburg, Virginia; comparable, but more or less fragmentary, specimens have been found at a number of other localities in Virginia. The fern genus

Acrostichopteris, which is very similar in vegetative character, is best retained for the somewhat similar remains of fronds until definite information is obtainable regarding their reproductive structures, although it is extremely probable that some at least of the species of *Acrostichopteris* should be referred to the Schizaeaceae, and the same remark is equally applicable to certain species ordinarily referred to the genus *Baiera*.[1] To mention a specific case, the homotaxial species *Baiera cretosa* of Schenk is very close to *Schizaeopsis expansa*, and was originally compared by Schenk with the modern *Schizaea elegans*.

As has already been mentioned, there are abundant theoretical reasons for expecting to find representatives of this family as far back at least as the later Mesozoic. Such still earlier ferns as have been supposed to exhibit affinities with the Schizaeaceae are too obscure and indefinite to be of much value, and it seems certain that the older Mesozoic and Palaeozoic ferns, at least the Leptosporangiate ones, were too generalized to admit of their being referred to the accepted families based, as the latter are to such a large extent, upon existing species. There is, however, abundant collateral evidence for the view that by the dawn of the Cretaceous the main lines of cleavage which separate the families as we now know them were rather clearly defined. In addition to the *Schizaea*-like species here described, the Schizaeaceae were represented in the lower Cretaceous rocks of both Europe and America by several species referred to the genus *Ruffordia*, which in the character of its fructifications and sterile fronds resembles the modern genus *Aneimia*. There is of course the well authenticated Jurassic genus *Klukia* of Raciborski,[2] which seems to fall within this family, and Professor Zeiller[3] has recently called attention to certain fern-remains from the Wealden of Peru which show sterile fronds similar to those of *Cladophlebis Browniana*, which bore annulate sporangia of the *Schizaea* type. Stopes and Fujii[4] have also described structural material from the upper Cretaceous of Japan sufficiently preserved to show some of the soral characters and to warrant the restoration of the sporangium of what they have named *Schizaeopteris mesozoica*. While the genus *Schizaeopsis* is thus far confined to the eastern United States, the fern genus *Acrostichopteris*, which so closely resembles it in vegetative habit and geological range, has been found in the Kootanie formation of the western United States and in the Wealden of England, and identical forms have been described as various species of *Sphenopteris* from the lower Cretaceous rocks of Portugal by Saporta.

[1] This is, of course, not true of all species of *Baiera*, some of which, in their fruiting characters, show conclusively a relationship with *Ginkgo*.

[2] Raciborski: Englers Bot. Jahrb., vol. xiii, 1891, p. 1.

[3] Zeiller: Comptes rendus, tome cl, 1910, p. 1488.

[4] Stopes and Fujii: Philos. Trans. Royal Soc., vol. 201 B, 1910, p. 6, text-figs. 1–3, pl. ii, fig. 1.

ACKNOWLEDGEMENTS.

I am indebted to Mr. W. R. Maxon, of the U.S. National Herbarium, for advice and for the use of specimens in his charge; to Dr. F. H. Blodgett, of Roanoke College, a former student at the Johns Hopkins University, for the camera lucida drawing of the spores; and to the U.S. Geological Survey for permission to reproduce the photograph of the specimen here figured.

SUMMARY.

1. The genus *Baieropsis* of Fontaine is shown to belong to the Filicales and not to the Ginkgoales.

2. Certain forms heretofore referred to *Baieropsis* show fructification characters which justify their reference to the family Schizaeaceae.

3. This affinity is corroborated by the identity of the frond characters between the fossils and certain modern species of *Schizaea* with which they have been compared.

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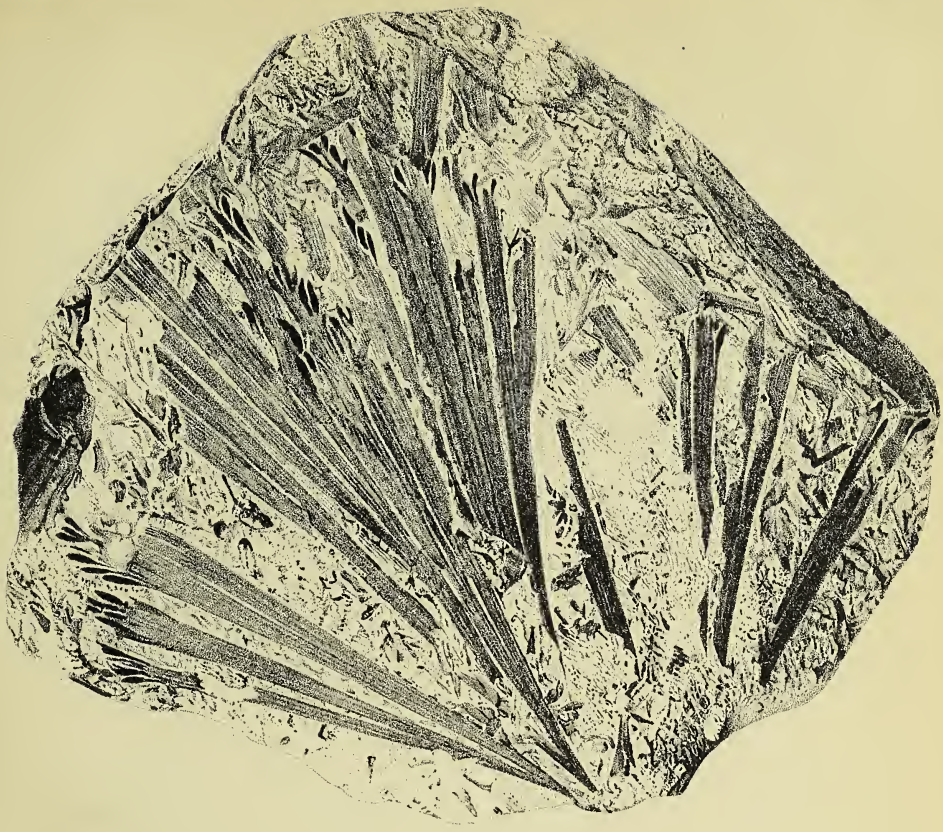
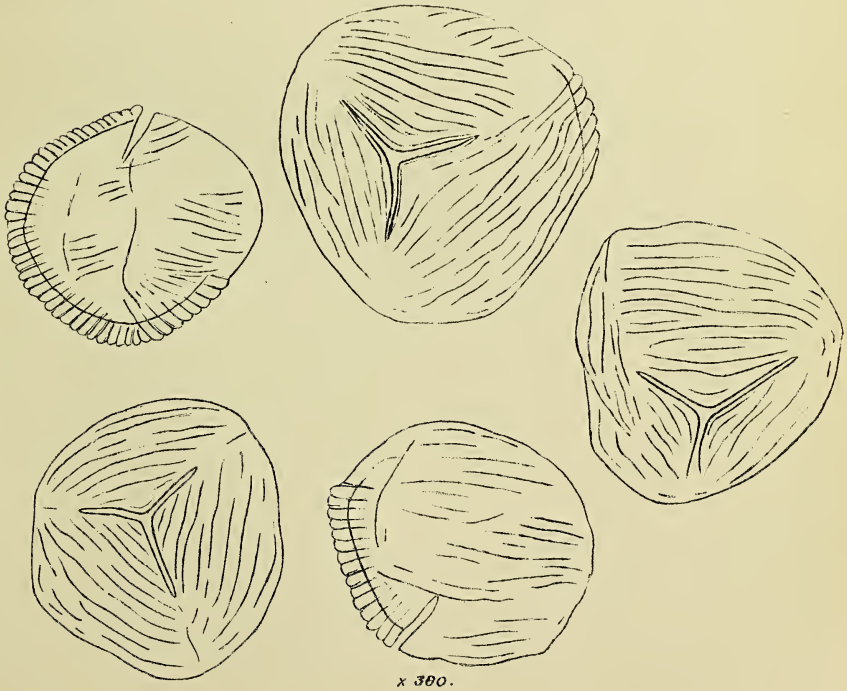
BALTIMORE, MD., U.S.A.

EXPLANATION OF PLATE XII

Illustrating Mr. Berry's paper on a Lower Cretaceous Species of Schizaeaceae.

Schizaeopsis expansa (Fontaine). Above, reproduction of the type from the Patuxent formation of Fredericksburg, Virginia, natural size. Below, camera lucida drawings of spores from the same specimen, × 380.





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BERRY — SCHIZAEOPSIS EXPANSA

Studies in Spore Development.

BY

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With Plate XIII.

Introduction.

DURING the eighties of last century great activity was displayed in the investigation of the structure and development of the spores and pollen-grains of a large number of plants. The first impetus to this activity was due to the splendid investigations of Strasburger upon this subject, which he published in his treatise 'Ueber den Bau und das Wachsthum der Zellhäute' in 1882 (16). Two years later Hubert Leitgeb issued his studies upon the structure and development of a number of spores with particular reference to those of the Hepaticae. In 1886 appeared Wille's 'Ueber die Entwickelungsgeschichte der Pollenkörner der Angiospermen', whilst in 1889 Strasburger published a further series of studies on spores and pollen-grains in Heft II of his 'Histologische Beiträge'.

After this date there was a temporary lull in the investigation of spore histology, and until the opening year of the new century the only important contribution to the subject—apart from the interesting microchemical work of Mangin—is yet another publication of Strasburger's in 1898 upon 'Die pflanzlichen Zellhäute' (18).

In 1900 an important account of the structure and development of the spores of *Isoëtes* and *Selaginella* appeared from the pen of Hans Fitting (5). In this work, crowded with a wealth of interesting details, probably the most unexpected feature was the demonstration that the spore-walls could carry on their growth although the protoplast of the spores was not in contact with them during this process. Fitting's discovery was very soon confirmed in *Selaginella* by Campbell (2), and a little later by Denke (4). Miss Lyon (10 *a*) has given a different interpretation of the growth of these membranes, but I think her objections have been sufficiently met by Fitting's reply in the 'Botanische Zeitung' (6). In 1905 I was able to find another case (*Oenothera*) in which the pollen-membranes possess independent powers of growth

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whilst the protoplast is not in contact with them. In 1908 Tischler (21) observed another and most striking example of this phenomenon. In the pollen-grains of *Mirabilis Jalapa* the protoplasmic contents degenerate and shrink to a scarcely noticeable quantity, and yet notwithstanding this the exine of these grains continues to grow very considerably in thickness and extent. For some time past I have been examining a large number of spores and pollen-grains belonging to many species of plants in order to find, if possible, other examples of membranes which are able to continue their growth without the direct co-operation of the protoplasm. I have, up the present, found no other such striking cases as those of *Selaginella*, *Isoëtes*, *Oenothera*, or *Mirabilis*, but a careful examination of so large a number of different spores could not fail to bring to light many interesting details which supplement our present knowledge of the subject.

I have already given a description in these pages[1] of two of these spores (viz. *Helminthostachys* and *Riccia*), and I now propose to add an account of some other pollen-grains and spores which I have had under observation. I intend to deal with the pollen-grains of *Ipomoea* in the present part.

I. Ipomoea.

The only account of the finer structure of the pollen-grains of a species of *Ipomoea* is the very short description given by Strasburger in 1889 (17). After having furnished a minute account of the spinous pollen of several species of Malvaceae, Strasburger devotes a few lines to the pollen-grains of *Ipomoea coccinea*, Moench., which appeared to him to be constructed quite after the manner of the Malvaceous type.

I have examined the pollen-grains of *Ipomoea purpurea*, Roth.,[2] in some detail, and as I find that these differ in several respects from the Malvaceous type of pollen, I will begin these 'Studies' with a description of them.

My material was fixed partly in Flemming's solutions and partly in chrom-acetic mixture without osmic acid. So far as possible I have checked my results by comparison with living material examined in 0·6 % NaCl solution, but the opacity of the structures did not render this method a very satisfactory one in the present case.

The pollen mother-cells of *Ipomoea* usually form two or sometimes three longitudinal rows in each pollen-sac; they are each surrounded by a wall which gives the reactions of callose and also of pectose, and they include a rather large nucleus (about 14 μ in diameter) which contains, as a rule, a single large nucleolus and a loose reticulum of fibres. The tapetal cells,

[1] Ann. of Bot., vol. xx, April, 1906, pp. 177–186, and Ann. of Bot., vol. xx, July, 1906, pp. 275–291.

[2] This plant is also known as *Pharbitis hispida* (Choisy).

which form a single layer round the mother-cells, are radially elongated structures which in the majority of cases enclose two nuclei, although cells with three or even four nuclei are met with. The stages of the division of the pollen mother-cells were but poorly fixed in my preparations, and, with the exception of the telophase of the second meiotic division, which was well shown in my sections, I will make no reference to the subject.

At the conclusion of the second division of the pollen mother-cells the chromosomes retain their individuality for some time after a nuclear wall has been reconstructed and a new nucleolus (or nucleoli) formed in each daughter nucleus (Pl. XIII, Fig. 1 *a*). The chromosomes are distributed throughout the nuclear cavity, but are connected with one another by a delicate linin threadwork. A little later the sharply defined, homogeneous chromosomes become more irregular in outline and apparently vacuolar in structure (Fig. 1 *b*), and it is easy to trace the gradual opening out of their substance and its distribution over the linin network until no trace of any individual chromosome can any longer be detected (Fig. 1 *c*). After the division of these nuclei is finally completed, therefore, nothing can be seen in the nature of the 'prochromosomes' which Rosenberg (14, 15), Overton (12, 13), Laibach (10), and others have described in the 'resting' nuclei of various plants.

I have examined the nuclei of the other tissues of the anther for prochromosomes, and in some, notably those of the young vascular tissue, chromatic aggregates are to be seen lying beneath the nuclear wall which resemble in appearance the prochromosomes of other writers (Fig. 1 *d*). Their number, however, appears to me to be too inconstant in these cells to have the significance which attaches to true prochromosomes.

There is evidently great variation in the behaviour of the chromosomes of different cells at the conclusion of nuclear division. In some, such as in the cases described by Rosenberg and others, the chromosomes appear to retain a large proportion of their material definitely aggregated as clearly distinguishable prochromosomes throughout a prolonged period of rest. In other cases, such as the pollen mother-cells of *Ipomoea*, the chromosomes retain their individuality as distinct bodies for a short, but yet quite definite, period, and then their substance becomes evenly distributed over the linin reticulum. Finally, in many other cells the chromosomes become vacuolated and their substance dispersed over the linin threadwork immediately at the conclusion of mitosis. In cells such as those of the young vascular tissue of *Ipomoea*, in which an inconstant number of chromatic aggregates occurs, it is not improbable that some of the chromosomes may remain visible as distinct bodies for a greater or less time, others may become distributed over the linin at once and lose their visible individuality, whilst others may become vacuolated and broken up into two or more smaller but still recognizable bodies.

The tetrads of young pollen-grains become surrounded by massive mucilaginous walls. Upon the periphery of each tetrad group is a granular deposit which stains with Bismarck brown but not with aniline blue or corallin soda. This is probably the remains of the primary wall which separates the sporogenous cells from one another. Within this a distinct, often rather massive, layer is seen which possesses the staining properties characteristic of Mangin's callose and of pectose.[1] This is the mother-cell wall already referred to above. Within this again is another mucilaginous wall which also gives the reactions of callose and pectose and which envelops the young pollen-grains and separates them from one another. For this innermost wall Strasburger (19) has recently suggested the convenient name of *Special wall* to replace the old term Special mother-cell wall with its false implication (Fig. 2).

In microtome sections which have been stained either with aniline blue or Congo red three radiating lines (really lamellae), often of a granular appearance, can be seen to traverse the middle of the special wall (Fig. 2). These granular bands are the first lamellae which are formed at the conclusion of the division of the mother-cell. In my sections stained with Heidenhain's iron-alum haematoxylin and Bismarck brown these lamellae are often quite unstained and appear as colourless clefts or lines in the middle of the brown special wall. Mangin (11) has described similar triradiate lines of granules in the case of *Althaea rosea*, and he states that they are nitrogenous in character. I was unable to determine their chemical nature in the case of *Ipomoea*.

At the time when the callose-pectose walls break down and set the pollen-grains at liberty it is often seen that the triradiate lamellae continue to exist for some time in the midst of the flocculent material derived from the rest of the wall (Fig. 3).

The callose-pectose layers of the special wall which immediately envelop the pollen-grains and which are the latest parts of this wall to be formed are denser than those in the neighbourhood of the granular lines.

There is evidence that the special wall of *Ipomoea* possesses a laminated constitution.

The young pollen-grains of *Ipomoea* surround themselves with a wall of their own—the exine. This is deposited by the pollen-protoplast as an extremely delicate layer upon the inner face of the callose-pectose wall which surrounds it. From the first it is marked off as an independent structure from the callose-pectose special wall, and there can be no doubt that it is a new membrane and not one derived from the transformation of the innermost lamellae of the special wall.

In its earliest stages it is an exceedingly delicate membrane, which is too thin to permit any structure to be seen in it even with the highest

[1] Compare Tischler (21), p. 48.

powers of the microscope and in the most delicate microtome sections. In Fig. 4 a young pollen-grain is represented lying within the special wall. Here the pollen-protoplast has contracted under the influence of the re-agents, and the young exine has also separated from the special wall from the same cause. Under these circumstances the newly developed exine can be seen exceptionally well as an independent membrane of great tenuity. In somewhat older pollen-grains a structural differentiation of the exine can be detected which even at this early stage exhibits some complexity. The exine can now be seen to consist of an outer lamella, upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this network the rudiments of the future spines already occur. Between the thickening bands and the outer lamella a narrow cleft or unstained space can be seen, and this is the position in which the rodlets of older pollen-grains are developed.

Fig. 5 shows the inner face of the exine at this stage in surface view. The more deeply staining system of thickening bands is seen to form a reticulum with polygonal (mostly hexagonal) meshes upon the lighter outer layer of the exine. At the angles of the network the spine rudiments are seen as deeply coloured dots.

Fig. 6 *c* represents the same stage in section. Here the alternation of thicker areas, where the thickening bands lie, with thinner intervals is seen. In very delicate microtome sections the separation of the thickening band from the outer lamella of the exine by a clear, unstained space or layer can readily be made out (Fig. 6 *b*).

Where the section has passed through the spine rudiments the appearance is somewhat different. In Fig. 6 *a* it will be seen that the thickening band appears to be pushed inwards (by the colourless layer) at each spine rudiment so as to form an internal spine. The spine rudiment itself appears as a deeply stained particle just within the apex of each of these projections. The external surface of the exine is still completely flat and smooth.

The mode of development which these component parts of the exine follow is a difficult matter to decide with certainty. There can be little doubt, I think, that the outermost lamella and the system of thickening bands are successive developments secreted by the pollen-protoplast one after the other. The thin structureless membrane of such stages as that represented in Fig. 4 I believe to correspond to the outermost lamella alone. Upon this the bands of thickening are laid down by the protoplast in somewhat older pollen-grains. At first these bands are so thin and faintly marked as to appear as little more than shadowy traces upon the inner face of the membrane, but they rapidly gain in distinctness as development proceeds and new material is added to them by the protoplast. Exactly how and when the spine rudiments and the rodlets are first developed is a more difficult problem to determine. The impression which I have

gained from the study of my preparations is that the clear space which is seen in the sections to lie between the thickening bands and the outer lamella represents a third and distinct layer of substance (with little affinity for stains) deposited by the protoplast previous to the development of the thickening bands. This layer subsequently becomes differentiated into the spines and rodlets. This interpretation of the layers of the exine of *Ipomoea* would be more or less in accordance with the views expressed by Strasburger (18) in the cases of *Knautia* and *Althaea*, and by Tischler (21) for *Mirabilis Jalapa*.

It is, of course, possible that the layer containing the rodlets and spines may only become differentiated later, after the thickening bands have already been deposited, but I think this is unlikely both from the appearances in the present case and from analogy with what occurs in other plants.

The thickening bands quickly increase in both thickness and breadth as fresh material is added to them by the protoplast. During the early stages the substance of the thickening bands appears to be soft and mucilaginous; their outer margin is ill defined and encroaches upon the clear spaces or layers referred to above, so that these become difficult to distinguish, and the spine rudiments have the appearance of being embedded in the substance of the bands (Fig. 7).

The external surface of the pollen-grain still remains smooth as the spine rudiments do not yet project above its surface.

It may be noted that during the time that the various layers of the special wall and of the pollen-wall are being laid down by the protoplast, kinoplasmic fibres can clearly be distinguished running between the nuclear membranes and the 'Hautschicht' at the periphery of the protoplast (Figs. 4 6 *a* and 7). These fibrils can be traced back to the kinoplasmic radiations which surround the nuclei during the telophase of the second meiotic division. The persistence of fibrillar differentiations of this kind is by no means uncommon during the earlier stages of the development of the pollen-grain, and I have met with it in several other plants besides *Ipomoea*.

It seems quite probable that influences of some kind are distributed along these fibrils from the nucleus to the 'Hautschicht' which is taking an active part in the formation of the new cell-wall lamellae.

Up to the present the young pollen-grains have remained enclosed within the special wall. Now, however, these walls break down into a diffuse, flocculent material which fills the cavity of the anther loculus. The triradiate middle lamellae of the special walls, which we previously recognized as granular lines in the middle of the callose-pectose walls, often remain intact for some time longer, and can be seen lying in the midst of the flocculent material derived from the degeneration of the rest of these walls (Fig. 3).

Soon after this time the spine rudiments have grown sufficiently centri-

fugally to project very slightly above the outer surface of the pollen-grain, and to give this a wavy appearance. Before long they project far enough beyond the periphery of the exine to give this a distinctly spinous character. At about this period the 'rodlets' can first be clearly observed as minute deeply stained structures lying in the position of the clear space or layer noticed at an earlier stage between the outer lamellae of the exine and the thickening bands. Both the 'rodlets' and the spines now stain much more deeply than the rest of the exine, and they are, therefore, very clearly distinguished in the sections (Fig. 8).

At this time the kinoplasmic fibres running between the nuclear membrane and the 'Hautschicht' become obscure, and appear to merge into and become lost in the alveolar substance of the cytoplasm.

In rather older pollen-grains the relation of the parts of the exine to one another becomes much clearer. The thickening bands have increased greatly in thickness and have become much broader, so that the thin areas of the exine between these bands are now reduced to a series of pores or narrow channels which represent the exit pores for the future pollen-tubes. Moreover, the substance of the bands appears to have undergone a change, for these are no longer diffuse and ill defined at their inner margins, but they are now sharply marked off from the distinct 'rodlet' layer. The spines have grown greatly in size. They are still limited to the angles of the network of thickening bands, and they are now seen to be spindle-shaped with their points projecting for some distance beyond the still very delicate outer lamella of the exine and their 'roots' occupying the whole thickness of the rodlet layers. These 'roots', moreover, are seen to be double, each consisting of two prongs (Figs. 9 *a* and *b*). A surface view of the pollen-grains at this stage shows that the rodlets are limited to the positions overlying the hexagonally arranged thickening bands, and that they themselves, therefore, form a hexagonal figure when viewed from above. This is shown clearly in Fig. 18, although this represents an older pollen-grain in surface view.

The protoplast of the pollen-grain, which completely fills the pollen-cavity, has meanwhile become much poorer in substance and more vacuolated than at earlier stages. As the pollen-grain has increased from about 32 μ to about 45 μ in diameter the decrease in protoplasmic density is at any rate partly due to its substance being distributed over a larger area, but I believe that there is also a real loss of substance by the protoplasm, which has contributed some material to the growing membranes. The nucleus has only increased very slightly in size; the average of a number of measurements showed only an enlargement of about 2 μ (from 10 μ of an earlier stage to 12 μ now). The nuclear reticulum has become somewhat coarser and stains more deeply: one, two, or often more rather small nucleoli may occur.

The pollen-grain continues to increase in size, and its wall grows both in surface and in thickness; in proportion as this growth proceeds the protoplast continues to become more vacuolated and poorer in substance, although it never contracts away from the pollen-wall, as in *Oenothera*. In pollen-grains which measure 70 μ in diameter the cytoplasm encloses a number of large vacuoles, and the nucleus, which now measures about 14 μ in diameter, contains one or more nucleoli and a rather scanty reticulum. By the time the pollen-grains have reached 80 or 90 μ in diameter the cytoplasmic lamellae which separate the large vacuoles from one another have become broken down, and the protoplast is reduced to a hollow shell with a single huge vacuole occupying its entire centre. This cytoplasmic shell consists of little besides a 'Hautschicht', except in the immediate vicinity of the nucleus, where some granular cytoplasm still remains (Fig. 10). The nucleus is now a flattened body measuring about 20 μ by 10 μ across its greatest and least diameters. It enclosed a rather scanty, somewhat faintly stained arrangement of threads, and one, two, or more nucleoli of large size. The great increase of nucleolar matter is certainly the most striking change in the nucleus from its earlier stages; these large nucleoli may measure as much as 8 μ across. Not infrequently the interior of the nucleoli has a vacuolar appearance. The alteration in the appearance of the nucleus which is just beginning to become evident ushers in the process of protoplasmic reconstruction. The cytoplasmic shell is seen to become slightly thicker, and the granular cytoplasm, which had been reduced to one small area near the nucleus, can now again be observed as a thin layer all round the inner surface of the hollow protoplast. In the meanwhile, the nucleus has increased in size to as much as 30 μ by 20 μ in its longest and shortest diameters. The pollen-grain itself still measures 90 μ across. This nucleus enclosed one extremely large nucleolus (rarely two) which on an average measures about 12 μ in diameter. The fibrils which traverse the nuclear cavity have become much more numerous; they are finely granular in appearance and diffuse, and irregular in outline (Fig. 11). The protoplasmic shell continues to grow in thickness, and before long the single central vacuole becomes bridged across by one or two cytoplasmic lamellae which divide it up into a few large vacuoles. These become progressively smaller as the protoplasmic lamellae grow more massive and more numerous. Starch, which hitherto was present only in comparatively small quantities, now occurs in great abundance. Granules, or more probably droplets, and irregular masses of material which are black in my preparations stained with Heidenhain's haematoxylin also accumulate in the cytoplasm of the pollen-grain. The distribution of this dark-staining material in the pollen-protoplast is of some interest. It is usually rather densely collected in the little peripheral finger-like cytoplasmic processes which project into the exit pores of the pollen-wall. From these points the material can be seen

to spread out irregularly into the interior of the protoplast (Fig. 12). It has the appearance of a material, derived from without, which is making its entrance through the exit pores of the pollen-wall, which is then taken up by the little pseudo-podium-like processes of the protoplast, and which from these points becomes diffused through the cytoplasm of the pollen-grain. I have been able to obtain but little information with regard to the chemical nature of this substance. From the fact that it is blackened by the osmic acid in Flemming's solution it is probable that the material is of a fatty nature, but beyond this I can say nothing at present.

It may be noted that just about the time when this dark-coloured material is making its appearance in the pollen-protoplast, a number of vacuoles of varying sizes are formed in the tapetal cells, and that these vacuoles are filled with a material which is also darkened by osmic acid. I have not succeeded in tracing this darkened material out of the tapetal cells into the cavity of the anther and establishing a direct connexion with the similarly blackened substance in the pollen-protoplast, but such a relationship between the two appears quite likely. Moreover, the tapetal cells can be seen to undergo a loss in the total amount of substance they contain. These facts taken together suggest that the pollen-protoplasts are growing and storing reserve bodies in their substance at the expense of materials derived, at any rate in part, from the tapetal cells. During the earlier stages of the growth of the pollen-protoplast we find that its nucleus divides, and that the very unequal cell-division which follows cuts off a small generative cell from the large tube cell. The cytoplasm of the generative cell is almost entirely composed of kinoplasmic fibres radiating from its nucleus (Fig. 13). In older generative cells the fibrillar constitution of the cytoplasm gives place to a dense, almost hyaline structure.

A distinct plasma membrane limits the generative cell peripherally, but no cell-wall is developed (Fig. 14). The nucleus of the generative cell measures about 14 μ in diameter, and contains a comparatively large nucleolus and a rather loosely arranged system of fibres.

The tube nucleus is large, irregular, or even amoeboid in outline, and is distinguished by the enormous nucleolus and the system of deeply staining chromatic threads which it contains (Fig. 15). Amoeboid tube nuclei have been described in a number of other plants ; for instance, in *Elodea canadensis* by Wylie (22).

The nucleolus of the tube nucleus is surrounded by a sheath of chromatin, and there are here no signs of the clear space (*heller Hof*) between the nucleolus and the chromatic reticulum of the nucleus which several writers have described (Fig. 15). In such cases as that represented in Fig. 16, where a slight contraction of the nucleolar substance has taken place at one spot, the relation between the nucleolar material and the chromatic sheath is particularly well seen. Martin Heidenhain, as long ago

as 1892 (and again in 1907) (9), described chromatic shells of this kind enveloping the nucleolus in several animal tissues, and his observation has been confirmed by other zoologists.

Cavara's (3) observations upon nucleoli may also be recalled in this connexion. This author described the nucleoli of higher plants as consisting of two parts: an external chromatic layer and an inner mass of plastin.

During this period of protoplasmic growth an intine has been formed on the inner surface of the pollen-wall. This layer is very thin over the general surface of the pollen-grain, but at each exit pore it is greatly thickened and protrudes towards the exterior. Where it is thickened the intine can clearly be seen to be composed of a number of lamellae, which suggests that its growth has taken place by the apposition of successive layers of material. An extension of the delicate outer layer of the exine covers the external surface of each protrusion of the intine at the exit pores of the pollen-grain (Fig. 17). The intine stains, although not very intensely, with those dyes which are characteristic of pectic bodies. Treated with calcium-chloride-iodine solution it gives at first no reaction, but after remaining in the solution for some days it is found to have coloured faintly violet. A preliminary boiling with dilute acid and alkali, according to Mangin's method, yielded no clearer cellulose reaction with the iodine reagents. From these reactions it may be concluded that the intine consists of pectic bodies associated with a little cellulose. The exine has meanwhile grown in thickness, and the relation of its parts to one another can now be very clearly seen.

The thickening bands of the exine have increased greatly in breadth and thickness, so that they now form a massive layer only perforated by the relatively small exit pores. This layer, which may be called the 'mesospore' according to Fitting's (7) terminology, possesses the reactions of a cuticularized structure (Fig. 17).

The outermost lamella of the exine (which we already saw at an early stage as an extremely delicate membrane) still remains very thin, and it can now be seen to possess an open structure perforated by countless little apertures which give it the appearance of a very fine reticulum in surface views. This perforated structure of the lamella is well seen in Fig. 18 and, in section, in Figs. 17 and 9. At the exit pores this reticulate layer dips down and covers over the protrusion of the intine. As at an earlier stage, we still find that the rodlets are limited to a hexagonal system of bands corresponding to the originally hexagonal disposition of the thickening bands (Fig. 18). At the angles of each hexagon is usually a spine. Both the spines and the 'heads' of the rodlets pass through the perforated outer lamella to reach the outer surface. The spines are spindle-shaped structures; their internal portions or roots are composed of two prongs, as already seen at an early stage. Fig. 18 shows that these

prongs have an hemispherical outline in transverse section. A stainable, homogeneous material lies between the rodlets under the reticulate outer lamella.

In sections which have been mounted in a drop of glycerine containing a little methylene blue and fuchsin mixture an interesting differentiation of the parts of the pollen-wall can be seen. The intine stains light red, the 'mesospore' is blue, the reticulate outer lamella of the exine, as well as the spines and the rodlets, is green, whilst the homogeneous sub-reticulate substance (between the rodlets) colours deeply red.

The mature spines of the exine measure between 12 and 14 μ in length; they are usually fusiform in outline, although I have occasionally found them with a dichotomously branched apex (Fig. 19).

The rodlets vary a good deal in size; their shape is usually like that of a drumstick with a part of the knob or head just projecting through the perforated outer lamella of the exine (Fig. 17).

The tapetal cells do not break down and scatter their contents between the pollen-grains, but they retain their membranes intact until the last. This tapetum, therefore, belongs to the 'secretion-tapeta' of Goebel.

Deeply staining fibres and granules occur in the cytoplasm of the tapetal cells of *Ipomoea* during the middle period of anther development. These are most probably similar to the chromidial structures which have been described in the tapeta of several other plants (*Nymphaea alba, Oenothera, Ribes, Lilium Martagon, Iris germanica, Syringa chinensis*). I have not succeeded in tracing their origin in *Ipomoea*, but these structures are frequently aggregated in the neighbourhood of a nucleus in a manner which suggests their origin from this body (Fig. 20). Two nuclei most often occur in each tapetal cell during the development of the pollen-grains, and these may still be seen as somewhat shrunken, degenerating bodies in stamens which are nearly mature.

From the foregoing account of the development of these pollen-grains it will have been seen that there is no contraction of the protoplast from the pollen-wall at any time, even though the cytoplasm of the pollen-grain is at one stage represented only by a thin, hollow shell of material.

Nevertheless, it is noteworthy that practically the entire growth of the spines and the rodlets takes place after the rudiments of these structures have been separated from direct contact with the protoplasm by the interpolation of the thickening bands of the exine ('mesospore'). That the growth of these structures is considerable will be seen from the fact that the spines increase in length from a rudiment which is too minute for measurement, to a comparatively massive spine with a length of 12 to 14 μ in the mature pollen-grain. It appears to me, therefore, that the growth of these spines and rodlets, which are in contact neither with the pollen-protoplast nor with

the tapetal cytoplasm, is of quite the same character as the growth of the entire membranes of *Isoëtes*, *Selaginella*, *Oenothera*, or *Mirabilis*. The present instance may not at once appear so striking as these latter cases are, but it is no less an interesting and clear example of the growth of a portion of the cell-membrane in entire independence of the direct influence of the living protoplasm.

In the case of the spines and rodlets of *Ipomoea*, as in that of the membranes mentioned above, the origin and first differentiation takes place under the direct control of the protoplasm, but, once formed, the further growth may continue and, moreover, maintain throughout the characteristic shape and structure of the part, quite independent of any immediate guidance from the living protoplast, provided only the material necessary for this growth is forthcoming.

In conclusion, I desire to express my indebtedness to the Government Grant Committee of the Royal Society for the loan of a Zeiss $\frac{1}{12}$-inch apochromatic objective (1·40 aperture), which has been invaluable throughout this research.

Summary.

1. At the conclusion of the second meiotic division the chromosomes remain distinguishable for a short time after the reconstruction of the daughter nuclei, but subsequently their substance becomes completely dispersed over the linin-reticulum.

Chromatic aggregations also occur in many of the nuclei of the anther tissues, notably in those of the young vascular bundle, but the size and number of these aggregations are quite inconstant.

2. The pollen-wall, when it first becomes recognizable, is a single, delicate membrane in which no structure can be distinguished.

3. The exine of slightly older pollen-grains consists of an outer lamella, upon the inner face of which is deposited a network of thickening bands. At the angles of the meshes of this reticulum the rudiments of the future spines already occur. Between the thickening bands and the outer lamella a narrow unstained space or layer can be seen ; this marks the position in which the rodlets of the older pollen-grains are developed.

4. The outer surface of the pollen-grain is at first quite smooth. The spine rudiments appear to project towards the pollen-cavity, so that they push the thickening bands inwards at these points into a series of short, internal spinous structures, but they do not extend beyond the outer surface of the grain.

5. During the earlier stages of development, whilst the layers of the special wall and the pollen-wall are being initiated, kinoplasmic fibrils connect the nuclear membrane with the ' Hautschicht ' of the pollen-protoplast. Influences of some kind are probably passing along these fibrils

from the nucleus to the 'Hautschicht' which is engaged in the organization of new cell-wall lamellae.

6. In older pollen-grains the spines have grown beyond the surface of the outer lamella of the exine, and the pollen-grain is now distinctly spinous externally. The inner parts or 'roots' of the spines occupy the rodlet layer, and they are double structures each consisting of two prongs. These spines, therefore, differ considerably in their development and structure from the purely superficial ones of such plants as *Althaea* or *Malva*.

7. As the pollen-grains increase in size the protoplast becomes vacuolated and relatively poor in substance, until it is finally reduced to a hollow shell enclosing one enormous central vacuole. In *Ipomoea* there is no contraction of the protoplast away from the pollen-wall, as is observable in the pollen-grains of *Oenothera* or in the spores of *Isoëtes*, &c.

8. The growth of the pollen-protoplast from a hollow shell of cytoplasm to the solid protoplasmic body of the mature pollen-grain is ushered in by changes in the appearance of the nucleus.

This body grows very considerably in size, and there is a relatively enormous increase in the amount of nucleolar matter which it contains.

9. The protoplasm of the older pollen-grain contains a quantity of reserve material. Starch, which in earlier stages was scanty, now occurs in great abundance. Also a material which blackens with osmic acid, and which probably is of a fatty nature, now occurs in some quantity. There is reason to believe that this fatty substance is derived from the tapetal cells, and that it passes from these through the exit pores of the exine into the interior of the pollen-protoplast.

10. The cytoplasm of the small generative cell which is cut off from the large tube cell is almost entirely composed of kinoplasmic fibres.

11. The tube nucleus is large, irregular, and amoeboid in form. It possesses a large nucleolus which is surrounded by a distinct chromatin sheath.

12. An intine develops within the exine. It forms a thin layer over the general surface of the exine, but at each exit pore it attains considerable thickness and protrudes towards the exterior. Its microchemical reactions indicate that it consists of pectic bodies associated with some cellulose.

13. In older pollen-grains the constitution of the exine is much more clearly seen than at earlier stages. It consists peripherally of an outer lamella which is very delicate in structure and perforated by countless little pores or apertures so that its substance is distributed as a delicate reticulum with open meshes. The thickening bands have grown greatly both in thickness and in breadth. They now form together a thick layer (the 'mesospore') perforated by the relatively narrow exit pores for the pollen-tubes. The outer lamella of the exine dips down slightly into the exit pores and covers over the protrusions of the intine at these spots. The

rodlets vary in size, and are usually drumstick-shaped with a part of their knobs just projecting through the perforations of the outer lamella of the exine. The spines are now large (12–14 μ) spindle-shaped structures with the two prongs of their roots lying beneath the outer lamella of the exine and just reaching to the 'mesospore', and their apices passing through the outer lamella to the exterior. Between the rodlets and spine roots a homogeneous, stainable material occurs; this material is not protoplasmic in nature as it does not give the reactions characteristic of this substance (e. g. no xanthoproteic reaction).

14. The tapetal cells do not disintegrate, and must, therefore, be classed with Goebel's 'secretion-tapeta'.

15. Deeply staining fibres and granules occur in the cytoplasm of the tapetal cells during the middle periods of anther development.

16. Almost the entire growth of the rodlets and spines takes place after these have become separated from direct contact with the protoplast by the interpolation of the thickening bands (mesospore). Neither are they in contact with the tapetal or any other cytoplasm. The conclusion may, therefore, be drawn that these structures possess a certain power of growth independent of any direct protoplasmic influence, and, moreover, during this growth they are able to maintain their characteristic form. The growth of the spines and rodlets of *Ipomoea* appears, therefore, to be of quite the same character as that of the entire membranes of *Isoëtes*, *Selaginella*, *Oenothera*, or *Mirabilis*.

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EXPLANATION OF PLATE XIII.

Illustrating Mr. Beer's paper on Spore Development.

All figures refer to *Ipomoea purpurea*, Roth., and were drawn with the aid of the camera lucida. For Figs. 1, 4, 6, 8, 9, 12, 15, 16, and 18, Zeiss' apochrom. objective $\frac{1}{12}$ inch (apert. 1.40) and compens. oc. 8 were employed, whilst for Figs. 5, 7, 11, 13, 14, 17, 19, 20, Leitz's $\frac{1}{10}$ inch object. and compens. oc. 8 were used. Magnification about 1500 and 1100 respectively.

Fig. 1. Telophase of second meiotic division. (*a*), (*b*), (*c*) show gradual vacuolization and dispersal of chromosome material. (*d*) Nucleus from tissue of young vascular bundle showing chromatic aggregates.

Fig. 2. Young pollen-cells surrounded by special walls. × about 640.

Fig. 3. Triradiate middle lamellae of special walls left after disintegration of this wall.

Fig. 4. Young pollen-grain with simple exine. The special wall still encloses the pollen-grain.

Fig. 5. Inner surface view of exine of young pollen-grain.

Fig. 6. (*a*), (*b*), (*c*). Exine of young pollen-grain in section. Same stage as Fig. 5.

Fig. 7. Young pollen-grain soon after its liberation from special walls.

Fig. 8. Older stage of exine than that shown in Fig. 7.

Fig. 9. (*a*) Still older stage of exine. The two prongs or roots of spine are clearly shown. (*b*) Slightly more enlarged view of spine.

Fig. 10. Protoplast of pollen-grain reduced to a hollow shell of substance. × about 480.

Fig. 11. Nucleus of pollen-grain in which the protoplast is just beginning to be reconstructed.

Fig. 12. A cytoplasmic projection into one of the exit pores in the exine. Dark stained material is shown apparently entering the pollen-grain at this point.

Fig. 13. Generative cell of pollen-grain being cut off. Cytoplasm of this cell, consisting chiefly of kinoplasmic fibres, can be seen.

Fig. 14. Generative cell and nucleus at a later stage.

Fig. 15. Tube nucleus of pollen-grain during the reconstruction of pollen-protoplast.

Fig. 16. Similar nucleus to that represented in Fig. 15. Note chromatic sheath round nucleolus has separated from nucleolar substance at one point.

Fig. 17. Wall of a nearly mature pollen-grain.

Fig. 18. The same in surface section.

Fig. 19. Spine from exine with dichotomously branched apex.

Fig. 20. Tapetal cell with deeply staining fibres and granules lying in its cytoplasm.


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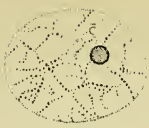
1b.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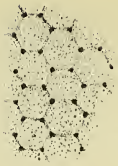
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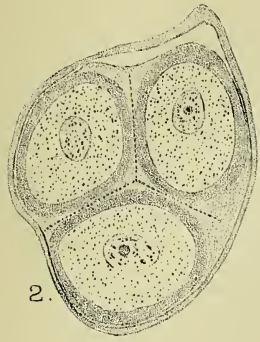
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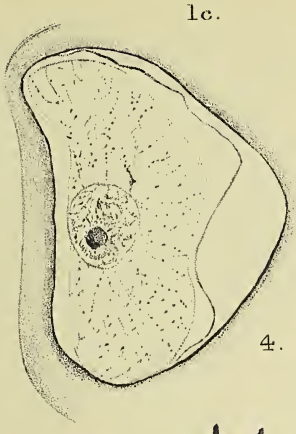
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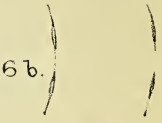
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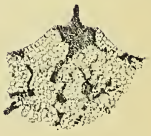
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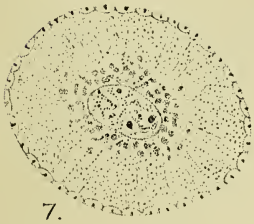
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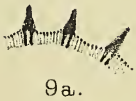
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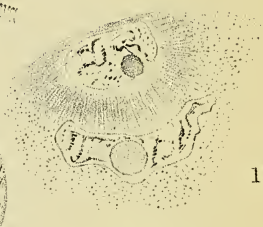
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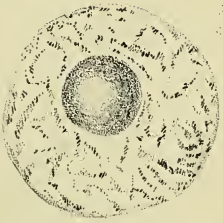
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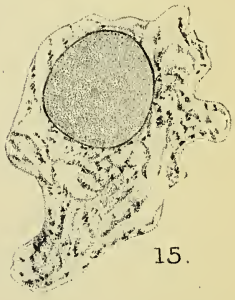
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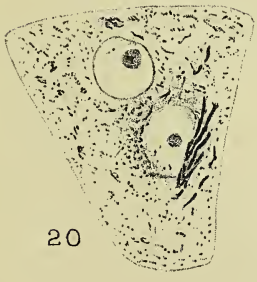
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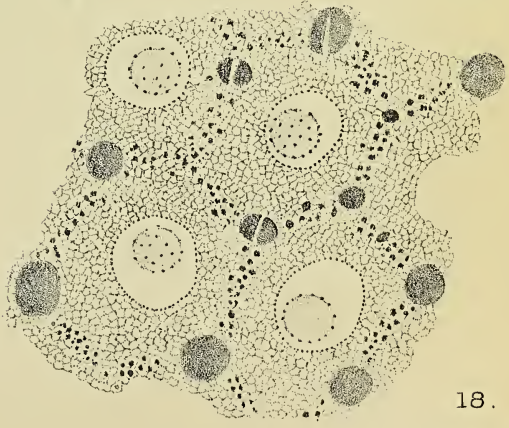
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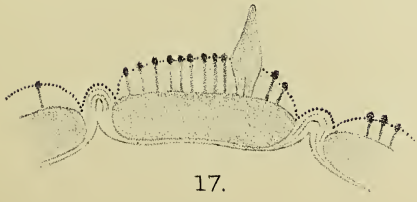
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BEER — SPORE-DEVELOPMENT.

On the Origin of the Herbaceous Type in the Angiosperms.[1]

BY

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With Plate XIV.

IN those dicotyledonous stems where increase in thickness results in the formation of a solid woody cylinder, the latter has been considered to be the result of the fusion of a ring of separate fibro-vascular bundles by the extension of the fascicular cambium across the intervening tissue, and by the formation of xylem by this interfascicular cambium. Thus, apparently, the woody type of stem arises directly by the increased lignification and the enlargement of a stem which is, in its early structure, typically herbaceous. We may cite Sachs' 'Lehrbuch der Botanik', p. 131 (1874), and Gray's 'Structural Botany', p. 73 (1879), as illustrating this method of development. This widely accepted view is evidently not the correct one, however. On the contrary, the herbaceous stem seems to be the higher type, and its separate bundles appear to have been derived from the woody cylinder by reduction, and by the dissection of the latter into a group of individual strands. In proof of this there is much evidence, both direct and indirect.

Palaeontological evidence, in that no undoubted herbaceous fossil remains are known from the older periods, points to the modern development of this type. Further, the only surviving representatives of the arborescent Cryptogams which flourished in the Palaeozoic are herbaceous or semi-herbaceous in habit and structure. Their survival is probably due to an adjustment to modern conditions, this adjustment involving the loss of secondary growth, and the acquisition of a low or prostrate habit. *Isoëtes* and *Lycopodium* may be compared with the ancient tree-like Lepidodendrids. As an herbaceous survivor of the arborescent Calamitean stock, *Equisetum* is another good illustration of the same principle. Secondary growth has practically disappeared and a stem structure simu-

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 39.

[Annals of Botany, Vol. XXV. No. XCVII. January, 1911.]

lating that of an herbaceous dicotyledon has been produced. Evidence has been brought forward[1] in proof that the bundles of this stem correspond to the primary structure of the Calamite stem. Such reduction appears highly significant.

Among the Cryptogams these changes seem to have produced more resistant plants—forms better fitted for the struggle, with new plant-groups and with new conditions. Likewise among the Angiosperms, the latest dominant vegetation, the herbs are the more adaptable and more efficient forms. Maturity is reached very early, with the least possible expenditure of material for vegetative structures ; and, further, a larger amount of seed, in proportion to the size of the individual and the space it requires, is produced. Evolutionary progress can be rapid among such forms. Among herbaceous plants the annual would then be the type of highest development. It is significant that those members of the Dicotyledons now generally considered the lowest are largely trees and shrubs, and that most of the Compositae outside the tropics are herbaceous. The vast majority of the Monocotyledons are herbs. Proof that the anatomical structure of the stem of the herbaceous Dicotyledons is the highest type within that group will add further evidence to that which leads to the opinion that the Monocotyledons are the more recent group of the Angiosperms.

If, from the solid cylinder, the stem with a ring of separate bundles has been evolved by reduction and dissection, it may be asked what factor has caused the change, or given the impulse that brought about so great a transformation. The process is to be attributed to the leaf-trace and its influence. It has been accomplished by the local transformation of secondary xylem into parenchyma below and above the entering leaf-trace ; by the elimination of segments of the protoxylem ; by the loss of the interfascicular cambium ; and, finally, in some Dicotyledons and in the Monocotyledons, by the disappearance of the fascicular cambium also. Along with the elaboration of interfascicular parenchyma by the transformation of secondary wood—essentially a development of huge rays—goes, *pari passu*, the gradual elimination of radial parenchyma within the xylem segments thus set off. The bundles of the Monocotyledons universally, and those of many herbaceous Dicotyledons, are thus entirely destitute of rays. This loss of rays is very gradual, and in some instances, at any rate, takes place by the transformation of the ray cells into a vertical parenchyma system, the elements gradually changing shape, so that the long axis, at first horizontal, ultimately becomes vertical. As a final step, these ray-like series break up into aggregate, or solitary, rows of wood-parenchyma cells, the ray form thus disappearing.

The investigations made by the writer on these points have so far been

[1] Eames, A. J. : On the Occurrence of Centripetal Xylem in *Equisetum*. Ann. Bot., vol. xxiii, 1909.

confined chiefly to the Rosaceae, with the examination of several vines, some members of the Ranunculaceae, and a few plants from other families.

A general ability to transform xylem into parenchyma exists in a number of families among the Dicotyledons. It has been shown[1] that the formation of the large rays in *Quercus* is due largely to such a change. Further, Mr. I. W. Bailey, in a paper contemporaneous with this, shows that the *leaf-trace* gives the impetus, by the influence of which woody plants form these additional masses of amyliferous parenchyma, and acquire thus the advantage of greatly increased storage capacity. This influence extends always for a considerable distance below, and also to a less extent above, the exit of the trace. The initial stage of parenchymatization is often the cutting out from the xylem cylinder opposite the trace of a segment which may ultimately be transformed more or less completely into parenchyma. In the woody Rosaceae similar conditions exist; whereas, in herbaceous representatives of the family, parenchymatization occurs in the same regions and in much the same way, but is carried much further.

Some of the woody members show conditions similar to those described by Bailey in the Cupuliferae. Species of *Rubus*, especially of the subgenera *Idaeobatus* and *Cylactis*, possess more abundant rays with some compounding in the regions below the leaf-traces, and *Rubus spectabilis* shows compounding well developed. *Potentilla palustris*, in its prostrate or procumbent stem, presents very suggestive conditions. In the segment of the xylem directly below the exit of each leaf-trace there is a decided lack of vessels and an agglomeration of rays (Fig. 1). Moreover, at these regions the annual ring dips inward strongly, as is always the case in the formation of radial parenchyma in mass in connexion with compounding and compounded rays. Further, this leaf-trace influence, so strikingly manifested in the corresponding inward dip of the annual ring, extends always down *through one*, and often through *several* of the *internodes*. Above the leaf-gap there is disturbance for only a very short distance. Conditions in the upright stem of this species will be discussed below.

In the species described above we are dealing with forms in which the woody cylinder is as yet fairly strong. Some of the herbaceous perennials of the Rosaceae hold perhaps the greatest interest in connexion with this problem—for example, certain species of *Potentilla*, the genera *Geum*, *Agrimonia*, *Sanguisorba*, and others. Among these plants we find two kinds of stems: a stout, subterranean, or creeping perennial stem, with large storage capacity; and more slender, erect, aerial, annual stems, arising from the perennial portion usually as lateral branches. The perennial stem is, in most cases, of the woody type, with certain modifications due to adaptations as a storage organ, whereas the erect annual stem is more or less completely of herbaceous texture. These two kinds of stems are of very

[1] Eames, A. J.: On the Origin of the Broad Ray in *Quercus*. Bot. Gazette, vol. xlix, 1910.

different anatomical structure, and serve to show transitional stages between the two extreme types.

Sanguisorba canadensis, L., illustrates several points very well. The woody cylinder of the rootstock consists of a series of bundles, very unequal in size, and somewhat irregular in shape, all connected by a strongly marked interfascicular cambium. Many of these bundles, however, are much modified; their cambium has ceased to form xylem at various stages and with much irregularity, and has laid down parenchyma thereafter instead. Fig. 2 illustrates this condition. In some of them, after the formation of primary wood is completed, parenchyma replaces the secondary xylem entirely, or with the exception of a very few scattered vessels or fibres. In the aerial stem the bundles are reduced in size, and the stem resembles the herbaceous type more closely. At the base the connecting interfascicular cambium is clear; in higher parts of the stem it gradually dies out. Fig. 3 shows a stage where only slight evidences of its activities are found; and in the upper portions of the aerial axis there is no trace of cambial activity between the bundles. (The plants studied were of mature growth.)

The genus *Geum* shows, in the few species examined, an interesting series in the aerial stems from forms with solid, though thin, woody cylinders to those possessing an herbaceous type of organization. The rhizomes of all species of *Geum* are similar to the underground stem of *Sanguisorba*. *G. virginianum*, L., the most woody of the species studied, has, throughout the most of its upright stem, a cylinder that is unbroken, save, of course, by brief leaf-gaps; but at the top there is a partial separation of the woody cylinder into fascicular segments. The upper portions of the stem of *G. canadense*, Jacq., have small, widely separated bundles, quite typically herbaceous, although the rest of the stem is structurally like that of *G. virginianum*. *G. triflorum*, Pursh, and *G. rivale*, L., have the aerial stem, with the exception of a very short piece at the base, dissected into separate bundles. This condition will be further discussed below.

The chief interest in the genus *Agrimonia* lies in the ray development. The structure of the central cylinder of the rhizome and aerial stem of the three species which were examined, *A. striata*, Michx., *A. gryposepala*, Wallr., and *A. mollis*, Britton, is not very different from that of *Geum virginianum*. The rays of the rootstock consist of ray cells, typical in size, shape, and position. Conditions in the aerial stem are very different however. A cross-section of such a stem of *A. striata* shows numerous, well-marked uniseriate rays. But in the tangential section no rays seem to exist. That which resembles ray tissue in transverse section appears in this section as wood parenchyma. Rows of parenchyma cells, narrow and much elongated vertically, extend for long distances in the stem—through one internode at least. The radial section shows, however, that these long rows

of cells are arranged in radial sheets like normal ray cells. In the genera and species mentioned above various stages intermediate between the typical ray cell and the condition found in *A. striata* occur; the change of position of the long axis from horizontal to vertical is very gradual, the cells apparently becoming square as a first step.

In the genus *Potentilla* we have a considerable range from the shrubby *P. fruticosa*, L., to the annual or biennial *P. monspeliensis*, L. But the reduction of the central cylinder has not been extreme; in the aerial stems of these plants separate bundles occur, so far as the investigations of the writer have gone (in fifteen species), only in the stolons of *P. Anserina*, and in the erect stems of *P. palustris*. However, the rootstocks of several species—for example, *P. monspeliensis*, L., *P. pumila*, Poir., *P. intermedia*, L., and *P. pennsylvanica*, L.—especially show a dissection of the central cylinder into alternating segments of xylem and parenchyma. These parenchymatous segments, however, are not entirely transformed, for each has a small group of xylem elements on its medullary border. This type of structure is somewhat similar to that of the rhizomes of *Sanguisorba* and *Geum*, mentioned above. The explanation of such a condition is best obtained from the seedling. Serial sections of the seedling stem show the small centrad groups of xylem which subtend the large segments of parenchyma to be leaf-traces. As in *Quercus*, a segment of the central cylinder is set off for some distance below the passing out of a leaf-trace by the formation of large rays resulting from xylem parenchymatization. A progressive change of the xylem of this segment into parenchyma occurs upwardly towards the point of exit of the trace; more and more of the secondary xylem becoming transformed until only the primary tissue is left. This now subtends pure parenchyma, through which it passes out as the leaf-trace. In the older plant with its crowded leaves these segments, which are somewhat longitudinally extended, overlap, giving the appearance shown in Fig. 5. That these parenchymatous segments in reality represent xylem-transformations is clear, not only because all stages of development can be followed, but because scattered groups of lignified elements and solitary vessels or tracheides often appear in them. All that is necessary to produce a typical herbaceous stem from this structure is to reduce the size of the normally lignified xylem segments; many semi-herbaceous forms have such a reduced xylem system. In a stem with a thin xylem ring, the setting off of the leaf-traces alone is sufficient to form the discrete bundle system of the herbaceous type.

That such is definitely the case sometimes is exemplified by two cases among the plants examined. For a short distance above the base the erect, fertile branches of *Potentilla palustris* and *Geum rivale* have a continuous woody cylinder. This becomes broken up into bundles very quickly—within a centimetre or less from the rhizome. Serial sections

through this region show that the dissection is due to the cutting out of the leaf-traces which are set off one, two, or three internodes below their exit; and that the traces of the lowermost leaf are first set off, then those of the others in succession. Fig. 4 shows this process at the base of the upright stem of *G. rivale.* That is, the bundles of the herbaceous stem thus formed represent leaf-traces and segments of the original cylinder, the common bundles of the stem. The short distance within which these traces first appear separately and the large number connected with each leaf complicate the interpretation of conditions here considerably. It is to be noted that some of the traces extend as such through two or three internodes. The condition here, especially in *Potentilla*, seems significant. The prostrate biennial or perennial stem has an unbroken central cylinder, a small, but thoroughly typical woody stem, and the same condition is found in the seedling. The central cylinder of the erect, annual stem, very soon after its derivation from the solid cylinder of the rhizome, breaks up to form the type of stem characteristic of annual plants.

Some members of another group of plants, the vines, deserve attention at this time, because they have been used as examples to show the supposed development of the solid woody cylinder by the fusion of originally separate and distinct bundles. In such forms as *Clematis* we find several large bundles separated by broad rays, but lacking within the bundles themselves all rays, even the uniseriate or primary rays. It is probable that this structure is one of adaptation to the habit of the plant. By the enlargement of the interfascicular rays sufficient ray parenchyma may have been acquired, causing the rest to disappear. In support of this view may be cited the case of *Vitis*. The mature wood of *Vitis* possesses ordinarily only large or secondary rays—for example, the species *V. vinifera*, L., *V. labrusca*, L., and *V. aestivalis*, Michx. The *seedlings* of the first two species, however, show distinct uniseriate rays rather abundantly in the substance of the bundle. Further, similar uniseriate or primary rays occur in the mature wood of *V. californica.* This appears to be clear evidence bearing on the suppression of these small rays. This condition in the fibro-vascular segments of *Vitis*, and probably also of *Clematis,* moreover, is homologous with that demonstrated above in the higher herbs, where the bundles have lost all internal rays, and the interfascicular parenchyma is very greatly developed.

We have thus considerable evidence pointing to the derivation of the herb from the woody plant. A solid tubular cylinder has been shown by Jeffrey,[1] from investigations made upon the Ranunculaceae, Nymphaeaceae, and Saxifragaceae, to be the primitive condition of the stele in the Angiosperms. Similar conditions have been disclosed by the study of the seed-

[1] Jeffrey, E. C.: The Morphology of the Central Cylinder in the Angiosperms. Trans. Canad. Inst., vol. vi, 1899.

ling in *Potentilla*, *Fragaria*, *Agrimonia*, and other Rosaceous genera. Inasmuch as these forms, especially those studied by Dr. Jeffrey, are herbaceous, the dissected cylinder seems to be clearly indicated as a recent modification. What, then, has given rise to the story of the fusion of the bundles into a ring, universal in our textbooks, and so commonly taught? A few forms only have been examined because these seemed to show well the desired relations; and it is true, indeed, that the illustrations chosen, viz. *Clematis*, *Aristolochia*, and *Quercus*, seem to vouch for the conventional view of the derivation of woody plants from herbs. Often diagrams are inserted in textbooks without specification of the plant used. *Clematis* and *Aristolochia*, which are apparently the usual basis of such diagrams, are *vines*—plants with a structure peculiar to themselves. They can hardly be considered types illustrating the growth of the xylem mass in woody Angiosperms in general.

In *Quercus*, another commonly used type, on the other hand, the writer could find no distinct bundle structure, as some diagrams would indicate. The protoxylem is not confined, as is usually stated, to a few projections of the xylem into the pith, but is distributed in many small groups, which appear nearly or quite simultaneously close together along the edge of the pith. The latter is strongly angled, and at some points along its edge slightly greater primary growth may occur, representing leaf-traces in the stem. Mr. Bailey's paper, which will appear with this, explains clearly the structural conditions in this genus which have led to its use to illustrate bundle-fusion. It is to be remembered that here we have a row of protoxylem groups, small, but close together, encircling the pith. External to these groups arises a continuous cambium ring, which forms xylem and medullary rays, the latter continuous with the pith between the protoxylem groups. Other rays not reaching the pith appear, of course, later in the wood.

In *Aristolochia*, the plant probably most used to illustrate bundle fusion, an examination of the very young stem showed that protoxylem groups arise at very nearly or quite the same time at a number of definite points (about twelve) where later the bundles form. A cambium ring arises outside these as in *Quercus*, forming first opposite the protoxylem, and then extending across the interfascicular tissue. This rapidly forms xylem in the bundles, and lengthens radially the wide medullary rays. It lays down, however, no new intermediate bundles. Those already formed become wider as the stem increases in size, and compress the rays somewhat. New large rays appear in the original bundles, dividing them externally. In comparison with *Quercus* we find the chief differences to be a restriction of the protoxylem to definite points, and the consequent formation of fewer, but very much larger medullary rays, that is, rays reaching to the pith. Moreover, this stem is marked

by the complete absence of primary wood rays in the fibro-vascular segments.

In *Clematis* the stem shows a definite number of bundles in the internode—twelve in *C. virginiana*, L., the species studied—alternately large and small. These lesser intermediate bundles are examples of those which are said by various writers to be laid down by the interfascicular cambium, and serve to fill the gaps between the original bundles, completing the solid cylinder. Lignification, indeed, appears first in the six large bundles (which are 'common' bundles), forming protoxylem. In the six smaller bundles the protoxylem is perhaps not formed within the internode proper, though in the node itself, and for a short distance above, it is well developed. Such a condition may be responsible for the view that the so-called 'interfascicular bundles' in general lack protoxylem. The cambial ring develops as before, appearing first in the bundles, and apparently only very slightly, if at all, sooner in the large than in the small ones, which can be seen outlined in the rapidly differentiating tissue. The interfascicular cambium is then formed, and increase in thickness proceeds as usual. No new bundles are formed, and only large rays occur. In comparison with *Aristolochia* we find the important differences to be an almost complete localization of the protoxylem in those large bundles from which the leaf-traces arise to pass out at the node directly above.

These three plants, so commonly chosen to illustrate the fusion of bundles in the formation of a woody cylinder, present a good series for the demonstration of the opposite view. The arrangement of the protoxylem in the stem of *Quercus* is very similar to that in the Gymnosperms, the larger leaf-traces, and ray changes influenced by them, causing the characteristic differences. The formation of large, originally compound, rays, setting off segments of the central cylinder, has been noted above as the cause of the misunderstanding of the course of development in this genus. In *Aristolochia* and *Clematis* we find the protoxylem more and more localized; but, as stated above, these two plants belong to a class generally admitted to be highly specialized. There seems to be evidence, in the unquestionable suppression of the small rays, of a considerable evolutionary advance in the vines. Of the two genera at present under discussion the writer has been unable as yet to examine seedlings for ray development and location of protoxylem. The evolution of vines seems to have been along a line somewhat similar to that followed by the herbs. Use has been made by both of localization of protoxylem and of ray-parenchyma. The herb has also extended the transformation of xylem into parenchyma, and so has widened its interfascicular rays and reduced the size of its bundles. These changes remove the necessity for intrafascicular wood rays, and they disappear gradually, becoming completely

obsolete only in small bundles. The reduction in size of the herbaceous stem, together with its slight development of xylem and lack of necessity for storage parenchyma, removes the demand for cambial activity. The cambium then disappears, first, naturally, in those regions where it gives rise to tissue no longer typically fibro-vascular. Its complete disappearance finally occurs when the bundles themselves have become much reduced.

Summary.

The prevailing view concerning the origin of the solid woody cylinder of the Angiosperms is, that its formation results from the fusion of a group of originally separate bundles; that this is accomplished by the extension of the cambium arising within those bundles across the interfascicular tissue, thus completing a cambium ring which subsequently develops the continuous cylinder. Instead of this, however, a reverse process seems to have occurred. A primitively solid cylinder has been reduced and dissected to form the type characteristic of dicotyledonous herbs—a ring of small, separate bundles. In favour of this view there is much evidence, both direct and indirect. The anatomical structure of fossil forms and parallelism in development in cryptogamic groups still living points in this direction. Direct development proof is supplied by some of the more herbaceous members of the Rosaceae, particularly the herbaceous perennials.

Further, there has apparently been a complete misunderstanding of the structure of some of the plants chosen to illustrate in textbooks the evolutionary development of the stem, as, for example, the case of *Quercus*. Diagrams of other plants of somewhat similar structure do not always indicate the particular plant they represent, and err perhaps too much on the diagrammatic side. Two genera very commonly illustrated in this connexion, *Aristolochia* and *Clematis*, seem at first glance to offer evidence in support of the older theory. But consideration of them as members of a class that is presumably highly developed, the vines, comparison with each other and with other chosen illustrative plants, together with a careful developmental and anatomical study, shows that whatever light is to be obtained from them in these matters aids in the formation of the opinion that the fascicular type of central cylinder has been derived from the unbroken woody cylinder of the lower Dicotyledons.

Herbaceous plants, especially annuals, are clearly very efficient forms. They have probably arisen as an adaptation to modern conditions, and their adaptability, together with rapid and greatly increased reproduction, has much facilitated their evolutionary progress.

The impetus for so great a change seems, in large part at least, to have been given by the leaf-trace. This has been effected by the transformation of small or large masses of secondary xylem into storage parenchyma in those segments of the woody cylinder directly related to the leaf-trace.

The first step in this process has been the formation of compound rays in connexion with the trace. In the next stage whole segments of the central cylinder in relation to the leaf-trace become transformed entirely into parenchyma with the exception of the primary wood (which is the trace in the stem). Thus there is obtained a stem with alternating segments of typical xylem and parenchyma, the latter with tiny groups of typical xylem elements on their centrad sides, or, in other terms, alternating large and very small bundles, the latter being leaf-traces. The leaf-trace segments, before their transformation, were first delimited laterally by transformation of xylem in narrow radial bands. This, in stems with thin xylem rings, is in itself sufficient to produce the herbaceous structure. When a cylinder is thus split up, the cambium ring remains unbroken for some time, the interfascicular portions actively forming parenchyma. As the plant becomes more and more herbaceous these segments of the cambial layer become less active and finally disappear. Later, the fascicular cambium, too, is suppressed, and the highest type of bundle, very small and without secondary growth, is formed. Along with this great increase of interfascicular parenchyma goes a gradual disappearance of rays from the diminishing bundles, so that the highest type lacks rays completely. Most of these points are exemplified by certain herbaceous perennials belonging to the Rosaceae; their two kinds of stems, subterranean and perennial, aerial and annual, have proved favourable for such demonstrations.

The writer desires to express his thanks to Mr. I. W. Bailey for opportunity to observe and compare ray development in many woody plants, and to Dr. E. C. Jeffrey for material and for suggestions and advice during the investigation.

DESCRIPTION OF PLATE XIV.

Illustrating Mr. Eames's paper on the Herbaceous Type in Angiosperms.

(It is suggested that a hand lens be used in the examination of these figures.)

Fig. 1. *Potentilla palustris.* Portion of cross-section of prostrate perennial stem, showing segment below exit of leaf-trace. × 40.

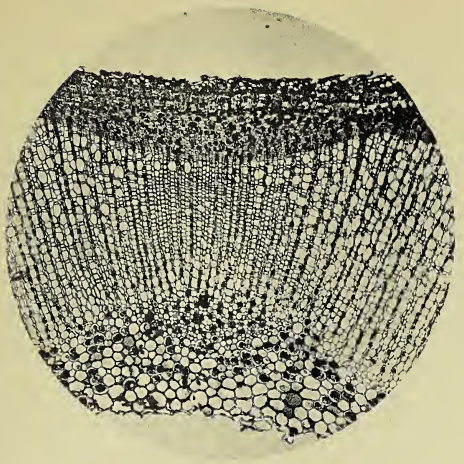
Fig. 2. *Sanguisorba canadensis.* Portion of cross-section of rootstock, showing two bundles connected by interfascicular cambium, that on the right consisting in large part of parenchyma. × 50.

Fig. 3. *Sanguisorba canadensis.* Portion of cross-section of mature aerial stem, showing portions of two bundles, with slight evidence of interfascicular cambial activity. × 100.

Fig. 4. *Geum rivale.* Cross-section of base of fertile aerial stem, showing dissection of a thin central cylinder by leaf-traces. × 15.

Fig. 5. *Potentilla pennsylvanica.* Cross-section of rootstock, showing alternating segments of xylem and parenchyma. × 12.

Fig. 6. *Potentilla pennsylvanica.* Portion of cross-section of rootstock, showing alternating segments of xylem and parenchyma, the latter with leaf-traces either passing out, or upon their medullary border. × 25.



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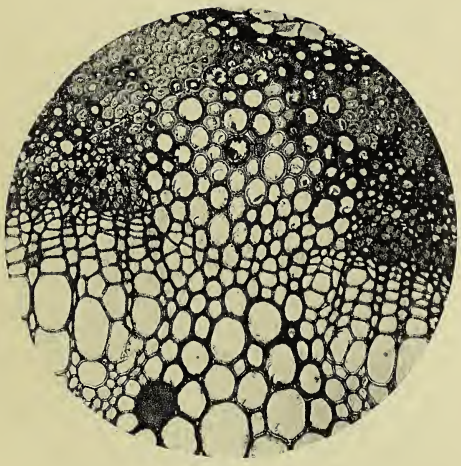

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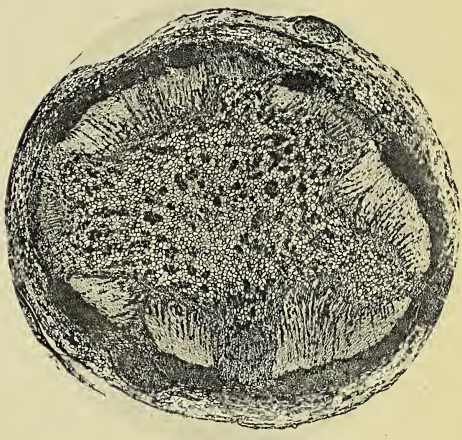

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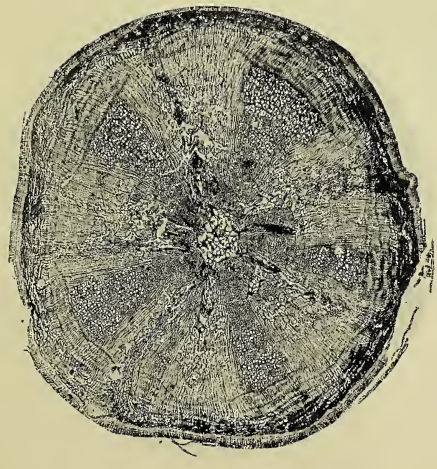

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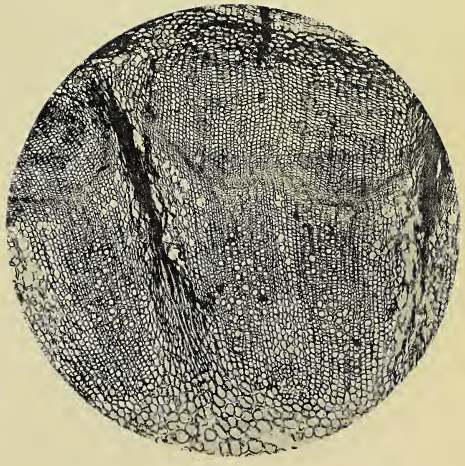

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EAMES — HERBACEOUS TYPE IN DICOTYLEDONS.

The Relation of the Leaf-trace to the Formation of Compound Rays in the Lower Dicotyledons.[1]

BY

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With Plates XV–XVII and one Figure in the Text.

THERE exists in the wood of dicotyledonous plants considerable variation in the size, shape, and structure of the radially disposed plates of parenchyma, commonly designated medullary rays. This diversity of structure is well illustrated by several well-known genera of the Cupuliferae. *Alnus* and *Castanea*, for example, are characterized by possessing numerous small linear or uniseriate rays, rays similar in form and structure to the small rays which are a distinctive feature of the wood of coniferous plants. Occurring with this type of ray, and in marked contrast to it, are the large fusiform masses of parenchymatous tissue, often called primary rays, which occur in *Quercus*. *Fagus*, like the oak, possesses both the small linear or uniseriate and the large multiseriate type of ray, and in addition smaller multiseriate rays which are graded in size between these two extreme types. *Betula*, *Carpinus*, *Ostrya*, and *Corylus* possess usually, in the mature wood, numerous bi- and tri-seriate rays, among which are to be found scattering rays of the linear type. Finally in the lower Cupuliferae, *Alnus*, *Betula*, *Carpinus*, and *Corylus*, bands or aggregations of uniserate rays occur, and have been described by certain writers as '*false rays*'.

The occurrence of 'false rays' in sections of a fossil oak from the gold gravels of California (Miocene) led Mr. A. J. Eames of this laboratory to investigate seedling and fossil oaks for evidence which might demonstrate what relation, if any, existed between the large multiseriate rays of living oaks and this 'false ray' of the Miocene oak. At the same time the writer carried on a series of investigations upon the distribution and origin of the 'false' and the large multiseriate rays in the Cupuliferae and other

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 27.

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families of dicotyledonous plants. The result of both investigations has shown that the large type of ray, normally found in living oaks, has been developed by the gradual aggregation and fusion of a number of small uniseriate rays.

The genus *Alnus* offers a particularly illuminating illustration of the steps by which the large multiseriate ray has been 'built up' by an aggregation and fusion of numerous smaller rays. The evidence which demonstrates this process of fusion occurs abundantly, both in the distribution of ray structures in the various species of the genus and in the life-history of the individual plant.

In Pl. XV, Figs. 1 and 2, tangential and transverse sections respectively of the mature wood of *Alnus acuminata*, H. B. K. (*A. oblongifolia*, Torr.), is shown a type of wood in which there is an entire absence of any tendency towards aggregation of the uniseriate rays. In marked contrast to this condition is that illustrated in Figs. 3 and 4, tangential and transverse sections of the mature wood of *A. incana*, (L.) Moench. In the central portion of the transverse section the entire absence of vessels from a band of tissue may be noted, and the uniseriate or linear rays are seen to be slightly approximated in this region. However, the tendency towards aggregation of the uniseriate rays is more clearly shown in the central portion of the tangential section, which should be examined with a pocket lens to demonstrate this feature more strikingly. In Figs. 5 and 6 a higher step of the compounding process may be seen in the mature wood of *A. rubra*, Bong. (*A. oregona*, Nutt.). Many of the aggregating rays have increased in size from uniseriate to bi- and tri-seriate, and are beginning to coalesce in places. Figs. 7 and 8 show an advanced step in the mature wood of *A. maritima*, (Marsh) Muehl. The aggregating mass has taken on the fusiform outline of a large oak ray, as may be seen in the tangential section, and is rapidly becoming a homogeneous mass of ray tissue, by the aggregation and fusion of the enlarged uniseriate rays, as well as by the transformation into parenchyma of fibres included in the coalescing mass of tissue. A completely fused *aggregate* or *compound* ray is illustrated in Fig. 9, a tangential section of the mature wood of *A. rhombifolia*, Nutt. This ray, upon comparison with the oak ray shown in Fig. 11, a tangential section of the mature wood of *Quercus rubra*, L., is seen to be homologous with the large multiseriate rays which occur in all American oaks with deciduous foliage. The transverse section of *A. rhombifolia*, shown in Fig. 10, is seen to illustrate a condition intermediate between those shown in Figs. 7 and 9. The transformation process has been completed on the left side of the aggregate ray, whereas the right side is still in incompleted stages of fusion. The elements which cross the axis of the ray diagonally are fibres in process of parenchymatization. This series of figures illustrates the development of the large compound ray from the uniseriate condition, but a great

number of intermediate steps of transformation exist between these characteristic ones picked to demonstrate the main steps of the compounding process.

The evidence derived from the study of the development of ray structures in the life-history of the individual alder plant affords as conclusive a demonstration of the mode of development of aggregate or compound rays as does the comparative study of ray structures in the mature wood of distinct species of the genus *Alnus*. In the early stages of the development of the plant primitive stages of compounding and fusion occur, and with subsequent growth more highly perfected stages of aggregation are progressively developed. Thus in the first-formed annual rings of the seedling plants of certain alders non-aggregated uniseriate rays occur exclusively, but in subsequently formed rings radiating bands of tissue arise, which are characterized by congeries of uniseriate rays and by the absence of vessels. In the radial extension of these aggregate rays during many years' growth progressively higher stages of compounding occur, such as have been illustrated in Plates XV and XVI. As in recent years, through the researches of Strasburger, Goebel, Jeffrey, Jackson, and others, the importance of seedling plants in the recapitulation of ancestral characters has become a firmly established canon of comparative anatomy, the evidence afforded by the examination of these alder seedlings is an important guide to the phylogenetic history of the development of aggregate or compound rays.

It is of interest to compare the condition just described in the genus *Alnus* with that which occurs in oak. Upon the examination of material of a large number of American oaks the interesting fact was discovered, that in most species of American Live Oaks the so-called 'false ray' occurs in a more or less advanced stage of development towards the large compound ray, characteristic of oaks with deciduous foliage. In Figs. 12 and 13 may be seen tangential and transverse sections of *Quercus virginiana*, Mill. (*Q. virens*, Ait.), the common Live Oak of the southern United States, which has reached a compounding stage similar to that seen in *Alnus rubra* in Fig. 5. Among other Live Oaks stages may be found in which fusion is even less well developed, and many advanced stages occur in the transformation of the aggregating into the broad, homogeneous type of ray. Strong evidence of the development of the large rays by a compounding process also exists in the American White and Red Oaks. Included fibres and wood parenchyma cells, vestiges of a compounding process, occur in the rays and display good evidence of their gradual transformation into ray parenchyma. A striking piece of evidence in this connexion is the fact that, whereas crystal cells are found abundantly in the large rays and in the wood parenchyma of many species of oak, these crystals are absent or extremely rare in the uniseriate rays. The presence of crystals in the large

rays is most easily accounted for on the basis of the inclusion in them of what was originally wood parenchyma. In the Live Oaks the stages of the process by which the crystal-bearing wood parenchyma cells are fused into the compound or aggregate rays may be seen clearly. Furthermore, as in the case of alder, seedling plants afford interesting evidence in regard to the phylogenetic history of the development of ray structures in oak. Eames[1] has pointed out that seedling White Oaks possess in their early wood only the primitive type of ray, the uniseriate ray. In subsequently formed layers the large homogeneous ray of the mature wood is formed by an aggregation and fusion of small rays and included fibre and wood parenchyma cells. The seedling Black Oaks display similar progressive stages of compounding (see Fig. 15), but in these oaks the aggregating tissue extends back to the earliest formed wood, indicating that the aggregate ray is more firmly established in the plant in this sub-genus. In contrast to this, in the early formed wood of the mature twigs of the Live Oak, there exist conditions similar to those which occur in seedling White Oaks, except that the aggregate ray develops further from the pith, and passes through the early stages of compounding less rapidly. Fig. 26 illustrates a twig of Live Oak in transverse section. The four aggregate rays at the top of the figure can be seen to originate in the central part of the section, and from the radius of curvature of the rings it can be seen that several years' growth intervene between the pith and the point where the aggregations originate.

Additional evidence of the formation of the large rays in oak by a compounding process has been pointed out by the writer in an article cited below.[2] In this article it is shown that the wounded wood of mature oaks may revert to primitive stages of compounding in the ray. Since, in recent years, it has become an established principle that traumatic areas may be the seat of reversion to primitive characters, the study of wounded regions in oak supplies valuable evidence in regard to the phylogenetic development of ray structure. Rays immediately external to the traumatic areas are uniseriate, but in passing out from the wounded area (see Fig. 14) there occur all the steps by which the small rays are aggregated and transformed into a homogeneous mass of ray parenchyma.

From the palaeobotanical evidence afforded by Miocene oaks with 'false rays', taken into consideration with that afforded by seedling plants, traumatic areas, and the distribution and development of compound rays in several genera, we see that primitive oaks and alders possessed only uniseriate rays. With the necessity for a large food reserve and storage system, due to the development of unequal seasonal temperature and rainfall

[1] Eames, A. J.: On the Origin of the Broad Ray in *Quercus*. Bot. Gaz., xlix, March, 1910, No. 3, pp. 161–7.

[2] Bailey, I. W.: Reversionary Characters of Traumatic Oak Wood. Botanical Gazette, November, 1910.

in later geological times, broad rays have been built up to meet this demand by a process of aggregation and fusion of numerous small uniseriate rays.

The evidence of the origin of large rays by a compounding process is not confined alone to the genera described above, but is amplified by a study of the development of ray structures in the life-history of certain species of *Betula*, *Carpinus*, *Corylus*, and Ericaceous and Rosaceous forms. In this connexion it should be noted that the aggregate ray, which was once apparently well developed, has been reduced in *Castanea* and certain Betulaceae and Ericaceae, and occurs in general only in portions of the plant which are known to reflect primitive characters. Several interesting features of this retrograde movement will be considered by the writer in a subsequent article.

It is of special interest to study the important part that the leaf-trace has taken in the origin and development of the type of ray under consideration in this article. This feature may be seen in small twigs of certain Fagaceae and Ericaceae in which the compounding rays are of more or less infrequent occurrence. These twigs show, upon the removal of the bark, that the aggregate rays occur in the form of longitudinal plates of tissue extending above and below the minute burls which occur at the node. These burls are disturbances formed through several of the first-formed annual rings, and are produced by the lateral and median traces of the leaf. In older and thicker twigs the aggregate rays are more numerously developed, and grouped in the nodal region. Obviously, the most accurate method of determining the exact relation of the leaf-trace to the compounding rays is the examination of transverse and tangential serial sections through the node. The writer, adopting this method, has constructed serial sections of the nodes of the Cupuliferae, Ericaceae, and other families of dicotyledonous plants. The transverse sections were constructed starting from a point well below the node and passing through the node, and to a considerable distance above it. Similarly, tangential serial sections were constructed through several annual rings to the pith. By the use of this method it was seen that the leaf-trace, in its passage outwards to the leaf, produces a disturbing effect upon the surrounding woody tissue. This is expressed by a diminution of the number of vessels and by the increase of storage tissue, particularly by the enlargement and multiplication of the rays. The extent to which parenchymatous tissue is developed about the entering leaf-trace varies with different species. In *Castanea*, *Ostrya*, and in certain alders and birches it is confined to the immediate vicinity of the leaf-trace, whereas in woods with well developed aggregate rays a large amount of storage tissue occurs above and particularly below the leaf-trace, as well as in radial extension beyond it. In Fig. 17, a transverse section of a small twig of *Alnus tenuifolia*, Nutt., is illustrated the condition in which lateral leaf-traces (marked *x*) produce in their vicinity a slight tendency for the exclusion of

vessels and the aggregation of rays, but this influence dies out radially after a few years' growth, and extends only slightly above and below the leaf-trace. In contrast to this, in the cross-section of a small twig of *A. iaponica*, Sieb. et Zucc., seen in Fig. 18, strongly developed aggregate rays (marked *x*) extend outwards from the lateral leaf-traces and extend vertically many centimetres below them. Owing to this extension of the compounding tissue below the trace, a section at the node shows, in addition to the aggregate rays which are related to the leaf-traces of this node, other compound rays which are related to the lateral and median traces of higher nodes. One of the lateral traces illustrated in Fig. 18 is seen under higher magnification on Pl. XVI. Fig. 19 shows the leaf-trace bundle and associated tissue below the node. The band of associated tissue extends in a radial direction towards the inner bark, and from it vessels are gradually disappearing. Fig. 21 illustrates the same trace bundle as it passes off to the leaf. Vessels have entirely disappeared from the associated tissue, and the uniseriate rays have been gradually approximated and increased in number. As the leaf-trace bundle moves outward a gap is left in the protoxylem elements of the central cylinder. Fig. 23 illustrates the associated aggregate tissue which extends above the leaf-trace. The gap left by the departure of the leaf-trace is marked by the absence of protoxylem elements, and the aggregate ray extends to this, and, in consequence, apparently originates at the pith. A tangential view of *A. japonica*, showing the lateral leaf-trace and a small portion of the associated compounding tissue, is seen in Fig. 24. The associated aggregate ray is seen to be composed at this point of somewhat loosely approximated uniseriate and biseriate rays. In serial tangential sections, cut through many subsequent annual rings, progressively higher stages of aggregation and fusion occur. Figs. 20 and 22 illustrate the relation of aggregate or compound rays to the lateral leaf-traces of *Quercus* and *Corylus*. Fig. 29, a transverse section of *Quercus velutina*, Lam., shows a condition which occurs frequently in *Alnus*, *Betula*, *Carpinus*, *Ostrya*, *Quercus*, and other genera, in which *two* or *more* aggregate rays are related to a single lateral leaf-trace. This condition occurs very frequently in tissue subtending the leaf-trace, whereas above the node there occurs usually but a single sheet of associated tissue.

The study of the leaf-trace in its relation to the origin and development of aggregate rays is very clearly shown in oak, as in this genus conditions exist which are extremely diagrammatic. The American Live Oak, *Quercus virginiana*, is of particular interest in this connexion, as it appears to retain primitive stages of the development of aggregate rays, and to indicate the influence which has made it advantageous for dicotyledonous plants to develop large storage systems in connexion with the leaf. Serial tangential and transverse sections, cut through the node of this oak, show that there is comparatively little specialized tissue above and below the leaf-trace during

the first few years' growth, but during subsequent growth the quantity of parenchymatous storage tissue related to the leaf-trace is constantly increased until it extends a considerable distance *below* it. Fig. 28 illustrates the former condition; the associated compounding dies out a short distance below the leaf-trace. Fig. 30 illustrates the leaf-trace at some distance from the pith; a long aggregate ray, a small portion of which can be seen in the figure, now subtends the leaf-trace. In transverse sections, as may be seen in Fig. 26, the aggregate rays appear to originate at some distance from the centre of the stem, and only in sections cut in the immediate neighbourhood of the leaf-trace does the aggregate ray extend to the vicinity of the pith. The condition, which exists in the primitive Live Oak, indicates that, in all probability, with the development of unequal seasonal temperature and rainfall, parenchymatous storage tissue has been increasingly developed in the vicinity of the leaf-trace. This tissue has subsequently been increased in amount until highly specialized sheets of storage tissue or aggregate rays result in the mature portions of the plant. As might be expected, American oaks with deciduous foliage possess higher stages of the development of storage tissue in relation to the leaf-traces. In seedling White Oaks, according to the law of recapitulation, a condition similar to that of the mature Live Oak stem exists. There is this difference, however, that although the rays do not extend to the pith above and below the leaf-trace, they pass through the stages of compounding abruptly, the phase of compounding being confined to one or two annual rings. In the mature stem of this sub-genus the lateral leaf-trace rays become gradually more firmly seated upon the plant, and originate in their internodal extensions in the vicinity of the pith. Fig. 25 illustrates the cross-section of a small twig of *Quercus alba*, L. It will be noted that there exists in oak a five-lobed pith. At each node a median trace passes off from the extremity of one lobe, and two lateral traces from the sides of the adjoining lobes. As more or less persistent aggregate rays are associated with the lateral traces, a section of the stem possesses usually ten aggregate rays which are grouped in pairs. Occasionally, however, aggregate rays develop in relation to the median traces, or one or more of the lateral traces may be abortive, in which case an odd number of compound rays exists in the cross-section, as is the case in Fig. 25. In the Red Oaks the aggregate rays are more firmly established upon the plant than is the case in the White Oaks. Thus, in seedling Red Oaks, as in the mature twigs of White Oaks, the associated tissue above and below the leaf-traces extends to the neighbourhood of the pith in the internode, as well as to the point at which the trace passes to the leaf. In mature twigs of Red Oak, as may be seen in Fig. 27, a transverse section of *Quercus velutina*, the tendency for the formation of aggregate or compound rays has become so firmly fixed upon the meristematic tissues that numerous rays, in addition to the charac-

teristic lateral leaf-trace rays, originate in the early formed wood of the twig. In other words, when the aggregate ray becomes firmly established upon the plant, as in the older wood of Live and White Oak and in the early wood of Black Oak as well, aggregate rays which are but indirectly related to the leaf-traces develop to meet the demands of the increased circumference of the stem. Just as in the case of coniferous woods, new uniseriate rays are continually formed by the cambium, at progressively greater distances from the pith, to maintain the proper proportion of ray tissue in the widening stem.

In the development of compound rays in the Cupuliferae the lateral leaf-traces have played the most important part. For, although compounding is often related to the median trace or traces (three in *Alnus*, *Carpinus*, *Corylus*, and *Ostrya*), the location of the bud directly over the median trace has apparently produced a retarding influence on the development of compound rays in this region. However, in the Ericaceae, where no lateral traces occur, the median traces have been the sole factors in developing the compounding rays. The fact that the Ericaceae are without lateral traces probably accounts in part for the primitive stages of aggregation found in the wood rays.

We may sum up the relation of the leaf-trace to compound rays as follows. In the development of the large storage systems necessary to plants living in regions of markedly unequal seasonal temperature characteristic of later geological time, the origin of storage tissue about the entering leaf-trace has proved a natural starting-point for the formation of compound or aggregate rays. By a gradual development of the amount of special storage tissue, above and below the traces, and its extension outward with each annual layer of growth, a larger and larger food-reserve system has been developed, until in the higher types, by the transformation of the compounding tissue, consisting of aggregated small rays and separating fibres, into ray parenchyma, homogeneous masses of ray tissue have been produced. In less highly specialized species of the Cupuliferae the primitive stages of the development of aggregate rays and their relation to the leaf-traces are clearly shown. In specialized species, in which the aggregate ray is firmly established, the phases of compounding and the importance of the leaf-trace, as the originating influence in the development of compounding, have been somewhat obscured except in the younger, seedling portions of the plant.

It is an interesting and important fact that in the development of the stem of plants which possess aggregate rays there is a marked difference between the general rate of growth of the woody tissues and that of the large aggregate rays. This is usually expressed by a strong 'dipping in' of the outline of the annual ring in the vicinity of the large ray. In other words, the compound ray produces usually a retarding influence upon

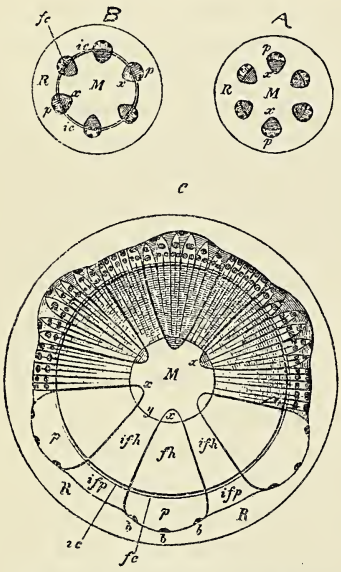
growth in its vicinity. This condition is shown in the cross-sections illustrated in Figs. 4, 6, 8, and 10, and it may be noted in addition that the sag in the outline of the year's growth increases *pari passu* with the development of the ray. With higher phases of compounding and the increase in size of the ray, the 'dipping in' of the compound ray becomes more strongly developed. This retarding influence of the aggregate ray upon the growth of the stem is well marked in the twigs of the Blue Beech (*Carpinus caroliniana*, Walt.) and *Betula pumila*, L. When the bark is removed from small twigs of these plants it is seen that the woody cylinder appears strongly crenulated in outline. The aggregate rays in these species are of large size and strongly developed, and produce pronounced sags in the periphery of the stem. Individually the aggregate rays produce a retarding influence upon growth, and when approximated several aggregate rays produce a general dip in the outline of the stem by their co-ordinated retarding influence upon growth. This condition is well illustrated by the bole of the Blue Beech (*Carpinus caroliniana*). As is well known, this small tree is characterized by a strongly fluted stem. A cross-section of such a stem reveals the interesting fact that the furrows in the stem correspond to bands of numerous closely approximated compound rays, and the ridges to areas in which the rays are nearly absent. The stem of other members of the Betulaceae and of *Quercus* and *Fagus* often show a similar condition. Where the compound rays are approximated there is a corresponding depression in the outline of the stem. The most striking illustration of the retarding influence of aggregate rays upon the development of the woody cylinder occurs in the branches of White and Red Oaks, and in *Clematis*. As may be seen in Fig. 25, the stem appears to be divided into ten segments, five of which are larger wedge-shaped segments projecting beyond five smaller depressed segments with parallel sides. The sunken segments, as will be noted on closer inspection, are separated from the larger ones by the lateral leaf-trace rays which have been described earlier in this article. In fact, it is the strong retarding influence of this storage tissue, associated with the lateral leaf-traces and longitudinally relayed from node to node in ten vertical lines up the stem, which has produced the depression representing the smaller segments. Where the rays are strongly developed the segments are strongly depressed, and when the aggregate rays are feebly developed the segments are correspondingly slightly depressed. Similarly, when the ray on one side of a small section is slightly developed or absent, the segment is seen to be unsymmetrically developed. Thus in Fig. 25 one of the small segments is depressed upon the right side by the aggregate ray (marked *a*), whereas on the opposite side (*b*), owing to the absence of a compound ray, the segment rounds up to the outline of the larger segment without well-marked sags in the annual rings, such as occur on the opposite side. A similar condition is shown in the lower portion of Fig. 27; the ray

at the left is at first feebly developed, and the segment is in consequence but slightly depressed upon the left side. In addition it should be noted in this figure that the segment becomes more strongly depressed as the aggregate rays become more firmly established in the older portion of the stem.

From this we see that the aggregate ray, as it possesses a different rate of growth from the rest of the secondary wood, has often a marked effect upon the development of the stem. This is expressed in most cases by a retarding influence upon growth, which produces a distinct sag in the outline of the annual rings in the vicinity of the large ray. The most striking illustrations of the effect of this retarding influence of the compound ray are seen in the fluted stem of the Blue Beech and in the depressed small segments of oak branches.

Conclusions.

The origin and development of aggregate or compound rays, as revealed by the study of the comparative anatomy and morphology of the Betulaceae and higher Cupuliferae, and by the phylogenetic evidence afforded by seedling plants and traumatic areas, is significant in a consideration of the development of the fibro-vascular cylinder of woody plants. The theory of the origin and development of the central cylinder of Gymnosperms and Angiosperms has remained practically unchanged, in the botanical literature of the Old and New World, since the publication of Sachs' 'Lehrbuch der Botanik'. Subsequent authoritative writers have adopted the theory, and the Sachsian figures illustrating it have been largely copied by European writers, as well as by American writers. This conception, which appears to have really originated with Sanio and to have been exploited by Sachs and De Bary, may be summed up as follows, using the Sachsian figures as illustrations. In herbaceous plants and in the 'embryonic stem' of woody plants (see A), there is developed a ring of primary collateral bundles. These are arranged in a circle, thus dividing the fundamental tissue into two portions, a pith *M* and a cortex *R*. Each open bundle possesses primary phloem, *p*, on the outside and primary xylem, *x*, on the inside, and these are separated by a meristematic tissue or fascicular cambium. In the development of



Text-fig.—Description in text.

trees, vines, and shrubs from this supposedly primitive condition, the fascicular cambiums of the bundles are extended in a tangential direction through the fundamental tissue enclosed between the bundles, or 'primary medullary rays' (see B). The fascicular cambiums are thus joined together, forming a cambium ring. The part of the cambium, *ic*, extending between the bundles is called interfascicular cambium in contrast to the cambium of the fascicular segments, *fc*. By the continual division of the cells of the cambium ring, secondary xylem is laid down on the inside of the ring and secondary phloem on the outside. Thus the primary phloem and xylem are forced further and further apart, and growth in diameter takes place. This condition is illustrated in C, in which *p* and *x* are the primary phloem and xylem, *fh* the secondary xylem formed by the fascicular cambium, and *ifh* the tissues formed by the interfascicular cambium. It is to be noted that no primary xylem subtends the segments *ifh*. In the primitive condition the entire fundamental substance between the bundles is supposed to be kept continuous from the pith to the inner bark by the divisions of the interfascicular cambium. In more specialized forms more or less of the tissue formed by the interfascicular cambium is composed of lignified elements or secondary xylem, and the 'primary rays' are thus confined to more or less restricted radii of the interfascicular segments. It has also been supposed that arborescent and shrubby plants have been evolved from herbaceous forms by the gradual fusion of separate fibro-vascular bundles into a compact woody cylinder.

A serious objection to the hypothesis which we have just outlined is the fact that, although arborescent and shrubby forms are supposed to have been evolved from herbaceous forms, the lowest dicotyledonous plants possess well established woody stems, and herbaceous plants occur mainly among the higher families of the Dicotyledons. Similarly the arborescent or shrubby condition is a distinctive feature of Gymnosperms to the exclusion of the herbaceous type. If the woody Phanerogams have been evolved from herbaceous ancestors the latter must have entirely perished. There is, moreover, no undoubted palaeobotanical evidence to indicate that such herbaceous progenitors ever existed. In this connexion it is of interest to note that the surviving representatives of the aborescent palaeozoic Cryptogams are herbaceous or semi-herbaceous in habit.

Furthermore, in accordance with the Sachsian theory, it would be reasonable to expect that in the individual development of the most primitive living dicotyledonous plants, the earliest formed tissues, which by the law of recapitulation reflect the phylogenetic history of the plant, should possess anatomical characters resembling ancestral characters more closely than do those tissues which occur in the mature portions of the plant. In fact, according to the Sachsian hypothesis, in the development of the stem of woody dicotyledonous plants, the transition from a separate ring of

bundles to a hollow woody cylinder is assumed to exist in the so-called 'embryonic stem'. This hypothetical condition, however, does not occur in woody plants, and the study of the development of seedling plants reveals the fact that in numerous species conditions exactly the reverse of those which should be expected in accordance with the Sachsian theory occur. The Cupuliferae illustrate the truth of this statement very clearly and diagrammatically. In the mature twigs of Red and White Oak there are, as has been shown in Fig. 25, five smaller depressed segments. These segments, and the rays which demark them, are considered interfascicular segments, and the aggregate rays are supposed to be continuations of portions of the fundamental tissue, included between the primary bundles. Furthermore, it has been supposed that masses of protoxylem subtend the larger, but not the smaller segments of the stem. However, we have seen above that seedling Red and White Oaks and the stem of the primitive Live Oak do not show this segmented character of the woody cylinder, but possess instead, in the first annual ring, a continuous woody cylinder. This unexpected condition is explained by a consideration of the origin and development of the large rays in oak. The so-called primary rays have been shown to originate in the vicinity of the leaf-trace, and to be formed from the secondary wood by an aggregation and fusion of numerous uniseriate rays and included fibres, and to be in no way related to inclusions of fundamental tissue between so-called primary bundles. As has been shown above, seedling plants elucidate the phylogenetic history of these ray structures, and illustrate the early stages of their development. In the mature portions of the plant the aggregate rays are seen to become highly perfected, and to extend considerable distances above, and particularly below, the leaf-traces. The depressed segments which occur in the mature twigs are, in reality, caused by the concentrated retarding influence of long lines of paired aggregate rays upon the growth of a portion of the stem. That these depressed segments do not correspond to tissue formed by an interfascicular cambium is further shown by the fact that they are subtended by protoxylem elements, similar to those which subtend the larger so-called fascicular segments. Similar conditions occur in other genera of the Cupuliferae. In fact the primitive central cylinder consists invariably of a continuous woody ring, and only with the development of highly specialized ray structures, in mature ramifications of the plant, does the central cylinder appear to originate from so-called fascicular and interfascicular segments. In Fig. 17 is illustrated the primitive condition of the central cylinder in the Cupuliferae. The protoxylem elements form an unbroken line around the pith, and there is no indication of the division of the central cylinder into the putative fascicular and interfascicular segments. However, as the two lateral leaf-traces pass off in this section, it may be seen that a gap is left in the protoxylem elements of the central cylinder. In the

species figured, which has only a slight amount of storage tissue associated with the leaf-trace, the foliar gaps are not persistent for any considerable distance above the node. In Fig. 18, on the other hand, is illustrated an alder in which long lines of storage tissue have been developed in relation to the leaf-traces. Above the node the storage tissue extends to the more or less persistent and elongated foliar gaps. This relation of the aggregate rays to the leaf-traces, which is found in the more highly specialized Cupuliferae with large aggregate rays, produces in the stem a segmented appearance of the central cylinder which has been erroneously taken to indicate the origin of the woody cylinder from a ring of originally separate, so-called primary bundles. In Figs. 19, 21, and 23 the formation of the aggregate ray and its relation to the enlarged foliar gap is illustrated in *Alnus japonica* by serial sections.

From this consideration of the origin and development of aggregate rays, and their modifying influence upon the primitive stele of the Cupuliferae, it appears that the Sachsian theory of secondary growth must be exactly reversed in order to agree with the anatomical evidence afforded by a comparative developmental and experimental study of the lower Dicotyledons. Furthermore, the general examination of the anatomy of fossil and extant plants reveals the fact that the primitive condition of the fibro-vascular system was a continuous hollow cylinder. Further, in the evolution of modern seed plants there has been a more or less pronounced reduction of the amount of primary xylem of the central cylinder. This reduction of the primary tissues has, in most cases, progressed unevenly along the inner circumference of the stele, and, as a result, in many cases well developed areas of primary xylem are separated by areas in which only traces of primary elements or none at all occur. In Angiosperms the primitive condition of the stele is a continuous tubular cylinder. This has been pointed out by Jeffrey[1] in a study of the Ranunculaceae, Nymphaeaceae, and Saxifragaceae, by the writer, as has been shown in this article, in the Cupuliferae, and by Eames, in an article which appears contemporaneously with this, in certain semi-herbaceous Rosaceae. In the development of the latest dicotyledonous plants, the protoxylem elements of the solid tubular cylinder have become gradually more or less localized, first into a primary ring with localized thickened areas, separated by areas from which the protoxylem has nearly disappeared, and finally into a dissected cylinder or ring of separate primary bundles. The origin and development of large sheets of storage tissue by the parenchymatization of certain radii of the secondary xylem, in connexion with the leaf, and the formation of elongated more or less persistent foliar gaps in relation to these structures, appear to have been the controlling factors in the localization of primary elements, and

[1] Jeffrey, E. C.: The Morphology of the Central Cylinder in Angiosperms. Trans. Canad. Inst., vol. vi, 1899.

in the dissection of the woody cylinder. By the development of these structures the woody cylinder becomes at first a network of xylem tissue filled in by large thin sheets of storage tissue. A higher step of this dissection process, in the Cupuliferae, may be seen in the mature twigs of Red and White Oak. In these highly specialized and diagrammatic stems, as also in *Clematis*, the aggregate rays formed by the leaf-traces at each node are relayed from node to node up the stem in long vertical lines. In these stems, therefore, the woody cylinder is actually divided into ten so-called fascicular segments, separated by sheets of vertically fused aggregate rays or interfascicular segments so called. Owing to the fact that the large rays are grouped in approximated pairs, five smaller segments are depressed by the concentrated retarding influence of these rays upon their growth. Among higher families of the Dicotyledons there exist still higher degrees of the dissection of the central cylinder by means of the parenchymatization of secondary xylem, in relation to the leaf. This expresses itself in the transformation of larger and larger segments of the central cylinder into parenchyma, and in the gradual decrease in thickness of the woody cylinder. The cylinder is thus split up into small segments or bundles, separated by broad radial stripes of parenchyma subtended by the so-called interfascicular cambium. In the progress of the herbaceous habit the interfascicular cambium also loses its activity. Eames, in an article which appears with this, has succeeded in demonstrating these progressive transitions in certain Rosaceous species. Thus, for example, the prostrate biennial or perennial stems of *Potentilla palustris*, 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 up into a typically herbaceous stem.

From this consideration of the comparative anatomy of living and fossil plants, particularly of the Cupuliferae, of the phylogenetic significance of seedling plants, and of the origin and development of storage tissue in relation to the leaf-trace, we come to the conclusion that the Sachsian hypothesis of the origin of the central cylinder of woody plants, based upon appearances rather than upon an adequate study of anatomical facts, must be reversed in order to agree with actual conditions among the higher seed plants. The most striking feature of this study has been the important part that the leaf-trace has played in the development of complex ray structures, and in the development of the herbaceous habit.

In view of the confused terminology of ray structures which exists in botanical literature, it seems desirable at this point to endeavour to unravel this tangle in the light of recent investigation upon ray structure. All ray structures occurring in the xylem portions of plants have been commonly called 'medullary rays'. Inasmuch as these structures originate only with secondary growth, and are in no sense related to fundamental tissue,

the term 'medullary' in this connexion appears to be inadmissible. It has been shown that the so-called 'primary' rays or aggregate rays are in no sense related to inclusions of fundamental tissue, but are produced with secondary growth by the aggregation and fusion of uniseriate rays, and by the parenchymatization of fibres separating these. Similarly the smaller multiseriate, bi- and tri-seriate rays, such as occur in *Betula*, *Carpinus*, *Ostrya*, and *Fagus*, are formed by the enlargement of individual uniseriate rays or by the dissection of aggregate rays. The primitive type of ray, the uniseriate ray, develops only with secondary growth, and is in no sense related to the medulla. This becomes very clear from a consideration of the comparative anatomy of living and fossil woody plants, particularly of the anatomy of plants which retain well developed primary structures. Fig. 16 illustrates a cross-section of *Lyginodendron oldhamium*. It will be noted at once that the rays which develop with secondary growth do not extend into the primary metaxylem at all, nor are they related in any way to the fundamental tissue of the pith. Similarly in living plants which retain well developed primary metaxylem, e.g. roots of certain Conifers, *Larix* and *Abies*, it can be seen that the rays originate only with secondary growth. It is only in more highly specialized plants, in which the primary structures have been much reduced, that the rays *appear* to connect with the medulla. In consequence it seems desirable to replace the term 'medullary ray' by *wood ray* in designating this parenchymatous tissue of the xylem. In the past it has been customary to divide ray structures into two classes, the so-called 'primary' and 'secondary rays'. As has been shown above, all *wood rays* are essentially secondary, in that they originate only with secondary growth. However, if these terms are to be retained in botanical literature the term *primary* might well be applied to the *primitive* or *uniseriate* ray, and the word *secondary* to the larger rays which have been evolved from them. The large ray may be divided into two classes according to its mode of origin. The most important large rays are the *compound*, *aggregate*, or *foliar* rays whose origin has been described in this article. In addition there are certain smaller multiseriate rays, usually bi- or tri-seriate, which originate by the increase in size of uniseriate rays or by the dissection of aggregate rays. These might well be called *multiseriate*. There is strong evidence for believing that this type of ray has been produced, as has the aggregate type, in connexion with the leaf. However, this will receive further investigation in this laboratory.

In conclusion, I wish to express my sincere thanks to Professor E. C. Jeffrey for valuable assistance and advice in conducting this investigation. To my colleague in Forestry, Professor J. G. Jack of the Arnold Arboretum, I am indebted for much carefully identified green material of

many species of woody plants, and to Mr. A. J. Eames for Figs. 12 and 15, and for the opportunity of examining serial sections of herbaceous and semi-herbaceous plants.

DESCRIPTION OF PLATES XV–XVII.

Illustrating Mr. Bailey's paper on Compound Rays in Lower Dicotyledons.

(It is suggested that a hand lens be used in the examination of the figures.)

PLATE XV.

Fig. 1. *Alnus acuminata.* Tangential section of the mature wood, showing non-aggregated uniseriate rays. × 40.

Fig. 2. The same. Transverse section. × 40.

Fig. 3. *Alnus incana.* Tangential section of the mature wood, showing in the central portion a band of aggregating uniseriate rays. × 40.

Fig. 4. The same. Transverse section. × 40.

Fig. 5. *Alnus rubra.* Tangential section of the mature wood, showing a higher step of the aggregating process in which the rays have increased in size to bi- and tri-seriate and are coalescing in places. × 40.

Fig. 6. The same. Transverse section. × 40.

Fig. 7. *Alnus maritima.* Tangential section of the mature wood, showing an advanced stage of the compounding process in which the coalescing mass has taken on the fusiform outline of a large oak ray, and in which the fibres included in the tissue are being transformed into parenchyma. × 40.

Fig. 8. The same. Transverse section. × 40.

Fig. 9. *Alnus rhombifolia.* Tangential section of the mature wood, showing a completely fused aggregate or compound ray which is composed of a homogeneous mass of parenchymatous tissue homologous to the large oak ray seen in Fig. 11. × 40.

Fig. 10. The same. Transverse section of the wood, showing an intermediate step between those shown in Figs. 7 and 9. × 40.

Fig. 11. *Quercus rubra.* Tangential section of the wood, showing the large aggregate ray which is characteristic of American oaks with deciduous foliage. × 40.

Fig. 12. *Quercus virginiana.* Tangential section of the wood, showing a compounding condition similar to that seen in *Alnus rubra* in Fig. 5. × 40.

PLATE XVI.

Fig. 13. *Quercus virginiana.* Transverse section of the wood, showing aggregating tissue in which fibres and wood parenchyma cells are being transformed into ray parenchyma. × 40.

Fig. 14. *Quercus alba.* Tangential section of traumatic wood, showing the reversion of wounded wood to primitive stages of compounding in the large rays. × 80.

Fig. 15. *Quercus velutina.* Tangential section of the seedling plant, showing primitive compounding phase similar to that which occurs in the mature wood of *Alnus rubra* and *Quercus virginiana.* × 40.

Fig. 16. *Lyginodendron oldhamium.* Transverse section of the primary and secondary wood, illustrating the fact that the wood rays originate with secondary growth and do not extend into the metaxylem or have any relation to the fundamental tissue or medulla. × 20.

Fig. 17. *Alnus tenuifolia.* Transverse section of a small twig, showing a primitive condition of the woody cylinder, which is seen to consist of a solid homogeneous tubular cylinder except at the points where the lateral leaf-traces (marked *x*) and associated tissue are interposed. × 15.

Fig. 18. *Alnus japonica.* Transverse section of a small twig, showing a somewhat specialized form of central cylinder in which long sheets of aggregated tissue, in relation to the leaf-traces, have produced a segmented appearance of the stem. × 10.

Fig. 19. *Alnus japonica.* Transverse section of the stem below the node, showing a leaf-trace bundle and associated tissue from which the vessels are gradually disappearing. × 40.

Fig. 20. *Quercus alba.* Transverse section of the stem, showing aggregating tissue associated with a lateral leaf-trace. × 40.

Fig. 21. *Alnus japonica.* Transverse section near the node, showing the leaf-trace just starting out to the leaf, the foliar gap left in the primary cylinder, and the aggregating tissue related to the leaf-trace. × 40.

Fig. 22. *Corylus rostrata.* Transverse section of the stem, showing the relation of an aggregate ray to the lateral leaf-trace. × 40.

Fig. 23. *Alnus japonica.* Transverse section above the node, showing the relation of the aggregate ray to the persistent foliar gap in the primary woody cylinder. × 40.

Fig. 24. The same. Tangential section of the wood, showing the aggregating tissue which occurs above and below the lateral leaf-traces. × 40.

PLATE XVII.

Fig. 25. *Quercus alba.* Transverse section of a small branch, showing the five small sunken segments which are depressed by the retarding influence of the approximated lateral leaf-trace rays upon the growth of the stem. × 10.

Fig. 26. *Quercus virginiana.* Transverse section of the stem, showing the aggregate rays which appear to originate at some distance from the pith. × 12.

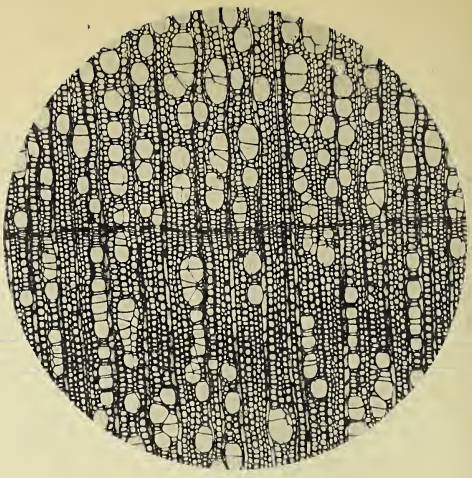
Fig. 27. *Quercus velutina.* Transverse section of the stem, showing a sunken segment and the retarding influence of the aggregate rays upon the growth of that portion of the stem. × 15.

Fig. 28. *Quercus virginiana.* Tangential section of the wood, showing the small amount of aggregating tissue that is associated with the lateral leaf-trace in the first-formed wood of the stem. × 60.

Fig. 29. *Quercus velutina.* Transverse section of the stem, showing two aggregate rays related in origin to a single lateral leaf-trace. × 20.

Fig. 30. *Quercus virginiana.* Tangential section of the wood at some distance from the pith, showing a portion of the long aggregate ray that is associated with the lateral leaf-trace. × 60.

1.



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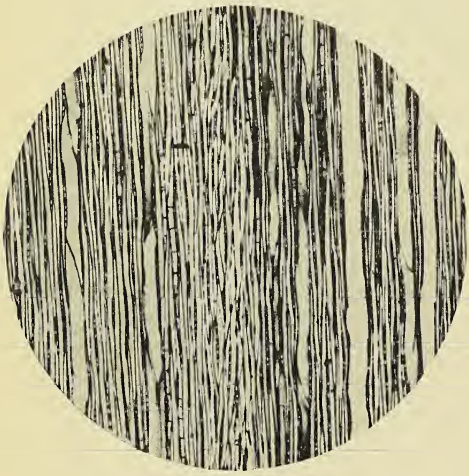

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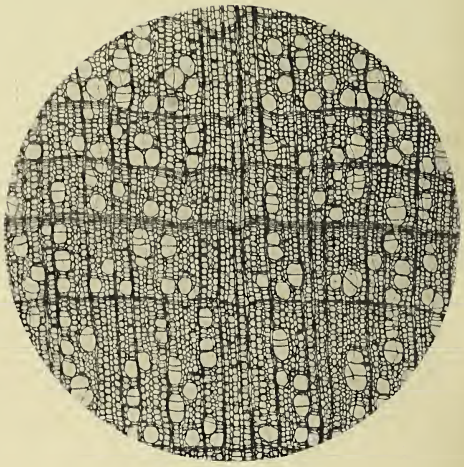

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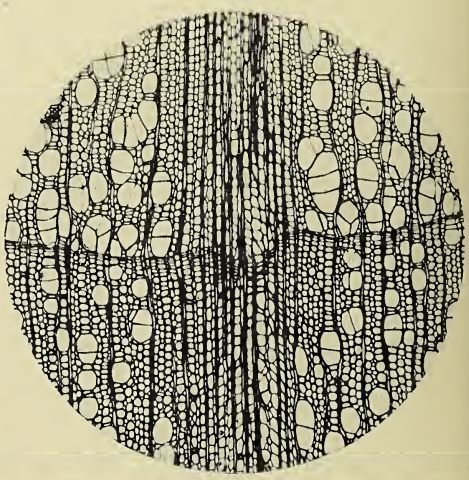

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BAILEY —— COMPOUND RAYS IN DICOTYLEDONS.

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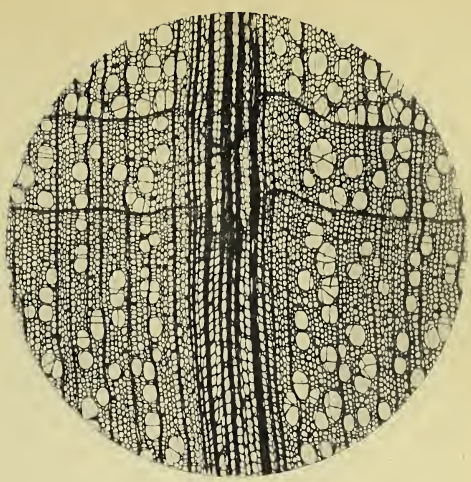

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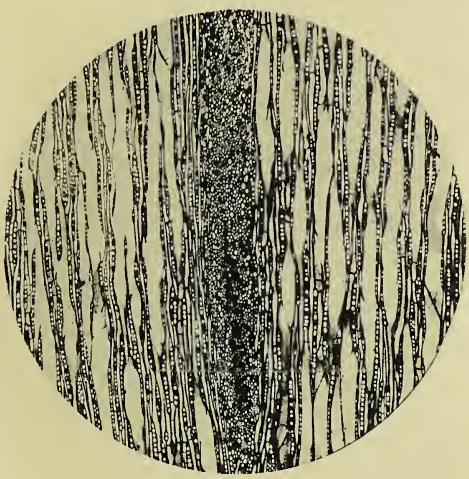

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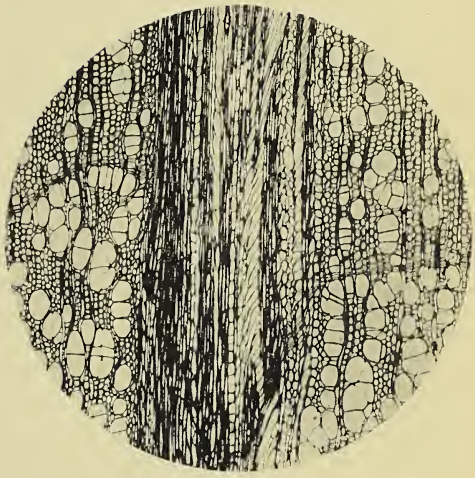

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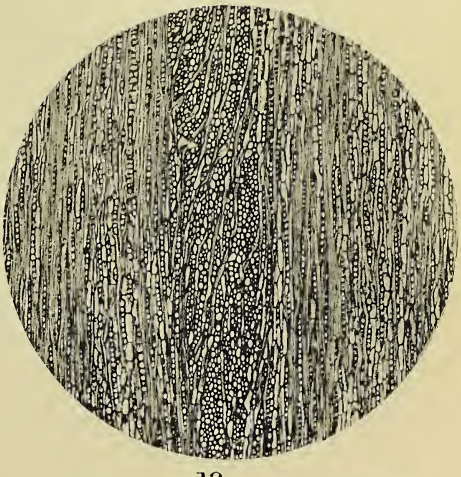

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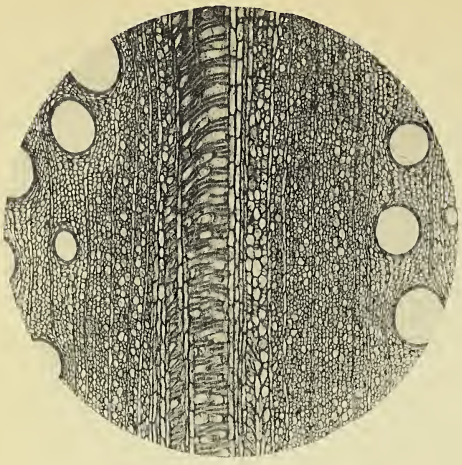

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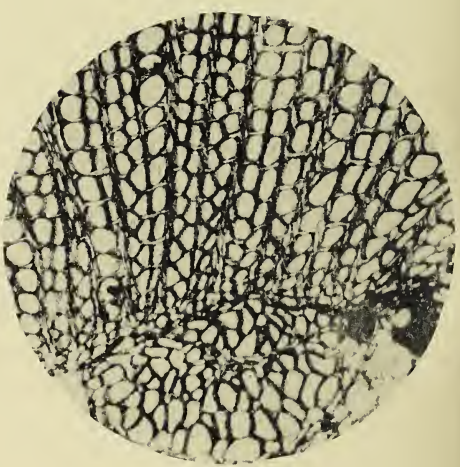

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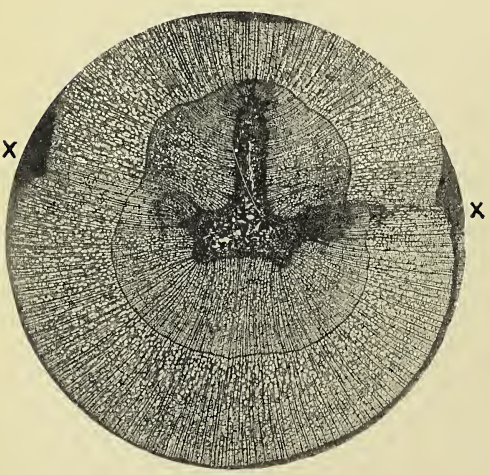

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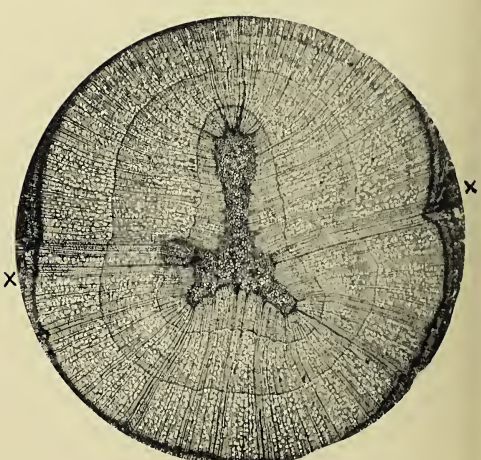

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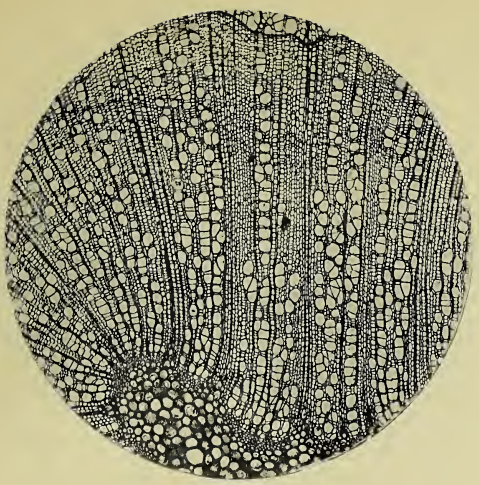

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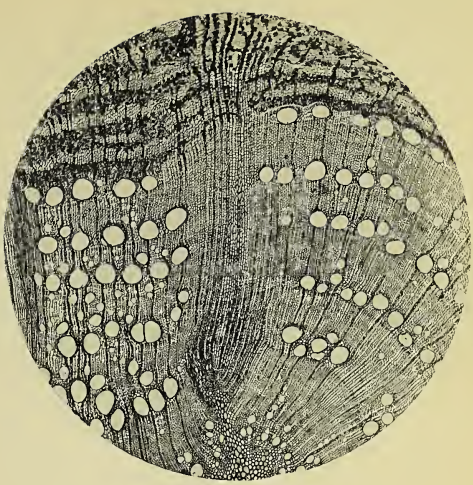

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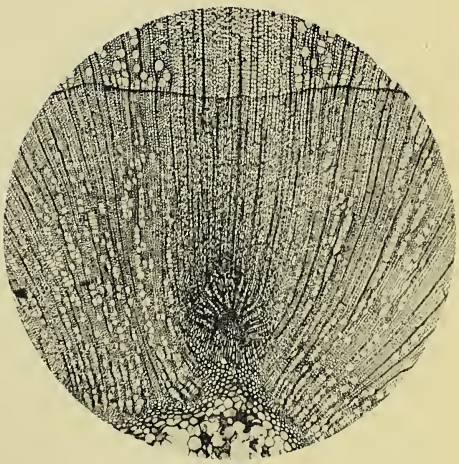

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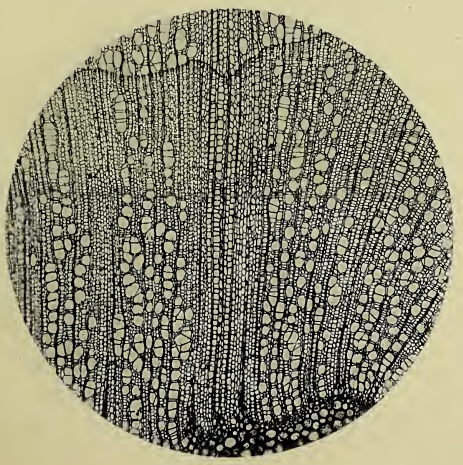

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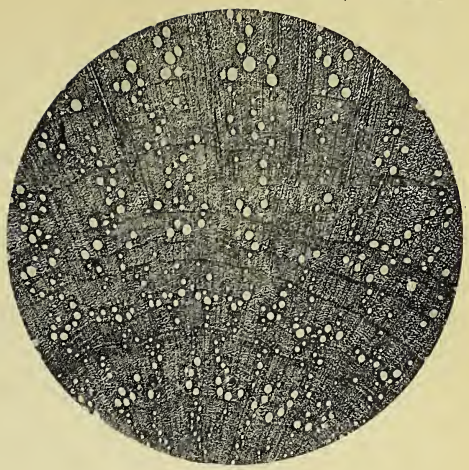

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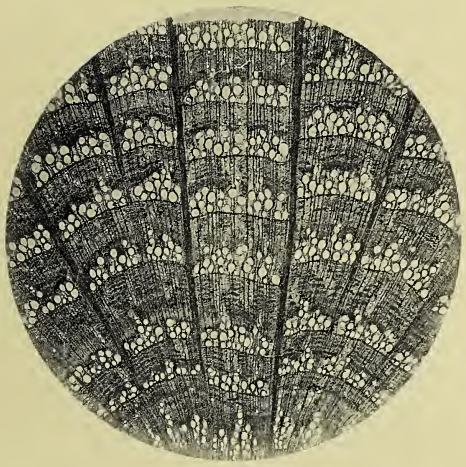

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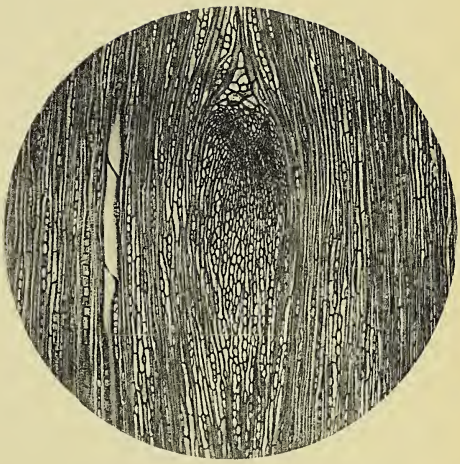

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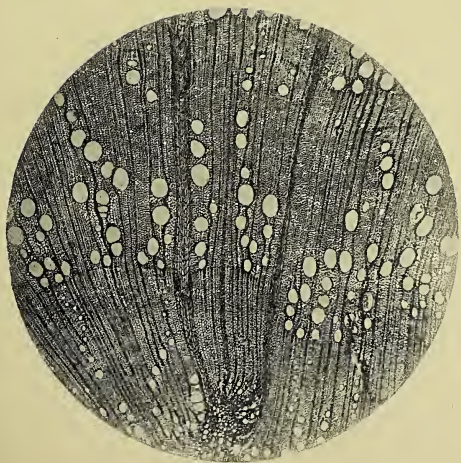

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BAILEY — COMPOUND RAYS IN DICOTYLEDONS.

Contributions to the Cytology of Helvella crispa, Fries.

BY

D. CARRUTHERS, B.Sc.

With Plates XVIII and XIX.

HELVELLA CRISPA, Fries, is a pale buff Ascomycete with a stalked ascophore, the head of which is much convoluted. Material was collected in the autumn of 1909 and was fixed in Flemming's fluid, the strong solution diluted with an equal quantity of water being most satisfactory.

Sections were cut from 3–8 μ in thickness, and were stained with Flemming's triple stain, or with Heidenhain's iron-haematoxylin, and in the latter case either erythrosin or *lichtgrün* was used as a counter-stain.

The research was carried out at Birkbeck College, and I wish to express my thanks to Dr. Fraser, at whose suggestion the work was undertaken, for valuable help and advice throughout its progress.

Vegetative Tissue.

The hypothecium is made up of a number of septate hyphae forming a loose tangle, the cells of which contain fairly abundant protoplasm and one, two, or several nuclei each. These nuclei are small; the chromatin appears to be scattered in small granules round the periphery without any definite reticular arrangement.

Certain of these nuclei were observed to fuse in pairs (Pl. XVIII, Figs. 1, 2), but no evidence of migration such as has been recorded in *Humaria* (7) and in *Nephrodium* (6) was obtained. In most cases the cells of the paraphyses are binucleate, while those of the fertile hyphae have a larger number of nuclei, but no exact differentiation can be traced between the two.

Formation of the Ascus.

The hyphae which are to give rise to the asci are generally larger than the paraphyses, and have scantier and less granular contents. Their nuclei resemble in size and appearance the fusion nuclei of the hypothecium.

A certain amount of evidence was obtained as to the mitoses in both

the vegetative and the fertile hyphae (Figs. 3, 4, 5, 6). The former show structures which have every appearance of spindles, with two chromosomes (Figs. 3, 4); the latter have four chromosomes (Fig. 5), and at a later stage eight are visible (Fig. 6). The nuclei are, however, so minute that it would be unwise to attach any great importance to these phenomena. It is conceivable that the 'spindles' are only a fibrillar arrangement of the cytoplasm, and the 'chromosomes' nothing more than some of the granules which are abundant in the hypothecial tissue. It is difficult to account for the occurrence of a bent hypha with two spindles, each bearing four chromosomes (Fig. 5), on the basis of coincidence, and it seems desirable to give the evidence for what it is worth, without accentuating its theoretical importance.

The ascogenous hypha bends over to form the characteristic crozier, and two nuclei pass into the bent portion; these divide simultaneously, and eventually a terminal uninucleate and a penultimate binucleate cell are cut off (Fig. 7). There is some evidence to show that the nucleolus of each nucleus now buds off a portion of itself, which is extruded at one end of the nucleus and is very conspicuous at this stage (Fig. 8).

The two nuclei of the penultimate cell fuse, and the nucleolar granules remain one at either pole of the resulting nucleus (Fig. 8). A projection is formed from the penultimate cell, and into this the large double nucleus passes; it grows rapidly, pushing up among the paraphyses, and forms the young ascus. The terminal cell often fuses with the stalk cell, their nuclei unite, and a second ascus is formed (Figs. 9, 10).

Meiosis.

The nucleus of the young ascus presents a characteristic double appearance. It is oval in shape, and at either pole is visible the granule which was extruded after budding off from the nucleolus. Shortly after fusion the chromatin contracts into two close masses, one at either end, and the granules, which have hitherto stained with nucleolar dyes, gradually become impregnated with chromatin (Fig. 12).

As contraction passes off the chromatic granules are expelled from the nucleus (Fig. 13), forming deeply staining pear-shaped bodies which may be seen traversing the nuclear membrane (Figs. 14, 15). In nearly all cases the original granules appear to be extruded, but there are also smaller chromatin bodies which are budded off directly from the chromatic substance of the nucleus. The larger bodies are evidently expelled with some force, leaving behind them a clear track in the cytoplasm (Fig. 14). At a somewhat later stage secondary buds may be formed from the nucleolus (Fig. 16), but their further history was not traced. The extruded bodies gradually degenerate and once more take up nucleolar stains, until finally they are visible only as refractive granules, which at this stage adhere closely to the nuclear membrane (Fig. 20).

As the nucleus passes out of contraction the thread is gradually unwound from either pole (Fig. 13), and in the spireme it is impossible to distinguish the two portions. The thread is distributed over the whole of the nuclear area, and indications of a longitudinal duplication become visible (Figs. 17, 18). The thread is very granular, and on the appearance of the duplication the granules are observed to lie in pairs (Fig. 18). Eventually the thread appears double throughout its length (Fig. 19).

The second contraction is not very marked; a certain amount of aggregation of the nuclear thread towards one side of the nucleus takes place, but it is rather a change in position than in length (Fig. 20). Very little actual contraction of the thread occurs, but the longitudinal fission is temporarily obliterated. Soon the thread loosens out again, the split reappears, and the thread is arranged in irregular loops, in each of which the split is often very evident (Fig. 21).

The spireme next breaks up to form four gemini, the limbs of which still show the longitudinal fission (Fig. 22). The limbs may be twisted together or remain divergent, and the chromosomes gradually contract into the typical heterotype forms, a figure 8 and an incompletely closed 8 being the commonest (Fig. 23). The tendency is, however, for the chromosomes to remain long and thin while they are free in the nuclear area. The final contraction occurs at about the time when the chromosomes pass on to the spindle (Figs. 24, 25, 26).

As the chromosomes begin to contract the nuclear membrane becomes less distinct, although the nuclear area is clearly evident throughout the division. The astral rays are quite distinct and spread round the nuclear area and out into the dense cytoplasm surrounding it (Figs. 24, 27).

The mature chromosomes are typically V-shaped, and in most the fission is obliterated (Fig. 26), but in some it is indicated by a cleft in each limb (Fig. 25). The limbs separate from each other so that each bivalent chromosome is divided transversely, and at this stage some of the daughter chromosomes are V-shaped, owing to the reappearance of the split (Fig. 27). As they pass towards the poles the chromosomes become much elongated (Fig. 28), contracting again as they reach the poles (Fig. 29).

On the reconstitution of the daughter nuclei distinct chromosomes are no longer visible, the whole chromatic substance forming a network (Fig. 30).

On the spindle of the second division four chromosomes reappear, each of which is V-shaped by incomplete fission. In most cases they are attached at the apex of the V, and here the final separation of the limbs occurs (Pl. XIX, Figs. 31, 32). The chromosomes may elongate during their passage to the poles, or they may remain as more or less rounded bodies.

The resulting four nuclei enter upon a short resting stage.

The Third Mitosis.

The prophases of the third division appear to be passed through very rapidly ; the nuclei increase very little in size during the resting stage and are very small at the time of the third division. The spindles, however, are exceedingly clear, and upon them reappear four chromosomes, at first corresponding in shape with those of the second division, but later becoming elongated and much curved or bent.

The chromosomes are at first closely grouped at the equator (Fig. 33), but at a slightly later stage they separate, two passing towards each pole (Fig. 34). Indications of two bent chromosomes at each pole were obtained (Fig. 35), and confirmation of this interpretation is afforded by the mitosis in the spore which shows two chromosomes passing to each pole (Fig. 39). Although instances in which the chromosome number is visible in the third telophase and in the spore or hypothecium are infrequent, yet the sum of the evidence from all these constitutes a strong argument.

The third mitosis is therefore a typical brachymeiosis.

Spore Formation.

Towards the end of the third division the astral fibres are very obvious (Fig. 36), and on the break-down of the spindle each nucleus develops a beak from which radiations proceed (Fig. 37). These rays completely delimit the spore, sweeping round the nucleus until they meet at the opposite pole (Fig. 38). As the spore wall is formed the nuclear beak elongates and finally becomes detached from the wall.

The nucleus then rounds itself up and the beak disappears. The spore nucleus now divides mitotically to form eight secondary nuclei (Fig. 39). Although this is the normal method of spore formation, there very frequently occurs an abnormality whereby the eight nuclei, instead of forming spores, divide again. Asci thus formed, containing sixteen to thirty-two free nuclei, are of fairly common occurrence (Fig. 41).

Abnormalities.

Abnormal hyphae were observed in which the nuclei of the penultimate cell had divided before fusion, and a second ascogenous cell was cut off (Fig. 40). Proliferation of the penultimate cell to form a second and even a third hook also occurs (Fig. 11). Occasionally the nuclei of the ascogenous cell fail to fuse, but pass together into the young ascus, and, at the stage observed, each was undergoing contraction and giving off a chromatin body (Fig. 42).

Morphology.

Our knowledge of the developmental stages of the Helvellineae was until recently restricted to the Geoglossaceae, and was due in the main to the work of Dittrich (4) on *Leotia* and *Mitrula*. He also studied *Helvella*,

but had little success, owing to the difficulty experienced in obtaining early stages. His work has recently been supplemented by McCubbin (11), who has described the morphology and development of the ascocarp of *Helvella elastica*, a form with which *Helvella crispa* has certain points in common.

In *H. elastica* there is a very marked difference between the fertile and vegetative hyphae, the former containing several nuclei in each cell, and the latter only two. In *H. crispa*, although there is generally a difference in the size of the hyphae, the number of nuclei is so variable that no definite separation can be based upon it.

Proliferation of the penultimate cell to form another hook, a phenomenon previously observed in *Humaria rutilans* (7), is described as common in *Helvella elastica*, and occurs, though less frequently, in *H. crispa*, as does anastomosis of the terminal and stalk cells. An abnormal hypha in which the penultimate cell has divided is of interest as the only instance observed in this species of an occurrence which appears to be fairly common in *H. elastica*.

Sexuality.

Helvella crispa is another example of the disappearance of normal fertilization and its replacement by the fusion of apparently undifferentiated nuclei. In this respect it is entirely comparable with *Humaria rutilans* (7). The second fusion occurs in the ascogenous hypha, and, as in the various forms already described (7, 8, 9), a double reduction takes place in the ascus.

Development of the Ascus.

Harper, in 1905 (10), describes the formation and further development of the ascus in *Phyllactinia* and *Erysiphe*. In these the ascogenous hypha does not bend over, but the same division and fusion of nuclei occurs as in the more usual hooked arrangement. In *Phyllactinia* there is no change in the chromosome number throughout the life-history, the chromosomes themselves fusing in pairs at nuclear fusion, so that each becomes quadrivalent.

In *Humaria rutilans* (7) no sexual organs are developed, but fusion of vegetative nuclei in pairs occurs. The usual second fusion takes place in the formation of the ascus and is followed by a double reduction. The first two divisions in the ascus are meiotic, but the third is of a simpler type, in which reduction is accomplished without any apparent pairing of the chromosomes. Similar processes have been described by Fraser and Welsford for *Otidea aurantia* and *Peziza vesiculosa* (8). In 1909 Fraser and Brooks (9) described the cytology of the ascus in *Humaria granulata*, *Lachnea stercorea*, and *Ascobolus furfuraceus*, in all of which there is a pseudapogamous fusion in the ascogonium and a subsequent fusion in the ascus. In each case the double fusion is followed by a double reduction in

the ascus, the first two divisions constituting a typical meiosis, and the third brachymeiosis of the type described for *H. rutilans.*

These results have been questioned by Guillermond, who doubts the existence of brachymeiosis. The numerical change in the third division has, however, been recorded, not only by those who accept the occurrence of two successive fusions, but by an observer so little predisposed in its favour as Dangeard (2), who considers that the fusion in the ascus alone takes place.

The essential phenomena in *Helvella* show a strong resemblance to those in *Humaria rutilans.* In both the normal sexual process is replaced by a fusion of vegetative nuclei, and in both meiosis and brachymeiosis occur in the ascus.

There are, however, differences which are of some interest. In *Humaria* the meiotic phase is initiated before the second fusion, and in the resulting nucleus the component nuclei cannot be distinguished. *Helvella* exhibits a less complete fusion, for the two nuclei in the penultimate cell of the ascogenous hypha show no sign of the coming meiosis, unless the casting out of a part of the nucleolus, the basis of the chromatin body, be regarded as a part of the process.

The association of nuclei in the asexual fusion in *Helvella* is less intimate than in any form yet described. The fusion is at first merely a disappearance of the boundary between two nuclear areas, and during the initial stages of meiosis there is no mingling of the chromatin. The first contraction takes place independently in the two masses of chromatin, and it is not until the formation of the spireme that the chromatin threads of the original nuclei cease to be distinct.

It may be possible to correlate this state of affairs with the fact that no pairing of the chromosomes takes place in brachymeiosis.

In *Humaria rutilans* pairing of the brachymeiotic chromosomes is also absent. In this case, although the chromatin masses do not as a rule remain obviously distinct, the first contraction is passed through before fusion, a fact which seems to indicate that the fusion itself is incomplete. Instances were also observed in *Humaria* in which the chromatin was visible in two separate masses in the post-synaptic stage.

There are four times the post-meiotic number of chromosomes in the definitive nucleus of the ascus, but as the first division is heterotype the chromosomes are paired before they appear on the spindle, and the quadruple number is therefore never seen.

Meiosis.

The details of meiosis conform to the method described by Farmer and Moore in 1905 (5). Two contractions occur, and while there is no evidence whatever of an approximation of spiremes, all the observed facts fall readily

into line with the process as described by Farmer and Moore and by subsequent workers for a great number of plants.

On any supposition other than the reappearance of a previous fission, the cleft ends of the chromosomes on the first spindle must be recognized as the manifestation of an entirely new development.

A comparison of the chromosomes on the heterotype spindle before and after the separation of the limbs leaves little doubt that transverse separation has occurred. On this view the heterotype division is to be regarded as interpolated into the stages of an ordinary karyokinesis, and the reappearance of the split is therefore to be expected.

The third or brachymeiotic division differs from meiosis in its greater simplicity and brevity. In *Helvella* no contraction takes place, and the process is very possibly to be regarded as merely the separation of the nuclei which fused in the ascogenous hypha.

The occurrence of brachymeiosis has been questioned by Strasburger, who accepts Claussen's (1) view that there is only one fusion, that in the ascus, and that this is the completion of a sexual process between two nuclei which have been long associated. Such a theory necessarily precludes the recognition of a double reduction in the ascus. The evidence concerning the absence of a previous fusion is, however, only negative, and so many cases have been recorded among the Ascomycetes of a sexual followed by an asexual fusion, as to make it evident that this state of things is normal.

Such forms as *Helvella*, with an apogamous and an asexual fusion, exhibit a readily comprehensible reduction from the typical sexual process. Each new instance of two successive fusions forms a further corroboration of brachymeiosis.

The 'Chromatin Bodies'.

The ejection of chromatic substance from the nucleus during the early stages of meiosis was described by Digby (3) in 1909 for *Galtonia candicans*. In *Galtonia* the bodies arise either as nucleolar buds which are later infiltrated with chromatin, or as outgrowths from the nuclear framework itself. They remain attached to the nucleus by long fine threads which persist even after the bodies have passed into a neighbouring cell. This phenomenon occurs most abundantly during synapsis, but frequently also in the earlier stages.

In *Helvella* the course of events is strikingly similar. The chromatin bodies may arise either from the nucleolus or nuclear framework, and in the former case they become gradually impregnated with chromatin. They are then forcibly ejected from the nucleus, often clearing a path in the cytoplasm, a fact which is of some interest, as the *Galtonia* bodies have usually a clear space round them. The somewhat pear-shaped body is

drawn out behind into a fine thread, by means of which it remains attached to the nucleus for a considerable time, but never at a great distance from it.

A relationship has been suggested between such bodies and the chromidia cast out during Gametogenesis in the lower animals, but it is at present still uncertain whether any special significance with regard to meiosis can be attached to the process. If the extrusion of a part of the chromatin could be shown to be a normal accompaniment of meiosis and to form an integral part of the process, it might perhaps be held that not only the qualitative halving of the chromosomes, but the loss of some constituent of the nucleus was necessary to the development of sexual cells. In *Helvella*, however, meiosis takes place many cell generations before any fusion, and sexual cells are never differentiated. In such a case it is possible that the chromatin bodies are no more than masses of nutritive substance, for which the nucleus has no further use. Against such a view is to be set the fact that the process is as well defined and elaborate in the Ascomycete as in the Angiosperm, and such a phenomenon occurring with striking similarity in widely separated groups is suggestive of some deep-seated analogy.

In drawing conclusions from these facts, it must, however, be remembered that *Helvella* is certainly a reduced form, and the expulsion of the bodies may be only a vestigial phenomenon. Even if it occurred in the ancestral type, which may reasonably be assumed to have possessed sexual organs as well developed as those of *Pyronema*, it is difficult to see what necessary connexion existed between the extrusion of chromatin masses and meiosis.

Summary.

1. There are no sexual organs in *Helvella crispa*, but fusion of nuclei in pairs occurs in certain hyphae of the hypothecium, and these cells produce the ascogenous hyphae.

2. There is evidence that mitoses in the vegetative and ascogenous hyphae show respectively two and four chromosomes.

3. A second fusion occurs in the formation of the ascus, but the chromatin of the two nuclei remains distinct until the spireme stage.

4. The first and second divisions in the ascus constitute a meiotic phase of the type described by Farmer and Moore.

5. During the early stages of meiosis chromatin bodies are extruded from the nucleus.

6. In the first two divisions four chromosomes appear on the spindle.

7. The third division is brachymeiotic; in the prophase four chromosomes appear and two pass to each pole.

8. The spores are outlined by radiations from the centrosome, and when mature contain eight nuclei; the divisions in the spore show two chromosomes.

List of Papers.

1. Claussen, P.: Zur Kenntniss der Kernverhältnisse von *Pyronema confluens*. Ber. der deutsch. bot. Gesellsch., xxv, 1907, p. 586.
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EXPLANATION OF PLATES XVIII AND XIX.

Illustrating Miss D. Carruthers' paper on the Cytology of *Helvella crispa*.

All figures were drawn with a Zeiss apochromatic immersion lens, apert. 1·30 and compens. oc. 12 (magnification about 2000), with the exception of Fig. 41, for which compens. oc. 6 was used.

PLATE XVIII.

Fig. 1. Apogamous fusions in the hypothecium.
Fig. 2. The same, later stage.
Fig. 3. Mitosis in vegetative hypha.
Fig. 4. Telophase of same.
Fig. 5. Prophase in ascogenous hypha, showing four chromosomes.
Fig. 6. Metaphase in fertile hypha, showing eight chromosomes.
Fig. 7. Crozier with two nuclei in penultimate cell.
Fig. 8. Fusion in ascus.
Fig. 9. Connexion between stalk and terminal cell.
Fig. 10. Nucleus migrating from terminal to stalk cell.
Fig. 11. Formation of successive croziers.

First Division.

Fig. 12. Definitive nucleus of ascus, with chromatin of two nuclei undergoing first contraction separately.
Fig. 13. Emission of chromatin body, spireme stage.
Fig. 14. Same, later stage.
Fig. 15. Same, showing thread passing through nuclear membrane.
Fig. 16. Secondary buds from nucleolus.
Fig. 17. Spireme with longitudinal fission.
Fig. 18. Ditto.
Fig. 19. The fission complete.
Fig. 20. Second contraction.
Fig. 21. Nucleus passing out of contraction, the fission once more visible in the loops.
Fig. 22. Spireme broken up into chromosomes.
Fig. 23. Immature chromosomes.
Fig. 24. Chromosomes on spindle, centrosome and aster visible.
Figs. 25, 26. Chromosomes on spindle.
Fig. 27. Metaphase with eight daughter chromosomes.
Fig. 28. Chromosomes passing to poles.
Fig. 29. Telophase.
Fig. 30. Reconstitution of daughter nuclei.

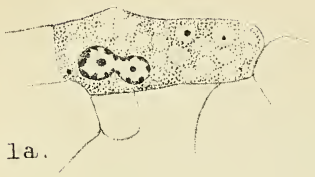
PLATE XIX.

Second Division.

Fig. 31. Chromosomes breaking apart.
Fig. 32. Spindle with eight daughter chromosomes.

Third Division.

Fig. 33. Prophase of third division.
Fig. 34. Metaphase, two chromosomes passing to each pole.
Fig. 35. Telophase.
Fig. 36. Late telophase with astral rays.
Fig. 37. Formation of nuclear beaks.
Fig. 38. Spore formation.
Fig. 39. Mitosis in spore.
Fig. 40. Proliferation of terminal cell of crozier.
Fig. 41. Secondary nuclei formed by division without spore formation.
Fig. 42. Two nuclei in ascus, which have failed to fuse.



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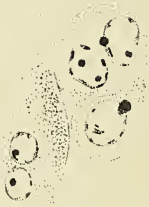

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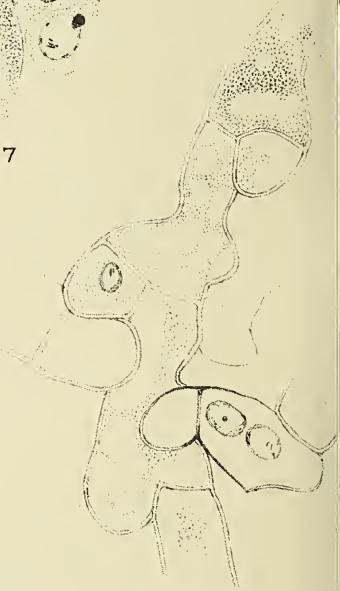

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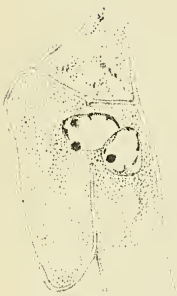

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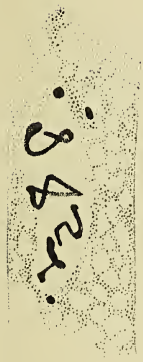

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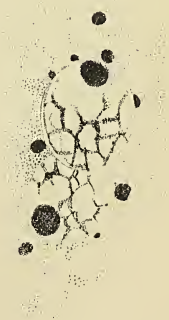

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Huth, lith. et imp.

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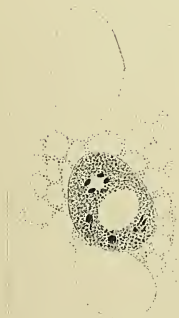

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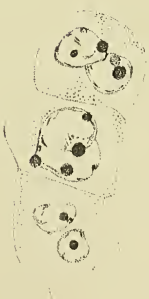

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Huth, lith et imp.

D. CARRUTHERS — HELVELLA CRISPA.

On the Structure and Biology of the Genus Hottonia.

BY

T. L. PRANKERD, B.Sc.

With Plates XX and XXI and seven Figures in the Text.

OF the two species comprised in this genus, the American representative, *Hottonia inflata*, has apparently never been studied, though the European form, *Hottonia palustris*, has received some amount of attention from several writers, and a few figures have been published. Since, however, there have been various misapprehensions, and interesting points of structure have been overlooked, an attempt may be made to give a connected account of some features in this aberrant genus of the Primulaceae.

As the stem anatomy varies somewhat in different individuals, and very greatly at different parts of the same plant, which may be several feet in length, much of the work was done by hand sections, but every point in structure has been invariably confirmed by microtome series. Chrom-acetic and acetic alcohol were used as fixatives, and the most satisfactory stains were found to be safranin, gentian violet, and orange G. for the seedlings and growing points, and Dr. Land's combination of safranin and aniline blue for the older parts.

I. External Morphology and Life-history.

In its typical development, i. e. in water a foot or more in depth, *H. palustris* consists of a long trailing rhizome, terminating at the surface of the water in a vertical inflorescence axis bearing numerous flowers arranged in whorls (Text-fig. 1). The oldest part of the rhizome is usually found embedded in the mud, and is often branched, the branches forming runners which frequently give off vertical, aerial branches—the so-called 'land forms'. This affords one method of vegetative propagation, since, by the dying off of the older parts of the stem, the young plants become entirely separate, and, should the water in the pond rise, may become new typically developed individuals.

These facts do not seem to have been recognized for *Hottonia*, probably because the brittleness of the stem usually causes it to break under the water (or the soil) when the plant is pulled at all, which perhaps accounts

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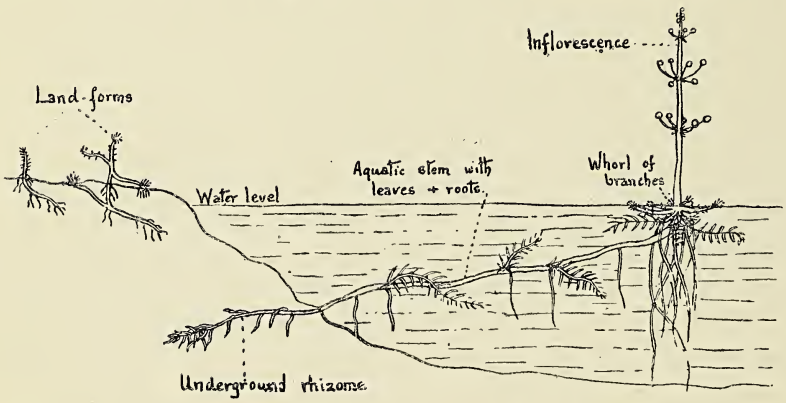
for the statement that *Hottonia* is ' free-floating ' (8), and the idea that land forms are quite distinct from ordinary aquatic individuals. A little care will show that the stem is nearly always attached, and where this is not the case, it has probably become detached from accidental causes (children, water animals, &c.). Similarly, in the case of the land forms, it is possible that they may sometimes be produced in some way other than that described, e. g. the germination of seeds; but I have never found them more than a few feet from the water, and frequently traced them to underground stems, various stages of decay often occurring in the intervening portions. The land form is sometimes considerably branched, the aquatic stem rarely so, except at the surface of the water, where a whorl of some six or eight branches is formed at the base of the inflorescence. In the young plant, these are often several centimetres in length, before the elongation of the inflorescence axis; and throughout the life of the latter undoubtedly help



TEXT-FIG. 1. Diagrammatic sketch of typical plants of *Hottonia palustris*.

to secure its vertical position. After fruit formation, these branches separate from the parent plant, rest through the winter, and form the starting-points for new plants the next spring, when they may be found truly free floating, and often without roots. This immature stage is the only one that may answer to Schenk's description of *Hottonia* as rootless (8), which is entirely incorrect for the full-grown plant. Of the scores of plants examined from various localities round London, I have never found a single mature rootless specimen, and Brokschmidt reports similarly of the specimens from the ponds of Dechsendorf, Germany. *Hottonia* produces very numerous adventitious roots along the whole length of the stem, particularly in the underground part and at the base of the inflorescence. They may appear in any position, but are most often found just above the insertion of the leaves, or of the axillary buds, if these are developed. They are long, slender, and unbranched, often reaching the soil and thus serving to anchor the plant.

A whorl of the characteristic pinnate leaves always occurs beneath the circle of branches, but along the length of the stem the insertion is very irregular. More than one is often found at a node, but Kamienski (7) states that they are spiral, and has calculated a divergence of $\frac{3}{8}$ for them. On the inflorescence axis the simple, entire bracts are certainly borne several at a node. The flowers are very similar to *Primula* in structure, with the trifling exception that it is in the short-styled forms that the stigma is found at the mouth of the corolla tube, while in the long-styled it projects. Cleistogamy has been attributed to *Hottonia*, but I have found no trace of it during three summers' field work. The idea is probably due to some small, closed flowers, which occur sometimes among those fully developed, but serial sections have shown that these are merely abortive.

The foregoing description applies also in the main to *H. inflata*, which differs chiefly from our own species in possessing a whorl of lateral inflorescence axes at the base of the terminal shoot. These take the place of the vegetative branches of *H. palustris*, though they are not horizontal, but rise nearly to the height of the main axis. The internodes are greatly swollen by the enlargement of the pith cavity, and if the general habit of the two species is similar, this reduction in the specific gravity may compensate for the loss of the aquatic branches. The material at my disposal was, however, confined to three specimens (kindly obtained for me by Dr. Coulter) which had passed through the post, and I therefore have no data for such points as the correlation of structure and function, the occurrence of land forms, &c.

II. Anatomy.

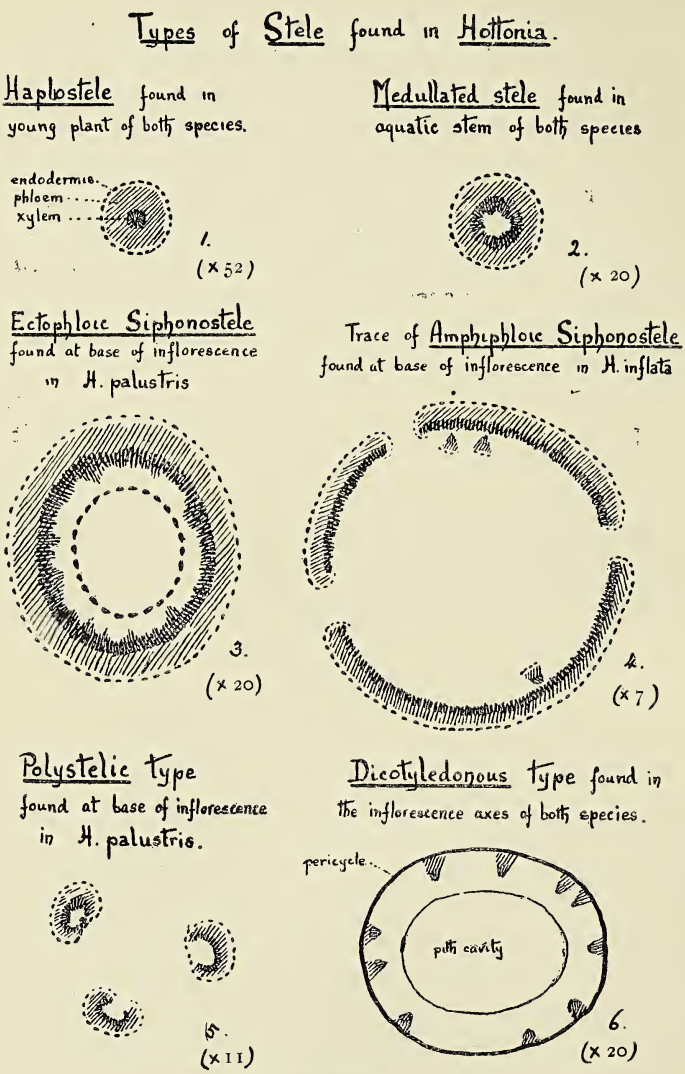
In the young plant of *H. palustris*, and occasionally in the very oldest part of the mature plant, the simplest type of stele is found, consisting of a central strand of xylem, surrounded by phloem, pericycle, and endodermis (Diag. 1, p. 256, and Fig. 21, Pl. XXI). With these exceptions a pith [1] is always present (Diag. 2), though it varies greatly in size, both in individuals and in different parts of the same plant. A cambium may usually be clearly distinguished (cf. Text-fig. 2), but there is little or no trace of secondary thickening. The cortex is lacunate, and in the subterranean stem, particularly in the thickly rooted parts, the three or four innermost layers often stain differently from the rest and are very regularly arranged round the endodermis; the constituent cells are tangentially elongated and fit closely with or without minute quadrilateral spaces between them (Fig. 12, Pl. XX). Thus in its underground part *Hottonia* shows an anatomical point of agreement with the terrestrial Primulas (cf. Gwynne-Vaughan (5), Fig. 1, Pl. XIV, and Solereder (10), p. 506, and Fig. 115 B).

Except in the subterranean stem, the epidermis bears numerous

[1] The term is used descriptively; its nature will be referred to later.

stalked, capitate glands. The stalks are unicellular on the aquatic and multicellular on the aerial stem (Fig. 13).

The structure of the land forms is in every way similar to that of the submerged, and not to the aerial part of the aquatic plants, which latter

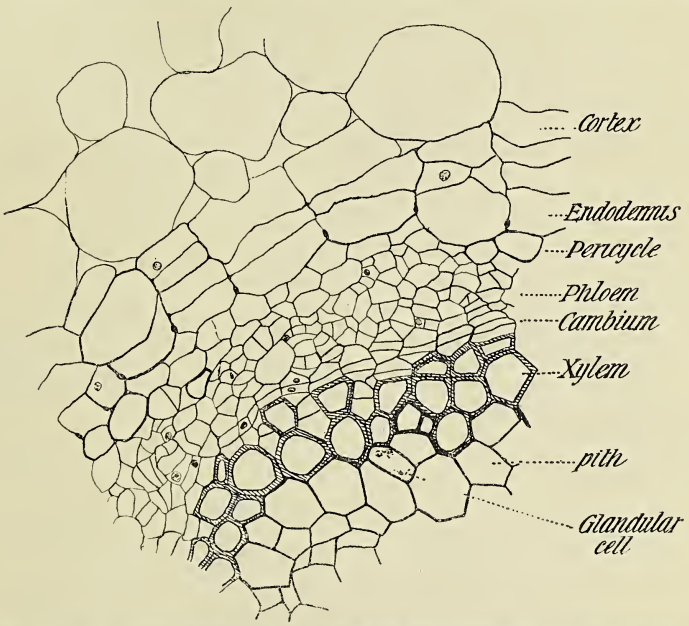


DIAGRAMS 1–6.

presents a striking contrast to that already described. Diagram 6, above, and Figs. 1 and 20, Pls. XX and XXI, illustrate the structure of the internodes, where we have a ring of V-shaped bundles separated by the wide medullary rays, surrounding a hollow pith. The cells of the innermost layer of the cortex fit closely together (*en*, Fig. 20), otherwise there is no characteristic

endodermis, but the pericycle is represented by a ring of lignified fibres (*pc.*)—a family likeness to the Primulaceae (Solereder (10), p. 504).

At the node the lacunae and fibres disappear, the bundles fuse, and a starch sheath forms externally (Fig. 13) around the vascular ring, and occasionally also internally to it (Fig. 14). A gap is left where the pedicel trace leaves the stele (*br. gp.*, Fig. 13), which is usually closed before the bundles separate. The leaf-gap does not occur in the central stele, but in the branch trace, as the procambial strand of the bud always unites with the trace of its subtending leaf at the extreme distal point of the latter, i.e.

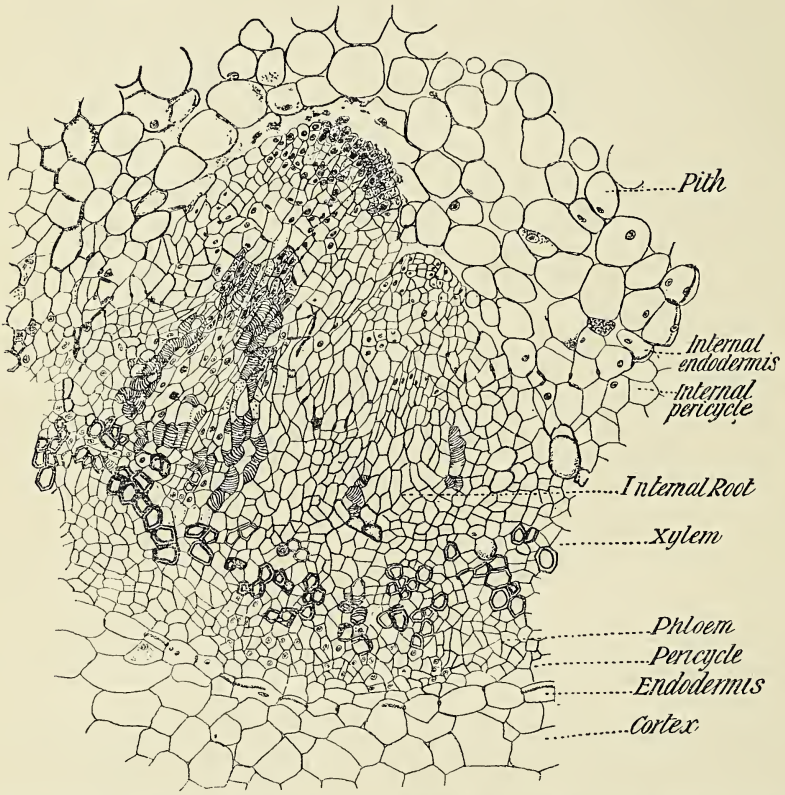


TEXT-FIG. 2. Part of a transverse section across the subterranean part of the rhizome. (× 240.)

just before it runs out into the leaf (cf. Text-fig. 4), as Gwynne-Vaughan (5), p. 314, describes for several species of *Primula*. Hence, when the bud develops into a branch, the leaf-trace is entirely fused with the vascular strand of the latter in its passage through the cortex, and is even occasionally carried up a slight distance into the branch itself, before passing out into the leaf—a link with *Samolus*.

At the base of the inflorescence the bundles again fuse into a more or less complete ring, which is almost immediately interrupted by the large branch gaps. At about this level, the starch sheath begins to show the characteristic endodermal thickening, and extends round the margins of the gaps, becoming connected with the cells internal to the protoxylem, which are seen to be irregularly but strongly lignified. The stele thus becomes broken up into several distinct band-shaped pieces, each of which

is surrounded by a distinct sheath, and shows a tendency to become a concentric structure by the strong incurving of the arms. Fig. 4, Pl. XX, shows a case where three almost complete steles were thus formed (cf. Diagram 5, p. 256), and though there is considerable variation in structure at this region, there is always more or less of a polystelic phase. At a lower level, the large branch traces, entirely concentric in structure, fuse with these central steles, and in so doing form a complete ring of xylem, surrounded by phloem and bounded externally and internally by a pericycle and endodermis,



TEXT-FIG. 3. Part of a transverse section across the aquatic stem near the base of the inflorescence. (× 108.)

or in Jeffrey's terminology, an ectophloic siphonostele (Pl. XX, Fig. 7, and Diag. 3, p. 256). In young plants, where the inflorescence is in bud, the internal endodermis is almost as regular as the external, exhibiting the Caspary dots on the radial walls (Text-fig. 3) in transverse section; but in the mature plant the thickening spreads over the whole of the cell-walls, and even extends to the walls of adjacent cells (Figs. 7 and 8).

As the pericycle has no special differentiation, it would be difficult to be sure of the presence of this layer internally if it were not that it frequently

produces roots which grow horizontally (morphologically) into the pith (*int. rt.*, Pl. XX, Figs. 5 and 6, and Text-fig. 3) and finally vertically upwards, passing out through one of the breaks in the vascular ring.

As in the case of the floral pedicels, the traces of the subtending leaves fuse with the branch steles before the latter unite with the main vascular supply, but the traces of many of the crowded leaves just below this point, which have no branches in their axils, enter the stem as shown in Figs. 5 and 6, Pl. XX, i. e. a complete gap is formed in the vascular cylinder through which the cortex and pith are in unbroken continuity and where the internal endodermis unites with the external. A very little lower down, however, no complete leaf-gap of this type is formed, as the external endodermis is not broken, and the internal parenchyma is thus never continuous with the external (Text-fig. 4). These two forms of leaf-gap characterize the two types of development described by Gwynne-Vaughan (5), p. 317, for the seedlings of *Primula japonica*, of which the latter, i. e. where there is a break in the vascular tissue only, is much the more prevalent. It is this type which is constant for the whole of the subterranean and aquatic stem with the exception of that just below the transitional region, and it is probably characteristic of at least aquatic Angiosperms, as I have found it in the stem of *Hippuris vulgaris*, *Myriophyllum proserpinacoides*, and *Menyanthes trifoliata*. It is interesting to note, as affording a transition between the two types, that for some distance below the point at which the internal endodermis ceases to unite with the external it is inclined to run out into the leaf-gaps as shown in Text-fig. 4 and Pl. XX, Fig. 8.

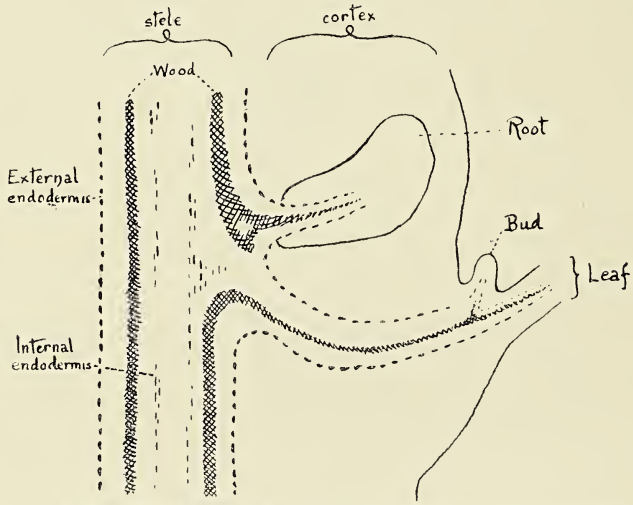
Just at the base of the inflorescence the vascular ring is relatively very large, the pith measuring about 3 mm. across, but as we pass down the stem it narrows rapidly till only a few cells are enclosed by the internal thickened ring (Pl. XX, Fig. 9), and finally none at all, the centre of the stele being occupied by a core of lignified cells (Fig. 10). Passing further down the stem, this core itself contracts till in transverse section it consists of two or three cells or even a single one (Fig. 11). At this point, which is usually several centimetres from the transitional region, the central lignified tissue may die out altogether, or it may persist for a long distance down the stem, though in this case it is almost always interrupted. Indeed, this interruption may take place at any level; the internal ring is often more or less incomplete, as well as irregular, and may be absent for several millimetres to reappear smaller a little lower down.

Thickened, lignified cells in the centre of the stele have been found as far as 18 cm. from the transitional region, and it seems a point worth noting that they tend to occur more often at the nodes, especially where these are crowded. They are never found in the subterranean stem. The whole internal lignified structure is thus comparable with a single 'endodermal pocket' described by Tansley and Chick (12), and Boodle (2), for

Schizaea sp., only that it is median and connected with many leaf-gaps instead of with one.

In general, the anatomy of the two species is similar, but at the base of the inflorescence in *Hottonia inflata* some variations are found owing to the fact that the vascular structure of the branches is here that of the aerial, and not, as in *Hottonia palustris*, the aquatic type. As the transitional region is reached, the separate V-shaped bundles of the main inflorescence approximate and fuse into an incomplete ring, the same thing taking place in the lateral inflorescences at a somewhat higher level (Text-fig. 5).

The separate pieces of the lateral branch traces tend to curve in horse-shoe fashion as in the main stem of *H. palustris*, the endodermis always



TEXT-FIG. 4. Longitudinal section of stem near the base of the inflorescence. × 20. (Diagrammatic.)

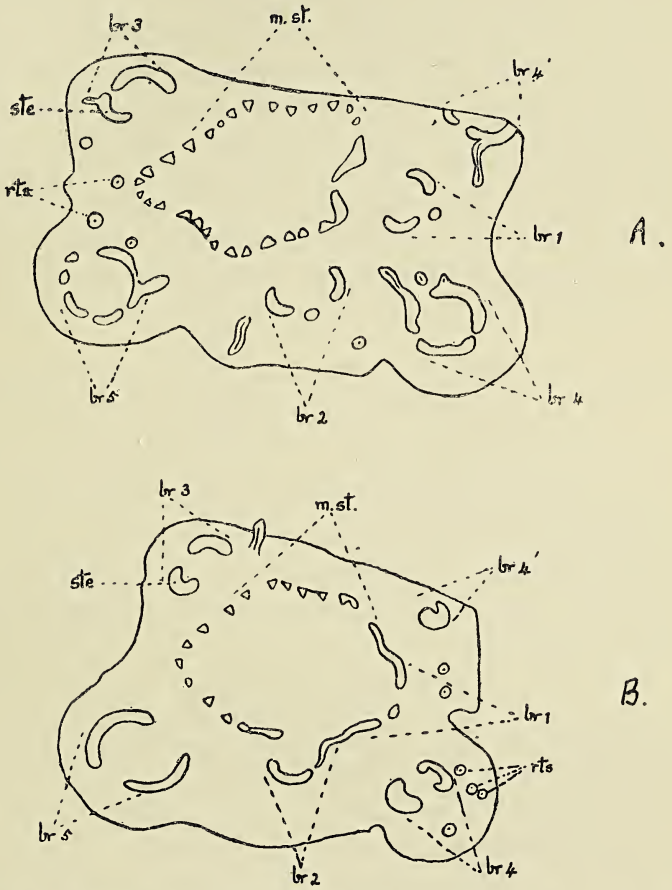
running round the incurved edges. The smaller of these meristeles sometimes form completely concentric structures—the xylem phloem, pericycle, and endodermis all fusing adaxially and enclosing a central mass of ground tissue (Pl. XX, Fig. 2).

Not one of the three specimens at my disposal showed any approach to this phenomenon in the main stem, hence (if these were typical) while *H. palustris* shows a phase of polystely where the constituent steles are imperfect, *H. inflata* exhibits the more complete type of individual stele.

The mode of insertion of the lateral upon the main stele varies greatly: in general, the upper branch traces enter the main vascular ring in several pieces (Text-fig. 5, B), while the lower unite as single concentric structures (*br.* 4′). In the latter case the subtending leaf-trace is completely fused with the branch trace; in the former it frequently unites with a portion of the lateral trace (*br.* 2), or may remain entirely separate till the trace has

entered the main stele (*br.* 1). After the fusion of a trace either with the main or lateral steles, the adaxial xylem, phloem, and endodermis may persist for a short distance down the stem, thus forming little pith strands with reversed orientation (Pl. XX, Fig. 3, and Diag. 4, p. 256).

If the tendency towards concentric structure were carried somewhat further, and the size of the main stele relative to that of the branches were



TEXT-FIG. 5. A. Diagrammatic transverse section across the base of the inflorescence of *H. inflata*. (× 20.) B. The same at lower level. (× 5.) *br.* 1–5 = successive branches; *m.st.* = main stele; *rts.* = roots; *ste.* = part of branch 3 incurving to form a complete stele.

reduced, we should have Jeffrey's amphiphloic siphonostele, as it is we have this suggested (Diag. 4, p. 256). These internal strands very soon die out, and there is apparently no trace of the lignified ring and core of *H. palustris*.

In both species the leaf anatomy is very simple ; the mid-rib is traversed by a single collateral vascular bundle, which has a tendency to become more or less concentric in its passage through the cortex of the stem. A

well-marked endodermal sheath always surrounds the leaf-trace, which is often very difficult to make out in the leaf bundle.

The roots are usually pentarch with a pith, and show a cortex composed of very regularly arranged cells with small quadrangular intercellular spaces, and a well-marked exodermis. They very often take their rise from points just above the leaf-gaps (Text-fig. 4), and the endodermis is always directly connected with that of the main stele (Text-figs. 3 and 4). Numerous roots are given off from branch and sometimes leaf traces in all directions, even as many as three from a single trace. In the case of those running more or less towards the centre of the stem, they sometimes encounter vascular tissue, in which case their direction is altered, and they find their way to the exterior by other routes. Instead of making their way straight to the exterior, the roots frequently bend and run through the cortex, or even the pith, parallel with the length of the stem either towards or away from the apex, and many are thus met in transverse section in a similar section of the stem (Text-fig. 5), recalling *Lycopodium pithyoides*, as described by Stokey (11), who mentions other plants in which this phenomenon occurs.

As might be expected from its habit, *Hottonia* is markedly sensitive to gravity, and an examination of the character and distribution of the starch shows a remarkable development of 'statoplasts' (4) whose appearance coincides with the first indication of graviperception, and which occur at those points where curvatures take place (Figs. 13, 14, and 15). So far as I know, mechanism for the perception of gravity has not been described for aquatics, but preliminary observations seem to indicate that certain of them are favourable objects for the study, which it is hoped may be continued in the near future.

III. The Seedling.

An unsuccessful search has been made for seedlings growing in nature, which are probably rare, as they do not seem ever to have been found, and we know that the plant has several means of vegetative reproduction. The following account is based on the examination of autumn seedlings obtained from seeds ripened on plants grown in the laboratory. These, in the case of both species, were extremely minute and fragile, the vascular system being very reduced, so that its interpretation is a matter of some difficulty. A seedling grown under water forms a bubble of gas between the cotyledons, which causes it to float to the surface, where it remains for some time, subsequently sinks, and takes root in the soil. Fig. 16, Pl. XXI, shows three seedlings in which the tips of the cotyledons are still embedded in the seed coat, and where *t* marks the transition from the hypocotyl to the root, indicated by the cessation of the green colour and the appearance of a ring of root-hairs—a lateral rootlet being frequently given off just above

this point. The characteristic glandular hairs occur on all parts of the seedling except the roots.

Fig. 18, Pl. XXI, represents a transverse section of *a* at Fig. 17 and shows the two cotyledons (*c*) and the remains of the seed coat, which is differentiated into a dark, structureless outer portion (*s*) and an inner parenchymatous layer (*int.*). In spite of a certain amount of shrinkage, due to the fact that the material was not fixed for cytological investigation, the cotyledons are seen to be surrounded by naked, nucleated protoplasm (*n.p.*), which can be traced to the inner cells of the seed coat. The cell-walls of the latter are partially dissolved, apparently by ferment action: and, as if to resist the softening action on their own tissue, the outer walls of the cotyledonary epidermal cells are here and there clearly cutinized (*c.t.*).

Each cotyledon is traversed by a single median bundle, consisting of very few vascular elements with no apparent bundle sheath. Immediately at the base of the cotyledons, the two bundles fuse to form the stele of the hypocotyl, which is root-like in structure, as the xylem groups unite to form

TEXT-FIG. 6. Xylem, shaded; phloem, white; protoxylem, ×.

a diarch plate in the cotyledonary plane, while the phloem groups each divide, the branches fusing in pairs, so that the new phloem groups occupy positions on each side of the xylem plate (Text-fig. 6).

There is little to show that the latter is, strictly speaking, diarch, since the few wood elements are all spiral in character and nearly the same size, but Fig. 19, Pl. XXI, which shows a consecutive series of sections (*a* . . . *e*) through the xylem of one bundle at the base of a cotyledon, indicates a transition to exarchy as the hypocotyl is reached (*e*). This is supported by longitudinal sections, for though none were obtained which passed exactly through the median plane of the xylem plate, those at right angles to this direction indicated that the tracheides at each pole were of a looser spiral type than those in the centre.

Text-fig. 6 is a diagrammatic representation of the transition from stem to root structure, which takes place in the space of about $\frac{1}{20}$ mm. at the base of the cotyledons, so that *Hottonia* seedlings conform to the type defined by Miss Thomas (13) as that 'in which a diarch root stele is formed at or immediately below the cotyledonary node, entirely by the two cotyledonary traces, so that the whole or nearly the whole of the hypocotyl possesses a central cylinder of typical root structure'. When this type was first described in 1903, it was thought to be uncommon and probably

primitive, but Miss Thomas kindly informs me that she has since often found it, and that it occurs in other members of the Primulales. Of the 'double bundle' (14) so widespread in its occurrence, I find no trace in *Hottonia*.

A little plantlet which has produced about fourteen leaves and an adventitious root is drawn in Fig. 16, Pl. XXI. Fig. 21 shows a transverse section of the stem, the stele of which consists of two xylem elements placed centrally, surrounded by small, delicate cells, presumably phloem, and this by a ring of large cells with well-marked endodermal thickening. The cortex is already developing lacunae.

IV. Theoretical Considerations.

From the anatomical standpoint, interest centres in the great divergence of structure shown in different parts of the plant, and especially in the polystelic phase exhibited in the region transitional from the aquatic to the aerial type. It may first be noted that the repeated statement to the effect that *Hottonia* has no true pith, but a central mass of wood parenchyma, in which may be found the remains of the first-formed tracheides (Solereder (11), p. 504), is incorrect. The ground of this statement is doubtless the thickened ring or core of cells in the pith described above. Had these structures been always as clearly defined as those shown in Figs. 9 and 10, instead of exhibiting great irregularity in transverse sections taken at different levels, they would probably never have been confused with protoxylem elements; though any transverse section shows that they are always placed more or less centrally, unless the section is taken quite close to the whorl of branches, and even here they are separated from the wood by several layers of parenchyma. Longitudinal sections, however, show conclusively that the cells are irregularly thickened, with no trace of spiral or annular markings, and clearly distinct from the protoxylem, which is quite characteristic, though often slightly disorganized by the stretching of the internodes. However 'true pith' may ultimately be defined, there seems no reason why the claim of *Hottonia* to its possession should be disallowed.

But if the thickened cells of the pith are not protoxylem, neither are they merely the lignified pith of frequent occurrence in Dicotyledons, for they have been shown to be in direct connexion with the internal thickened ring found at the base of the inflorescence, which when young is a characteristic endodermis. Physiologically, the appearance of this tissue may be due to the great strain placed upon the stem at the insertion of the whorl of branches, and the importance of strengthening their connexion with the main stele to prevent detachment. It is true that *H. inflata* does not possess an internal endodermis, but here both the structure and mode of

insertion of the branch traces differ. They are similar to the main stem, with which they unite at a very slight angle, so that they are at first mere bulges of the great central ring of vascular tissue (Text-fig. 5) which higher up get nipped off; whereas in *H. palustris* the branches leave much larger gaps relatively to the size of the main stele, with which they unite almost at right angles.

Whether the above is the true explanation of its presence or not, we undoubtedly have for a centimetre or more below the inflorescence of *H. palustris* a siphonostele, which, by the union of the internal with the external endodermis at the branch gaps, together with the incurving of the arcs of vascular tissue, results in a transient phase of polystely. More than twenty years ago Scott (9) suggested that the origin of polystely in Dicotyledons might lie in their descent from aquatic ancestors, whose reduced vascular system was not sufficient for a renewed terrestrial existence, and which supplied this deficiency by increasing the number of steles, rather than the size of the single central cylinder. In support of this view, he pointed out that the polystely of the ferns has undoubtedly arisen in this way, and that the two natural orders of Dicotyledons then known to exhibit the phenomenon (Halorraghidaceae and Primulaceae) both possessed aquatic representatives—*Myriophyllum* and *Hottonia*. It is interesting to note that most of the cases of polystely since described occur in aquatic and marsh plants, or in their near relatives (6) (*Nymphaea*, *Parnassia*, *Ranunculus*), and in *Hottonia* we seem to have the phenomenon in its very inception, as in the individual we pass from the haplostele, through siphonostely to polystely. The fact that the haplostele is not primitive does not alter the value of the illustration afforded of the elaboration such a structure may undergo, and the significance of the nodal traces of the 'endodermal pocket' is illumined by Boodle's view (1) that 'advance in complexity probably begins at the nodes, and is afterwards continued through the internodes'. Followed upwards we have in all likelihood the evolutionary course of development; traced downwards we may say, again to quote Boodle, there is taking place from the crowded nodes of the transitional region 'a downward progressive modification', which if continued would result in a polystelic form, such as we find in the Auricula section of the closely allied genus *Primula*.

Probably the best test of the theory lies in experimental work, and cultures have been started with this end in view; meanwhile, on the lines of comparative anatomy, the occurrence of a trace of polystely in the two geographically separated species of this water plant, taken in connexion with its variable habitat and plasticity of structure, seems to afford a link in the chain of evidence that an aquatic ancestral existence is the origin of at least some, if not all, cases of polystely in Dicotyledons.

Summary.

1. The anatomy of the seedling is very simple. There is no trace of the 'double bundle', and root structure is present in the hypocotyl.

2. The mature plant consists of subterranean, aquatic, and aerial portions, and exhibits considerable divergence of structure. *H. palustris* shows a trace of 'polystely' at the base of the inflorescence axis, and more or less perfect steles are formed from arcs of vascular tissue in the lateral inflorescence axes of *H. inflata* near their junction with the main stem.

3. The plant is markedly sensitive to gravity, and the special development of statocyte tissue corresponds in time and position with the occurrence of graviperception.

4. The roots show some peculiarities of position.

Dr. Fritsch first suggested that I should undertake this work, which has been mainly carried out in Prof. F. W. Oliver's laboratory, University College, London. I wish heartily to thank Dr. Coulter and his staff, particularly Dr. W. J. G. Land, for their kindness to me while working at Chicago University. My acknowledgements are also due to Dr. Agnes Arber for the kind and ready assistance she invariably afforded me.

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EXPLANATION OF PLATES XX AND XXI.

Illustrating Miss Prankerd's Paper on the genus *Hottonia*.

The photographs were taken with a Gordon's Photomicro-camera, and Figs. 18–21 drawn with the Abbé camera. All figures and photographs except 2 and 3 are from *H. palustris*. The following abbreviations are used:—*p.* = pith; *p.cav.* = pith cavity; *x.* = xylem; *p.x.* = protoxylem; *int.x.* = internal xylem; *ph.* = phloem; *int.ph.* = internal phloem; *v.b.* = vascular bundle; *pc.* = pericycle; *en.* = endodermis; *int.en.* = internal endodermis; *ext.en.* = external endodermis; *cor.* = cortex; *cor.l.* = cortical lacuna; *ep.* = epidermis; *g.* = glandular hair; *ste.* = stele; *rt.* = root; *int.rt.* = internal root; *br.* = branch; *br.gp.* = branch gap; *l.tr.* = leaf-trace; *l.gp.* = leaf-gap; *t.s.* = transverse section.

PLATE XX.

Fig. 1. Part of transverse section across internode of inflorescence axis. × 40. Cf. Diag. 6, p. 256, and Fig. 20.

Fig. 2. Transverse section of a complete stele at base of lateral inflorescence axis of *H. inflata*. × 40. Cf. Text-fig. 5, *ste.* The arrow shows the direction of the centre of the stem for Figs. 2 and 3.

Fig. 3. Transverse section of part of stele at base of main inflorescence of *H. inflata*, showing two internal strands with reversed orientation. × 40. Cf. Diag. 4, p. 256.

Fig. 4. Transverse section of base of inflorescence, showing three incomplete steles. × 11.

Fig. 5. Transverse section a little lower than Fig. 4 to show complete type of leaf-gap. The pith is in direct communication with the cortex through the gap, which is lined by the fused internal and external endodermis. × 18.

Fig. 6. A very little lower than Fig. 5. Shows union of leaf-trace with vascular ring and a root springing from the internal pericycle. × 18.

Fig. 7. Transverse section lower down to show internal thickened ring and incomplete leaf-gap. × 18.

Fig. 8. Part of stele to show transitional type of leaf-gap where the internal endodermis runs out towards the external. × 38.

Fig. 9. Transverse section of aquatic stem about 3 cm. below the base of the inflorescence to show contracted internal ring. × 52.

Fig. 10. About 2 cm. lower than Fig. 9. *C* = central core of thickened lignified cells. × 38.

Fig. 11. Considerably lower down. *C'* = the core of Fig. 10 reduced to a single element. × 52.

Fig. 12. Transverse section of subterranean stem, showing several layers of cortical cells grouped regularly round the endodermis. × 40.

Fig. 13. Transverse section across a node of the inflorescence axis to show branch gap and starch sheath (*st.sh.*). × 18.

Fig. 14. Part of a section similar to Fig. 13 on a larger scale to show the internal starch sheath (*st'.sh'.*) which fuses with the external at the gap. Note the contrast of the statoplast with the ordinary trophic starch scattered over the ground tissue. × 40.

Fig. 15. Transverse section of stele just below the transitional region from aerial to aquatic type to show the whole of the ground tissue, pith, and cortex as statocyte tissue. × 18.

PLATE XXI.

Fig. 16 *a–e*. Three seedlings. × *circa* 2½. *d* = young plant (natural size); *cot.* = cotyledon; *pr.rt.* = primary root; *adv.rt.* = adventitious root.

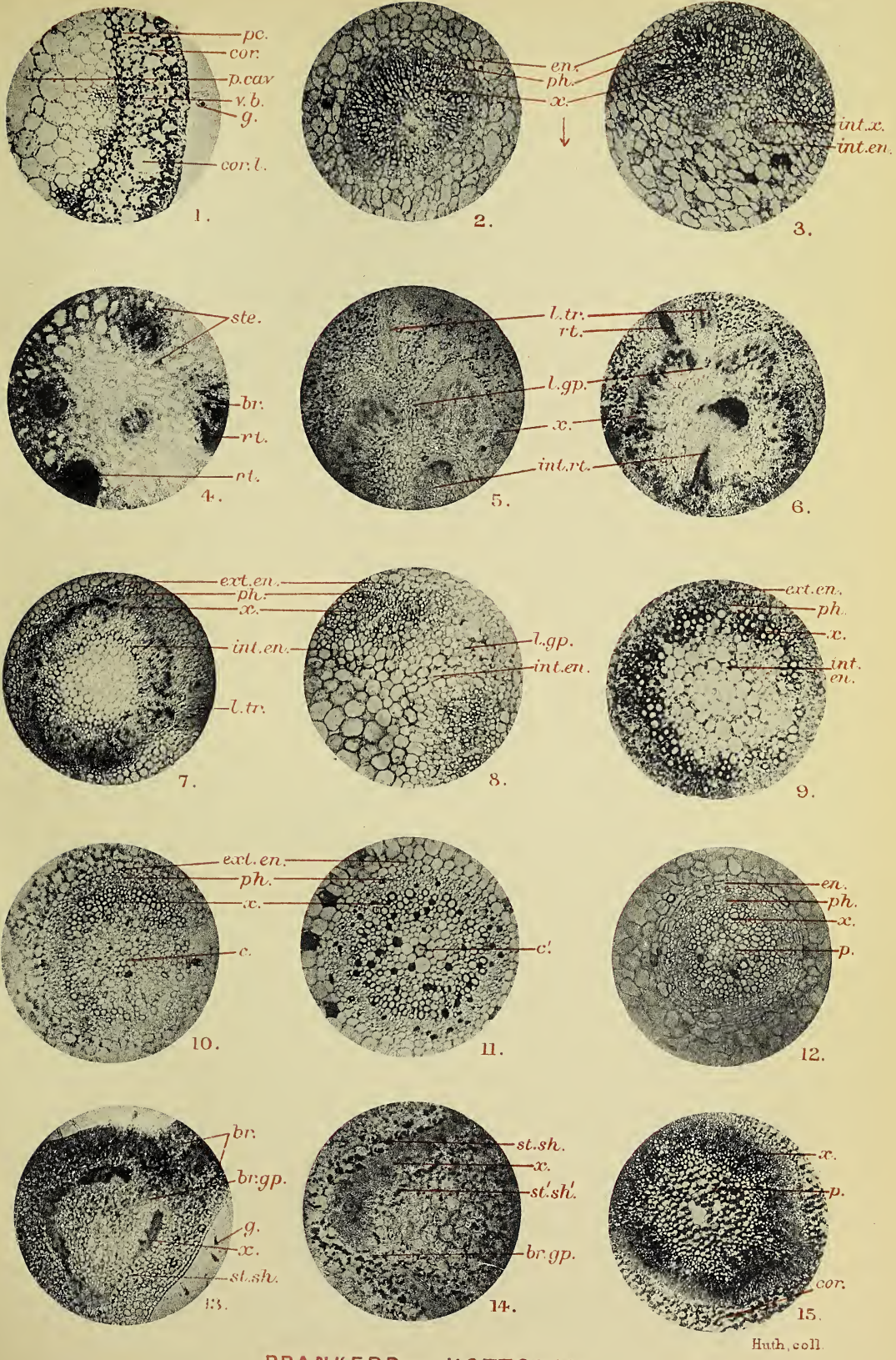
Fig. 17. Diagrammatic median longitudinal section through a seedling to show plane of section of Fig. 18. *Hyp.* = hypocotyl.

Fig. 18. Transverse section of tips of cotyledons (*C.*) enclosed in disorganized remains of seed coat (*S.*). *Ct.* = cutinized walls; *N.p.* = nucleated protoplasm from cells of inner layer of seed coat (*int.*). × 130.

Fig. 19 *a–e*. Serial transverse sections through the wood at the base of a cotyledon (*a*) passing to hypocotyl (*e*). The arrow indicates the direction of the centre of the stem. × 650.

Fig. 20. Transverse section of small part of inflorescence axis to show detailed structure of bundle. × 70.

Fig. 21. Transverse section of stem of young plant (*d*, Fig. 16). × 130.



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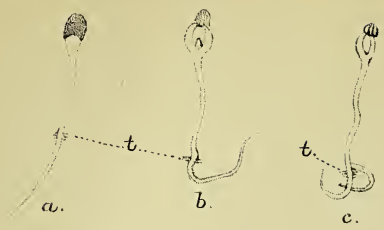
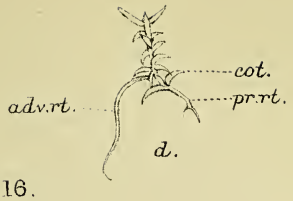
PRANKERD — HOTTONIA.





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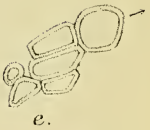

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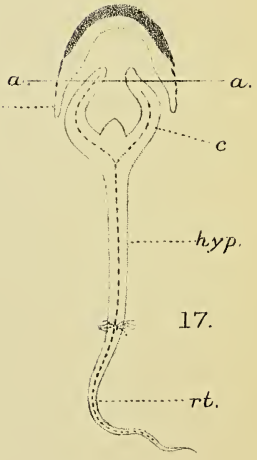

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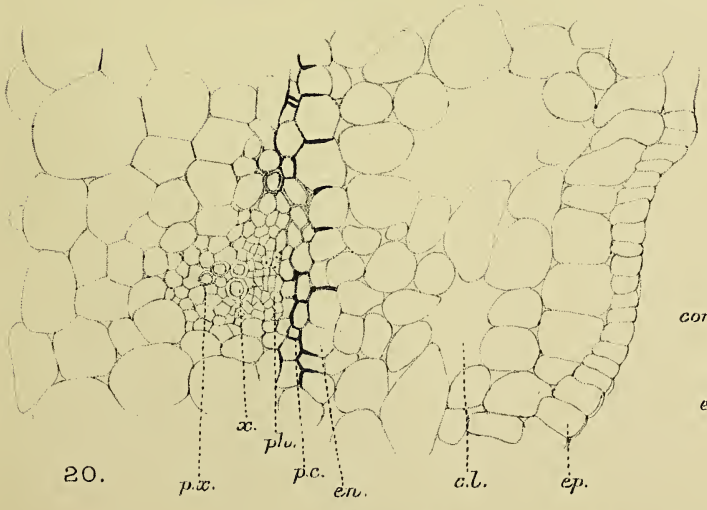

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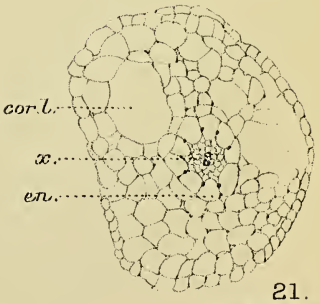

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PRANKERD — HOTTONIA.

NOTES.

A REPLY TO PROF. JEFFREY'S ARTICLE ON YEZONIA AND CRYPTOMERIOPSIS.—Prof. Jeffrey's article in the last number of the 'Annals of Botany', pp. 767–73, would have created a different impression in the minds of some readers if he had stated that 'the large memoir' by himself and Dr. Hollick 'so often quoted' in that article had not appeared till the paper by Prof. Fujii and me had been read at the Royal Society. We put a note regretting that fact, and that we had not had the advantage of comparison with his work, at the end of our bibliography.

At a future date I am intending to treat the subject at greater length, but the publication of Prof. Jeffrey's paper calls for some immediate recognition. I have only seen a few sections of *Brachyphyllum*, kindly lent me by Prof. Oliver of London and Prof. Seward of Cambridge, and they certainly show considerable similarity to the plant described by us as *Yezonia*. All the data necessary, and in particular the fructifications, are not available however.

The cone figured and described by Jeffrey and Hollick ('Studies of Cretaceous Coniferous Remains from Kreischerville, New York,' 1909, p. 37), so far as it goes, does not coincide at all with the petrified seeds and scales described by Stopes and Fujii (Phil. Trans., London, 1909, p. 33), our fructification showing many points of structural similarity to *Yezonia*, which make its connexion with that genus almost certain. Other cones more or less indefinitely associated with *Brachyphyllum* do not help to bring the plants together.

The further information as regards vegetative structure given by Prof. Jeffrey in his recent paper in the Annals certainly tends to support the view that *Yezonia* belongs to the genus *Brachyphyllum*. The fructifications, however, can alone determine this conclusively.

As regards *Cryptomeriopsis* Prof. Jeffrey's conclusions seem less sound. We have no evidence of fructifications. The points which he mentions as like *Geinitzia* in the vegetative parts of our plant are not sufficient to unite it to that genus. *Geinitzia* (*Sequoia*) *Reichenbachi* is put among the plants with Araucariaceous affinity by Jeffrey and Hollick, and when the Japanese fossil is swept into that all-embracing group, I must protest. Except for the lack of stone cells in the phloem, *Cryptomeriopsis* shows no feature that would justify more than specific distinction from the living monotypic genus *Cryptomeria* now endemic in Japan. Prof. Jeffrey's objection to *Geinitzia* being put with the genus *Sequoia* is justified, and I agree with him, but it is beside the point in the present argument. We have never suggested that *our* genus

has affinities with *Sequoia*, but I maintain, from detailed comparison with the living material of *Cryptomeria*, that our fossil is exceedingly like this living genus.

The general conclusion reached by Prof. Jeffrey is that 'it is clear that regions so widely separated geographically as Southern New England and Northern Japan were characterized during the Cretaceous period by a similar and characteristic Coniferous flora'. The pairs of genera above considered are from different horizons in different continents, and the fructifications are unknown. Palaeobotanists have been too much given in the past to generalizations from data of this sort. Prof. Jeffrey adds, 'The validity of this conclusion is much strengthened by the nature of the Abietineous remains recently described by Miss Stopes from the same deposits, since these correspond closely, so far as they go, with remains . . . from . . . Kreischerville.' Now, one of the two Abietineous leaves I then described from my Japanese material is notable in being exactly like the leaf of a modern *Pinus*, transfusion tissue, endodermis, infolded mesophyll and all (see Stopes and Kershaw, Ann. Bot., xxiv, p. 399 et seq.). The facts in this paper go directly against the previous conclusions of Prof. Jeffrey, who (Ann. Bot., 1908) had said for the study of the American leaves, 'One feature which, in general, serves to distinguish all of the Cretaceous Pines thus far examined . . . is the very wide zone of transfusion tissue surrounding the leaf-bundles', and other features of endodermis, mesophyll, &c., unlike modern pines.

It must be obvious that the time is not yet ripe for 'general conclusions' about the flora of the Cretaceous epoch.

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A NEW PARASITIC FUNGUS FOUND IN THE ROOTS OF GRASSES.—PRELIMINARY NOTICE.—I have found tubercles or swellings on the roots of *Poa annua* and other grasses. These tubercles, and to a greater extent the roots themselves, when microscopically examined, revealed the presence of a fungoid parasite allied to *Sorosphaera Junci*. This fungus, which is, I believe, new to botanical science, I propose to call *Sorosphaera Graminis*. There are several interesting points of difference between these two parasites, the amoebae of the latter of which, for instance, send out protoplasmic threads which penetrate through the cell-wall from one cell into its neighbour. One means by which infection is effected is by the entrance of an amoeba into a root-hair.

I hope shortly to publish a detailed account of the life-history of *S. Graminis*, including its cytology, which in most respects agrees with that of other members of the Plasmodiophoraceae.

E. J. SCHWARTZ.

A PRELIMINARY NOTE ON THE LIFE-HISTORY AND CYTOLOGY OF SPONGOSPORA SUBTERRANEA, WALLROTH.—Though the life-history of *Spongospora subterranea*, the organism producing the 'Powdery' or 'Corky Scab' of the potato, has to some extent been described already,[1] no account of its cytology has yet appeared.

The organism is first apparent in young potato cells just below the surface of the tuber, as a uninucleate amoeba. This increases in size, while its nucleus divides by a special method of amitosis, not unlike that described for *Sorosphaera*[2] and *Plasmodiophora*.[3] The amoeba itself divides by fission, so that a late stage shows many multinucleate amoebae in a single host-cell. Fresh cells are infected, not by a passage being bored through their cell-walls, but by the passing of amoebae into the daughter cells in the meristematic tissue. On the conclusion of the vegetative phase a plasmodium is formed, and the nuclei undergo a reconstruction. The chromatin passes out into the protoplasm as chromidia (akaryote stage), while later new nuclei are formed, apparently on different sites from the old ones. These nuclei fuse in pairs, the fusion being followed by a condition suggestive of synapsis. Following this state the nuclei divide twice by karyokinesis, the first of these divisions being marked by a longer spindle than the second, while in the latter eight chromosomes can be counted. These mitoses probably correspond to heterotype and homotype divisions. During the karyokinesis the protoplasm of the plasmodium has developed numerous cleavages; subsequent to the divisions it rounds itself off about the nuclei and forms spores. These are about 5μ in diameter, uninucleate, and are united in irregularly spherical masses, characterized by depressions and fissures giving the structure a sponge-like appearance.

The main conclusions reached as a result of these observations are that *Spongospora* should be united with *Plasmodiophora*, *Sorosphaera*, and *Tetramyxa* in the family Plasmodiophoraceae.

The account of the karyogamy[4] in the cysts of *Plasmodiophora* is not substantiated by more recent work,[5] while no nuclear fusion has as yet been observed in the life-history of *Sorosphaera*. In the latter organism synapsis and reduction divisions are recorded just prior to spore-formation, and it is not impossible that a nuclear fusion has been overlooked.

The karyogamy described for *Spongospora* and the two subsequent mitoses present a striking resemblance to the occurrences in the plasmodia of *Arcyria* and *Trichia*,[6] and serve to strengthen the relationship between Plasmodiophoraceae and the Mycetozoa.

T. G. B. OSBORN.

The Cryptogamic Research Laboratory,
Manchester University,
November 1910.

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PRELIMINARY NOTE ON SPONGOSPORA SOLANI, BRUNCH.—The organism named *Spongospora Solani* by Brunchorst (1), which has become generally distributed in Great Britain and Ireland, causes, as the direct result of its parasitic habit, the formation of scars and scabs of a more or less cankerous nature, in potato tubers, according to the conditions and virulence of the attack. These injuries have been called by various names, i. e. Corky Scab, Powdery Scab, Canker, &c. At the same time the potatoes may be stimulated to produce abnormal outgrowths.

In spite of the interest aroused by Nawaschin's (2) paper on *Plasmodiophora*, *Spongospora* has remained practically where Brunchorst left it nearly a quarter of a century ago.

Brunchorst saw in this parasite an endophytic Myxomycete, possessing a plasmodium capable of entering and living within the cells of its host. This plasmodium at some period of its life-history becomes changed into a spongy, coherent mass of walled spores—a single mass or spore-ball in each cell.

Spongospora, during what may be safely regarded as its earliest stage in the host tissue, appears in the form of uninucleate myxamoebae, so that the penetration of living host-cells, where this occurs, is accomplished, not by plasmodia, but by uninucleate bodies. The method of penetration, however, is still unknown. Massee (3), in 1904, announced the discovery of myxamoebae, but supposed them to belong to some other organism: in a subsequent paper (4), however, these bodies are described as the myxamoebae of *Spongospora*. The myxamoebae increase in number and their nuclei divide by the method observed by Nawaschin for the amoebae in *Plasmodiophora*. Their extension into newly forming tissue is brought about owing to the fact that some of the myxamoebae enter each of the two daughter cells arising—by normal mitosis—from the originally infected tissue cell.

Towards the close of the vegetative phase of the parasite the separate amoeboid bodies become approximated and grouped about the nucleus of the host-cell, so that a condition obtains analogous more to that occurring in the pseudo-plasmodia of the organisms enumerated by Olive (5) in the section Acrasieae of the group Sorophoreae, Zopf, than to that of the true (or fusion) plasmodia of the Myxogasteres.

Nawaschin states that plasmodia are formed just prior to the generative phase in *Plasmodiophora*. It is impossible at present to state definitely whether this is the case or not in *Spongospora*. At all events the pseudoplasmodium (colony)—or perhaps the plasmodium—becomes converted into a single spongy spore-ball. This structure is not hollow as stated by Massee (6), and on this account cannot be formed in the way described by him. During the generative phase the nuclei divide karyokinetically, there are many striking nuclear appearances, some of which resemble the figures published by Harper (7) for *Fuligo* and by Jahn (8) for *Ceratiomyxa*.

Encysted individuals or groups of individuals may be found which are similar to the microcysts of other Myxomycetes.

Structures comparable to the chromidia figured by Prowazek (9) for *Plasmodiophora* also occur during both phases of the life-history, but their character and mode of grouping vary during nuclear division.

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AN ARRANGEMENT FOR USING THE BLADES OF SAFETY RAZORS IN THE MICROTOME.—The safety razors now in general use have cheap blades of thin steel with a keen edge suitable for cutting sections. The price of a blade is so low that it can be discarded as soon as the edge becomes dull, and the labour of sharpening is thus dispensed with. The writer has used blades of this type for some years for cutting sections by hand, and has recently designed an arrangement for holding the blades in a rigid support, so that they can be used in the microtome.

A blade found very convenient for the purpose is that of the Gillette razor. It has two edges. The blade appears to be cut out of a sheet of steel with parallel surfaces, so that, apart from the actual cutting edge, the two surfaces of the blade are parallel; it is thus more easily held in a support than a blade with inclined surfaces. The arrangement for holding the blade consists of two blocks of steel (A) shown in surface view in Fig. 1 and in section in Fig. 2. Each block is wedge-shaped in section, and has two plane surfaces inclined to meet at an acute angle. The blocks are fastened together at each end by the screws (B) and thus form what is practically a razor blade made in two pieces, but without the actual cutting edge. The latter is supplied by inserting one of the above blades (C, Figs. 2 and 3) between the two blocks. The edge of the blade should project a little beyond the blunt edges of the two supporting blocks, and be parallel with them. The adjustment is made easier by the fact that the other side of the blade projects beyond the corresponding side of the supports. The actual amount of cutting edge that should project can easily be found by trial. The less the edge projects, the greater its rigidity; but it must project just far enough for the paraffin block to clear the edges of the steel supports. After the adjustment has been made the supports are screwed tightly together. To ensure the two supports having a uniform grip of the blade two steel washers (D) of the exact thickness of the blade are placed on the screws between the two blocks. Such washers are easily made from a discarded blade which has convenient holes in it.

The blade is cut in two, and each half has a central hole and can be trimmed to form a washer, and placed in the position indicated in Fig. 1.

The resulting composite microtome blade can be at once inserted in the microtome in the ordinary way. The arrangement as described is adapted to the Minot-

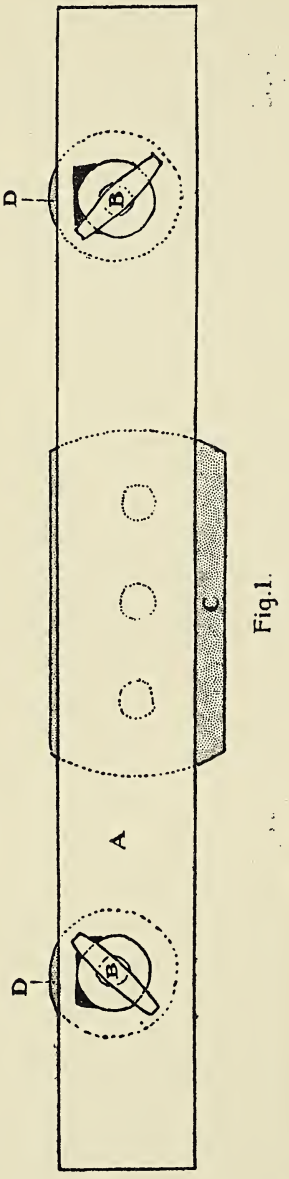

Fig. 1.

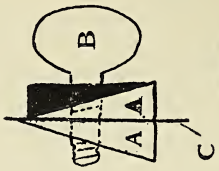

Fig. 2.

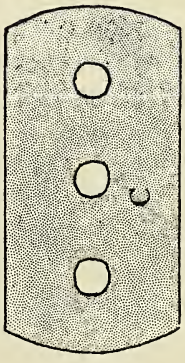

Fig. 3.

Zimmermann microtome, but could be adapted with slight variations to many others. It cuts sections as thin as 2 μ with success, and gives results equal to those obtained with any blade.

Several advantages may be claimed for this device. The blades may be bought anywhere, and the best two-edged blade costs only 4*d*. They can be stropped by one of the automatic stroppers already on the market, or else thrown away as soon as they become dull. For this reason the arrangement should be of great service where large classes of students have to be taught the use of the microtome and would have to provide their own blades. A further advantage is that hard or gritty objects can be cut at the sacrifice of only a cheap blade, without fear of doing damage to an expensive microtome blade of the usual pattern.

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SHEFFIELD.

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Notes on the Morphology of Ophioglossum (Cheiroglossa) palmatum, L.

BY

F. O. BOWER, F.R.S.

Regius Professor of Botany in the University of Glasgow.

With Plates XXII–XXIV.

OPHIOGLOSSUM PALMATUM is the most outstanding type among the remarkable family to which it belongs. At the same time it is one of the least fully investigated. Its habitat on rotting tree-trunks suggests a saprophytic tendency. Its swollen stock provides storage capacity beyond that of any other member of its genus. Its leafage, with its irregularly lobed sterile region and its varying number of fertile spikes, readily earns for it a position in a distinct section of the genus *Ophioglossum*, which has been styled § *Cheiroglossa*. Up to the present time little is known of its methods of propagation, while its gametophyte has never been observed. It was, then, with special interest that efforts were made to secure material of this peculiar plant on a recent visit to Jamaica. Certain decaying tree-trunks in the Blue Mountains on which it was known to have grown were visited. But the plant was only found in small quantity, and one plant alone was retained whole as a herbarium specimen. It is represented in Pl. XXII, Fig. 1, A, kindly drawn for me by Dr. A. A. Lawson, who took the specimen himself in the living state. It shows a medium, well-grown plant, with the tuberous stock characteristic of the species, supported by numerous fleshy roots. The upper part of the stock is covered by a dense mat of pale-coloured hairs, from which emerge the two leaves. One of these is simple in form, and barren. The other is larger, and is divided into two divergent lobes, one of which shows a rudimentary third lobe. Upon the adaxial face are three fertile spikes, of which the lower two are seated as a pair side by side; the third is inserted rather higher up, and in a lateral position from the median line. A second larger specimen had a very broad leaf divided distally into three large lobes, and imperfectly subdivided again into eight minor lobes, while six fertile spikes were borne on the adaxial face. From external observation the lowest of these is approximately median; those which follow upwards are disposed right and left,

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but as regards external contours all of them are distinctly intramarginal. Their succession is not alternate, as the specimen represented in 'Studies', ii, Fig. 122, might lead us to expect: the lower spike is slightly to the left, the second and third to the right, the fourth and fifth to the left, and sixth to the right. This is not according to the rule for pinnae, for they usually alternate. The stock of this plant, which was the largest found, was preserved for anatomical study.

It thus appears that the leaves of the two fertile specimens collected in Jamaica fall in generally as regards their external characters with those previously compared.[1] There is a reasonable, though not an exact numerical parallel between the lobes of the sterile blade and the fertile spikes. The insertion of the latter is distinctly within the margin of the leaf, as defined by the strongest curvature of the surface, but there is no regularity of alternation. In the example here figured there are two fertile spikes, right and left, seated as a pair on either side of the median line. This is like what is seen in the specimen shown as Fig. 118 in my 'Studies', ii, Pl. VIII. It is a much less common condition than that where the lowest spike is solitary and median in position.

Numerous other plants of smaller size were found, some of them very minute. By carefully rubbing down the decayed humus in which the plant was growing, Dr. Lawson was able to collect a considerable number of tubers too small to bear leaves. Some of them were attached to roots, and it is plain from a comparison of them that the plant is chiefly multiplied by buds formed on the roots of older plants, just as in other species of *Ophioglossum*. Fig. 1, B, shows such a young plant with tuberous stock, bearing its first leaf, and still attached to the parent root.

Anatomy of the Stock and the Leaf-trace.

The anatomy of the stock of *O. palmatum* has never yet been described, so far as I am aware. Nevertheless Dr. Chrysler [2] remarks of it that 'as in *Ophioderma*, the vascular supply of the leaf arises as several strands', and refers to my paper on *O. simplex* [3] as his authority for the statement. But in that paper [4] it is specifically laid down that 'the case is still open for § *Cheiroglossa*, in which I am not aware that the stock has been examined anatomically'. And again in the 'Origin of a Land Flora', which Dr. Chrysler also quotes in his list of literature cited, it is stated on p. 462, after a description of the way in which the strands of the leaf-trace of *Ophioderma* are separately inserted upon the vascular system of the stock, that 'it is still uncertain whether or not § *Cheiroglossa* shares this character'. I am now able to supply some data of the anatomy of the

[1] Studies, ii, pp. 27–32.
[2] Ann. of Bot., 1910, p. 10.
[3] Ann. of Bot., 1904, p. 205.
[4] p. 215.

stock of this species, and they are in the main such as I had anticipated: their demonstration is, however, given for the first time, so far as my information goes.

The large stem investigated was about $\frac{3}{4}$ in. in diameter, and a little over an inch in length, bearing one large matured leaf, while younger leaves constituted a terminal bud covered in by numerous hairs. Following the trace of the large leaf downwards, it was found to consist at the base of the petiole of about twenty-five strands arranged in a ring, with their xylems all directed inwards (Fig. 2, I). This is in accordance with what has already been described for this species.[1] Passing downwards, they show frequent and irregular fusions, by which their number is diminished (Fig. 2, II), but they still constitute an unbroken series across the adaxial face. In that region there is evidence of repeated fusions, which bear at first no relation to any definite break in the series. Lower down, however, the strands gather into groups right and left of the median plane of the leaf (Fig. 2, III), and these condense by further fusion into a few, and finally into two relatively large strands (Fig. 2, IV), which diverge widely apart. Following these deeper into the axis, they attach themselves to the reticulum of the axial system, their phloem naturally fusing first with it (Fig. 2, V). At this level a vascular commissure traverses the space between the points of attachment of the two leaf-trace bundles, while from the centre of this usually springs the vascular supply to a root (*rt*, Fig. 2, V–IX). In sections again lower down the fusion of the two leaf-trace strands with the axial system is seen to be complete (Fig. 2, VIII, IX), but the commissure has faded out, showing the broad leaf-gap upon the divergent margins of which the two leaf-trace strands are inserted (Fig. 2, IX). The root (*rt*) which has been seen to be attached to the commissure above the leaf-gap is now free to pursue its course, which is usually downwards and obliquely outwards.

It is thus seen that in *Cheiroglossa* also, as already shown for *Ophioderma*, the diagnosis will apply 'petioli fasciculi numerosi separatim in rhizomae fasciculos inserti'. The number in *Cheiroglossa* is, however, the lowest plurality, viz. two. In this character these two sections of the genus *Ophioglossum* differ from all other Ophioglossaceae, in which the leaf-trace is gathered at its base into a single strand. In this they illustrate an anatomical condition which, on the basis of general comparison among the Filicales, is held to be derivative, as compared with the section of the genus designated *Eu-Ophioglossum*.

The numerous successive leaves of a large stock are found to behave constantly like that above described. Upon this basis the vascular system of the stock is built up. The condition accordingly seen in the upper region of the stock is as shown in the photograph, Fig. 3. Numerous roots are seen on their oblique course outwards, and the section is strewn with

[1] Ann. of Bot., xviii, p. 210.

vascular strands of the leaf-traces, which at first sight appear indefinite in position. But comparison with the diagrammatized drawing in Fig. 4, in which the roots are omitted, shows that the scheme above described is adhered to. There is a central pith of moderate dimensions, round which are disposed three meristeles with their xylems directed inwards, and separated by wide foliar gaps. On either side of one of these gaps the two distinct strands of a leaf-trace (No. 1) are coming away, but not yet detached. In the case of No. 2 one of the strands is detached, but the other is still connected with the meristele of the axis. In leaf-trace No. 3 both strands are clear, while that on the anodic side is already branched. It may be noted that constantly the anodic branch takes precedence in time, and is also the larger of the two. Following on through the succession of leaves—which incidentally it may be remarked show no exact regularity of divergence—the strands become wider and wider apart, owing to parenchymatous swelling, and break up into a number of strands, which behave after the manner already shown in Fig. 2.

This being the condition in an old stock, it became a matter of interest to see what is the vascular arrangement in those young buds formed upon the roots, which in default of the seedling plants may be taken as giving evidence of the ontogenetic history. The roots correspond to those of other species of the genus, in growing with a single initial cell, and in showing frequent dichotomy. On these, close to the apex, the adventitious buds arise endogenously. The details, so far as they have been observed, correspond to what has already been demonstrated by Rostowzew[1] for *O. vulgatum*. The bud soon swells into an almost spherical tuber, which shows at first little differentiation, but as it grows a deep indentation becomes apparent, lined with closely grouped hairs. At the base of this is the *punctum vegetationis* which gives origin to the young leaves. The first of these is shown emerging from the terminal tuft of hairs in Fig. 1, B.

Sections through such tubers show at first little differentiation of the turgid parenchymatous mass, but as the leaf formation is advanced the vascular system develops, and in a case where several leaves were already initiated the condition was traced from below upwards, with the results shown in Fig. 5, I–VIII. The vascular system of the bud is inserted upon that of the root from which it sprang. From the first the stele of the bud was in this case distinctly medullated, though it does not appear to be always so. Fig. 5, I, shows it at the level of its attachment to the vascular tissue of the root, the tracheides projecting right and left forming the junction with it. Externally the stele is delimited by a definite endodermis; the xylem is, however, only poorly developed, and the phloem is hardly recognizable as such. This condition is continued upwards (II), but shortly the continuity of the xylem-ring is broken by a gap, which resembles the

[1] Recherches sur l'*Ophioglossum vulgatum*, L., Copenhagen, 1891, p. 12.

foliar gaps formed later (III), but in this case no strands of a leaf-trace were recognized as passing off. A little higher up a second leaf-gap appears on the opposite side of the stele, the xylem being thus separated into two equal bands, while from the middle of each of them a root-strand is given off (IV). It is at this level that the endodermis becomes indefinite, and it is not recognizable higher up. Nor is there at any point any indication of an internal endodermis. Shortly the first foliar strands are separated from the margins of the second gap (V) as two quite distinct strands, widely apart from one another in their origin, and showing further divergence as they pass outwards (VI, VII). Meanwhile the meristeles of the axis again extend, and close the leaf-gaps, so that the ring is reconstituted (VII). Other leaf-traces follow after the same plan, though not with any exact or constant angle of divergence. The two strands entering a leaf soon divide up according to the plan shown for the older leaves (Fig. 2), but naturally the branching is here carried out to a less degree.

A condition is found in the lower region of the old stock above described, which may be regarded as a natural consequence of the parenchymatous swelling of it for storage purposes. As shown in Fig. 6, the pith becomes greatly distended, as compared with Fig. 3; the meristeles pass further apart, and the stelar condition becomes accordingly disturbed, and less readily intelligible. The large pith is traversed by a number of roots. Four of these are shown in Fig. 6, but as many as six have been observed. The roots are usually triarch, but sometimes diarch. They appear to pass into the pith from outside, traversing the foliar gaps. Though this condition is unusual, if not actually unique among the Pteridophytes, it is similar in principle to what is seen in *Lycopodium Selago* and some other species, where the roots traverse the softer middle cortex. In the present case all the tissues except the vascular skeleton are soft, and entry into the pith is as easy as a course through the cortex, while access to the soil is gained through the equally soft basal region of the tuberous stock.

An examination of the roots which traverse the humus in which the plant grows shows the presence of endotrophic mycorrhiza, though not in such a profuse development as the habit of the plant might lead one to expect.

Comparing what is seen in the stock of *O. palmatum* with the type for *Eu-Ophioglossum*, the differences appear such as would be natural in a tuberous development of that type, having a saprophytic tendency. The general development of the vascular tissue is poor, both as regards quantity of xylem and of phloem, especially the latter. The stelar construction is, however, fundamentally on the same plan, with its basket-like disposition of meristeles round a bulky pith. If the normal system of *O. vulgatum*[1] be imagined as abbreviated and distended the condition would not be unlike

[1] Land Flora, Fig. 256 (5), after Rostowzew.

that of *O. palmatum*. The attachment of the roots is essentially the same. A difference of some importance is, however, seen in the leaf-trace. Whereas in *Helminthostachys*, *Botrychium*, and *Eu-Ophioglossum* the trace consists of a single strand springing from the base of the foliar gap, here there are two strands which originate widely apart, and laterally from the edges of the foliar gap. The leaf-trace thus divided is found also in *O. pendulum* and *simplex*.[1] It has already been pointed out that these species, all of which are of saprophytic tendency, may be regarded as specialized offshoots of the Ophioglossaceous stock. And just as in the Ferns the single strand is characteristic of those types which may be held to be more primitive, while the divided leaf-trace is probably a derivative condition, so also here the condition seen in *Eu-Ophioglossum* and others of the family is probably the primitive state. This conclusion should have its bearing on the comparative study of the fertile spikes in those representatives of the genus. It should dispose us to inquire whether the elaboration of the spikes which is seen among them is not also derivative as compared with the simpler condition of *Eu-Ophioglossum*.

Morphology of the Fertile Spikes in *O. palmatum*.

Hitherto the development of the spike of *Ophioglossum palmatum* has never been described. Fortunately the terminal bud of the large plant under investigation provided two young leaves which were developing as fertile leaves, and gave the opportunity for observing early stages of the spike as seen in transverse sections of the blade (Figs. 7–10). This has a semilunar outline, with relatively blunt margins which are unequal, one being habitually more massive than the other. The cell cleavages at the margin are of the T-type, and are repeated often with some degree of regularity at the point of greatest convexity. The result is a characteristic cell-net such as is shown in Figs. 11, 12. But this structure is most clearly seen in sections of the lamina above the insertion of the spikes. Lower down, where the margin is more rounded, the T-division still holds, but not being repeated in rapid succession the structure appears less characteristic.

It is naturally impossible to give any exact account of the segmentations involved in the origin of the spike from two leaves only, and both of them cut in transverse section. But as the details are fairly well known for *O. vulgatum*, this species will serve as a basis for comparison of the less perfect data from *O. palmatum*.[2] The spike arises as an outgrowth in which several cells appear to take part (Fig. 13). As the convexity increases, segmentation is seen which is compatible with the type described for *O. vulgatum*, having an initial cell with the shape of a four-sided pyramid. A comparison of the drawings, meagre though they are, indicates

[1] Ann. of Bot., 1904, p. 205.

[2] Compare Land Flora, pp. 447–8, Fig. 246.

that the early stages are substantially similar to those described for the Adder's Tongue (Figs. 13, 14).

As regards position upon the leaf, the lowest spike is as a rule median, though this is not without exception, as is seen in mature specimens (Fig. 7 also, 'Land Flora', Fig. 238, D). In the younger of the two fertile leaves observed the lowest spike was perfectly median (Fig. 14). Passing upwards, younger spikes in the first stage of initiation were seen right and left, but they were distinctly intramarginal as regards the external contours (Fig. 13). It thus appears that there is an acropetal succession in the origin of these intramarginal spikes, and it is possible that the succession here seen might have been continued further had the development of the leaf been more advanced.

In the older fertile leaf the number of spikes was greater. Figs. 7–10 represent sections successively at higher levels. The lowest (Fig. 7) shows a single median spike as before. Higher up indications of at least four other spikes are seen (Figs. 8, 9); they are disposed right and left of the median line, but all are distinctly intramarginal so far as the external form is concerned. One of these is represented on a larger scale in Fig. 15, and the spike appears seated at a considerable distance from the margin. It may be added that, following up the region of greatest curvature in this section into the upper region of the leaf, it is found to be continuous with that marginal segmentation already described (Figs. 11, 12). One further point is worthy of note: that in Fig. 8, on the left-hand side, the section, though it is in a transverse plane, traverses two spikes. They are both intramarginal, but one more deeply than the other. In Fig. 16 three such spikes are traversed in the single transverse section. Here the group in question lay near to the middle of the adaxial face. In point of origin it seems impossible to refer such developments, varying as they do in degree of intramarginal insertion, to the margin of the leaf. The plain fact is that, whatever the vascular connexions may be, the spikes of *O. palmatum* are in their prime origin intramarginal.

The later phases of the discussion on the morphological nature of the fertile spikes in *Ophioglossum palmatum* have turned so largely upon the anatomical facts that, while not neglecting other data, it is necessary at the start to be clear as to the vascular connexions. It is common knowledge that the leaf-trace in *Helminthostachys*, *Botrychium*, and *Eu-Ophioglossum* leaves the stele as a single strand. Already Holle[1] had shown that while in *Botrychium* that strand divides into two equal parts, in *Eu-Ophioglossum* there is a central strand which maintains its identity between the lateral strands which spring from it, even up to the apex of the blade.[2] The lateral strands vary in number in the different species.

[1] Bot. Zeit., 1875, p. 269.

[2] See also Prantl, System der Ophioglosseen, Pl. VII, VIII.

Observations have from time to time been made on the vascular supply running from the petiole to the fertile spike in *Ophioglossum*. Prantl showed in *O. lusitanicum* how two strands come off laterally from the marginal strands of the petiole, and unite to form the single strand of the spike.[1] A somewhat similar state, though simpler still, was traced in *O. Bergianum*,[2] where branches coming off laterally from the single petiolar strand fuse to form the supply of the spike. In such cases the vascular system of the spike is evidently a secondary derivative of that of the petiole. In larger species of *Ophioglossum* the plan is the same, only with more profuse branching. But no sufficient series of drawings has been published giving the whole story of origin of the supply to the spike, though it has been suggested for various species by several authors. Accordingly, the changes have been depicted as seen in *O. reticulatum* (Fig. 22, I–X), from which it is clear that the five strands of the spike seen in X arise by successive branchings and fusions from the marginal strands of the petiole, while these are themselves derived by lateral branching from the original leaf-trace strand. This is set down explicitly to meet the statement of Campbell, 'that the bundles which supply the spike are not secondarily given off from the main bundles of the petiole, but are themselves the adaxial bundles which can be traced from the base of the petiole into the spike.'[3] I can only read the origin of the vascular supply of the spike in the species quoted as secondary, indicating that the spike is a subsidiary part in its relation to the whole leaf.

Passing from the relatively stable type of *Eu-Ophioglossum* with its single spike to those of *Ophioderma* and *Cheiroglossa* with less stability of the fertile tract, it is to be remembered that it has been shown that the leaf-trace is in them a divided one, a condition which comparison with the Ferns indicates to be probably later and derivative. This suggests that the instability of the spike seen in them is also derivative, a view which had already been developed before the anatomical facts came to hand. It has led to the seriation of the various forms already known for *O. palmatum* as showing a progression of complexity from the type of the single spike. The occasional branchings of the spike seen in *O. pendulum* are held to be incipient developments in a similar direction.[4] It will now be seen how the facts of vascular supply will relate these aberrant forms with the type of *Eu-Ophioglossum*.

It has already been shown[5] how the strands of the fertile leaf of *O. pendulum* form at the base a semicircle open on the adaxial side (Fig. 3), which, however, closes higher up (Fig. 4), and then becomes flattened as the leaf expands (Fig. 5). This is in accord with Campbell's drawings.[6] But

[1] loc. cit., Pl. VII, Fig. 1. [2] Studies, ii, p. 68, and Land Flora, p. 463.
[3] American Naturalist, vol. xli, No. 483, p. 157.
[4] See Studies, ii, p. 28, &c., and Land Flora, p. 435, &c.
[5] Ann. of Bot., 1904, Pl. XII, Figs. 3–5. [6] loc. cit., p. 154, Fig. 13.

it is necessary to be clear how complete the fusion of the marginal series is on the adaxial side. This is shown in Fig. 21, *a*, *b*, from which it appears that all possibility of discriminating between the confluent marginal strands is lost. The supply to the spike is afforded by the median adaxial region of the flattened circle. The same is the case with *O. intermedium*,[1] but here the actual coalescence of the marginal strands appears to be delayed till the base of the spike is reached, while the proportion of the strands of the fertile to those of the sterile region matches the relative size of those parts. It may be concluded from my own drawings of *O. simplex* that the condition there is in principle the same.[2] Hence it is seen that the identity of the margins as marked by the vascular strands is entirely obliterated, while the vascular supply to the spike in *Ophioderma* comes off from the indeterminate vascular supply on the adaxial side of the petiole.

Turning now to *Cheiroglossa*, in the leaf-stalks of the weaker and first-formed sterile leaves the margins as defined by the vascular strands are widely apart, which is naturally in accord with their flattened form. But as the plant strengthens and works up to the production of propagative organs the leaf-stalks become more nearly cylindrical, while the marginal strands come nearer together, till in the fertile leaf they constitute a complete circle (Fig. 2, I–III). Frequent fusion of strands is seen on the adaxial side, so that the identification of the margins by means of the strands is quite impossible. This obliteration of the margins takes place close to the leaf-base, and it is far above the point where it occurs that the fertile spike or spikes arise. The source of their vascular supply has been indicated for a case where there were three spikes, the lowest of them median.[3] But fresh drawings have been made from the old sections, giving the details more satisfactorily (Fig. 17, I–IX). From these it is seen that the supply to the lowest spike, which was median, comes off from the adaxial region of the circle of strands, where the marginal characters had been completely obliterated by the repeated fusions during their course through the elongated cylindrical petiole (I, II). By the passage of the median strands outwards the remainder of the strands appear ranged in a semicircle (III). Fig. 17, III–VII, shows successive steps in the separation of the vascular supply for the two higher spikes, which are borne right and left. The origin of the strands is by segregation from the margins of the semicircle ; it may, however, be noted that their derivation is not by any uniform scheme, though the number ultimately arrived at is in each case three. The sections show further that at the level of separation of the spikes their position on the leaf was intramarginal (VII–IX). It is worthy of note as having some bearing on their morphological character that the number of

[1] Campbell, loc. cit., p. 153, Fig. 12.

[2] loc. cit., Figs. 24–9.

[3] loc. cit., Pl. XII, Figs. 14–23.

strands traversing the stalk in all the three spikes is equivalent, the number being three, of which the median strand is the largest.

As the knowledge of the vascular supply to the spikes in *O. palmatum* is still very limited, being hitherto restricted to the above observations together with a very imperfect representation of similar facts by Bertrand,[1] the portion of the large dried specimen described above, bearing the two highest spikes, was cut out (Fig. 18). After swelling with caustic potash it was halved, embedded, and cut into sections. These provided the essential points in the origin of their vascular supply. Fig. 19 shows successive sections from below the spike to the left in Fig. 18. It will be seen that the marginal strand from the leaf passes directly into the stalk of the spike without any complications. The next intramarginal strand does the same, but just before the separation of the stalk from the leaf a vascular commissure runs across, almost transversely connecting it with the third strand from the margin of the leaf (IV). On complete separation of the stalk, a third strand, but weak and imperfectly developed, is seen in the section of the stalk (V), occupying the place of the lateral strand as seen in Fig. 17 (VIII–IX). Evidently the vascular system of this spike, which is small, is incompletely developed as compared with the larger examples. The still smaller spike on the right-hand side, which was the highest borne on this leaf, showed the vascular connexions seen in Fig. 20 (I–VII). Here the small marginal strand of the leaf first fuses with the rather stronger strand next adjoining it (I–III). This then passes out into the base of the spike (IV), and subsequently separates again into two (V); then a vascular commissure, as in the previous example, passes from the next inner strand almost horizontally outwards to join it (VI). The stalk then separates from the leaf (VII), its vascular supply being represented in its further course up the spike by a single band-shaped strand, which shows signs of being composed of three strands as in other cases. It may be held to be a reduced example of the same type of structure.

A careful comparison of the five examples thus described shows that in no two of them are the vascular connexions at the base of the stalk exactly the same, though the structure of the stalk is essentially alike in them all, allowance being made for their varying size. These facts indicate two things. First, that the spikes are probably all morphologically equivalent parts. Secondly, that their attachment is not according to strict rule, a condition that readily accords with the interpretation put upon them in my 'Studies'.[2] They were there held to be referable in origin to interpolation or chorisis of a single spike, in which case their position would not necessarily be regular. It would then be natural to expect less regular vascular connexions than in the case of parts produced in the normal sequence. A comparison of the specimens here described with those figured in

[1] Travaux et Mémoires de l'Université de Lille, t. x, Mém. 29, Fig. 97. [2] ii, pp. 43, 44.

'Studies', ii, Figs. 120–129, indicates that greater variations still may occur in the vascular connexions of the spikes than those above noted.

In order to check my former statements and verify the drawings, a re-examination has been made of the specimens at Kew. This confirms the accuracy of the description and drawing already given as Fig. 120, in point of the insertion of the median branched spike which it shows; its vascular connexion also is clearly intramarginal relatively to that of the lower-lying, but more marginal spikes. The specimen of Miers from S. Brazil, represented in my Fig. 121, was also re-examined. I state specifically that my former representation of it is substantially correct. The spike fourth from above is inserted in an almost median position, and its vascular connexion, which can be quite easily followed since the specimen is young and transparent, runs internally (i. e. more near to the median line), as compared with those of the next two spikes on the right, which are more marginal in insertion. To show that these are not isolated cases, it may be stated that there is a second specimen at Kew, also collected by Miers from S. Brazil, very like that of Fig. 121, bearing two spikes in approximately a median position: these are the fifth and sixth counting from below, and they are inserted between those which are lateral, and they have their vascular connexions visibly joining a strong strand which runs up the median region of the leaf. This strand lies clearly in a more median position than the vascular connexion of the next lower lateral pinna.

Another specimen collected by L'Herminier, but without locality, shows on a leaf bearing ten spikes a median insertion of the spike sixth from the base; those directly below it are inserted right and left of it, one of them being branched. At almost the same level as this median spike are other lateral spikes, while the vascular connexions, which are readily recognized in the dried condition of this specimen, pass internally to those of the next lower marginal spike. This is again a clear case of intramarginal insertion, with the vascular connexion also intramarginal relatively to that of a lower spike. It is to be noted that these examples of pronounced intramarginal insertion are all seen in cases where the spikes are numerous. It is precisely in such cases that the clearest departure from the marginal position would be probable if the origin of the plurality of spikes were by some process of interpolation or chorisis.

Dr. Chrysler, in his paper,[1] makes certain deprecatory remarks on 'inspection of the external surface'. This is at least better than no examination at all. It is to be borne in mind by those who criticize, that specimens in the Herbaria of Kew and the British Museum cannot be cut up at will. Nevertheless, evidence from examination of them without cutting them into sections, though neither final nor so convincing as this would be, has its value. It cannot be summarily dismissed, however great may be the

[1] loc. cit., p. 11.

preference for evidence by sections. But Dr. Chrysler has not only ignored the evidence afforded by my drawings,[1] he has also neglected the theory which was based upon them. This theory is not even mentioned by him. But on the basis of observations on other genera, combined with the citation of my own observations on a certain specimen of *O. palmatum* above quoted,[2] he concludes that 'according to the reasoning here employed it may be inferred that the lowest spike in this specimen represents two fused lobes of the leaf, while the next two spikes represent single lobes'. He continues thus: 'Bower lays much stress on the observation that the spikes do not generally arise from the margin of the leaf, yet the only transverse sections which he figures (6, p. 463) clearly show that in the case of the three spikes so represented, the origin of the vascular supply at any rate is truly marginal, i. e. derived from the free edges of a curved leaf-trace made up of a number of separate strands. Probably most morphologists would place more reliance on the disposition of the vascular skeleton than on the superficial "flesh" which clothes the skeleton.'

This rough and ready way of deciding a rather intricate morphological problem will hardly commend itself to morphologists who take other facts besides those of vascular anatomy into their view, least of all to those who have personally examined a large series of specimens of *O. palmatum*. Most morphologists have little or no personal knowledge of the plant in question, and Dr. Chrysler does not bring any evidence that he has studied specimens of it himself. Under these circumstances it would only be reasonable to expect some degree of reticence in amending conclusions which are explicitly based upon examination of a large series of specimens. In explanation of the facts derived from such study in 1896, a theory of chorisis or duplication was advanced.[3] But as this theory is not criticized, but passed over in silence by Dr. Chrysler, it seems necessary to state it afresh, and to recapitulate the reasoning upon which it is still held to be the correct interpretation of the facts for *O. palmatum*.

It is found in such normal types of *Eu-Ophioglossum* as *O. vulgatum* that the spike is susceptible of occasional branching, especially when the conditions are hypertrophic.[4] In *O. pendulum* the branching is more frequent, and sometimes involves the lower sterile stalk of the spike (Figs. 130–2). It was suggested that the condition which appears as an occasional hypertrophic abnormality in *O. vulgatum* became frequent in *O. pendulum*, and has become fixed as an almost constant condition in well-nourished plants of *O. palmatum*. The comparison was drawn with those Angiospermic flowers in which an increase in number of the parts from the probable primitive type is referable to interpolation or chorisis. Comparison may be made with the androecium of Hypericaceae, Malvaceae, or Rosaceae.

[1] loc. cit., Figs. 120–9.
[2] Ann. of Bot., 1904, Pl. XV.
[3] loc. cit., p. 43; see also Land Flora, p. 439.
[4] Studies, ii, Figs. 133, 134.

Some indication of a like process is seen in the Sphenophyllales, while it is not going too far to trace a distant parallel in the spread of the sori over the enlarged leaf surface in many phyla of Ferns. The following are the facts and considerations which make me think that we may find in duplication in its widest sense, or *pleiogeny*,[1] as it might better be termed, the correct interpretation of the peculiarities of *O. palmatum*.

I. Frequent branched conditions of the fertile spike occur in *O. palmatum*. This is especially common in specimens where the number of spikes is large ('Studies,' ii, Figs. 120–9). The details in some of the spikes near the margin in *O. palmatum* are closely similar to the branchings observed in *O. pendulum*, in cases where the single though branched spike is inserted in a median position ('Studies,' ii, Figs. 131, 132). Thus the branching is not a feature of the median spike only, nor is the branching always a simple bifurcation, as Dr. Chrysler's interpretation would demand.

II. A parallelism has been traced between the number of the lobes of the sterile leaf and the number of the spikes in *O. palmatum*, showing that the number of the fertile spikes bears a general relation to the nutritive leaf area. As the plant grows stronger the higher complexity in both regions is attained. There are signs of a like though less exact parallelism in *O. pendulum*. These facts readily accord with a theory of pleiogeny.

III. Certain specimens have been described, such as those shown in 'Studies', ii, Figs. 120, 121, which are difficult to harmonize with any pinna-theory, though they fall in readily enough with a theory of pleiogeny. They show certain of the higher-seated spikes in an approximately median position, nearer, in fact, to the centre than others which are almost at the same level but more nearly marginal in their insertion. The vascular connexions of these have not been examined by sections, but the specimens are often sufficiently transparent to give opportunity for tracing them, and from such evidence it seems highly improbable that they would turn out to be constantly marginal like the rest.

IV. The superficial origin of the spikes upon the adaxial face of the leaf is now demonstrated developmentally, and even the later spikes of the leaf have, so far as observed, an intramarginal insertion (Figs. 8, 9, 13, 15), while numerous cases can be quoted where the spikes show no regular alternate arrangement as pinnae usually do.

V. The identity of the margins of the leaf, so far as these are defined by the vascular strands, is entirely merged by the repeated fusions of the strands on the adaxial face of the elongated petiole. It is, therefore, a question how far the marginal strands above the first spike really represent the original (phyletic) margins to which normal pinnae might be referred.

VI. Dr. Chrysler, following and extending the observations of Bertrand and Cornaille, has shown that marginal vascular origin is not a necessary

[1] See note explaining this term, p. 296.

criterion of pinna-nature within this family, for the supply both to spikes and to pinnae may be intramarginal. It may then be doubted how far a mere marginal origin of the vascular supply can be used as a criterion of pinna-nature, as Dr. Chrysler does in the present case. It cannot be accepted as outweighing other considerations, such as arise from comparison based on a large series of specimens.

VII. It has been shown that the vascular supply to the median spike in the leaf examined in 1904 consists, like that of the succeeding two spikes, of three strands, of which the median one is the largest. All the three spikes are in fact equivalent in this respect (Fig. 17, I–IX). Dr. Chrysler's suggestion on the basis of the vascular connexions alone is 'that the lowest spike in this specimen represents two fused lobes of the leaf, while the next two spikes represent single lobes'. The fact that the vascular supply to all of these is equivalent in all except the source, being in the one case median, in the others marginal, should carry some weight with vascular anatomists, raising a doubt of the validity of the conclusion. The vascular supply to two fused pinnae might be expected to be more complex than that to a single lobe. On the other hand, the structure actually observed is such as might be expected on a theory of pleiogeny.

VIII. The species which show the peculiarities under discussion belong to the two sections of the genus generally admitted to be specialized. It is in such forms that one may most readily expect developments which will diverge from the usual type and follow a line of their own. Both species are partially saprophytic, a condition often associated with unusual developments, reducible with difficulty to the ordinary schemes of construction. In plants biologically so restricted as these half-saprophytic dwellers on decaying trunks and humus a large spore-output is almost a necessary condition of survival, or at least of spread. This utilitarian reason for amplification is easily intelligible, and the ready means appears to have been amplification and irregular lobing of the sterile lamina, and repetition of the fertile spike. Both of these were probably special and phyletically late occurrences. There seems to be no reason to hold that they should necessarily be retrospective developments. Dr. Chrysler appears to assume that they were. I have suggested, on the other hand, that both the sterile and the fertile regions of the leaf have progressed along new lines of development, and that their parts are not necessarily reducible to terms of pinnae such as are seen in the pinnate members of the family.

The conclusion arrived at may be summed up as follows. The spike of *Eu-Ophioglossum* is regarded as the unit upon which further development has played. The facts, both developmental and anatomical, fall in readily with a theory of amplification of that unit, or pleiogeny, as explaining the complex forms observed in *O. pendulum*, and more pronouncedly in *O. palmatum*. The sterile lamina enlarged and formed irregular lobes,

which are not held as pinnae in the usual sense of the word. The spikes underwent a parallel pleiogenetic amplification. Sometimes this amplification took the form of repetition of spikes like the original one, but seated at points apart: this is duplication or interpolation. Sometimes two spikes are seated near together, or upon a common stalk; in the latter case they may merely show distal branching. These are conditions indicative of chorisis. Other variants may also be found. But in all these amplifications the spike is the unit throughout, and the branchings are not to be interpreted in terms of pinnae as normally understood.

Morphology of the Ophioglossaceous Spike.

The above discussion has been concerned with the more elaborate types of the spike-development in the genus *Ophioglossum*. It has not touched the question of the morphology of the spike itself, which has been held as the unit in the amplifications seen in *Ophioderma* and *Cheiroglossa*. In my 'Studies. II. Ophioglossaceae (1896)', the evidence up to that date was summarized and weighed with a view to tracing the probable affinity of the family, and incidentally the morphological character of the fertile spike which is its most notable feature. This required a careful balancing of evidence for and against alternative views. Of these the one indicated affinity with the Sphenophyllales and Lycopodiales, the spike being regarded as a result of amplification from the sporangiophore, or ultimately from the sporangium. The alternative indicated an affinity with the Filicales, in which case the morphology of the simple spike would be referable in some form or another to a pinna or a coalescence of pinnae. The evidence up to 1896 appeared to me to favour the former alternative, and that conclusion was stated with ample illustration and discussion of the details on which it was based.

Since then considerable advance has been made in various directions which bear upon the question. The researches of Lang [1] and of Bruchmann [2] have greatly increased the knowledge of the gametophyte and of the embryology. The discovery by Lyon [3] of the suspensor in *Botrychium obliquum* (followed recently by the description of a like body in *Helminthostachys* by Lang [4]) at first sight appeared to strengthen the Lycopod affinity, but the demonstration of a like organ in *Danaea* by Campbell [5] put matters back *in statu quo*.

A second line along which recent advances affect the question is the anatomy of some of the earlier types of the Filicales. Kidston and Gwynne-Vaughan, by comparison of fossils which are plainly of Osmundaceous alliance, whether or not they are Osmundaceae in the modern sense, have shown it to be highly probable that the structure seen in the modern Osmundaceous stem is in the main the result of an up-grade development with medullation from a protostele. This has paved the way for similar

[1] Ann. of Bot., xvi, 1902. [2] Bot. Zeit., 1904, and Flora, 1906. [3] Bot. Gaz., Dec., 1905.
[4] Ann. of Bot., 1910, p. 611. [5] Ann. of Bot., 1909, p. 691.

comparisons in the anatomy of the Ophioglossaceae, and the analogy between the vascular condition of the Ophioglossaceous stock and that of *Thamnopteris*, for instance,[1] is, to say the least, very suggestive.

In the next place, the work of Bertrand and Cornaille[2] has shown that the origin of the vascular supply to the pinnae in certain relatively primitive Ferns was intramarginal on the abaxial side. This line of inquiry has been followed up by Chrysler,[3] and applying it to the Ophioglossaceae, he has demonstrated that in *Botrychium virginianum* the intramarginal origin holds not only for the sterile pinnae, but also for the fertile spike. He concludes from this and other facts that the normal spike is the result of fusion of two basal pinnae. In fact, he upholds the old theory of Roeper, and maintains that the Ophioglossaceae are related to the Ferns, and especially to the Osmundaceae and Marattiaceae, springing from a primitive stock at a remote period. But in arriving at this conclusion, Dr. Chrysler does not, in his own observations, travel over ground outside that of the anatomy of the leaf, combined with a comparison of external form of certain abnormalities in *B. obliquum*. This is a rather hazardous course in such questions. While due weight must be accorded to his anatomical results, his conclusions must stand the test of reference to other sources of information before they can be considered as amounting to more than interesting suggestions.[4]

It must be admitted that since 1896 the trend of the evidence has been decidedly such as to strengthen the Filical alliance of the family. This follows in the first place from the comparison of the gametophyte, when due allowance is made for the similarity of underground habit which they share with the Lycopods having led to a probable parallel development. It comes out also in some degree (which recent work on the Osmundaceae and Botryopterideae has distinctly strengthened) in the anatomy of the stock. Lastly, the observations of Dr. Chrysler have contributed in marked degree to strengthen the comparison with Ferns on the basis of the anatomy of the leaf. All this indicates that a reconsideration is necessary of the Lycopod-Sphenophyll-alliance previously recognized for the Ophioglossaceae. But before coming to a definite conclusion there are three other points to be taken into our view, two of them favourable, the other adverse to the Fern alliance. In the first place the comparison has been made in previous discussions with the Sphenophyllales (including Psilotaceae) in respect of the behaviour of the sporogenous cells. It was pointed out that in the Psilotaceae a proportion of the spore-mother-cells became abortive before the tetrad

[1] Fossil Osmundaceae, Part iii, Pl. I.

[2] Travaux et Mémoires de l'Université de Lille, x, Mém. 29. [3] Ann. of Bot., 1910.

[4] The recognition of affinity of the Ophioglossales with certain early Filicales is no new conception. It was indicated in unmistakable terms by Renault in 1875 (Ann. Sci. Nat., Sér. 6, tom. i, pp. 232–4), while Prantl, in 1884, pointed specifically to the Botryopterideae and the Osmundaceae as possible lines of relationship with the living Ophioglossaceae (Beitr. z. Syst. d. Ophioglosseen, p. 345). See also Scott, Studies in Fossil Botany, ii, p. 640.

division, acting as a diffused tapetum. In the Memoir of 1896 [1] the results of Rostowzew were accepted, without sufficiently critical examination of my own preparations, as showing that in *Ophioglossum* also cells scattered through the sporogenous group became disorganized without undergoing tetrad division. It has since been found that this is not so,[2] and accordingly the comparison with the Psilotaceae on this feature falls away.

Secondly, the comparison with the Psilotaceae-Sphenophyllaceae alliance was also based on the method of insertion of the sporangiophore on the adaxial face of the leaf. But it now appears that the similarity does not extend to the mode of origin of the vascular supply to the fertile regions in the plants compared. Dr. Chrysler has laid weight upon the marginal attachment of the vascular supply to the spike. The fact is now quite plain that, with certain possible exceptions in the specialized *O. palmatum*, the supply comes off in the Ophioglossaceae either from the margin of the petiolar system, or on the abaxial side from an intramarginal gap. It may be seen, on the other hand, in any series of sections of the sporophyll of *Tmesipteris* that the marginal portions of the strand of the leaf-stalk pass off right and left into the leaf-lobes, while the central portion enters the synangium. The origin of the supply to the sporangiophores in *Cheirostrobus* is described as coming off from the middle strand that supplies the sterile region of the leaf, by a branching, so that one of its branches lies above and inside the other.[3] That is, the origin is median and adaxial. A similar branching appears to be the rule also for *Sphenophyllum*. These facts indicate a real difference between the marginal or slightly abaxial origin of the vascular supply to the spike in the Ophioglossales, and the median adaxial supply to the sporangiophores in the Sphenophyllales, which if it prove to be constant will strengthen the alliance of the former with the Filicales. It is of course a possible view, which might be based on the mere anatomical facts, that in *Tmesipteris* the synangium represents the whole terminal region of the leaf, which has remained fertile, as in *Osmunda regalis*. But against this are the facts of development ;[4] moreover, that explanation would not fit for *Sphenophyllum* or *Cheirostrobus*. For these reasons I prefer to accept the anatomical distinction as a real and a valid one.

On the other hand, one of the most impressive features in the Filicales is the extraordinary constancy of the progression by which the sporangium suffers reduction in size and complexity of structure, in the thickness of its gradually elongating stalk, and in the numerical output of its spores as we pass from the more primitive to the more specialized forms, while the precision of the mechanism for scattering them increases. This matter has been treated at length in the 'Land Flora' (pp. 637–46, &c.). But if the

[1] p. 20. [2] Compare Land Flora, p. 451, Fig. 251.
[3] Scott, Phil. Trans., Series B, vol. clxxxix, p. 12.
[4] Land Flora, p. 414.

Ophioglossales were derived from a primitive Fern stock, and the sequence be, as the anatomical comparison indicates, from a type like *Botrychium virginianum* with relatively numerous smaller sporangia through types such as *B. ternatum* and *Lunaria* with fewer and larger sporangia, to *Ophioglossum* itself, it is plain that we are contemplating a progression which is the direct converse of that illustrated by the Ferns at large. Such a progression is of course possible. The question is whether it is probable. The apparent improbability of it weighed with me strongly in previous discussions on the phyletic position of the Ophioglossales.

There is, however, a further circumstance which makes this difficulty less serious than it at first appears. It lies in the fact that in *Ophioglossum* a single initial cell is found at the apex of its stem and root. The leaf-apex and the structure of its margin, on the other hand, approaches that of the Marattiaceae, and is in accord with the bulky sporangial structure. A comparison may be drawn of the meristematic conditions seen in the various groups of Ferns.[1] In the Marattiaceae all the segmenting parts are bulky and complex in their cleavages; in fact the centre of construction lies deep. In the Osmundaceae a middle position is seen between the state of the Marattiaceae and that of the Leptosporangiate Ferns, and this applies here also to all the segmenting parts. In the Leptosporangiate Ferns themselves a more definite scheme of cleavage is found in all the meristems, which is in accord with the definite segmentations of the attenuated sporangium; in fact in them the centre of construction is more superficial. In all of these the various parts share the character consistently of a definite or a less definite reference to single initials. But the Ophioglossaceae show a discrepancy which does not find its match in any of the true Filicales. For the leaf and sporangium resemble the Marattiales in their more complex cleavages, while the apices of stem and root have each a single initial as in the Leptosporangiate Ferns. This discrepancy might be referred either to a progressively more bulky modification of the leaf and sporangium in a plant derived from a type resembling the Osmundaceae, or to a conservatism by which the plant has retained the characteristics of some remote ancestry in its leaf and sporangium, while the axis and root have taken on the characters of later forms. In view of the peculiar biological conditions of the family, and especially of the tendency towards saprophytism in the genus *Ophioglossum*, I am inclined to the former alternative, and to look in the direction of the Osmundaceae and Botryopterideae for the nearest relatives of the Ophioglossaceae.

There are various other comparative points which indicate collectively a relation, however distant, with early types of the Filicales rather than with the Sphenophyllales, such as the hairiness in the apical region, especially as seen in *O. palmatum*, the conformation of the leaf-base and the vascular

[1] See Land Flora, p. 650.

structure in the family at large. But these must be left over at the moment. It is obvious that there are difficulties in assigning any definite place to the family at present. But sufficient has been said to show how clearly the balance of evidence is setting in the direction of an alliance of the Ophioglossales with the early Filicales.

A natural consequence of adopting a Filical alliance for the Ophioglossaceae would be a recognition of the pinna-nature of the spike, probably in most cases with a coalescence of two pinnae as in the old theory of Roeper. I should, however, attach less weight in arriving at this conclusion to such abnormalities as those quoted by Dr. Chrysler than he appears to do.[1] For branchings of very various character may be found, which all deserve equal consideration with those which support a hypothesis of fusion. The analogy with the Marsiliaceae also becomes obvious. But probably the condition there seen is an instance of parallel development, and arises along a different phyletic line. For the two families belong to distinct types of Fern-development. The Ophioglossaceae appear to be naturally referable to the Coenopterid[2] type, characterized by less definite specialization of the sporangium. But the Marsiliaceae are pronouncedly Leptosporangiate, and, as Campbell has so convincingly shown, their relations appear to be in the direction of the Schizaeaceae.[3]

Summary.

1. The tuberous stock of *Ophioglossum palmatum* is traversed by a stele showing a bulky central pith and transversely widened leaf-gaps. It is of the same type as *Eu-Ophioglossum*.

2. The leaf-trace originates as two distinct strands inserted widely apart, right and left of the leaf-gap.

3. The spikes originate like that of *Eu-Ophioglossum*. The lowest is usually median, but not always. The insertion of the rest is usually intramarginal, and shows no regular alternation. The spikes branch frequently and irregularly.

4. The facts indicate that the spike of *Eu-Ophioglossum* is the morphological unit, and that the conditions seen in less degree in *O. pendulum*, and more clearly in *O. palmatum*, are due to 'pleiogeny', that is an increase from that unit in various ways, such as by partial or completed branching, or by interpolation of accessory spikes. The numerous spikes of *O. palmatum* are not then directly referable to normal pinnae, any more than are the irregular lobes of its sterile lamina.

5. Phyletically *O. pendulum*, *intermedium*, and *simplex* form a derivative series from *Eu-Ophioglossum*.[4] *O. palmatum* represents a parallel, but probably a distinct line, which has carried amplification of the leaf further.

[1] loc. cit., pp. 7–9 and Pl. II.
[2] Seward, Fossil Plants, ii, p. 433.
[3] Campbell, American Naturalist, xxxviii, 1904, p. 761.
[4] See Land Flora, p. 441.

The divided leaf-trace which they all show confirms the derivative character of both lines.

6. The balance of evidence acquired since 1896 has distinctly favoured an alliance of the Ophioglossaceae with the Filicales. It is in the direction of the Coenopterideae, and of living Ferns the Osmundaceae, that we may look for their true place.

7. The normal spike of the Ophioglossaceae will, in relation to this comparison, and especially in accordance with the anatomical facts, be held to be ultimately of pinna-nature; perhaps in most cases a result of pinna-fusion, according to the theory of Roeper. But this unit is subject to repetition in *Ophioglossum palmatum*.

8. The vascular supply to the Ophioglossaceous spike being normally marginal, or from an abaxial pinna-gap, it differs from that to the spore-producing organ in the Psilotaceae and Sphenophyllaceae, where it comes off from the adaxial face of the foliar strand, or is the middle region of it. This difference, if it be found to be constant, may provide a real and valid anatomical distinction.

Note.

I know only too well the undesirableness of introducing new terms into the vocabulary of the science. But any one who has read attentively those interesting pages of Professor Goebel's 'Organography' in which he deals with the phenomena of increase and decrease in number of parts in the flower [1] must have felt the want of some expression which, without connoting any detailed view as to the method, shall still convey the conception of increase or decrease in number of the parts from that which is regarded as normal or typical. The old terms 'chorisis', 'dédoublement', 'fission', or 'splitting', all have had special applications, and convey meanings relating to the method or manner of the increase. In the use of them there is a danger of conveying more than the simple conception of increase. 'Negative chorisis' and 'ablast' suffer under the same disability. The readiest way of avoiding such difficulties is by the introduction of some new term which carries no preconceptions, and while stating the fact does not imply any method. I venture therefore to suggest that the term 'pleiogeny' should be used to connote those phenomena which involve increase in number of parts beyond the normal or typical. The term 'meiogeny' would similarly connote a decrease from the normal or typical.

It is not only in the case of floral structure that such terms may apply. In the present case it would be a phenomenon of 'pleiogeny' that we have been studying in *Ophioglossum palmatum*. And similarly the increase of sori so frequently traceable in Ferns would be a phenomenon of the same class. Having recognized this, the next step will be by comparison and

[1] English Edition, pp. 528-42.

analysis to decide the method by which it is brought about. It will then appear that more than one method may be involved in any given example. For instance, where one primordium is already initiated, and the activity of tissue-formation becomes localized at more than one centre in relation to it, thus producing more than one part in place of the original one, the method of the pleiogeny would be chorisis. But if two centres of activity originate apart from one another where usually only one exists, or if an additional one appeared in a position usually untenanted, that would be interpolation. But both would fall under the general expression of pleiogeny.

DESCRIPTION OF FIGURES IN PLATES XXII–XXIV.

Illustrating Prof. Bower's paper on *Ophioglossum palmatum*.

Fig. 1. A, Fertile plant of *Ophioglossum palmatum* represented complete, about three-quarters of the natural size, from a drawing by Dr. A. A. Lawson. B, A bud inserted upon its parent root, showing the tuberous stock (*t*), surmounted by a dense tuft of hairs (*h*), and a single young leaf. × $2\frac{1}{2}$.

Fig. 2, I–IX. Successive transverse sections of a leaf-base, following the leaf-trace strands downwards to their insertion on the stele. I and II show the very numerous small strands, forming a more or less circular series, with frequent signs of fusion across the adaxial face of the petiole. III and IV show stages of segregation of the strands into two lateral groups, which in V and VI have united into single strands. V–X show the successive stages of insertion of these, right and left, at points widely apart upon the margins of a leaf-gap. In V–VII a commissure of the vascular network is seen just above the leaf-gap, and from the middle of it arises a root (*rt*), which takes its course obliquely outwards (VII–IX). The leaf-trace strands do not fuse completely with the meristeles of the axis till the level of the gap is reached (VIII, IX). × about 6.

Fig. 3. Photograph of a transverse section through the old stock, about half-way down its length, showing centrally the pith surrounded by three meristeles. From the edges of two of these a leaf-trace is coming off, as in Fig. 2, VIII. Externally numerous leaf-traces in various stages of advance are traversing the bulky cortex, and several roots are also seen. × 4.

Fig. 4. A slightly diagrammatized key to the vascular system shown in Fig. 3. The roots are omitted for clearness, while the several foliar traces are connected by dotted lines and numbered. It will be seen that they conform to the stages shown in Fig. 2; also that the divergence between the successive leaves is not a constant one.

Fig. 5, I–VII. Successive transverse sections of the stock of a young adventitious bud. I shows the insertion on the vascular system of the parent root; II, the vascular ring surrounding the central pith, which is present from the first. In III the ring opens, but no leaf-trace was given off from the gap. In IV the ring has opened on the opposite side also, while each vascular meristele is giving off a root-trace. The first leaf-trace is shown coming off in V, as two separate strands from the edges of the second gap. In VI the strands are separated, but the first gap has closed again by a vascular commissure. In VII the second gap has also closed, reconstituting the ring, while the strands of the trace are diverging widely. Drawn freehand. × about 50.

Fig. 6. Photograph of a transverse section of the old stock near to its base, showing the pith greatly dilated, the meristeles separated, and stretched transversely, while four roots are traversing the pith; others are seen in the cortex. × 4.

Figs. 7–10. Photographs of successive transverse sections through a young fertile leaf. Fig. 7 is at the level of insertion of the lowest spike, which is seen to be median. Fig. 8 shows four spikes in two groups, right and left of the median plane. Fig. 9 shows a higher section traversing two spikes only, which are clearly intramarginal. Fig. 10 is a transverse section of the leaf above the spikes, showing projecting margins similar to those already seen in Fig. 9. × 48.

Figs. 11, 12. Detail of segmentation of the marginal region of a fertile leaf, showing the T-type of division, but not with constant results. × 125.

Fig. 13. A very young spike, as seen in a transverse section of the parent leaf, from which it arises in an intramarginal position. It results from the outgrowth of a number of cells. × 125.

Fig. 14. A spike from the same leaf, but lower down, and inserted in a median position. It shows a rather more advanced state, thus indicating that there is an acropetal succession in the formation of the spikes. × 125.

Fig. 15. Transverse section from an older leaf, showing a young spike in a distinctly intramarginal position. × 125.

Fig. 16. Transverse section of the middle region of a fertile leaf, cutting obliquely through the insertion of three spikes. This indicates the irregularity of their position while young. × 125.

Fig. 17, I–IX. A series of fresh drawings from the sections of the fertile leaf described in the 'Annals of Botany', 1904, p. 210, Pl. XV. Figs. I–III show the origin of the vascular supply to the lowest median spike. Figs. IV–IX show the same for the two lateral spikes. It will be noted that in either case the number of the strands is ultimately three.

Fig. 18. Portion of the leaf of the large dried specimen from Jamaica, bearing to the left the fifth spike, and to the right the sixth, the latter being the smaller. The transverse sections of their stalks are also shown; the fifth is traversed by three strands, the weaker sixth only by one. × 2.

Fig. 19, I–V. Successive transverse sections showing the origin of the vascular supply to the fifth spike.

Fig. 20, I–VII. Successive transverse sections showing the origin of the vascular supply to the sixth spike.

Fig. 21 *a*, *b*. Transverse sections through the petiole of *O. pendulum*, *a*, lower down, *b*, higher on the same part, showing how completely the margin as marked by vascular characters is obliterated. Drawn freehand.

Fig. 22, I–X. Successive transverse sections through the petiole of *O. reticulatum*, showing origin of the vascular supply to the fertile spike. Section I is the lowest. Drawn freehand.

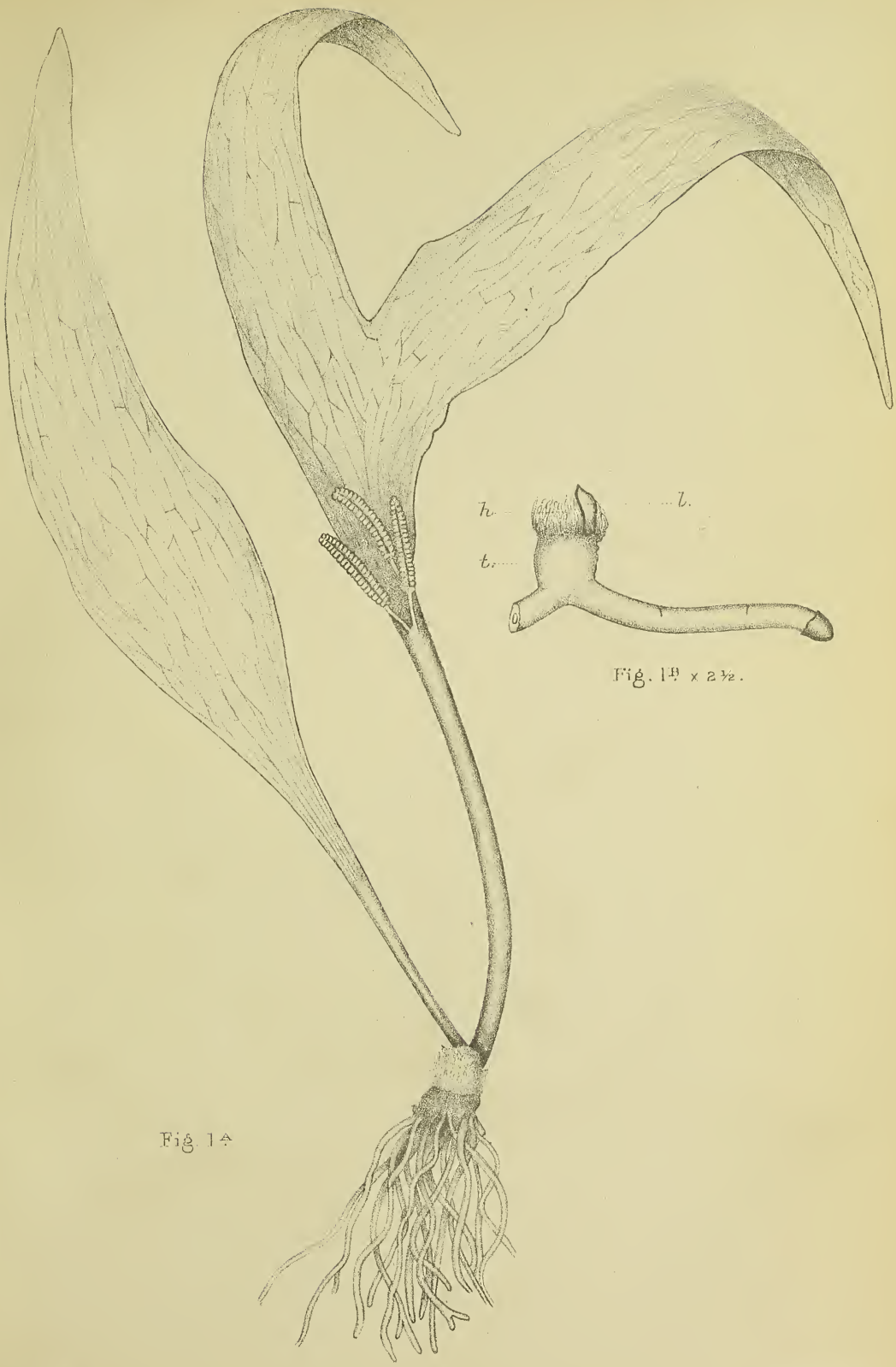


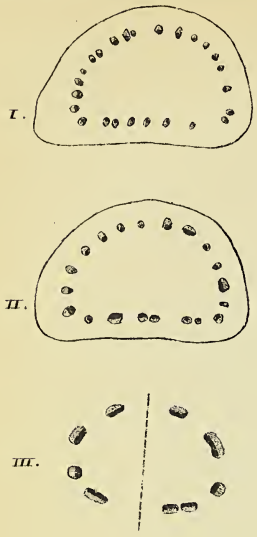
Fig. 1B × 2½.

Fig 1A

A. A. L. del.

Huth. Lith et imp.

BOWER — OPHIOGLOSSUM.



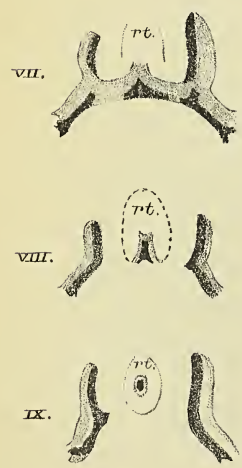
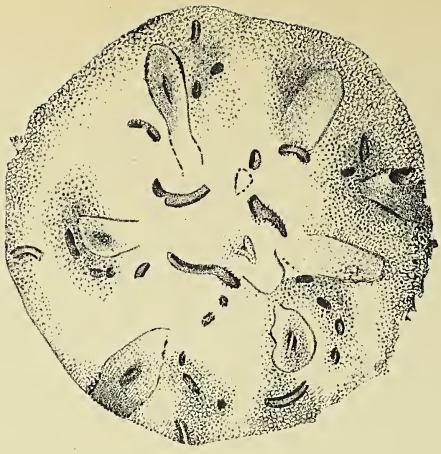


Fig. 2. *(I–IX)*



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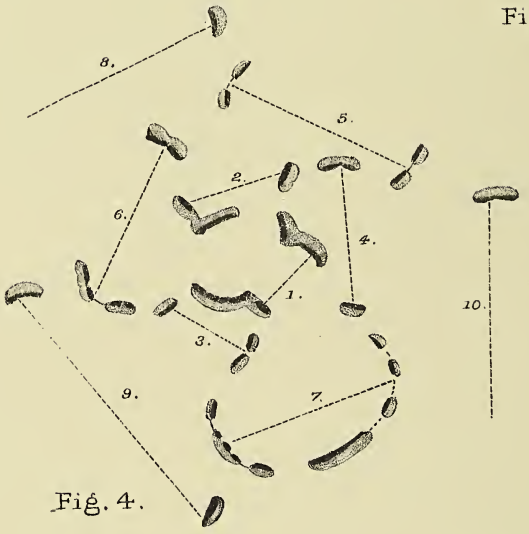


Fig. 4.

Fig. 5.

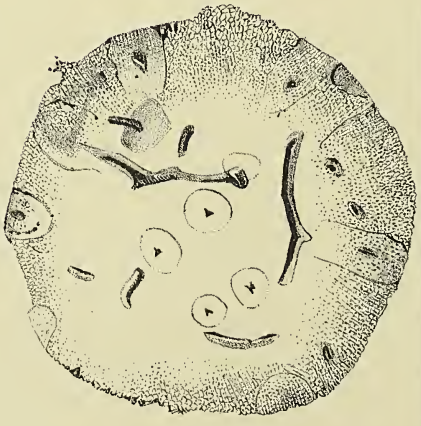
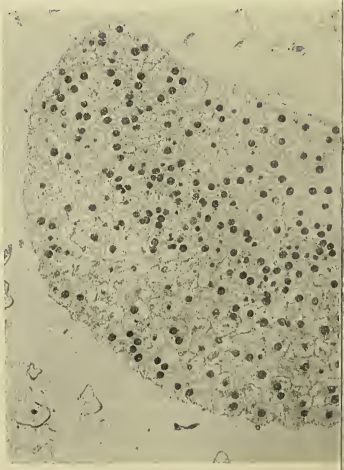


Fig. 6.



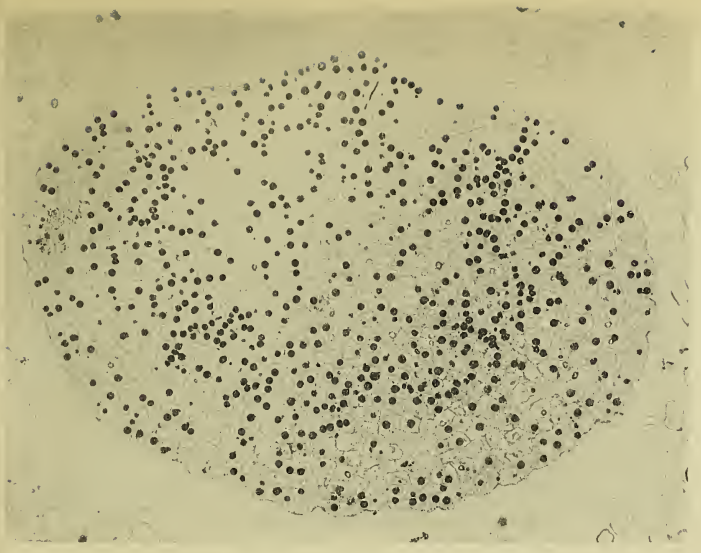


Fig. 7.

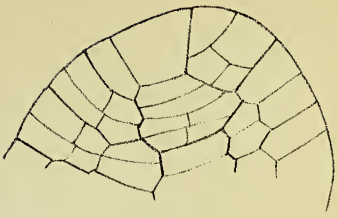


Fig. 11.

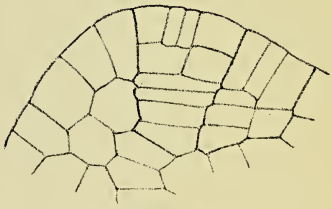


Fig. 12.

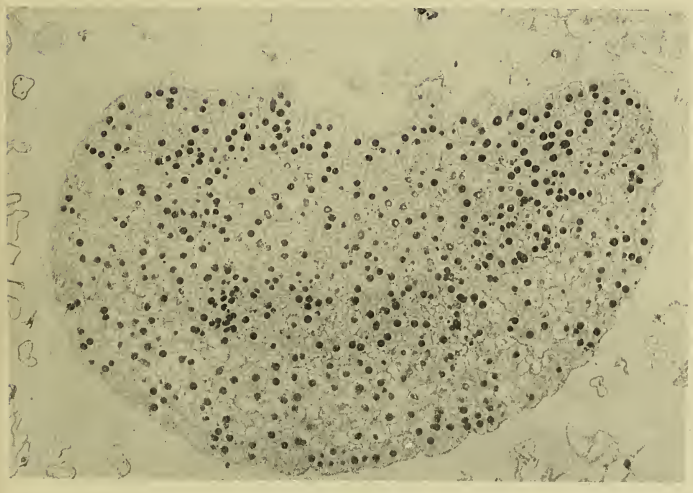
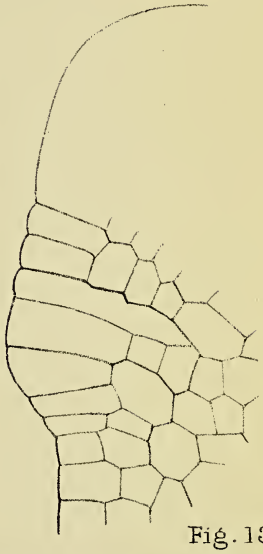


Fig. 8.



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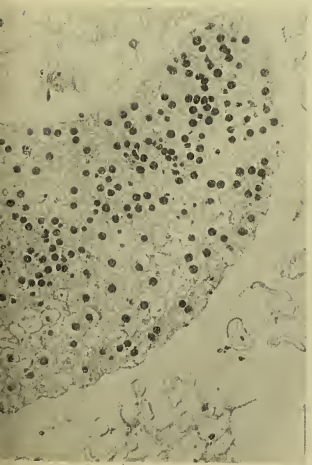

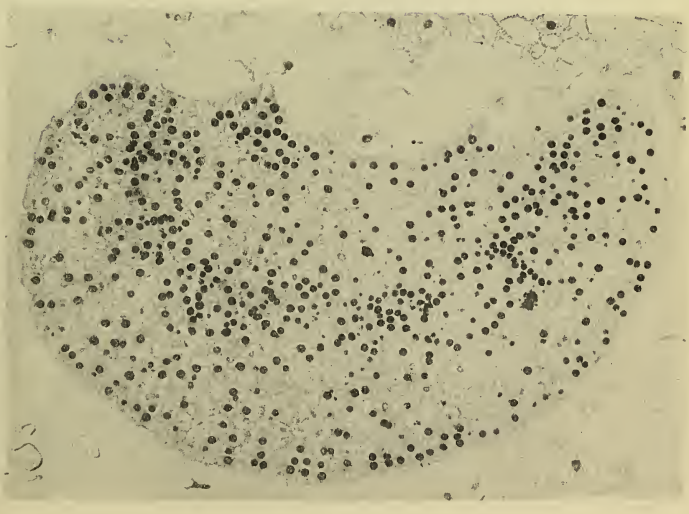

Fig. 9.

Fig. 10.

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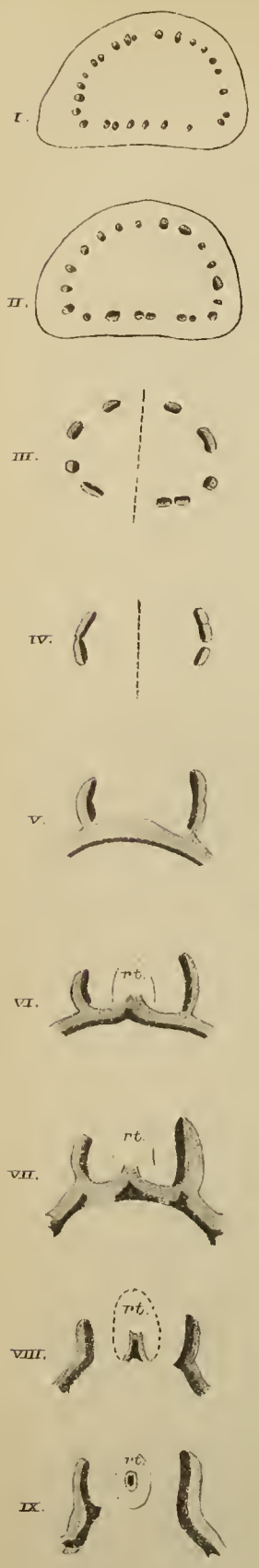
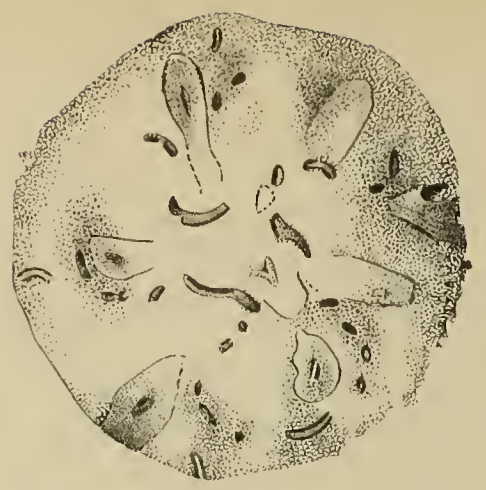


Fig. 2. *(I–IX)*



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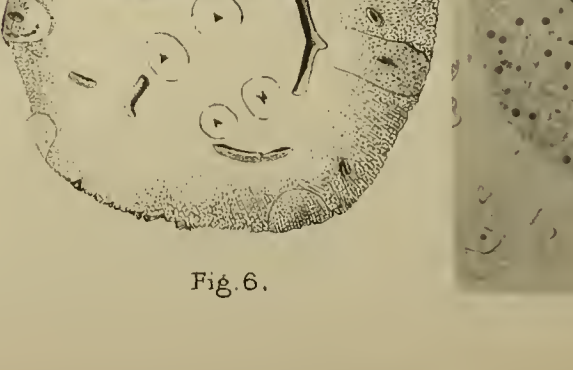

Fig. 4.

Fig. 5.

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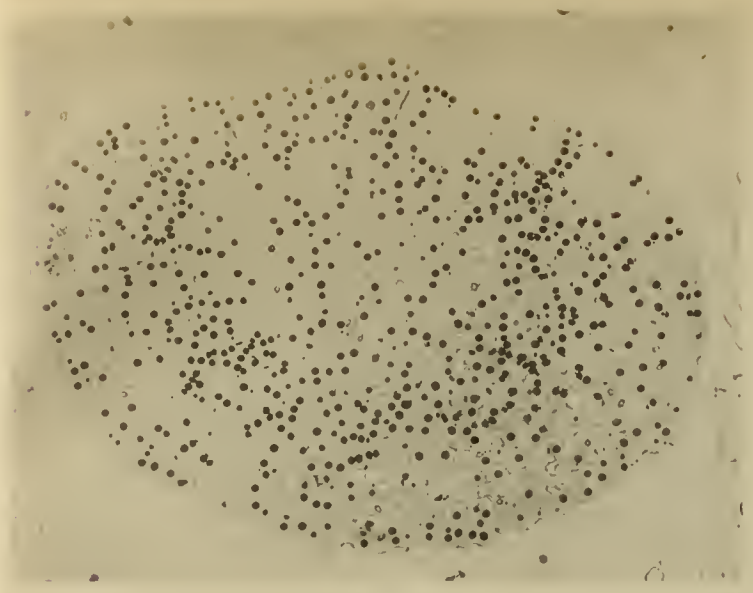

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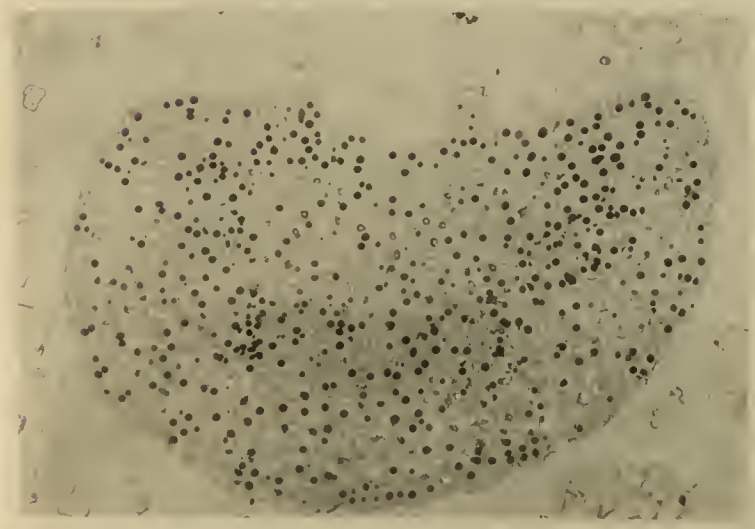

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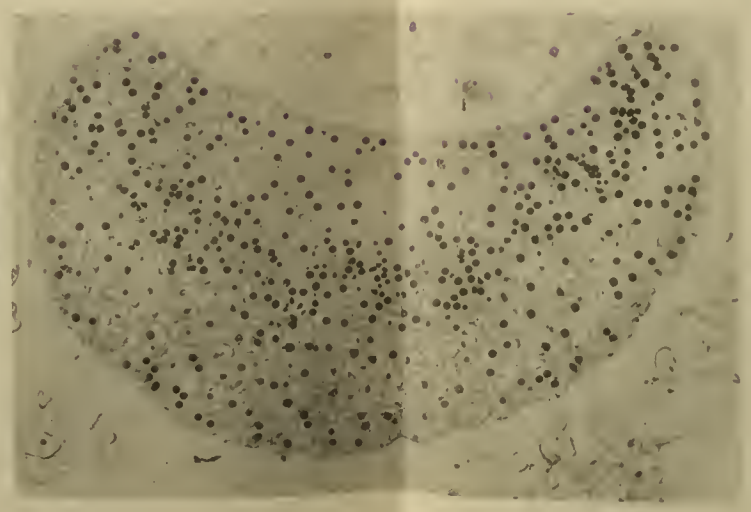

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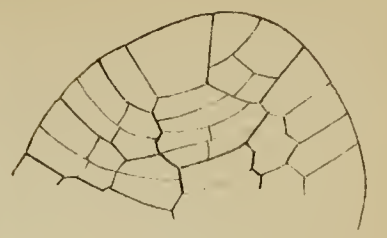

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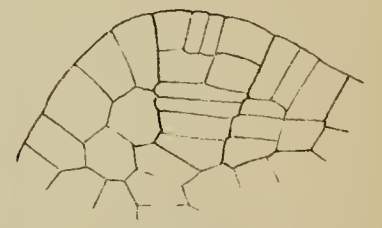

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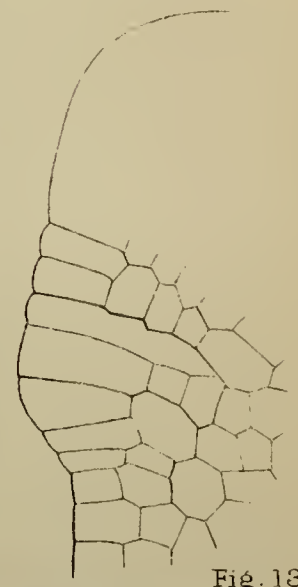

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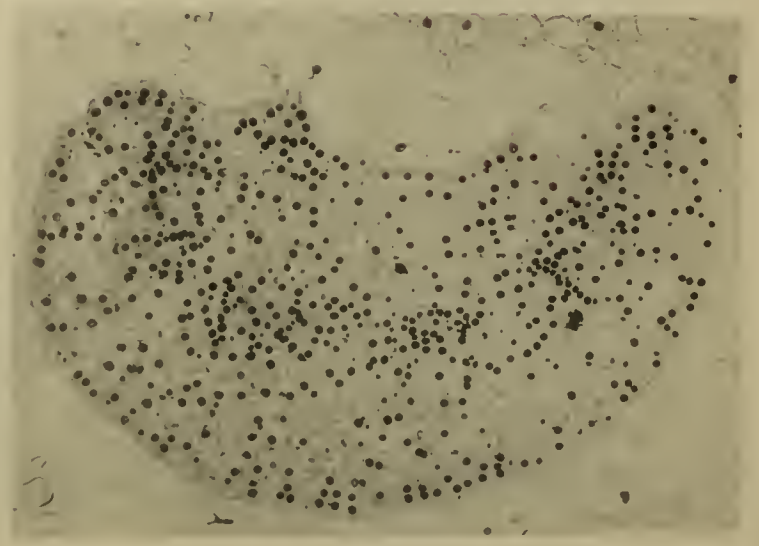


Fig. 9.

Huth, London.

Fig.14.

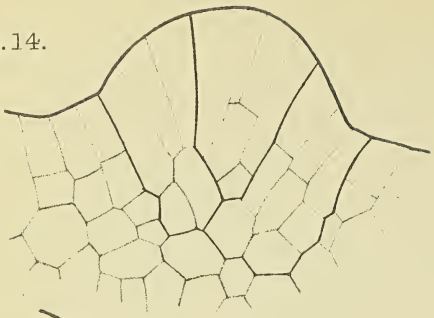


Fig.17. (I–IX)

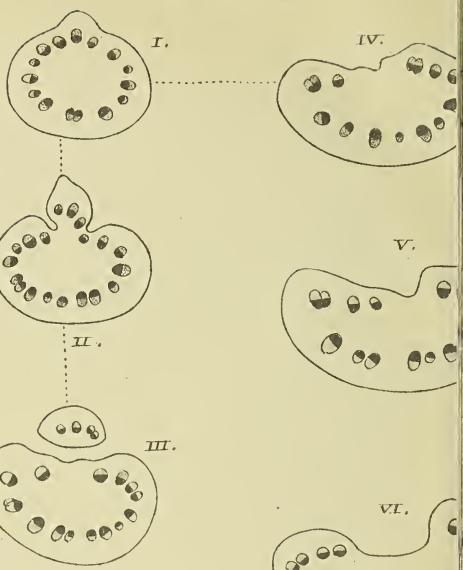
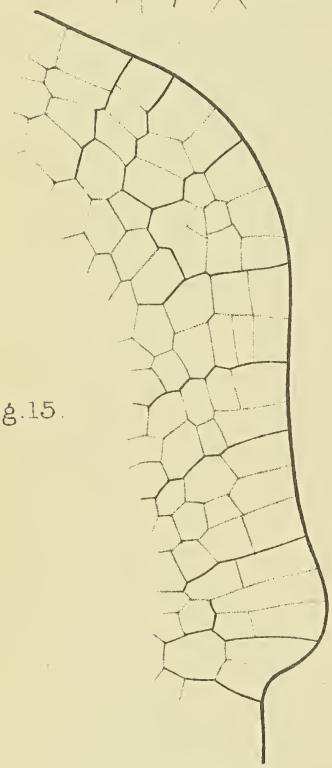


Fig.15.



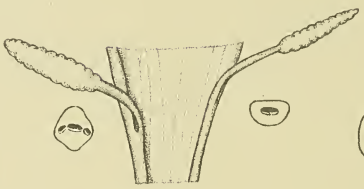


Fig.18. ×2.

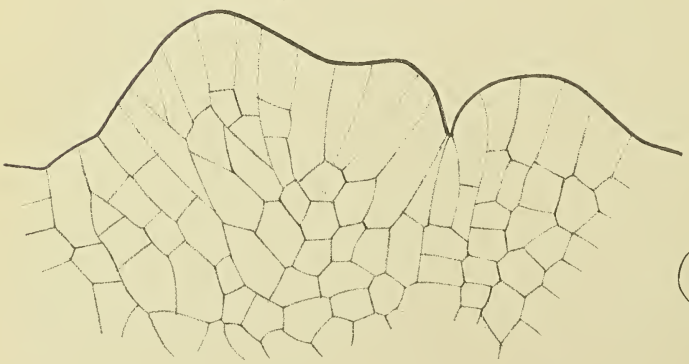


Fig.16.

Fig. 19. (I-V)

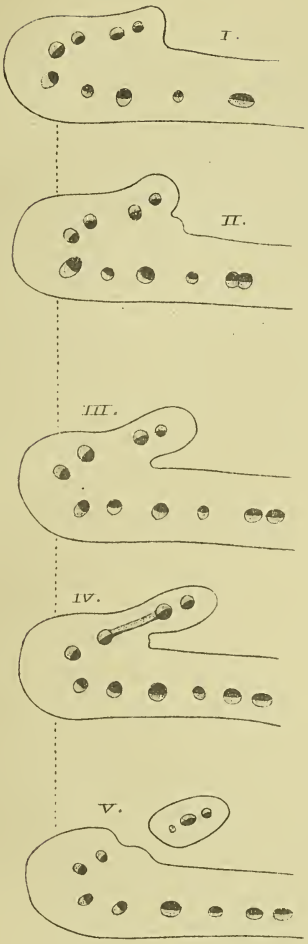


Fig. 21.

Fig. 20. (I-VII)

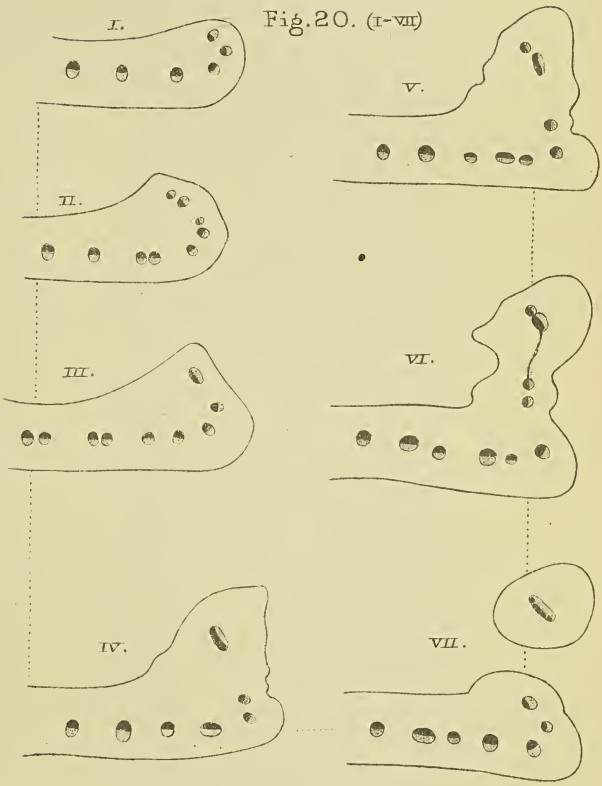
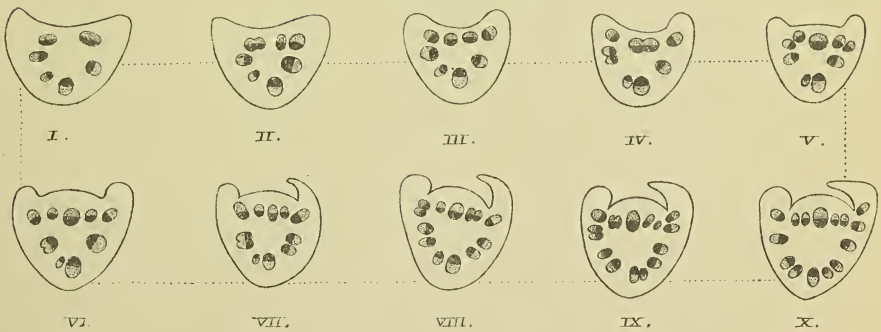


Fig. 22. (I-X)



Huth, lith. et imp.

Fig. 14.

Fig. 15

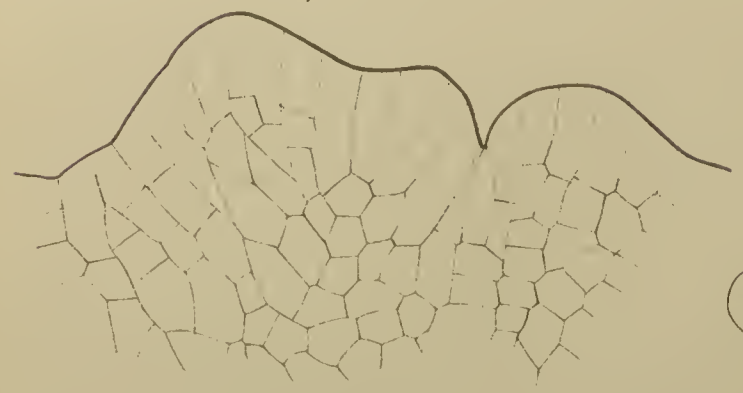


Fig. 16.

Fig. 17. (I–IX)

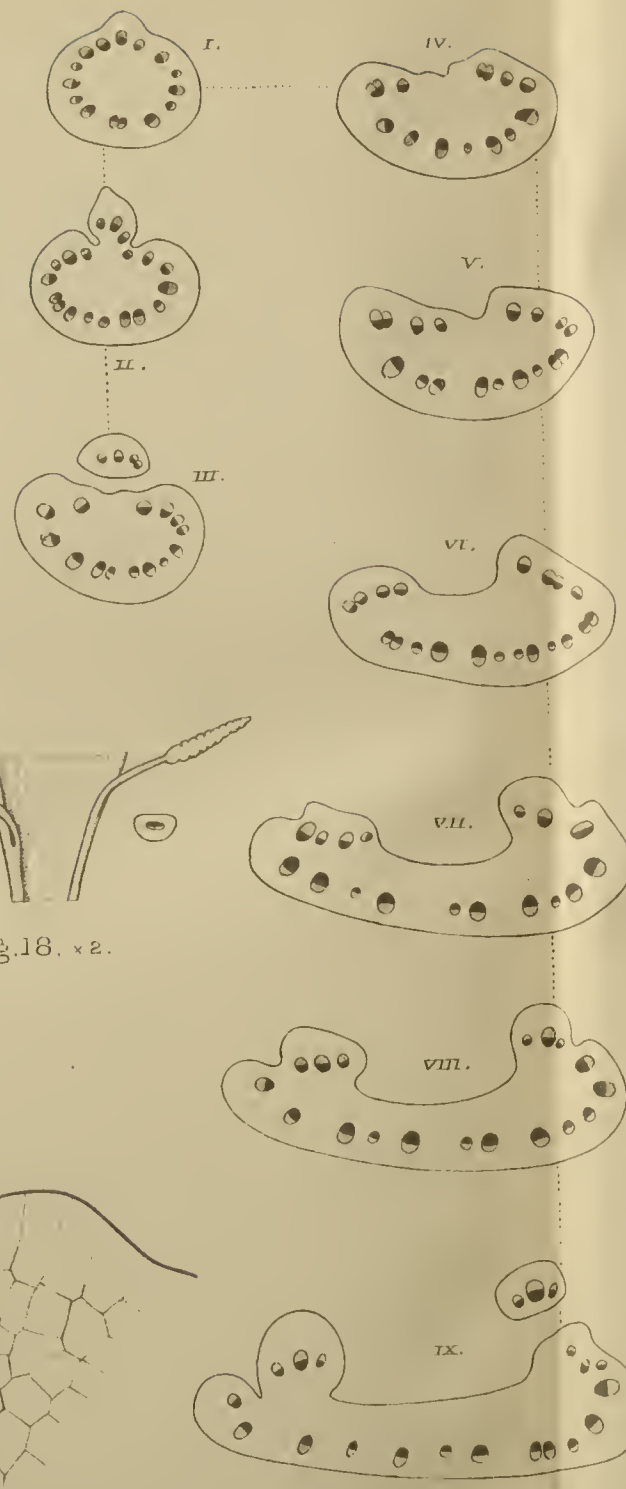


Fig. 18. ×2.

Fig. 19. (I–V)

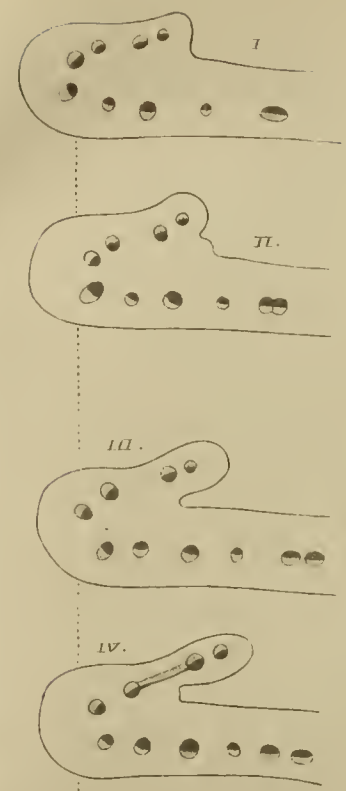


Fig. 21.

Fig. 20. (I–VII)

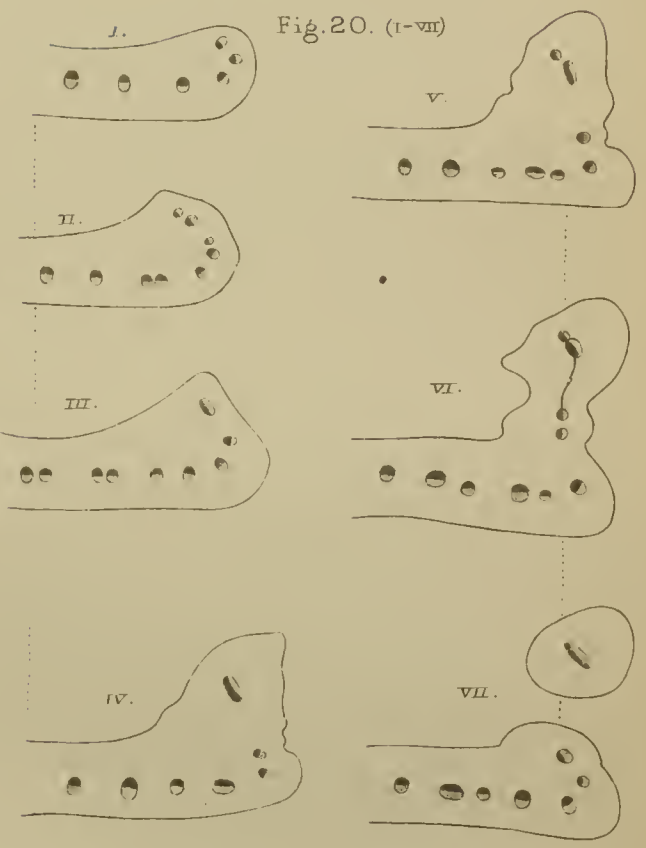
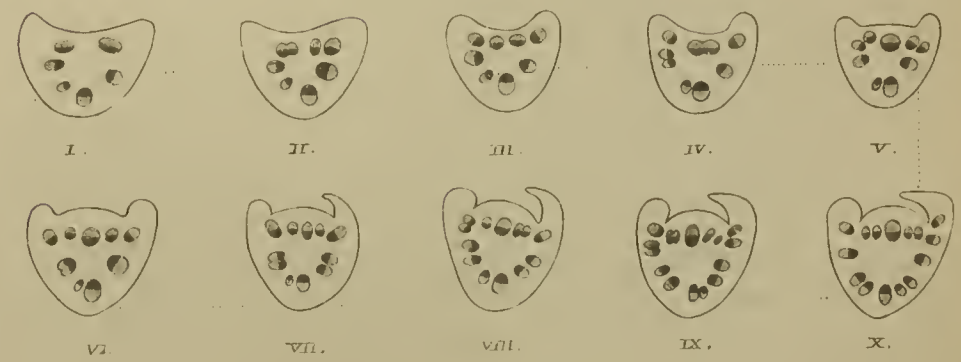


Fig. 22. (I–X)



Spermatogenesis in certain Hepaticae.

BY

WILLIAM LOGAN WOODBURN.

With Plate XXV.

DURING recent years considerable interest has been shown in the study of spermatogenesis in the Bryophytes, Pteridophytes, and other cryptogams, as evidenced by the contributions on this subject. It has been difficult to arrive at correct conclusions in regard to the true nature and history of spermatogenesis in some of these forms, especially in the Bryophyta. Two difficulties in particular have, as a rule, been encountered. In the first place the cells are small, and certain important details of their contents are hard to differentiate. In the second place the process of spermatogenesis involves such a complete, and at times rapid, transformation of the individual cell that the origin and fate of certain parts are difficult to trace, and the nature and function quite as hard to determine. These difficulties are evidenced by the various opinions in regard to the process presented by the different investigators.

It was with the hope of obtaining further facts which might clear up somewhat the problems encountered in tracing the development of the sperm in some forms of the Hepaticae, that a study of spermatogenesis in *Porella* and other Liverworts was undertaken.

The first detailed account in recent years, treating of spermatogenesis in the Hepaticae, was given by Ikeno ('03) for *Marchantia polymorpha*. He discusses the early divisions in spermogenous cells, the last or oblique division, and the development of the spermatozoids. In all divisions of spermogenous tissue he finds a body which he calls a centrosome occupying each pole of the spindle. This centrosome he describes as separating from the chromatic mass in the nucleus, moving towards the nuclear membrane, and passing through the latter into the cytoplasm. Here it divides and the daughter bodies move to opposite sides of the nucleus, where they apparently take part in the formation of the spindle. They occupy the poles of the spindle until late anaphase or telophase, when they disappear, but reappear in the prophase of each successive division. In the last spermogenous division, the spindle lies diagonally, and the cell is consequently separated

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into two triangular sperm cells, which lie in pairs, and are not separated by a cell-wall. During this division the centrosome does not disappear, but persists to function as the blepharoplast. It remains, at first, in the corner of the sperm cell originally occupied by the pole of the spindle, then it moves into one of the acute angles of the cell. Shortly thereafter, a large body (*Nebenkörper*) appears in the cytoplasm for a short time, and then, during the formation of the blepharoplastic band, disappears. Whether, like the centrosome, this body is of nuclear origin or not, Ikeno did not determine with certainty.

After the centrosome has taken up its position in one corner of the sperm cell, it lengthens slightly, keeping in contact with the plasma membrane, and soon develops two cilia. Then the nucleus moves so as to come in contact with the plasma membrane on the same side along which the centrosome has stretched out to function as a blepharoplast. In this position, the nucleus changes both in form and structure. It becomes homogeneous in staining capacity, and begins to flatten or draw out in a bow-like form. In the meantime, a protoplasmic projection has grown out from the nucleus in the direction of the blepharoplast until the two are united. The nucleus continues to lengthen until it becomes a narrow band, and the cilia long slender threads. At the same time the cytoplasmic mass decreases in size until only a small vesicle is left at the posterior end of the nucleus, while from the anterior end of the latter a very narrow band extends forward to the diminished blepharoplast. The latter grows only a short distance towards the nucleus, and then becomes smaller, and practically disappears as the cilia develop, so that the forward end of the sperm projecting beyond the nucleus consists of a short, scarcely perceptible blepharoplast, bearing two cilia, and connected to the nuclear portion by a band of cytoplasm. Posterior to the nuclear portion is a cytoplasmic vesicle. The whole body of the sperm is more or less loosely coiled; it is formed of three metamorphosed elements of the cell—the centrosome, which has become the blepharoplast, the nucleus, which forms the main body of the sperm, and the cytoplasm, which forms the posterior vesicle and the band connecting the nucleus with the blepharoplast. The cilia may consist partly of material derived from the blepharoplast, and partly from the cytoplasm or plasma membrane of the sperm cell.

Bolleter ('05) described briefly the spermatogenesis of *Fegatella conica*. This description agrees mainly with that of Ikeno for *Marchantia*, except that Bolleter failed to see the centrosome-like body at the poles of the spindle in the prediagonal divisions of the antheridial cells. However, since he found such a body occupying the region of the spindle pole of the last division, and also observed its nuclear origin, he concludes that it must be present also in the earlier divisions.

Lewis ('06) found centrosome-like bodies at the poles of the spindles

in the earlier, as well as in the diagonal, divisions of the spermogenous tissue of *Riccia natans*. These bodies, which he is not inclined to consider as true centrosomes, do not have genetic continuity, but disappear after each cell-division, except the last, when they persist in the sperm cells and function as blepharoplasts. He finds no evidence of their nuclear origin, but considers them as arising *de novo* with each cell-division. In the formation of the spindle, cytoplasmic fibres radiate from the nucleus and converge at the centrosome-like body. While the behaviour of this body seems more like a blepharoplast than a centrosome, the fact of its occurrence in so many cell generations before the formation of the sperm leads to some difficulty in determining its nature.

Escoyez ('07) describes the behaviour of the chromatin and the formation of the spindle in diagonal and prediagonal divisions of *Marchantia polymorpha*. Contrary to Ikeno's observations, no centrosome-like body, according to Escoyez, is apparent until during the prophase of the diagonal division. Then in each corner of the sperm-mother-cell a darkly stained body appears, which will lie at the pole of the spindle. A body of similar appearance may occur also in each corner of the cell, which will be in the plane of the cytoplasmic division. He found no evidence of the nuclear origin of either of these bodies, but states that the body, or corpuscle, which occupies the pole of the spindle, persists in the sperm cell and functions as the blepharoplast.

In early divisions of antheridial cells of *Fegatella*, he found no centrosome-like bodies. The last division of the spermogenous tissue was not studied.

Escoyez concludes that centrosome-like bodies are present in the spermogenous tissue of *Marchantia* in the last division only, and that these corpuscles are the blepharoplasts; that they are not true centrosomes, functioning secondarily as blepharoplasts, but organs *sui generis* of the antheridial cells. They are the 'cilia bearers' and play no rôle in karyokinesis. If they were true centrosomes they should also be found in the earlier divisions. The mere fact that they occupy the poles of the spindles does not imply that they take part in the spindle formation.

In his studies, Schaffner ('08) found bodies, which he called centrosomes, occurring in all spermogenous divisions of *Marchantia*. These bodies, he says, persist through the last divisions and function as blepharoplasts. The latter are modified centrosomes, and their ontogeny can be traced back through the preceding cell-divisions of the antheridium. He does not state the origin of these bodies, nor whether they persist during all phases of karyokinesis.

Miss Black, while working on *Riccia Frostii*, Anst., during the winter of 1909–10, found no centrosome-like bodies in any phase of the prediagonal divisions or immediately after the diagonal division. But at a later stage,

in the cytoplasm of the sperm cell, a dense body appears which grows out into a densely staining slender cord around one side of the cell. This densely staining cord is the blepharoplast and bears very near one end—the anterior end of the mature sperm—two cilia, while the nucleus draws out into a narrow band along the posterior region of the blepharoplast.

So far I have not found that any work has been done on the spermatogenesis of *Porella*. The observance of large nuclear figures in this Liverwort suggested that it might be suitable for such a study. Accordingly material was fixed at various times in the chromic-osmic-acetic killing fluid as formulated by Mottier ('97). Antheridial branches were fixed entire. At almost any time of the year, especially when cool and damp, various stages in the development of the antheridia were found in acropetal succession on the same branch—from very small antheridia to almost mature sperms. But only occasionally were nuclei found in the process of division. Resting conditions may be of long duration, but the process of division seems to occur very rapidly. This fact is no doubt correlated with the ability of *Porella* to dry up for long periods and then revive with rapid cell-division. Along with *Porella* careful studies were made in the spermatogenesis of *Marchantia* and *Fegatella*.

The material, after fixing ordinarily for about twenty-four hours, was washed, brought up through the various grades of alcohol, embedded in paraffin, and sectioned, for the most part two microns thick. The following combinations of stains were used: anilin safranin and gentian violet, usually without the orange G; Heidenhain's iron alum-haematoxylin; anilin safranin, iron alum-haematoxylin, and gentian violet; iron alum-haematoxylin and gentian violet; and iron alum-haematoxylin and Bismarck brown.

In what follows I shall discuss the mitosis in spermogenous tissue, the last, or oblique division, and the development of the sperm.

Mitosis in Spermogenous Tissue.

As already suggested, the spermogenous cells of *Porella* are usually quite large. Compare Fig. 3, Pl. XXV (*Porella*), with Fig. 27 (*Marchantia*) and Fig. 46 (*Fegatella*). In an early stage of the antheridium, the nucleus presents a large nucleolus surrounded by a clear space, and, nearer the nuclear membrane, an open linin network with scattered chromatin granules (Figs. 1 and 2). The cytoplasm is more or less alveolate or fibrillar in nature, and quite granular. The granules vary in size, number, and disposition, as do also the vacuoles if present (Figs. 1 and 3).

In early prophase the chromatin lumps are larger and more drawn out along the linin threads. More than one nucleolus may be present at this stage (Fig. 3). If the latter figure be compared with Figs. 4, 5, and 6, it is evident that the chromatin continues to collect along the linin threads until a more or less irregular spireme is formed, which lies near the nuclear mem-

brane. The details of the formation of the chromosomes from this condition were not observed. Fig. 7 shows a cell in which the nuclear membrane has disappeared and the chromosomes are differentiated. This count shows only six, which is two less than the gametophytic number for *Marchantia* and *Fegatella*. However, this was the only cell found cut in such a plane through a dividing cell that the chromosomes could be counted. I am not certain, therefore, that six is the exact number.

The formation of the spindle was not observed, but many sections were found showing metakinesis (Fig. 8). The cytoplasm is a little coarser in texture than in preceding stages, but otherwise apparently the same. Fig. 8 shows numerous granules in the cytoplasm quite similar to those shown in Fig. 3. There is nothing to warrant the conclusion that any one granule, or body, differs from the numerous other ones, except in size and density. All are scattered promiscuously throughout the cytoplasm, some in vacuoles, others embedded in the cytoplasmic structure (Figs. 3 and 8). Nothing was found in the cytoplasm or at the pole of the spindle which resembled a centrosome. The spindle (Fig. 8) stands out clear and distinct. The fibres are collected into rather dense, irregular strands which are attached to the chromosomes at the plate, while they come to a sharp point at each pole. Sometimes one of the irregularly placed granules happens to lie near the pole, but no body similar to those shown by Ikeno ('03) and Schaffner ('08) for the same stages in *Marchantia*, and by Lewis ('06) for *Riccia*, was found. Neither were there any radiations from the region of the poles.

Fig. 9 represents an anaphase of one of the earlier spermogenous divisions. The cells in one half of the antheridium have reached this stage simultaneously. Sharply defined spindle fibres are still attached to the ends of the retreating chromosomes. The cytoplasm is more coarsely granular, but the largest granules are doubtless oil-drops. The number of chromosomes apparent here would indicate that the previous count was correct. Fig. 10 represents a telophase following one of the divisions shortly before the last. The cells and nuclei are smaller than in earlier stages of the antheridium. However, too much significance must not be placed in size alone, as this varies considerably for cells of corresponding stages in different antheridia. It will be observed that the nucleus which shows most nearly the median section has a chromatin network quite similar to that in Fig. 3, save that a nucleolus is not visible.

In *Marchantia* the resting condition (Fig. 25 A) shows an evident linin network containing relatively large lumps of chromatin in a clear nuclear sap without any nucleolus. The nucleus seems to pass this stage very quickly, while those stages represented by Figs. 25, 26, and 27 persist for a longer period. Fig. 25 A is quite similar to Escoyez's Figs. 7 and 8. Ikeno ('03) does not figure the chromatin in this condition (Fig. 25 A), neither

does he find at this, or a later stage, the irregularly granular nature of the cytoplasm. The latter's figures all show a very smooth homogenous cytoplasm. At times (Fig. 28 A) I found the cytoplasm quite finely granular, but, as a rule, the structure was coarser and more irregular, the smaller granules tending to collect into larger masses.

The chromatin then collects into one or more lumpy, irregular masses in the centre of the nuclear cavity. This latter now stains rather densely, as if filled with some homogenous or very finely granular substance. This appearance of the nucleus continues during the formation of the chromosomes and the early stages of spindle formation. The two latter processes were not observed in detail, but Fig. 27 shows a chromatin mass, shortly before the differentiation of the chromosomes, still surrounded by the same darkly staining substance. This homogenous material around the chromatic mass seems to indicate a preparatory step in the formation of the spindle. The spindle fibres (Fig. 28) apparently occupy the former nuclear area and stain quite similarly to the homogenous nuclear content, so that it seems quite possible that the fibres have had their origin in this latter substance. If this be so, it agrees with the origin of the spindle in the spore-mother-cell of *Pellia epiphyllia* as described by Farmer ('95), with the exception that there are no centrosomes or centrospheres present (Figs. 28 and 28 A). There is nothing that indicates the presence of centrosomes, as claimed by Ikeno ('07) and Schaffner ('08) for similar spermogenous stages in the same plant. Fig. 28 shows two granules, or bodies, in the region of one pole, but the latter is broad, a condition that does not obtain when true centrosomes are present. Even when these bodies do chance to be in such a position, it seems quite arbitrary to designate them as bodies specially differing from others which occur just as frequently in various places throughout the cell. Usually no special body of any sort occupies the spindle pole (Fig. 28 A). So far my observations agree with those of Escoyez ('07), except that I did not find the spindle fibres forming definitely outside of the nuclear membrane as represented by his Figs. 13–15.

The earlier mitotic conditions in the spermogenous cells of *Fegatella* are much the same as in *Marchantia*, but as metakinesis approaches there are some differences, so that a brief description will be in place. The nuclear figures are, as a rule, larger and more distinct. The nucleus in a resting condition (Fig. 45) shows a linin network with small lumps of chromatin quite evenly scattered throughout, and one or more relatively large, centrally placed bodies. Fig. 45, which is typical of this stage, shows one body which resembles a nucleolus, and another body which has the appearance of a mass of chromatin. The number of the larger bodies may vary from one to several. During prophase the chromatin becomes scattered rather evenly in the nuclear network as irregular lumps of nearly uniform size (Fig. 46). From this condition an irregular spireme develops which is

evidently of a double nature (Figs. 47 and 48). Since this is not a reducing division the double nature is certainly the result of a split. The chromosomes are now quickly differentiated. Whether the large loops and enlarged portions of the spireme (Fig. 48) separate as individual chromosomes must be left for further investigation. In sections cut in the plane of the equatorial plate the chromosomes are easily counted, and, as already stated by other investigators, the number is clearly eight.

During the prophase the nuclear cavity is not so often found to be filled with the homogenous or very finely granular substance as observed regularly in *Marchantia*. This condition, however, prevails to some extent (Fig. 48). But in this figure the nuclear membrane has practically disappeared, and there are indications in the surrounding cytoplasm that spindle fibres are beginning to form. These are at first very fine and evenly and closely distributed ; later they collect into coarser strands attached to the chromosomes at the equatorial plate (Figs. 50 and 51). The appearance of the chromosomes in this position is quite similar to that in *Marchantia* (Fig. 28 A) and *Porella* (Fig. 8). The individual chromosomes cannot always be distinguished. The spindle fibres are evidently active in laying down the cell plate (Fig. 52, *Fegatella*, and Fig. 10, *Porella*), as is the case in the higher plants.

The cytoplasm is quite similar to that of *Marchantia*. At no time is there any evidence of a centrosome-like body. This agrees with Escoyez's ('07) observations on the same plant. Bolleter ('05), however, concluded that, since he saw bodies in the sperm cells resulting from the diagonal division, and that, following Ikeno's conclusions for *Marchantia*, these bodies were centrosomes, therefore they must surely be present in the preceding spermogenous divisions, although he did not observe them.

In *Asterella* my studies were less extensive, but some sharply defined spindles were found (Figs. 61 and 62). Very minute dots were sometimes seen occupying the poles of the spindle, but these were very insignificant as compared to the size and appearance of granules lying in various places throughout the cytoplasm. Nothing was found in this study as far as pursued which resembled centrosomes in appearance or behaviour.

The Oblique Division.

Fig. 11 shows two cells from an antheridium of *Porella*, which is evidently ready to enter the last division, as indicated by the size of the organ and by the number and size of the cells. The nuclear network is quite coarsely granular, with small chromatin particles, and contains a small nucleolus. The cytoplasm is rather finely and evenly granular. The last division was not found, although hundreds of sections were examined for this particular stage. Pairs of sperm cells, however, just succeeding the last division were found, but whether these are the result of a diagonal division

is not shown by their position, as the rounded pairs lie variously placed within the antheridial cells, whose walls are also somewhat rounded off (Fig. 13).

As already described by Ikeno and others, the last division of the spermogenous tissue, in *Marchantia*, is a diagonal one which separates the cell contents into a pair of triangular sperm cells not separated by a cell-wall. The oblique position of the spindle has also been accurately described. In some respects, however, my observations differ from those of the other observers. Cells that are ready to enter upon this division have usually a more coarsely granular cytoplasm than those in early stages of spermogenous development. The cytoplasm becomes more vacuolate, and the denser areas tend to collect in aggregations of granules, or in definite bodies (Fig. 30). There are usually two rather larger, dense bodies of about equal size, lying on opposite sides of the nucleus, and usually opposite the angles of the cell-wall. Smaller ones, which apparently differ only in size, are also found in the cytoplasm. Fig. 29 shows spindle fibres beginning to form from the immediate region of one of these larger bodies. Comparing Figs. 31 and 32, we see that the fibres reach down around the nuclear membrane, which soon disappears, and the fibres will enter the nuclear cavity and become attached to the chromosomes. Fig. 32 shows an intermediate stage in the formation of the spindle, in which the nuclear membrane is evidently gone, but where the chromosomes are neither fully differentiated nor attached to the spindle fibres. During this stage the bodies occupying the poles of the spindle are quite conspicuous. In Figs. 33 and 34 the chromosomes, while each one cannot be distinctly made out, are evidently arranged in an equatorial plate. Nearly every spindle has at least one body at the pole. Sometimes (Fig. 34) more than one body occurs with the spindle fibres attached to each. On the other hand, a single body may be present with few of the fibres attached to it, while the rest evidently extend beyond, often forming a broad pole (Fig. 33). These bodies as a rule do not stain as deeply in metakinesis as during prophase. Figs. 29–37 were drawn from the same slide, and consequently had received the same treatment in staining. After metakinesis these bodies do not persist with any regularity. Sometimes they may be seen in anaphase and telophase, but more frequently they cannot (Figs. 35, 36, and 37). I could find no evidence that the body, which occupies the pole of the spindle during the diagonal division, persists as an individual organ in the resulting sperm cell, as stated by Ikeno ('03), Escoyez ('07), and Schaffner ('08) for *Marchantia*, Bolleter ('05) for *Fegatella*, and Lewis ('06) for *Riccia natans*. At a later stage a definite body is found in the sperm cell, but no certain connexion can be traced between this body and the one which earlier occupied the spindle pole in the diagonal division, further than the fact that they both appear in the cytoplasm of the cell concerned. The cytoplasm

often shows bodies of various sizes and collections of granules (Fig. 30). Some of these seem to be merely dense globular masses of cytoplasmic material and the two which eventually occupy the spindle poles are apparently similar. There has been no evidence produced which would indicate their nuclear origin as claimed by Ikeno ('03). The drawings of the latter do not confirm his opinion that the intranuclear body seen in his Figs. 1 or 2 is identical with the body seen outside the nucleus in his Fig. 3.

The oblique division of the spermogenous tissue in *Fegatella* occurs in essentially the same manner as in *Marchantia*. The spindle fibres extend from a small point or body in the cytoplasm towards the nuclear membrane (Fig. 53). The latter disappears, the fibres enter the nuclear cavity, and the chromosomes become arranged in an equatorial plate (Fig. 53 A). During this process the body at each pole of the spindle persists; at the same time other bodies may be scattered through the cytoplasm or in the region of the spindle (Fig. 53 A). By late anaphase the polar bodies have disappeared and the cytoplasm may or may not contain granular bodies (Fig. 54). As in *Marchantia* there is no evidence that the bodies which occupied the poles of the spindle during metakinesis persist as individuals in the sperm cells.

Development of the Sperm.

The nuclear network of the sperm cell of *Porella* soon after the last division shows no nucleolus. The nucleus is smaller than in earlier stages of the antheridium, and the amount of cytoplasm is less (compare Fig. 12 with 1). The cytoplasm is granular and shows a denser aggregation in one end of each cell. Around the nucleus it is lighter, but in the opposite ends of the cells there are also denser areas (Fig. 12). The densely stained body in each sperm cell, as shown in Fig. 13, corresponds exactly in position to the smaller dense collection of cytoplasmic granules in Fig. 12. These figures give strong evidence that the body shown in Fig. 13, from which the blepharoplast develops, is of cytoplasmic origin. Fig. 14 shows one of the pair of sperm cells viewed from the side, if we speak of the view in Figs. 12 and 13 as edgewise. The chromatin lumps of Figs. 12 and 13 seem to be drawn out into longer and irregular pieces. Otherwise the cell has changed but little. The densely staining body now begins to develop directly into the form of the mature blepharoplast. It lengthens as a cord, following quite closely the plasma membrane (Fig. 15), until, as shown in Fig. 17, it may extend half-way or more around the periphery of the cell. Fig. 18 shows a pair of the sperm cells with the blepharoplast extending along the edge past the nucleus. The forward end of the blepharoplast, or the one from which the development has proceeded, remains slightly larger for a short distance backward than the remaining posterior portion. The

cytoplasm in the peripheral region of the cell through which the blepharoplast grows is denser than in the interior (Figs. 15, 16, and 17).

Frequently a very dense band of cytoplasm extends somewhat beyond the posterior end of the developing blepharoplast (Figs. 17 and 18), as if possibly contributing to the growth of the latter. The blepharoplast throughout seems to be composed of similar material; at least, no differentiation could be brought out in the staining. The conditions observed in the development of the blepharoplast indicate that the body shown in Fig. 13 represents cytoplasmic constituents; that this body begins to grow into a cord, and enough material is added from the cytoplasm to produce the mature threadlike blepharoplast. The position of the blepharoplast is quite similar (Figs. 17–20) to that of *Chara*, as described by Mottier ('04), but the form and development differ. The blepharoplast of *Chara* arises as a differentiation of the plasma membrane with the posterior end the thicker, while in *Porella* it grows from a spherical body, and the forward end is the larger.

The chromatin grauules gradually lose their identity, forming a homogeneous mass which begins to draw out along the blepharoplast into a curved or crescent-shaped band (Figs. 19, 20, and 21). The nuclear material now becomes so closely applied to the blepharoplast that the exact extent of the latter cannot be determined. The nuclear band continues to lengthen, describing eventually a little more than one turn within the cell-wall. The blepharoplast, or as much of it as can be seen extending from the nucleus, becomes thinner, but the forward end from which the cilia spring remains slightly thicker than the rest (Figs. 22 and 23). Fig. 22, which is still enclosed within the cell membrane, shows the cilia coming off from the rear of this thickened forward end of the blepharoplast and extending around the body of the sperm, and at certain points in contact with the cell membrane. Fig. 23 shows more distinctly the point of insertion of the cilia. The cytoplasm collects in coarse granules within the concave side of the sperm, mainly within the posterior or nuclear part (Figs. 21, 22, and 23). Mature sperms (Fig. 24) were secured by dissecting out antheridia from the antheridial branches, and allowing the sperms to escape in water on a slide. In about thirty minutes after escaping, they were killed with 2% osmic acid and stained with anilin safranin and gentian violet. The blepharoplast is readily distinguished, extending forward from the nuclear portion and bearing close behind its tip a pair of cilia, which are somewhat longer than the entire body of the sperm. The blepharoplast is nearly straight, but the nuclear part is curved and forms a little more than one complete turn. The blepharoplast cannot be traced back along the side of the nuclear portion, but a distinct line extends beyond, which, together with the posterior end of the nucleus, encloses a small vesicle of apparently homogeneous cytoplasm. On comparing Fig. 24 with Figs. 17 to 20, we can readily see that this line

around the vesicle may be the posterior end of the blepharoplast; however, this is only a conjecture and cannot be proved from the material thus far observed. The mature sperm, then, consists of a nuclear portion which makes up the main body, a blepharoplast bearing two cilia, and a small cytoplasmic vesicle. The evidence so far brought to light on *Porella* favours the view that the blepharoplast and cilia represent specialized cytoplasmic material, which is first distinguishable in the origin of the blepharoplast, as a spherical granule or body, in the cytoplasm of the sperm cell; that it represents, as suggested by Mottier ('04), individualized parts of the kinoplasm which arise *de novo* in the spermogenous cells.

As already stated, the sperm cell of *Marchantia* does not contain a centrosome-like body which has persisted throughout the last division of the spermogenous tissue. However, it is possible at almost any stage occasionally to locate granules or bodies within the cytoplasm. But it is only after the last division of the spermogenous cells that any body or granular collection can be shown to persist as an individual throughout the subsequent metamorphosis of the resulting sperm cell, and to become a part of the mature sperm. This has already been described for *Porella*, which, on account of the slow development of the sperm, gives better opportunity for the observance of a complete series of stages.

Soon after the last division in *Marchantia* a globular or slightly elongated body appears in one point of the sperm cell (Figs. 38, 39, and 40). This body, as in *Porella*, is the beginning of the blepharoplast, and in appearance and subsequent behaviour is practically the same. As it proceeds around the side of the cell, denser areas, or aggregations, of cytoplasm evidently contribute to its growth (Fig. 43). The direction which the blepharoplast takes around the cell is shown by comparing Figs. 41, 42, and 43. In Fig. 41 it comes around next to the observer, while in Fig. 42 it goes behind the nucleus. When the pairs of sperm cells appear as shown in Fig. 41, the blepharoplast does not usually extend around the edge, but more or less next to, or away from, the observer. The mature sperm is quite similar to that of *Porella*, except that it is relatively thicker and shorter and not so much coiled. The cytoplasmic vesicle attached to the posterior end is more distinct. The greater rapidity with which the sperm of *Marchantia* develops would probably account for all of these differences.

Just after the oblique division in *Fegatella*, we find the daughter nuclei lying in a more or less granular cytoplasm, which does not immediately divide (Figs. 55 and 56). The latter figure shows a number of granules in the cytoplasm, but it would be entirely arbitrary to select any particular one as the future blepharoplast, or as the body which occupied the pole of the last spindle. A little later, however, when the two sperm cells have separated, a dense body similar to the one found in *Marchantia* or *Porella*, at first spherical then slightly elongated, appears in one angle of the cell

(Fig. 57). Fig. 58, which is a side view, shows this body developing as a cord along one edge of the cell. So far as stages were observed, the blepharoplast developed and the nucleus changed in shape and structure as in *Porella* and *Marchantia*. Fig. 59 A compares very favourably with Figs. 18, 19, and 20 of *Porella*, although the blepharoplast cannot be traced throughout its entire course. The rapidity of development, however, compares with that of *Marchantia*. The mature sperm (Fig. 60) is somewhat larger than that of *Marchantia* (Fig. 44), otherwise they appear quite similar. The cilia arise from nearer the tip of the blepharoplast in *Marchantia* and *Fegatella* than in *Porella* (compare Figs. 44 and 60 with Fig. 24). The cytoplasmic vesicles in the two former differ slightly from the latter, and all three differ in size ; but the general form and structure is the same in all three plants.

Conclusions.

The evidence furnished by these studies seems to warrant certain conclusions. In the first place there is no evidence that centrosomes occur in the spermogenous tissue of *Porella* and *Asterella*, or in *Marchantia* and *Fegatella*, as has been claimed by certain investigators. When occasional bodies are found in the cytoplasm or the region of the spindle they do not present the behaviour or appearance of centrosomes. Miss Black found the same conditions to prevail in *Riccia Frostii*, as did also Escoyez in *Marchantia*.

During the last division of the spermogenous tissue of *Marchantia* and *Fegatella*, which results in the formation of the sperm cells, a darkly staining body, as a rule, occupies each pole of the spindle. There is no evidence leading one to believe that this body is of other than cytoplasmic (kinoplasmic) origin. There are no indications that it arises in the nucleus. No radiations except the spindle fibres extend from this body. Van Hook ('00) found centrosomes with profuse radiations extending into the cytoplasm in dividing vegetative cells of *Marchantia*. I compared my slides with those of Prof. Van Hook, but found that my preparations of the spermogenous tissue showed no figures similar to his of the vegetative tissue. I also compared my slides with those of Prof. Mottier showing centrosomes in *Dictyota*, but found in mine no similar structures. No figures similar to those found by Farmer ('95) in the germinating spores of *Fegatella* and *Pellia*, and by Farmer and Reeves ('94) in *Pellia*, were found.

Furthermore, there is no conclusive evidence that this body, which occupies the poles of the diagonal spindle, persists as an individual in the resulting sperm cell. No genetic continuity can be traced throughout even two cell generations. Whatever may be the nature of this body, its appearance and behaviour do not agree with that of a true centrosome in those places where this latter organ is found.

The fact that a body does sometimes occupy the pole of the spindle does not necessarily imply anything more than a probable concentration of cytoplasmic or kinoplasmic materials.

The development of the blepharoplast in all these plants proceeds from a dense granular mass or spherical body, located usually in the most distant angle of the sperm cell. Evidence so far obtained indicates its origin as a condensation or aggregation of cytoplasmic material. The blepharoplast becomes a cord, growing in close contact with the plasma membrane and entirely past the nucleus. The latter then draws out along the posterior portion of the blepharoplast and may extend beyond it, while from near the forward and slightly enlarged end two cilia develop. My observations lead me to believe that the 'Cytoplasmatischer Fortsatz' of Ikeno is merely a part of the blepharoplast.

No body corresponding in size and appearance to the 'Nebenkörper' of Ikeno was found.

According to some authors, the mature sperm represents three metamorphosed elements of the cell, each of morphological rank, namely, the nucleus, the cytoplasm, and the blepharoplast or centrosome; the centrosome and blepharoplast being held by some to be homologous. In the light of my observations I believe that no centrosome exists in *Porella*, *Marchantia*, or *Fegatella*, and that the blepharoplast arises *de novo* in the cell in which it is to function as the cilia-bearer. Escoyez takes the same view, save that he describes the blepharoplast as originating in the spermogenous tissue just previous to the last division, and occupying the pole of the diagonal spindle. These considerations tend to confirm the view expressed by Mottier ('04), that the fundamental substance known as kinoplasm possesses genetic continuity, and that the blepharoplast represents individualized parts of the kinoplasm arising *de novo* in certain spermogenous cells. We cannot at all times, by present cytological methods, differentiate the kinoplasmic part of the cytoplasm.

We may thus consider the mature sperm to represent the two constant cell elements, nucleus and cytoplasm; the main body, or the nuclear portion, representing the nucleus, the blepharoplast and cilia representing specialized parts of the cytoplasm, and the remainder of the latter being found in the cytoplasmic vesicle.

These studies were carried out under the direction of Professor D. M. Mottier, to whom I wish to express my thanks for his kindly assistance and for his valuable and timely criticisms.

Indiana University,
August 31, 1910.

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EXPLANATION OF FIGURES IN PLATE XXV.

Illustrating Mr. Woodburn's paper on Spermatogenesis in Hepaticae.

All figures were drawn with the aid of a camera lucida and with a Leitz apochromatic 2 mm. objective, 1.30 apert. with compensating ocular 18. Magnification 3000 at table level.

Figs. 1–24. *Porella.* (Figs. 1–11 previous to the last division of the spermogenous tissue.)

Fig. 1. Cytoplasm and nucleus in young antheridium. Nucleus in resting stage.
Fig. 2. Nucleus in resting stage.
Fig. 3. Cell in very young anteridium. Nucleus in early prophase.
Figs. 4–6. Nuclei in various stages of prophase.
Fig. 7. Cell showing number of chromosomes.
Fig. 8. Metakinesis in young antheridium.
Fig. 9. Anaphase.
Fig. 10. Telophase.
Fig. 11. Two cells shortly before entering upon last division.
Fig. 12. A pair of sperm cells without the enclosing cell-wall.
Fig. 13. A pair of sperm cells enclosed in cell-wall. Seen from the edge.
Fig. 14. One sperm cell, seen from the side, showing blepharoplast primordium.
Fig. 15. Sperm cell. Blepharoplast beginning to develop in length.
Fig. 16. Blepharoplast slightly longer than in Fig. 15.
Fig. 17. Blepharoplast half-way around the sperm cell.
Fig. 18. A pair of developing sperms, showing blepharoplast.
Figs. 19 and 20. Showing nucleus becoming more homogeneous than in Fig. 18.
Fig. 21. Nucleus beginning to draw out along the blepharoplast.
Figs. 22 and 23. Later stages of the sperm with the cilia partly developed.
Fig. 24. Mature sperm escaped from the antheridium.

Figs. 25–44. *Marchantia.* (Figs. 25 A to 28 A are drawn from spermogenous tissue.)

Fig. 25 A. Spermogenous cell in resting condition.

Fig. 25. Chromatin in a central mass. Nuclear cavity very finely granular or filled with a homogenous substance.

Fig. 26. Nuclear cavity as in Fig. 25. Chromatin in two or three lumps.

Fig. 27. Nuclear cavity a little more coarsely granular. Chromatin nearly ready for the formation of chromosomes. Cytoplasm unevenly granular.

Figs. 28 and 28 A. Spindle stages.

Fig. 29. Just previous to the last division. Cytoplasm containing two dense bodies.

Fig. 30. The same stage as 29, but more bodies in the cytoplasm.

Figs. 31 and 32. Formation of the oblique spindle with the cytoplasmic bodies occupying the poles of the spindle.

Figs. 33 and 34. Spindle stages. Chromosomes in equatorial plate.

Fig. 35. Anaphase. Bodies at poles disappearing.

Figs. 36 and 37. Anaphase and telophase, bodies at poles having disappeared or nearly so.

Fig. 38. A pair of sperm cells. One showing blepharoplast primordium.

Figs. 39 and 40. Single sperm cells. Blepharoplast beginning to lengthen.

Fig. 41. Two antheridial cells containing respectively a pair of sperm cells seen from the edge.

Figs. 42 and 43. Single sperm cells showing the course of the blepharoplast. The stage is about the same as in Fig. 41, but from a different view.

Fig. 44. Mature sperm after escaping from the antheridium.

Figs. 45–60. *Fegatella.* (Figs. 45–52. Before the diagonal division.)

Fig. 45. Nucleus of a cell in resting condition.

Fig. 46. Cell with nucleus in early prophase.

Fig. 47. Later prophase. Chromatin forming an irregular spireme of a double nature.

Fig. 48. Spireme about as in Fig. 47. Cytoplasm showing indications of spindle formation.

Fig. 49. Showing number of chromosomes.

Figs. 50 and 51. Spindle stages. Chromosomes in equatorial plate.

Fig. 52. Telophase.

Fig. 53. Formation of spindle in oblique division.

Fig. 53 A. Oblique division. Equatorial plate and bodies at poles.

Fig. 54. Anaphase. No definite bodies persisting in region of poles.

Fig. 55. Pair of sperm cells. Blepharoplast cannot be identified.

Fig. 56. Pair of sperm cells. Numerous granules scattered throughout cytoplasm.

Fig. 57. Blepharoplast primordia. Sperm cells seen from the edge.

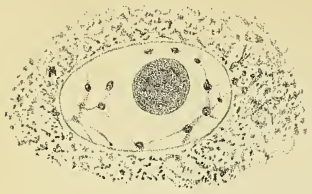
Fig. 58. One sperm cell seen from the side. Blepharoplast beginning to lengthen.

Fig. 59. A pair of sperm cells. Blepharoplast much elongated and nuclei drawing out along the rear portion of the blepharoplast.

Fig. 59 A. Somewhat later than Fig. 59. The blepharoplast disappears behind the nucleus.

Fig. 60. A mature sperm after escaping from the antheridium.

Figs. 61 and 62. Two spindles from spermogenous tissue of *Asterella.*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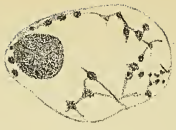
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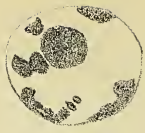
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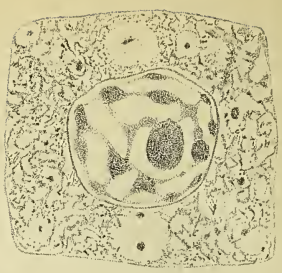
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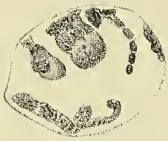
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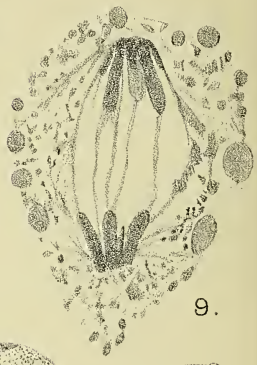
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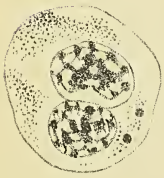
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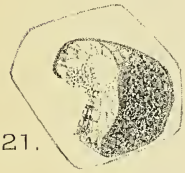
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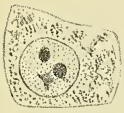
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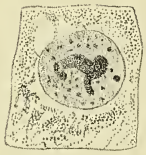
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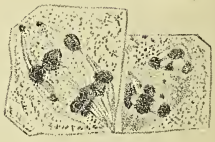
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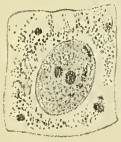
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WOODBURN — SPERMATOGENESIS IN HEPATICAE.

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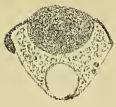

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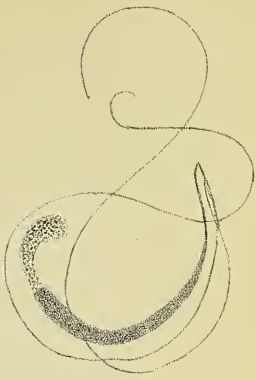

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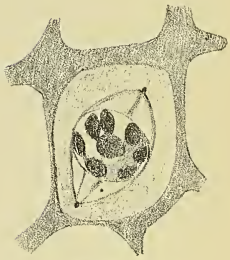

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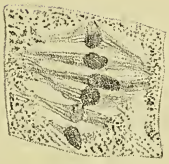

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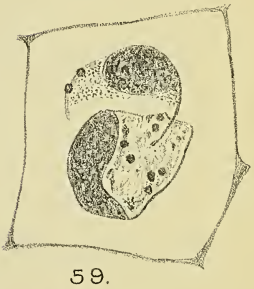

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Huth, lith et imp

A Cretaceous Pityoxylon with Marginal Tracheides.[1]

BY

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With Plate XXVI.

IN their description of succiniferous *Pityoxyla* from the Cretaceous of Staten Island and Scituate, Jeffrey and Chrysler called attention to the fact that the wood of the pinelike Conifers of the Mesozoic, unlike that of the modern members of the genus, is characterized by the absence of ray tracheides. This condition was considered ancestral, inasmuch as among living pines ray tracheides do not occur in regions of phylogenetic significance, namely, the cone axis and the earliest formed wood of the stem. It was further inferred by the writers that the evolution of these structures, occurring in all probability in the Tertiary, explains the greater development of the genus in recent geological times.

In view of this well-marked peculiarity of the pines which antedate the Tertiary, the occurrence of ray tracheides in a *Pityoxylon* from the Upper Cretaceous of Morgans, New Jersey, specimens of which have been submitted to me for investigation, is of interest in affording a connecting link between modern pines and their Mesozoic ancestors.

Anatomical Structure of the Specimen.

The material in the form of lignite consisted of one large fragment, about 30 cm. long, 10 cm. wide, and 8 cm. thick, and several smaller pieces. The former, the core of a larger stem which had been flattened by lateral pressure, exhibited in selected areas an admirable state of preservation. A small lateral branch, which fortunately possessed a well-preserved medulla and brachyblasts or short shoots, was embedded in this fragment, and together with the other anatomical characters present in the lignite affords conclusive evidence of the affinities of the specimen.

Fig. 1, Pl. XXVI, shows the structural features of the larger fragment in transverse section. The annual rings are distinct and conspicuous, as is the

[1] Contributions from the Phanerogamic Laboratories of Harvard University, No. 41.

[2] Jeffrey, E. C., and Chrysler, M. A. : On Cretaceous *Pityoxyla*. Bot. Gaz., vol. xlii, July, 1906, pp. 1–15.

case in living pines. The abrupt transition from small thick-walled summer tracheides of one annual ring to the larger thin-walled spring tracheides of the succeeding ring is clearly shown at the base of the figure, and the more gradual transition from spring to summer tracheides which takes place during the year's growth may be seen in the annual ring in the central portion of the photomicrograph. The resin canals, which are occluded by numerous tyloses, are surrounded by large masses of thick-walled and highly resinous parenchyma cells, a feature which is without parallel among living pines, but which greatly resembles the condition found by Jeffrey and Chrysler in *Pityoxylon scituatense.*

Fig. 2 shows a resin canal under higher magnification, and illustrates more clearly the thick-walled character of the parenchyma cells and their dark resinous contents.

In these photomicrographs it may be noted that the wood rays contain the resinous substance, and that cells adjacent to them are frequently filled with it. The latter, however, are not wood parenchyma, since they possess bordered pits, but are tracheides into which a portion of the resinous contents of the adjacent ray parenchyma cells has been poured. That this is the case is clearly shown by longitudinal sections, in which the resinous matter may be found passing through the ray pits into the neighbouring tracheides. This appears to take place by means of tyloses more or less completely filled with resinous secretions. Chrysler [1] in his study of the occurrence of tyloses in the tracheides of Conifers came to the conclusion that they occurred only in *Pinus*, in the heartwood of the root and in the cone axis. Since these regions are often the seat of ancestral or primitive characters, the occurrence of tyloses in the stem of a Cretaceous *Pityoxylon* seems to indicate that these structures are ancestral, and occur only in primitive regions of modern pines. This conclusion, however, is not entirely substantiated by a study of the stem wood of living pines, as I have found indications of tyloses in the tracheides of several species. Fig. 5, a transverse section, illustrates the occurrence of tyloses in the stem wood of *Pinus Strobus*, L.

Fig. 3 shows the tangential section of the lignite under a low magnification. The rays are obviously of two kinds, linear and fusiform, which are characteristic of *Pityoxylon*, Kraus. The fusiform rays are seen to contain horizontal resin canals, occluded by numerous tyloses. The highly resinous character of the ray parenchyma is clearly shown in this section, and it may be noted that the walls of the tracheides have been crushed together by lateral pressure, whereas the ray parenchyma cells have retained their natural form. This condition is probably accounted for by the resinous contents of the rays.

[1] Chrysler, M. A.: Tyloses in the tracheides of Conifers. The New Phytologist, vol. vii, No. 8.

Fig. 4 shows the tangential section under higher magnification, and illustrates with greater distinctness a portion of the structures shown in Fig. 3. It may be noted that the marginal cells of the linear ray in the central portion of the figure are narrower and devoid of resinous contents. Similarly, two cells of the next ray to the left are of small size and without the dark-coloured substance which fills the ray parenchyma cells. This is in marked contrast to the other Cretaceous *Pityoxyla*, especially *Pityoxylon statenense* and *P. scituatense* of Jeffrey and Chrysler, in which the marginal cells are not noticeably narrower than the central cells of the ray. In radial section the cells are seen to be ray tracheides, *since bordered pits occur between adjoining cells of this form, and half-bordered pits between these cells and the succiniferous ray parenchyma cells.*

Fig. 6 shows in radial section a linear ray composed of succiniferous ray parenchyma and ray tracheides. The latter, which occur in the middle of the ray, are noticeably narrower than the ray parenchyma cells, and possess bordered pits between their end walls. The lateral pits of the ray parenchyma cells are seen with difficulty in this photomicrograph owing to the resinous character of the cells, but are narrow lenticular openings, with circular borders on the tracheide side. This type of pit, which occurs characteristically in all Abietineous Conifers with the exception of certain large pitted and highly specialized modern pines (see Figs. 11 and 12), has been conveniently called 'piciform'. In the *Pityoxylon* under consideration in this article, the pits, although variable in size and somewhat larger than the most primitive type (see Fig. 10), are uniformly distributed, one to each crossing field. Fig. 7, a radial section of the root wood of *Pinus Taeda*, L., illustrates pits of similar size, shape, and structure, and in the central portion of the photomicrograph the distribution, one pit to each crossing field, is also similar.

The sclerified condition of the pith is shown in Fig. 8, a longitudinal section of the small branch found embedded in the larger fragment of lignite. The clusters of sclerenchymatous cells are arranged in more or less well-defined horizontal bands.

Fig. 9 shows a cross section, cut near the base of one of the brachyblasts or short shoots. Clusters of sclerenchymatous cells, such as occur in the pith of the branch, are also present in the medullary tissue at the base of these structures. The short shoots are thicker and shorter than those of most species of living pines.

The Identification of Coniferous Woods.

With this consideration of the salient anatomical characters of the lignite, we may turn to a consideration of the affinities of the specimen. One of the earliest scientific classifications of coniferous woods, and one which in its main features has been adopted by most modern anatomists

and palaeobotanists, was advocated by Kraus,[1] and divides the Conifers into five distinct groups or 'genera', separated by marked structural differences. The 'genus' *Araucarioxylon*, framed to include the Araucarian Conifers, of which *Agathis* and *Araucaria* are living representatives, possesses closely approximated and mutually flattened bordered pits in the radial walls of the tracheides, pits which when in more than one row alternate with one another. The remaining Conifers in contrast to this possess unflattened and non-alternating or opposite pits. Of the four genera which occur in this general group, the most distinctive is *Pitoxylon*, which possesses resin canals and is represented among living Conifers by *Pinus*, *Picea*, *Larix*, and *Pseudotsuga*. *Taxoxylon*, with *Taxus* for its type, possesses throughout the year's growth tracheides with well-developed tertiary spiral thickenings. *Cedroxylon*, represented by *Cedrus* and allied forms, is separated less easily from *Cupressoxylon* (*Cupressinoxylon*), which includes the Cupressineae and Taxodineae, &c., but in contrast to the latter is characterized by the absence or feeble development of resin parenchyma.

According to the classification of Kraus, our lignite would undoubtedly be classified under *Pityoxylon* since it possesses well-developed resin canals.

The 'genera' of Kraus have been somewhat modified by Gothan[2] who points out that the distribution of resin parenchyma is not a satisfactory criterion upon which to separate *Cedroxylon* and *Cupressinoxylon*, since abundant resin parenchyma occurs in certain forms commonly referred to the former. He asserts that the Abietineae possess a distinctive type of pitting (*Abietineentüpfelung*) which he uses as a diagnostic character in separating the two 'genera'. The latter 'genus' is divided by him into five divisions upon the basis of differences in ray pitting. Similarly, *Pityoxylon* is divided into *Pinuxylon*, to which are assigned the ligneous characters of living pines, and *Piceoxylon*, which includes *Picea*, *Larix*, *Pseudotsuga*, and similar forms.

The classification of coniferous woods as originally framed by Kraus, and as modified by Gothan and others, affords from the point of view of the anatomy of modern representatives a satisfactory basis for dividing Conifers into natural groups of closely related forms. In addition it has been customary in the past to study the affinities of the ligneous remains of primitive Conifers by similarity of structure with the genera of Kraus. Thus the absence of so-called Araucarian pitting has been considered a sufficient criterion for excluding relationship with the Araucarian Conifers. The occurrence of heavily pitted ray parenchyma and resin canals has been taken as conclusive evidence of relationship with the Abietineae, &c.

[1] Kraus, G., in Schimper's Traité de paléontologie végétale, Bd. v, pp. 363–85.

[2] Gothan, W.: Zur Anatomie lebender und fossiler Gymnospermen-Hölzer. Abhandl. der königl. preussisch. geolog. Landesanstalt, Neue Folge, Heft 44, Berlin, 1905, pp. 101–103.

However, a review of recent investigation upon the coniferous remains of the American Cretaceous, and a study of the comparative anatomy and phylogeny of modern and fossil Conifers, leads to the conclusion that in the identification of the fossil remains of primitive Conifers, the structural characters which separate the 'genera' of Kraus are not of constant diagnostic value, and may be utilized only in connexion with numerous other anatomical features in the study of the affinities of ancestral forms.

Thus, for example, the Araucarineae, which have been considered an isolated group of Conifers,[1] are represented in the American Mesozoic by forms which possess well-developed Abietineous structures. *Brachyoxylon*, described by Hollick and Jeffrey,[2] from the Cretaceous deposits of Kreischerville, comprises several genera of Araucarian Conifers which differ from modern members of the family in the scarcity of alternating pitting and in the presence of numerous traumatic resin canals. *Araucariopitys*,[3] although an undoubted Araucarian Conifer, approaches even more closely to the structures of the Abietineae, since it possesses short shoots, thick-walled and heavily pitted ray parenchyma (*Abietineentüpfelung*), scarcity of alternate pitting, and traumatic resin canals. More recently Sinnott has described[4] an Araucarian Conifer from the Cretaceous of Scituate, Mass., in which the bordered pits of the radial walls of the tracheides occur in a single row, and only infrequently are the pits flattened somewhat by mutual contact. Evidently there exists then a strong similarity between the ligneous characters of the older Araucarian Conifers and the Abietineae.

The difficulty in separating the wood of living forms included under *Cedroxylon* and *Cupressinoxylon* has been referred to above. We are not able to follow Gothan, who separates the genera upon the basis of ray pitting, since his so-called 'Abietineentüpfelung', which occurs in the primitive Araucarians, is nearly identical to the ray pitting which exists in certain specimens of *Juniperus*, *Libocedrus*, and *Cupressus* which have recently come under my observation. Furthermore, there is strong evidence for believing that the older Abieteae, Taxodineae, and Cupressineae possessed ligneous characters which resembled those of *Pityoxylon*. The well-known occurrence of resin canals in *Sequoia* and the Abieteae, in regions which reflect ancestral characters, points strongly in this direction. Similarly, the

[1] Seward, A. C., and Ford, S. O.: The Araucarineae, recent and extinct. Phil. Trans. Roy. Soc., London, B. 198, 305–411, 1900, pp. 23, 24.

[2] Hollick, A., and Jeffrey, E. C.: Affinities of certain plant remains commonly referred to the genera *Dammara* and *Brachyphyllum*. Am. Nat., vol. xl, 1906, pp. 189–215. Studies of Cretaceous Coniferous remains from Kreischerville, New York. Mem. of the N. Y. Bot. Gardens, vol. iii, 1909.

[3] Jeffrey, E. C.: *Araucariopitys*, a new genus of Araucarians. Bot. Gaz., vol. xliv, 1907, pp. 435–44.

[4] Sinnot, E. W.: *Paracedroxylon*, a new type of Araucarian wood. Rhodora, vol. ii, No. 129, Sept., 1909.

sporadic occurrence of ray tracheides which has been noted by De Bary, Penhallow, and Gothan in certain Taxodineae and Cupressineae leads to the same conclusion as does the traumatic occurrence of ray tracheides which Jeffrey has described in *Cunninghamia sinensis.*

From this we see that a careful consideration of the comparative anatomy and phylogeny of living and ancestral Conifers is necessary in studying the affinities of the remains of primitive Conifers; that the anatomical features which characterize the genera of Kraus are not of constant diagnostic value in separating Conifers into natural groups of closely related species, and that in many cases a large number of anatomical characters must be considered in order to make an accurate identification of the remains of primitive forms.

Before attempting to classify our fossil, therefore, it seems to be necessary to point out certain anatomical lines of evolution which are very significant and have been made clear by recent researches in the comparative anatomy of living Conifers. Primitive Araucarian and Abietineous Conifers, which strongly resembled one another, were characterized by the entire absence of wood parenchyma except where associated with resin canals, and by the presence of resin canals. In these forms the rays were characterized by the absence of ray tracheides, by thick, usually heavily pitted, ray parenchyma walls, and by small lateral ray pits with distinct borders upon the tracheide side. The disappearance of resin canals, the development of wood parenchyma, the reduction in the thickness of the ray parenchyma walls, the development of 'Eiporen' or non-piciform lateral ray pits, and the development and subsequent loss of ray tracheides are all lines of evolutionary modification in the development of modern Conifers.

Lines of evolution which appear significant in the Araucarian Conifers are—the disappearance of resin canals and thick-walled, heavily pitted ray parenchyma, and the development of wood parenchyma.[1] In the case of the Taxodineae and Cupressineae similar lines of phylogenetic interest exist—the disappearance of resin canals, the development of wood parenchyma, and the reduction in thickness of the ray parenchyma walls; but to these must be added the loss of ray tracheides, structures which have never been observed in Araucarian Conifers.

The woody structure of modern Abietineous Conifers has been evolved from that of their Mesozoic ancestors by the development of ray tracheides, by the development of wood parenchyma, except in *Pinus*, and by the disappearance in the Abieteae of resin canals.

In the evolution of modern White and Hard Pines certain highly

[1] In regard to the alternate and flattened bordered pits of modern Araucarians which are strongly contrasted to the more Abietineous type of pitting which occurs in Cretaceous genera, it seems to be impossible to determine in the light of present investigation whether this type of pitting is really homologous or merely analogous to that of the *Cordaites.*

specialized structures have been evolved. Thus modern Hard Pines are characterized by the absence of tangential pitting in the summer tracheides, and by possessing ray tracheides with dentate and reticulate thickenings, structures which occur only in these pines. Recently the writer has called attention[1] to the evolution in modern Hard and White Pines of large irregular lateral ray pits or 'Grosseiporen' (see Figs. 11 and 12) from piciform pits (see Fig. 10) such as occur in certain primitive living pines (the Nut and Foxtail Pines of the south-western United States, *Pinus Bungeana*, Zucc., and *P. Gerardiana*, Wallich, of Asia) and Cretaceous pines. The 'Grosseiporen' were shown to be formed by the fusion of several small piciform pits into a single large pit. This fusion takes place either by the enlargement of the lenticular openings of the piciform pits and their subsequent fusion as is shown in Figs. 7 and 11, or by the gradual reduction in thickness of a large portion of the tracheide and parenchyma walls. In the latter case the lignified secondary wall of the tracheide often falls away first, leaving the ghost-like pit partition intact on the parenchyma side. This condition is illustrated in Fig. 12, a radial section of *Pinus flexilis*, James. The development of 'Grosseiporen' or fusion pits does not appear to the writer a character of great diagnostic importance, since it is a character which is likely to be evolved in all Conifers in which there is a reduction in the thickness of the ray parenchyma walls. In defence of this statement it is only necessary to call attention to the occurrence of fusion pits or 'Eiporen' in certain Podocarpineae, notably in *Dacrydium* and in certain ligneous remains referred to *Araucarioxylon latisporosum* by Kraus and Conwentz,[2] to *Cupressinoxylon Barberi* by Seward,[3] and to *Xenoxylon phyllocladoïdes* by Gothan.[4]

If we now turn to the lignite under consideration in this article we see that the mere presence of resin canals, of thick-walled, heavily pitted ray parenchyma (*Abietineentüpfelung*), of piciform lateral ray pits, and the absence of wood parenchyma and so-called Araucarian pitting are not in themselves sufficient evidence for inferring that we have not to deal with a primitive member of the Araucarineae, Abieteae, Taxodineae, or Cupressineae. The occurrence of ray tracheides appears, however, to exclude the Araucarineae, since these structures have never been observed in living or fossil forms of this family. Furthermore, the occurrence of bars of Sanio between the radial bordered pits in the tracheides of our Conifer confirms this supposition. Miss Gerry has made a careful study of the distribution

[1] Bailey, I. W.: Anatomical characters in the evolution of *Pinus*. Am. Nat., vol. xliv., May, 1910.

[2] Fossile Hölzer aus der Sammlung der königl. preuss. geolog. Landesanstalt, 1882, p. 170.

[3] Jurassic Flora, pt. ii, 1904, p. 61, t. vii, Figs. 1, 4, 6.

[4] Fossile Hölzer aus dem Bathonien Russ. Polens. Verhandl. kais. russ. mineral. Gesellsch., 1906, p. 454. Die fossilen Hölzer von König Karls Land. Kungl. Svenska Vetenskaps-akademiens Handl., Bd. 42, No. 10, 1907.

of these structures [1] in the Coniferales, and although unable to discover their presence in living or fossil Araucarians, noted their presence in all other genera of living Conifers, and in a number of fossil Abietineous forms, including *Prepinus*.

The short shoots, which are a very characteristic feature of our lignite, indicate that the material is the stem of a pine-like Conifer, since these structures occur in Conifers only among pine-like forms and primitive Araucarians. Furthermore, the distribution, structure, and general appearance of the ligneous characters strongly resembles that of living and Cretaceous pines, and is quite unlike that of other Abietineous genera. It appears to be quite evident that we have to deal with a primitive pine-like Conifer, and it is therefore necessary that we should compare in greater detail the structures of the lignite with those which occur in other living and Cretaceous pines. As has been pointed out earlier in this article, this pine possesses the highly resinous ray parenchyma which is characteristic of *Pityoxylon statenense* and *P. scituatense*, and resembles the latter in possessing large masses of resinous parenchyma associated with the resin canals. It likewise possesses the primitive thick-walled ray parenchyma and piciform pits which are present in Cretaceous pines, including the very primitive *Prepinus*, and in the living Nut and Foxtail Pines of the southwestern United States. It is, however, distinct from other Mesozoic pines in possessing well-developed ray tracheides, which are present, however, in all living pines. Thus we see that in its anatomical characters our lignite appears to occupy an intermediate position between *Pityoxylon scituatense* and *Pinus edulis*, Engelm., since it possesses the highly resinous parenchyma and abundant epithelium of the former, combined with the ray tracheides of the latter, and resembles both species in possessing numerous tangential bordered pits in the summer wood, and thick-walled ray parenchyma with piciform lateral ray pits.

Owing to the strong similarity between the woody structure of the *Pityoxylon* and that of certain primitive living pines, the American Nut and Foxtail Pines, we propose to follow the precedent set by Conwentz [2] in naming the succiniferous remains of Baltic *Pityoxyla*, and refer the lignite to the genus *Pinus*. We appear to be justified in this course, particularly as the foliar structure of the pines of the Upper Cretaceous, as shown by Stopes and Kershaw,[3] is similar to that of living pines. The leaves of pines in the Lower Cretaceous, as has been shown by Jeffrey,[4] differed from modern pines in the probable absence of an endoderm and in possessing a double transfusionary sheath. We therefore suggest for our fossil the

[1] Gerry, E.: Bars of Sanio in Coniferales. Annals of Bot., vol. xxiv, No. 93, Jan., 1910.

[2] Conwentz, H.: Monog. d. balt. Bernsteinbäume. Danzig, 1890.

[3] Stopes, M. C., and Kershaw, E. M. Annals of Bot., vol. xxix, No. 94, April, 1910.

[4] Jeffrey, E. C.: On the structure of the leaf in Cretaceous pines. Annals of Bot., vol. xxii, No. 86, April, 1908.

name *Pinus scituatensiformis*, since it possesses highly resinous ray parenchyma and abundant epithelium about its resin canals, characters which occur in *Pityoxylon scituatense.*

Conclusions.

The occurrence of ray tracheides in this Mesozoic Conifer throws interesting light upon the origin and phylogeny of these structures. As has been mentioned above, Jeffrey and Chrysler have pointed out that ray tracheides do not occur in Lower Cretaceous pines, nor in the primitive regions of modern pines, namely, the cone axis and the first-formed wood of the stem. From this they inferred that ray tracheides are of comparatively modern origin, and that in all probability they were evolved early in the Tertiary. The distribution of ray tracheides in our lignite confirms these writers in their conclusion that the absence of ray tracheides is a primitive condition, but shows that these structures were evolved during the latter part of the Cretaceous rather than at the beginning of the Tertiary. This is shown by the fact that ray tracheides are feebly developed even in the older wood of the stem, and do not occur during the first ten to fifteen annual rings. Among modern pines ray tracheides are often absent or feebly developed in the first few years' growth of the stem, but the condition which we have described is a more primitive one and indicates the recent origin of ray tracheides in the plant under consideration in this article.

There are two theories which have been advanced recently to explain the origin of ray tracheides. Penhallow[1] holds the view that these structures, at least in *Pinus*, have been formed from ray parenchyma cells by the thickening of their walls. The transition from thin- to thick-walled parenchyma, which occurs in certain pines, culminates according to this writer in the formation of ray tracheides. We are not able to follow Professor Penhallow in this supposition, since, as has been shown by the writer, thick-walled ray parenchyma is the primitive condition in *Pinus*, and the gradations from thick-walled to thin-walled parenchyma occur in pines which are becoming specialized by the reduction in thickness of the parenchyma walls and by the formation of 'Eiporen', which is a natural concomitant of the process.

Thompson[2] has more recently advocated the theory that ray tracheides were originally derived from short tracheides which have assumed a horizontal position parallel to the axis of the ray. His investigations, however, have been confined to *Pinus resinosa*, Sol., and *P. Strobus*, two perhaps of the most highly specialized and most modern pines. Among the more

[1] Penhallow, D. P.: A manual of the North American Gymnosperms, ch. vi. Ginn & Co., Boston, 1907.

[2] Thompson, W. P.: The origin of ray tracheides in the Coniferae. Bot. Gaz., vol. 50, No. 2, Aug., 1910, pp. 101–16.

primitive Hard and White Pines, and the American Nut and Foxtail Pines, and the very primitive pine under consideration in this article the writer has been unable to find conclusive evidence in support of this theory. In many specimens of stem wood, conditions similar to those figured by Thompson were observed, but occurred invariably where the wood was of slow growth and twisted grain, and the tracheides in consequence of irregular shape. Root wood, as is well known, possesses an extremely short and twisted 'fibre' which may account for the irregularly shaped tracheides found by Thompson in the root of *Pinus Strobus* and *P. resinosa*.

Summary.

1. In the identification of the remains of primitive Conifers the absence of alternate pitting, wood parenchyma, or 'Eiporen', or the presence of resin canals or 'Abietineentüpfelung' are insufficient data in determining whether we have to deal with a primitive member of the Araucarineae, Abietineae, or Cupressineae.

2. The fact that bars of Sanio and ray tracheides are well-developed features of the lignite under consideration in this article indicates that we are not concerned with an Araucarian Conifer.

3. The presence of short shoots and the general pine-like appearance of the fossil indicate strongly that the specimen is a primitive member of the genus *Pinus*.

4. It is intermediate in structure between the older Cretaceous pines and the most primitive of living pines.

5. It affords additional evidence that primitive pines possessed thick-walled ray parenchyma with piciform lateral ray pits, abundant tangential pitting in the summer tracheides, and highly resinous ray parenchyma.

6. The somewhat infrequent occurrence of ray tracheides in the older portions of the stem and their entire absence from the younger wood indicate that these structures are of recent origin and are not strongly fixed upon the plant.

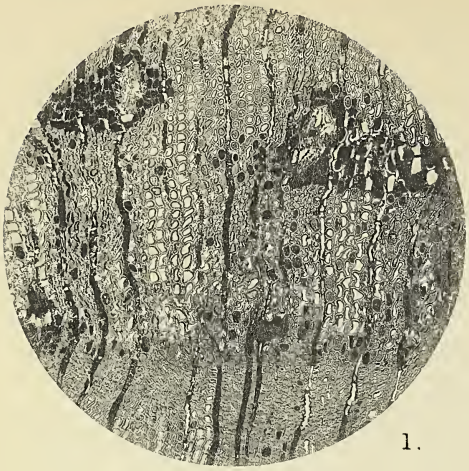
7. The occurrence of ray tracheides in this Cretaceous pine indicates that the development of these structures occurred in the Upper Cretaceous, and not, as has been supposed, in the Tertiary.

This investigation has been conducted in the Phanerogamic Laboratories of Harvard University, and to Professor Jeffrey the writer is indebted for material and kind assistance in the course of the work. To Professor Conwentz the writer is much indebted for his kindness in sending valuable material of the Baltic *Pinus succinifera*, and to Professor Jack of the Arnold Arboretum for material of Asiatic pines.

DESCRIPTION OF PLATE XXVI.

Illustrating Mr. Bailey's paper on a Cretaceous *Pityoxylon*.

Fig. 1. *Pinus scituatensiformis*: transverse section of stem wood. × 60.
Fig. 2. The same: higher magnification. × 100.
Fig. 3. The same: tangential longitudinal section of stem wood. × 60.
Fig. 4. The same: higher magnification. × 100.
Fig. 5. *Pinus Strobus*: transverse section of the stem wood. × 300.
Fig. 6. *Pinus scituatensiformis*: radial section of stem wood. × 500.
Fig. 7. *Pinus Taeda*: radial section of root wood. × 300.
Fig. 8. *Pinus scituatensiformis*: longitudinal section of the pith. × 60.
Fig. 9. The same: transverse section of the base of a short shoot. × 40.
Fig. 10. *Pinus Balfouriana*: radial section of stem wood. × 500.
Fig. 11. *Pinus palustris*: radial section of stem wood. × 500.
Fig. 12. *Pinus flexilis*: radial section of stem wood. × 300.



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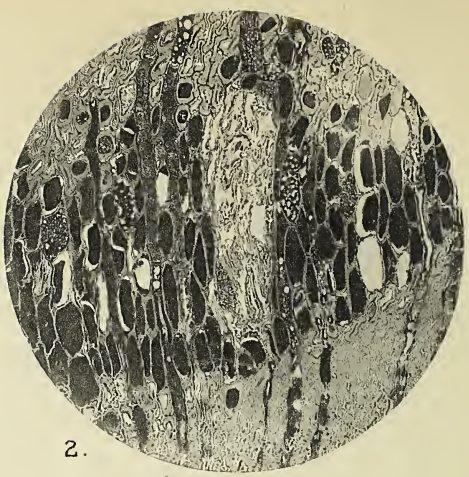

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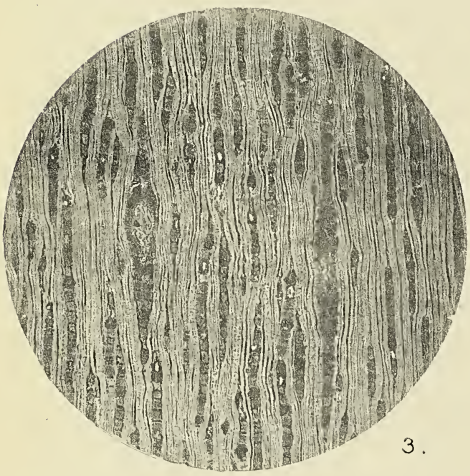

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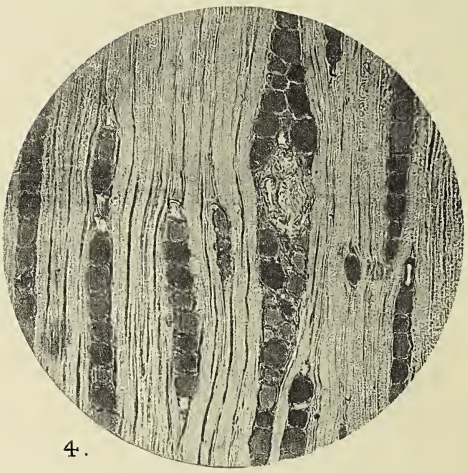

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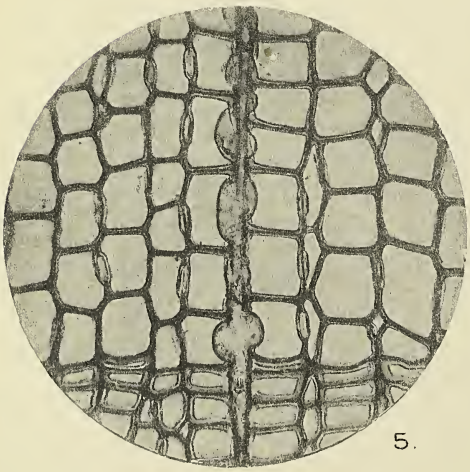

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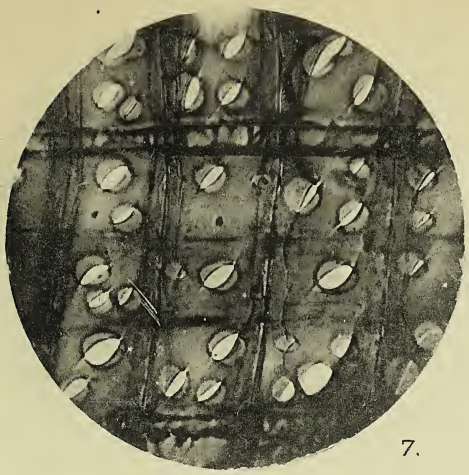
BAILEY —— PITYOXYLON.



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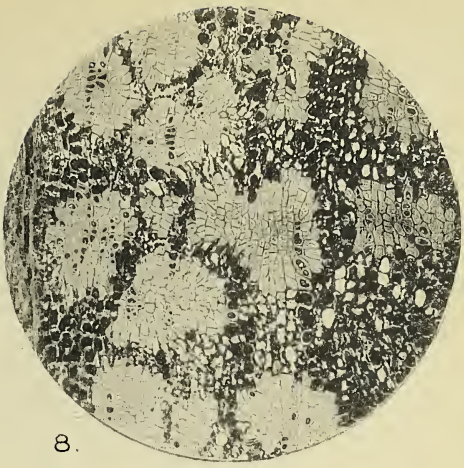

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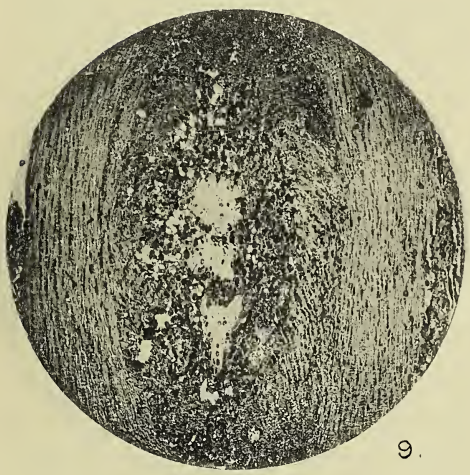

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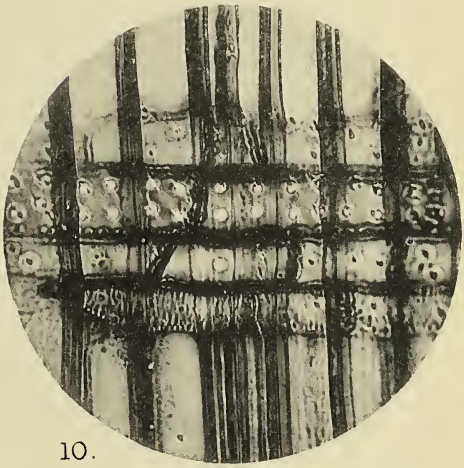

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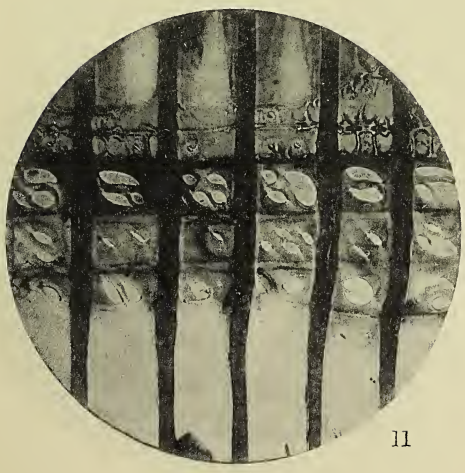
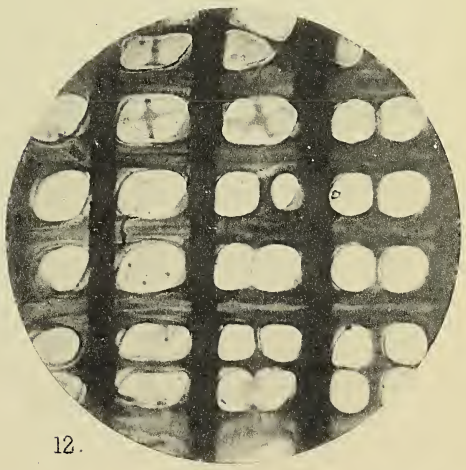


11



12.

Huth, coll.

Spongospora subterranea, (Wallroth) Johnson.[1]

BY

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With Plate XXVII.

THE organism producing the 'Corky' or 'Powdery Scab' of potatoes (*Spongospora subterranea*) has recently been described by both Johnson[2] and Massee,[3] but, as the results contained in these investigations are not in agreement, further work on the subject seemed desirable. In the present paper I shall confine myself to giving an account of the life-history and cytology of *Spongospora* so far as I have been able to trace them, together with some remarks on its affinities. Into the disagreement that has arisen between the two previous workers on the subject of nomenclature, I do not propose to enter. The name I have adopted would seem to be justified on the ground of priority,[4] and it is sufficient to refer to the discussion in the papers quoted.

The plants used in this investigation were grown in the experimental greenhouses of the Botanical Department of the University of Manchester, and I wish to thank Mr. J. M. Hector, of Leeds University, for some of the 'seed' potatoes used. The laboratory work has been carried out in the Cryptogamic Research Laboratory here, and I should like to express my thanks to Professor W. H. Lang for the facilities afforded there, and for his kindly interest and help.

Introductory.

The disease known as 'Powdery Scab' or 'Corky Scab' of potatoes is produced by an internally living parasite, *Spongospora subterranea*, hitherto regarded as a member of the Mycetozoa and having affinities with *Plasmo-*

[1] A summary of these results was given in the January number of this Journal (p. 271), and in the same number Mr. A. S. Horne gave a preliminary account of his work on *Spongospora* (p. 272).

[2] Johnson, T.: Econ. Proc. Roy. Dublin Soc., vol. i, pt. 12, Apr., 1908; Sci. Proc. Roy. Dublin Soc., vol. xii, N. S., No. 16, July, 1909.

[3] Massee, G.: Journal of the Board of Agriculture, vol. xv, 1908, p. 592.

[4] *Erysibe subterranea*, Wallroth, 1842; redescribed by Brunchorst (who was unaware of the earlier description) in 1886 as *Spongospora Solani*.

diophora Brassicae, Woronin. It is stated by Johnson to be rampant in the West of Ireland; references to its occurrence in this country have not been infrequent during the last few years in the 'Journal of the Board of Agriculture', and I have obtained specimens from more than one locality in the neighbourhood of Manchester.

In its earliest stages, which are visible on young tubers not larger than hazel-nuts, the disease is apparent by small slightly raised pimples, and a slight discoloration of the surface. When cut open, the infected areas appear faintly purplish and extend from approximately the outermost cells of the tuber towards the deeper layers. As the organism matures the surface of the potato above the diseased portions becomes ruptured. If the soil is dry, wound cork is formed, and the extent of the injury is quickly limited. In damper soils cork formation being checked, the infected area becomes hollowed out, this hollowing being continued as the tuber develops until large cavities over an inch in diameter and of considerable depth are produced.

There is not any apparent hypertrophy of the tissues such as is caused by *Plasmodiophora* and *Sorosphaera Veronicae*.[1] The disease, moreover, would seem to be limited to the tubers, though I have occasionally found small scabs on the rhizomes as well, but never on the aerial portions of the plant.[2]

My own observations as to the transmission of the disease from infected 'seed' or soil to sound 'seed' potatoes have been entirely negative. I have planted tubers of 'Up to Date', 'Factor', and 'Conquest' in pots of infected soil and side by side with infected 'seed', under varying conditions of moisture and temperature, but in no case was I successful in inducing the disease.

It has not been a part of the present investigation to test any remedies or chemical checks to the disease.

Methods.

Material was fixed in almost every case in the weaker Flemming's solution. Acetic alcohol was tried, but this was not successful, owing to a shrinkage of the tissues and an apparent hardening of the starch grains which interfered with the section-cutting. The material was brought through ten per cent. glycerine to absolute alcohol, cleared in chloroform and embedded in wax with a melting-point of 54° C. Microtome sections were cut at thicknesses varying from 2–10 μ (4 μ was most frequently used) and

[1] Bloomfield, J. E., and Schwartz, E. J.: Annals of Botany, vol. xxiv, 1910, p. 35.

[2] Schwartz, E. J.: Annals of Botany, vol. xxiv, 1910, p. 511. *Sorosphaera Junci* is limited to the roots of certain species of the Juncaceae, and does not produce hypertrophy of their tissues. I am much indebted to Mr. Schwartz for giving me material of *S. Veronicae* and *S. Junci* for comparison with *Spongospora*.

were stained with Flemming's triple stain, gentian violet and orange, or Heidenhain's iron haematoxylin. As counterstains with the last-named, orange G, erythrosin, or light green (*Lichtgrün*) dissolved in clove oil were tried, also one per cent. aqueous Congo red. In spite of its poor keeping qualities, light green was found to be the most generally useful, as it clearly differentiated the host protoplasm from that of the parasite.

Summary of Present Knowledge.

Briefly stated, our present knowledge of *Spongospora* is as follows. Uninucleate myxamoebae are observed in young potato cells,[1] though this is disputed by Johnson.[2] These subsequently fuse to form a plasmodium, while it is stated that fresh cells are invaded by a passage being bored through their walls.[3] At the approach of spore formation the plasmodium becomes very vacuolar, and then, according to Massee, a hollow sphere is formed, in the walls of which lacunae appear, while later polygonal cells (spores) are cut off, arranged in a single layer. Johnson [4] has corrected this statement, pointing out that the spore mass is a 'sponge-like' body. He further states [5] that each spore contains a number of nuclei (4 or 8), comparing this with Jahn's [6] and Olive's [7] observations on *Ceratiomyxa*. Massee saw only a single amoeba, which escaped on the germination of the spore.

Life-history.

Vegetative phase. Actual infection of the potato tuber by *Spongospora* has not been seen, nor have infection experiments been successful. The earliest stage in the life-history that has been observed is that of a single uninucleate amoeba in a young potato cell near an eye (Pl. XXVII, Fig. 1). The amoeba is somewhat rounded in outline, and consists of finely granular protoplasm, which has different staining properties from that of the host cell, so that it can be clearly differentiated from it. The nucleus appears to conform to the well-known Mycetozoon type, described by Lister [8] and others.

It has a membrane and linin network bearing chromatin granules, as well as a deeply staining body occupying a central position (Fig. 17). This central body retains the safranin with triple stain, and acquires an intense black with iron haematoxylin. As at times it appears to contain all the chromatin of the nucleus, it is, perhaps, best referred to as the karyosome, rather than as the nucleolus.

The nucleus divides in a manner to be described later, and this is generally followed in the early stages of infection by a division of the amoeba

[1] Massee, G.: loc. cit., p. 596.
[2] Johnson, T.: loc. cit., 1909, p. 171.
[3] Massee, G.: loc. cit., p. 597.
[4] Johnson, T.: loc. cit., 1908, p. 455.
[5] loc. cit., p. 456.
[6] Jahn, E.: Ber. d. deutsch. bot. Gesell., vol. xxvi *a*, 1908.
[7] Olive, E. W.: Trans. Wiscon. Acad. Arts. Sci. Litt., vol. xv, pt. ii, 1907, p. 753.
[8] Lister, A.: Journal Linn. Soc. Bot., vol. xxix, 1893, p. 529.

itself. This process continues for some time, so that a number of myxamoebae are to be found in one cell (Pl. XXVII, Fig. 2).

The amoebae are to be found in the cambium of the tuber, generally in the outer layers, though, in advanced stages of the disease, apparently also in the medullary cambium.[1] On the division of the host cell (Fig. 3) it is a purely fortuitous circumstance whether each resulting cell shall contain an amoeba, and so be infected or not. As far as my observations go, the whole spread of the organism from cell to cell takes place in this way. I have never seen any signs of the migration of an amoeba to a neighbouring cell, nor any continuity of protoplasm, such as Massee has described. This passive infection of fresh cells, or rather, handing on of the parasite to daughter cells in a dividing tissue, is like that described by Nawaschin [2] for *Plasmodiophora*, and Bloomfield and Schwartz [3] for *Sorosphaera*.

The amoebae continue to divide as has been described, while the host cell increases in size, so that a late stage of infection will show many amoebae, now not infrequently multinucleate, occupying the greater part of its area (Fig. 4). The nuclei during this period divide in an amitotic manner much the same as characterizes the divisions in a similar stage in *Sorosphaera* [4] and *Plasmodiophora*.[5] The chromatin arranges itself in the form of a ring around the karyosome, giving an appearance that has been referred to as the 'Saturn stage'. This ring of chromatin now splits into halves which travel apart (Fig. 18, *b*), from which it will also be seen that the nuclear membrane has become drawn out into an elliptical shape. The karyosome divides by becoming elongate, then dumb-bell shape, the halves subsequently pulling apart; this does not occur until the chromatin ring has split, a slight point of difference from the occurrences recorded in the other genera (Fig. 18, *c*, *d*, &c.). As the chromatin approaches the poles of the much elongated nucleus its constituents, derived from the halves of the ring and of the karyosome, blend together, and in the concluding stages appear as single, rounded, deeply staining masses near the poles (Fig. 18, *f*). Nuclear membranes form around these daughter nuclei, part of the membrane being derived from that of the parent nucleus (Fig. 18, *g*). It will thus be seen that at this stage the karyosome apparently contains all the chromatin of the nucleus. The chromatin granules of the latter appear later, and are apparently given off from the karyosome (Fig. 18, *h*). The linin network is not distinguishable until the granules are formed. I have not been able to determine the presence of spindles or centrosomes during this type of division, nor have I seen polar radiations during this or any

[1] Read, T.: Annals of Botany, vol. xxiv, 1910, p. 537.

[2] Nawaschin, S.: Flora, vol. lxxxvi, p. 404.

[3] Bloomfield, J. E., and Schwartz, E. J.: loc. cit., p. 40.

[4] See also Maire, R., and Tison, A.: Ann. mycolog., vol. vii, 1909, p. 226.

[5] See also von Prowazek, S.: Arb. aus dem kaiserl. Gesundheitsamte, vol. xxii, 1905, p. 396.

other of the nuclear divisions. Maire and Tison[1] and Prowazek[2] record these in *Sorosphaera* and *Plasmodiophora,* and the former regard this type of nuclear division as an 'intranuclear karyokinesis combined with an amitosis', a statement which is in accordance with their advocacy of the 'dual hypothesis' of nuclear structure.

Reproductive phase—akaryote stage. When the amoebae have exhausted most of the food material in the cell (though the host nucleus and occasional starch grains are still to be seen at this stage), they coalesce to form a plasmodium (Fig. 5). The plasmodium is thus the product of the fusion of a number of vegetative amoebae, and this fusion is the first step to spore formation. It would seem to be usual for only one plasmodium to form in a cell, though exceptions are to be found, and in one case as many as eight mature spore balls were seen (Fig. 16).

The formation of the plasmodium is followed by an akaryote condition in which the nuclear matter appears to be scattered throughout the whole plasmodium. The chromatin granules on the network, hitherto a prominent feature, disappear in all the nuclei of a plasmodium at the same time, being possibly conducted to the nuclear membrane along the linin threads and there extruded. In the same way the karyosome diminishes in size and is gradually lost (Fig. 19), while the protoplasm of the plasmodium (now rounded in shape wherever conditions of space permit) becomes filled with deeply staining granules which may be termed chromidia. This appearance is shown in Fig. 7, which is a drawing of a plasmodium at this stage. It will be seen that the sites of the nuclei are not lost to view, but remain as circular areas free from any trace of chromidia and showing up in marked contrast to the surrounding protoplasm. This appearance might at first sight be thought to be suggestive of vacuolation; the nuclear areas, however, when examined in sections stained with iron haematoxylin and light green for instance, are perfectly distinct. A similar occurrence is recorded for both *Sorosphaera* and *Plasmodiophora,* while a chromidial state is well known for certain Protozoa.[3] It is impossible to give any idea of the time of duration of the akaryote condition, but to judge from my preparations I do not think it to be very long.

The nuclei as they are developed the second time are of a very different appearance from the previous vegetative ones. There is a membrane, network, and chromatin granules staining an intense black with haematoxylin, but no karyosome. It is not easy to trace the development of the new nuclei, but there is evidence that they are constructed *de novo,* rather than reconstituted on the sites of the old ones. The evidence for such a statement rests on such stages as are figured in Figs. 8 and 20. By the side of the clear spaces, representing the previous vegetative nuclei, there may be

[1] Maire, R., and Tison, A.: loc. cit., p. 230. [2] von Prowazek, S.: loc. cit., p. 398.
[3] See literature quoted by Dobell, C. C.: Q. J. Micro. Sci., vol. liii, N. S., 1909, p. 279.

seen rods and granules of deeply staining chromatin surrounded by a non-staining area. These gradually become more marked until in the new nucleus there may be seen a considerable mass of chromatin in a lump, often lying to one side of the membrane (Pl. XXVII, Fig. 21), while at the same time the protoplasm appears less granular.

The most satisfactory explanation of these facts would appear to be that on the reconstruction of the nuclei the chromidia are reduced in number, though they do not totally disappear, and thus there is a certain wastage of chromatin, which ultimately degenerates. The chromatin mass in the new nucleus gradually becomes less contracted, and a network arrangement is to be seen (Fig. 22), though there is no sign of a karyosome or nucleolus. Bloomfield and Schwartz, when describing a similar stage in *Sorosphaera Veronicae*, were unable to state where the fresh nuclei appeared in relation to the old ones, but in *S. Junci* Schwartz says he observed 'granules and irregular masses of chromatin forming fresh nuclei in the vacuoles'.[1]

Karyogamy and spore formation. The reproductive nuclei are to be seen at first irregularly scattered through the whole plasmodium. It is soon to be noticed, however, that there is a very definite association in pairs (Figs. 9 and 10). This in itself is suggestive of a fusion, and all stages of the occurrence have been observed in numerous plasmodia (Fig. 11). In a pair of fusing nuclei, the membrane at the point of contact breaks down, and their contents merge one into the other (Fig. 23). The union occurs at approximately the same time for all pairs of nuclei in a plasmodium, so that the various stages are by no means rarely to be seen (Fig. 24). Such nuclei as are unable to pair degenerate and are quickly lost to sight.

The fusion nucleus has an appreciably increased diameter (5 μ), while its chromatin matter appears in the form of threads (Fig. 25). It is at this period that there is the greatest difficulty in arranging the various stages in their proper sequence. The plasmodia are so small that no progressive series of changes can be seen in the single plasmodium, as has been recorded for various Mycetozoa, but careful comparative study of different plasmodia of *Spongospora* leads to the following account. The chromatin matter contracts to form a dense irregular mass lying within the enlarged membrane. The appearance at this stage is strongly suggestive of a synapsis (Fig. 26). On emerging from this state the chromatin is in the form of granular threads (Fig. 27) arranged along a diameter of the cell (Fig. 12), but unfortunately I cannot state with certainty the various stages that must intervene between this condition and the first karyokinesis, nor am I able to say, from my own observations, whether a condition of diakinesis occurs or not.

The first karyokinetic division is marked by a well-defined but very dense plate of chromatin lying equatorially on a spindle which is relatively long compared with the diameter of the plate (Fig. 13). The spindle, how-

[1] Schwartz, E. J.: loc. cit., p. 516.

ever, is very small (7 μ), as may be seen by a comparison with that of *Badhamia utricularis*, which is about 18 μ in length. The spindle in *Spongospora* shows two clearly-defined poles with centrosomes, but the actual threads of the spindle in this division are not easy of differentiation, nor can the individuality of the chromosomes be made out. It is, of course, possible that there are no definite chromosomes in this division, the chromatin existing in a granular state, as Blackman has described in *Coleosporium*. The nuclear area often persists as a clear space during this first division (Fig. 28, *a*), though this is not so in all cases (Fig. 28, *b*). It is surprising how infrequently the ana- and telophases have been observed. A series of sections showing many plasmodia with their nuclei in the metaphase will show but few in the later stages of division, and this is true, moreover, for material of widely differing dates and hours of fixation. It is, perhaps, to be accounted for by a rapid movement of the chromatin masses towards the poles. Harper[1] found it otherwise in *Fuligo varians*, for, he says, 'all stages in the separation of the daughter chromosomes and their migration to the poles of the spindle can be observed in the greatest abundance.' All that can be said of the later stages of the first karyokinesis in *Spongospora* is that the chromatin splits into two apparently equal portions, which travel to the poles, retaining for some distance their plate-like character (Fig. 29). As they near the poles this appearance is lost, while the spindle fibres in the middle of the spindle disappear, though the apices and centrosomes are well marked (Fig. 30). The chromatin rounds itself off, while a new nuclear membrane appears. The second division is characterized by a shorter spindle (5 μ) and by the absence of any sign of the nuclear area at the metaphase (Fig. 14). The later stages of this division resemble the preceding one, the chromosomes travelling to the poles in a mass (Figs. 33 and 34). In the plate stage, however, the spindle fibres can be differentiated with less difficulty, while sections transverse to the long axis of the spindle show a number of chromosomes—eight in those cases in which it has been possible to count them (Fig. 32).

By the time that this division is completed the protoplasm has become rounded about each nucleus, so that there is a condition in which the organism consists of a number of uninucleate masses of protoplasm, the young spores, about which the spore wall then forms. The mature spores are spherical bodies about 4 μ in diameter, with a cell-wall, single nucleus, and a certain amount of oil (Fig. 35). The spore wall does not give a cellulose reaction with chlor-zinc iodine.

The individual spore thus bears a strong resemblance to that of *Plasmodiophora*, as described by Nawaschin. The spores are loosely aggregated to form the structures referred to as 'spore balls'. These are of a shape varying from spherical to ovoid, while the diameter also varies, though 50 μ

[1] Harper, R. A.: Bot. Gaz., vol. xxx, 1900, p. 233.

may be taken as an average size. The 'spore balls' are marked by numerous depressions and fissures (Pl. XXVII, Fig. 16), as has been described, arising from the formation of clefts in the plasmodium. Harper has described minutely the progressive development of the cleavages that characterize the development of the spores in *Fuligo*. The whole plasmodium is so small in *Spongospora* that no such full description can be given, but since cleavages are to be noticed in the plasmodium at any time after its formation (Figs. 7 and 10), though not generally till the time of the second mitosis (Fig. 14), it is obvious that in *Spongospora*, as in the Mycetozoa, the segmentation of the protoplasm is independent to a great extent of nuclear division.

The spore in all cases that I have observed is uninucleate, the nucleus having a karyosome and other chromatin matter.

Unfortunately all my cultures of the spore balls have proved intractable, so that I have not been able to observe the germination of the spores. Professor Johnson has not seen this either, Massee's account [1] being the only one published. He records that the contents of the spores escape intact, 'and are irregularly globose in form, with a few small projections. They show a very sluggish movement for some time, after which they become stationary. The diameter of the amoeboid body after its escape from the cell is about 3 μ.'

Effect upon the host plant. The effect of *Spongospora* upon its host has to some extent been described already. Under dry conditions of the soil the external appearance is limited to small circular patches about 5 mm. across. Under wet conditions the damage is more serious, and the scabs may be as large as 3–4 cm. in diameter and as much as 2 cm. in depth. This is the only external appearance; there is no sign of hypertrophy nor any distortion other than that caused by the pitting.

A definite cork cambium is formed below the seat of injury, though amoebae are to be observed in the deeper layers of the tuber under the cork.

In the host cells it is apparently the starch that is especially attacked, and starch grains of any size are generally absent at the time of spore formation. This is certainly not so in the initial stages, nor even up to the time of plasmodium formation. It is, of course, possible that starch does not develop in those cells that are attacked when young, the soluble carbo-hydrate material being absorbed as it enters the cell by the parasite. The subject, however, needs further investigation, which it is hoped to carry out.

About the time that the plasmodium is formed, the host cell appears to be exhausted, and most of its cytoplasm has disappeared. This is not the case with the nucleus, which has become much enlarged and unhealthy in appearance as the attack has proceeded. Many of the nuclei show remarkable lobing and indentations (Figs. 5 and 12), and they are often closely applied to the plasmodium of the invader (Figs. 7 and 12). The nuclei

[1] Massee, G.: loc. cit., p. 598.

may possess more than one nucleolus, and have densely granular nucleoplasm. By the time of the first karyokinesis, the host cell nucleus would appear to have degenerated or to have been absorbed by the parasite; it is not generally to be observed later than the akaryote stage. No multinucleate host cells have been observed, nor do the host cells appear to be much enlarged, which is an interesting point of difference from the cells attacked by *Sorosphaera Veronicae* and *Plasmodiophora*, though in *S. Junci* their behaviour is similar.

In the last few years a number of papers have appeared adding to our knowledge of the Plasmodiophoraceae and the allied groups. These have been summarized recently in the 'Progressus Rei Botanicae',[1] but it is necessary to review some of the salient points here.

Nawaschin's[2] account of *Plasmodiophora* in 1899 described a method by which the amoebae infected new host cells, which is similar to that given here. The nuclei of the amoebae were found to divide in a special manner, differing markedly from indirect nuclear division. The ring of chromatin matter around the karyosome is described, also something in the nature of an achromatic spindle, but neither centrosomes nor polar radiations are mentioned. Spore formation was found to be preceded by plasmodium formation, followed by a reconstitution of the nuclei and their subsequent karyokinetic division.

In 1905 Prowazek's[3] description gave still further details of the divisions in the vegetative phase. Centrosomes and asters were described, and a distinction drawn between the tropho- and idiochromatin stated to be present in the divisions. The chromidial stage was described in great detail, also the presence of two karyokinetic divisions subsequent to it, which are referred to as generative divisions. Following these divisions the protoplasm is stated to round itself off about the nuclei. These uninucleate bodies unite in pairs before encystment. The spore membrane then forms, and the nuclei within the cyst divide; one nucleus from each pair then degenerates, while the remaining two nuclei fuse. Prowazek regards this division in the cyst as the reduction division, the two karyokineses in the plasmodium having no such significance.

Maire and Tison,[4] in their memoir on the group, state that they are unable to agree with Nawaschin that a plasmodium formation occurs, since they have several times observed in the same cell an amoeba in the metaphase and another in the anaphase of the sporogenous division. They agree with Prowazek on the matter of a double karyokinesis, but are quite unable to accept his statement as to the autogamy succeeding encystment, which they consider simply as an abnormal occurrence.

In addition to *Plasmodiophora*, Maire and Tison have in the same

[1] Pavillard, J.: Prog. Rei Bot., vol. iii, 1910, p. 474.
[2] Nawaschin, S.: loc. cit., 1899.
[3] Prowazek, S.: loc. cit., 1905.
[4] Maire, R, and Tison, A.: loc. cit., 1909, p. 239.

paper described *Sorosphaera Veronicae* in detail. The myxamoebae found in the leaves and stems of *Veronica* spp. contain, at first, a single nucleus with a karyosome, and chromatin on a linin network. The division of this nucleus is 'une mitose d'idiochromatine combinée avec une amitose de trophochromatine'. In this division an intranuclear spindle is described with centrosomes and polar radiations more or less visible. This stage is regarded as a 'schizont' condition of the organism, since small amoebae are constricted off from the larger ones. At the conclusion of this stage the nuclei are reconstituted, the chromatin passing out into the protoplasm, where it is found as chromidia. Later the contents of the nuclei reappear, synapsis follows, the nucleoli disappear, and chromosomes form. There is a double karyokinesis, the divisions of which are regarded as heterotypic and homotypic, eight double chromosomes being visible in the former. During the second division the protoplasm becomes rounded about each nucleus, forming a spore, a number of which are arranged in a hollow sphere.

Very similar results were obtained by Bloomfield and Schwartz,[1] only recorded in less detail. The point of infection was found to be at the growing apices, and the infection of fresh host cells was produced, not by the penetration of the cells by the amoebae, but by the subsequent divisions of the cell originally infected. In *S. Veronicae* there is much hypertrophy of the tissues, and the infected cells of the host become multinucleate.

Schwartz [2] has more recently described a new species, *S. Junci*, the life-history of which is essentially the same, but no hypertrophy of the tissues of the host is produced.

Before entering upon a discussion of the points of difference between *Spongospora* and the other members of the Plasmodiophoraceae, it will be useful to consider the recent work on the Mycetozoa. Following the early work of Strasburger and Lister [3] (who suggested an amitosis of the vegetative nuclei in *Badhamia utricularis*), Harper [4] gave a detailed account of the cell and nuclear divisions in *Fuligo*. In regard to the cell-divisions, his work was of importance in showing that they are independent of those of the nuclei, and result from a progressive cleavage of the plasmodium.

In 1907 Fräulein Kränzlin [5] described the development of the sporangia in *Arcyria* and *Trichia*. Previous to spore formation the nuclei associate in pairs and a fusion occurs; any nuclei that do not fuse quickly degenerate and disappear. The fusion is interpreted as karyogamy, and is followed by a temporary enlargement of the nucleus and a synapsis. The nuclei, on regaining their normal size, show an arrangement of eight double chromosomes that is described as diakinesis. At the conclusion of this stage

[1] Bloomfield, J. E., and Schwartz, E. J.: loc. cit., 1910.
[2] Schwartz, E. J.: loc. cit., 1910.
[3] Lister, A.: loc. cit., 1893. [4] Harper, R. A.: loc. cit., 1900.
[5] Kränzlin, Helene: Archiv f. Protistenkunde, vol. ix, 1907, p. 170.

a simultaneous division of all the nuclei occurs, which, on the ground of the prophases, is regarded as heterotypic. This first division is immediately followed by spore formation. 'Le processus de réduction se trouve ainsi interrompu, pendant toute la période de la vie ralentie de la spore mûre. La réduction s'achève à la germination,'[1] the succeeding homotypic division being the one described by Jahn[2] in the swarm spore.

In the exosporous genus, *Ceratiomyxa*, Olive[3] and Jahn[4] have described a karyogamy. This occurs, according to Olive, towards the close of the segmentation of the protoplasm, and is followed by synapsis, each potential spore mass receiving a nucleus in that state. Two divisions follow, giving the typical four-nucleate spore.

Jahn's account is quite different. Following nuclear fusion, which he places at an earlier stage, there are stages regarded by him as synapsis and diakinesis, though it is to be regretted that his figures of the latter are not more convincing. There is, then, one (heterotypic) division of the nucleus, followed by a degeneration of half the daughter nuclei. The remaining nuclei form the spores, in which a double karyokinesis occurs. Yet another nuclear division takes place on germination, so that eight swarm spores are freed. It is not easy to homologize this account with what is known of the endosporous genera. Jahn regards the nuclear reduction as completed by the heterotype division and the subsequent nuclear degeneration, the two divisions in the spore, and that on its germination as of no special significance. In his review of the work, Pavillard[5] suggests that probably the division on the germination of the spore is a true homotypical mitosis (comparable to that in the *Endosporae*), the two preceding divisions being ordinary mitoses interpolated between the heterotype and the homotype divisions, and of no special significance.

Discussion and conclusions. The formation of a definite plasmodium has been described in the life-history of *Spongospora*. Prior to spore formation the host cell is found to contain a large multinucleate mass of protoplasm in the place of separate amoebae. All the nuclei in each mass divide at the same time, and the spores resulting from these divisions are united as a rule in a single spore ball.

The question of the formation of a true plasmodium in *Plasmodiophora* is the subject of a disagreement among the various workers on the organism. In *Sorosphaera* there is stated to be no plasmodium formation.

If these three genera are to be united, either in the Plasmodiophoraceae according to Maire and Tison, or in the Sorophoreae of Schröter, the defini-

[1] Pavillard, J.: loc. cit., p. 510.

[2] Jahn, E.: Ber. d. deutsch. bot. Gesell., vol. xxii, 1904, p. 84.

[3] Olive, E. W.: Trans. Wiscon. Acad. of Arts, Sci. and Litt., vol. xv, pt. ii, 1907, p. 753.

[4] Jahn, E.: loc. cit., vol. xxvi *a*, 1908, p. 342.

[5] Pavillard, J.: loc. cit., p. 511.

tion of these groups as one in which a plasmodium does not form must be suitably modified.

It is hardly safe to express any definite opinion on the significance of the dissolution of the nuclei and on the occurrence of chromidia, in view of the small amount of evidence at present in our possession. It may well be that the nuclei rid themselves of a portion of their trophochromatin before entering upon a reproductive phase. However, in the present state of our knowledge, such a deduction, involving, as it does, the 'binuclearity hypothesis', is unjustifiable as anything more than the merest speculation until further facts give the theory a firmer basis. As far as the present observations on *Spongospora* go, this loss of chromatin at the time of the formation of the reproductive nuclei is the only one to be observed. No constant stream of chromatin leaving the karyosome has been seen as described for *Sorosphaera.*

The karyogamy observed in *Spongospora* shows a striking similarity to that described by Fräulein Kränzlin in *Arcyria* and *Trichia*; the comparison with *Ceratiomyxa* is less easy. The nuclear fusion cannot be confused with a direct division, since, apart from the direct evidence as to its nature, it has none of the features of the very definite amitosis that occurs in the vegetative amoebae.

The observations on the peculiar method of karyogamy described for *Plasmodiophora* have not been confirmed by more recent workers, while as yet no fusion of nuclei has been observed in the life-history of *Sorosphaera.* It is not impossible that a karyogamy has been overlooked in these two organisms, and that further work on them will complete this gap in the knowledge of their life-histories.

It is unfortunate that a more definite account cannot be given of the prophases of the two karyokineses preceding spore formation in *Spongospora*; since, except for the enlargement of the nuclei and a contraction of the chromatin contents, no further details are known. Maire and Tison figure a synapsis in *Sorosphaera Veronicae*, and they are further of the opinion that the two mitoses are those of a reduction. Fräulein Kränzlin has described synapsis and diakinesis in the *Mycetozoa*, so has Jahn in *Ceratiomyxa.*

Personally I incline to the view that there is a synapsis in *Spongospora*, and that the two following mitoses are the heterotype and homotype of a reduction division. However, in default of further evidence, this must be stated as an opinion rather than as a fact, but as an opinion that receives considerable support by comparison with the occurrences in *Sorosphaera* and *Trichia* and *Arcyria.*

Accepting, then, the validity of this assumption, it is seen that the life-history of *Spongospora* resembles in the main a Mycetozoon as regards its nuclear constitution. The nuclei throughout the whole vegetative period are of the haploid form (x generation); the diploid state ($2x$) being only

attained for a short time just previous to spore formation. The reduction in *Spongospora* is completed before this occurrence; in the Mycetozoa the homotype division does not take place till the spore has germinated.

Should the observations of Prowazek on *Plasmodiophora* be confirmed, the case of that organism is wholly different from the Mycetozoa or *Spongospora*. It is diploid from spore to spore, the x generation being limited to a short period within the cyst. This may be more in accordance with 'all Protozoa', as Hartmann[1] has observed, but it does make it more difficult to trace the homologies between the plasmodia of *Plasmodiophora*, on the one hand, and those of *Spongospora* and the Mycetozoa on the other. Thus, though the evidence regarding *Plasmodiophora* is to some extent conflicting, it may be assumed that karyogamy in the plasmodium preceding the karyokinesis is the normal occurrence in the Plasmodiophoraceae; it is interesting to note that Pavillard[2] has already forecast this in his summary of the work upon the group.

Summary.

1. *Spongospora subterranea* is an intracellular parasite of the potato tuber, living in the cells in an amoeboid condition, and invading the daughter cells as they form in the process of cell-division.

2. The nuclei of the amoebae divide in an amitotic manner during the vegetative phase; on its conclusion the amoebae fuse to form a plasmodium.

3. Plasmodium formation is followed by a degeneration and disappearance of the vegetative nuclei, chromidia appearing in the protoplasm. This is the akaryote stage.

4. On the conclusion of the akaryote stage the nuclei are formed on different sites to the previous ones, some of the chromidia being used in the process while the remainder degenerate.

5. Karyogamy occurs between pairs of the nuclei.

6. Karyogamy is succeeded by a temporary enlargement of the nuclei and a contraction of the chromatin, which is possibly a condition of synapsis.

7. Two karyokinetic divisions of the nuclei follow each other rapidly; the first is marked by its length of spindle; the spindle of the second is shorter, with more sharply defined fibres, and has eight chromosomes.

8. The spores are uninucleate, and are aggregated in rounded masses traversed by fissures and marked by irregular depressions, but remaining attached in structures known as 'spore balls'.

9. *Spongospora* is a member of the Plasmodiophoraceae, which group has many points of relationship to the Mycetozoa, differing chiefly in the parasitic habit, the method of division of the vegetative nuclei, and by the less constant presence of a flagellum on spore germination.

[1] Hartmann, M.: Archiv f. Protistenkunde, vol. xiv, 1909, p. 284.

[2] Pavillard, J.: loc. cit., 1910, p. 506.

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EXPLANATION OF PLATE XXVII.

Illustrating Mr. Osborn's paper on *Spongospora*.

All figures were drawn in outline with a Zeiss camera lucida at the table level, with a tube length 160 mm. Except where stated, a Zeiss 3 mm. apochromatic oil-immersion objective (1·30 aperture) was used with (Figs. 1–15 inclusive) a × 6 compensating ocular and (Fig. 17 ad. fin.) with a × 18 comps. occ. The magnification was thus roughly 900 and 2,750 diameters respectively.

Fig. 1. Single uninucleate amoeba in young host cell.

Fig. 2. Three amoebae in a cell, one binucleate; note the young starch grains which are also present.

Fig. 3. Host cell which has recently divided, showing cell-plate formation with amoebae in each daughter cell.

Fig. 4. Several large amoebae in a cell. Starch grains and host cytoplasm are still present; the host nucleus is becoming enlarged.

Fig. 5. Plasmodium formation. The host nucleus is here considerably enlarged and shows marked indentations.

Fig. 6. Plasmodium showing the vegetative nuclei in process of degeneration.

Fig. 7. The akaryote and chromidial stage.

Fig. 8. Conclusion of akaryote condition, the new nuclei forming apart from the old nuclear sites. Cleavages in the protoplasm are shown in this plasmodium.

Fig. 9. Nuclei approximating. The plasmodium still contains a number of densely staining particles.

Fig. 10. Nuclei pairing.

Fig. 11. Fusion of nuclei in pairs (Figs. 9 and 11 are from contiguous host cells).

Fig. 12. Nuclei after pairing in synapsis state. The host nucleus is still visible, much lobed, and closely applied to the plasmodium.

Fig. 13. First karyokinesis.

Fig. 14. Second karyokinesis; the cleavages are more apparent and the segmentation of the protoplasm is also marked.

Fig. 15. Section through a mature spore ball, showing the rounded spores, and the cleavages between them which give the spongy appearance when seen in surface view.

Fig. 16. Host cell with eight mature spore balls, and a starch grain. Under this power the spaces between the individual spores are not clearly visible, the cysts appearing polygonal, not rounded. (Reichert objective 7 *a*.)

Fig. 17. Nucleus of a myxamoeba in a resting condition.

Fig. 18. Division of nucleus in myxamoebae. *a*. Ring of chromatin around karyosome. *b*. Splitting of chromatin ring before the karyosome has elongated; the nuclear membrane is becoming elliptical. *c*. Karyosome dumb-bell shaped. *d*. Later stage. *e*. Halves of karyosome and the chromatin of the plate nearing the poles. *f*. Karyosome and plate chromatin blending to form the fresh karyosomes. *g*. Nuclear membrane disappearing between the two chromatin masses. *h*. Two daughter nuclei. The extra karyosome chromatin is appearing in the form of granules.

Fig. 19. Degeneration of the vegetative nuclei, showing the diminished karyosome and the extrusion of chromatin granules.

Fig. 20. Nuclei in process of reconstruction, showing the dense chromidia-containing protoplasm and the sites of the vegetative nuclei.

Fig. 21. Dense mass of chromatin matter in reconstructed nuclei.

Fig. 22. Chromatin network in pre-fusion nuclei, showing the absence of a karyosome.

Fig. 23. Fusion of nuclei (Fig. 11 enlarged).

Fig. 24. Late stage in fusion of the nuclei.

Fig. 25. Post-fusion nuclei showing increased diameter.

Fig. 26. Synapsis.

Fig. 27. Spireme emerging from synapsis.

Fig. 28. *a*. First division, metaphase showing nuclear area. *b*. First division, metaphase nuclear area not visible.

Fig. 29. First division, anaphase.

Fig. 30. First division, telophase.

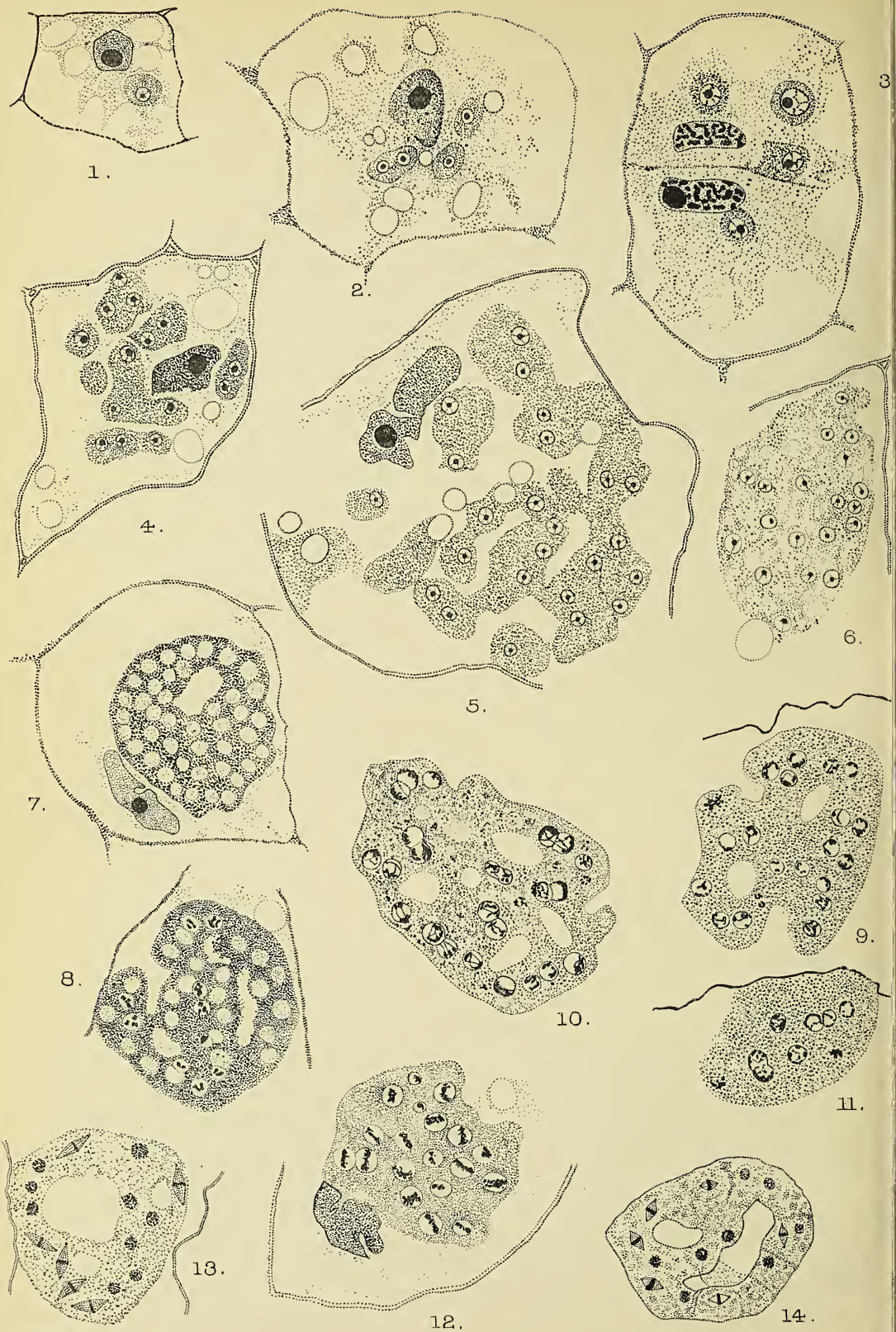
Fig. 31. Conclusion of first mitosis.

Fig. 32. Second division, showing eight chromosomes in plates cut transversely to the long axis of the spindle.

Fig. 33. Second division, metaphase.

Fig. 34. Second division, anaphase; the proplasm at this stage shows marked segmentation.

Fig. 35. Mature spores showing their rounded shape and single nucleus.



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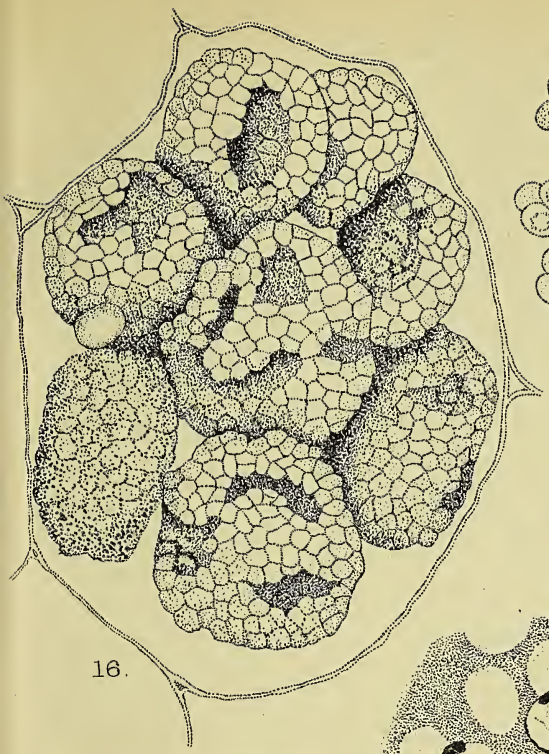
OSBORN — SPONGOSPORA SUBTERRANEA.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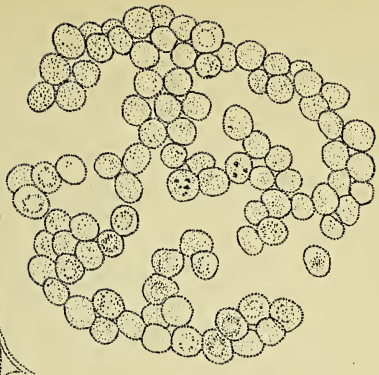
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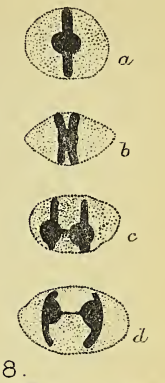
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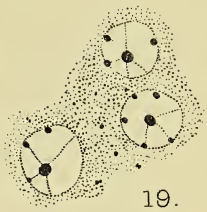
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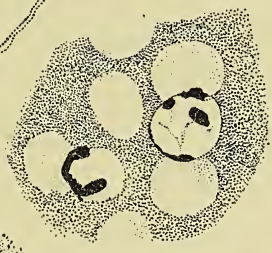
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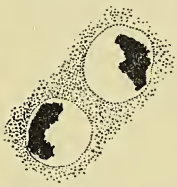
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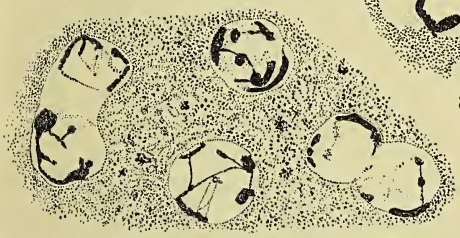
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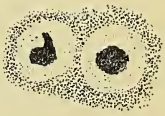
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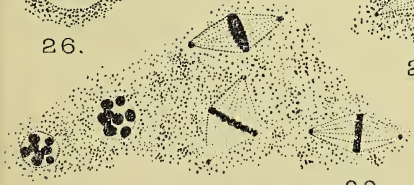
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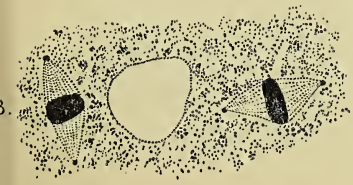
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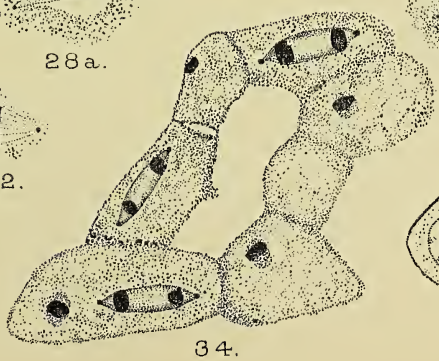
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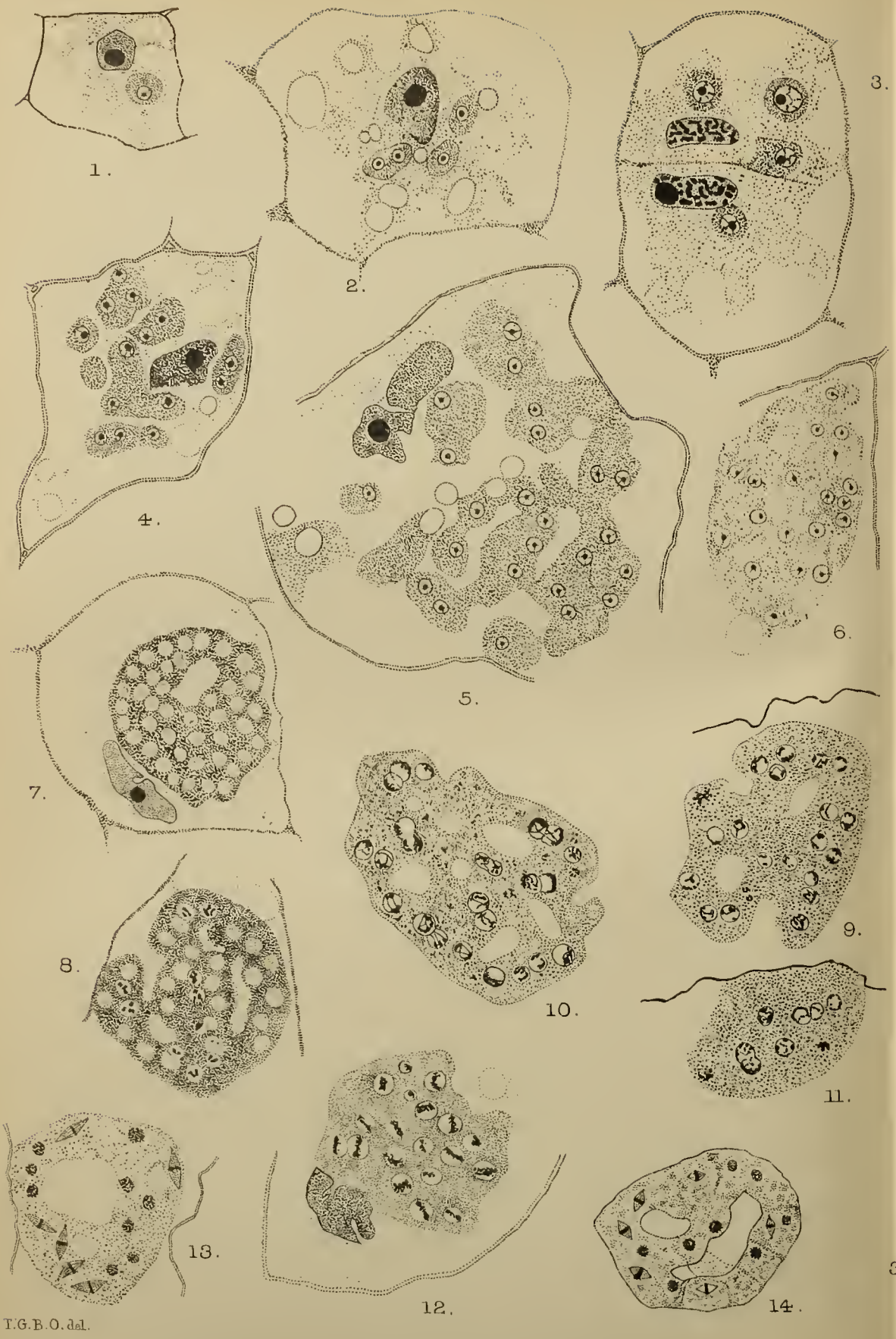

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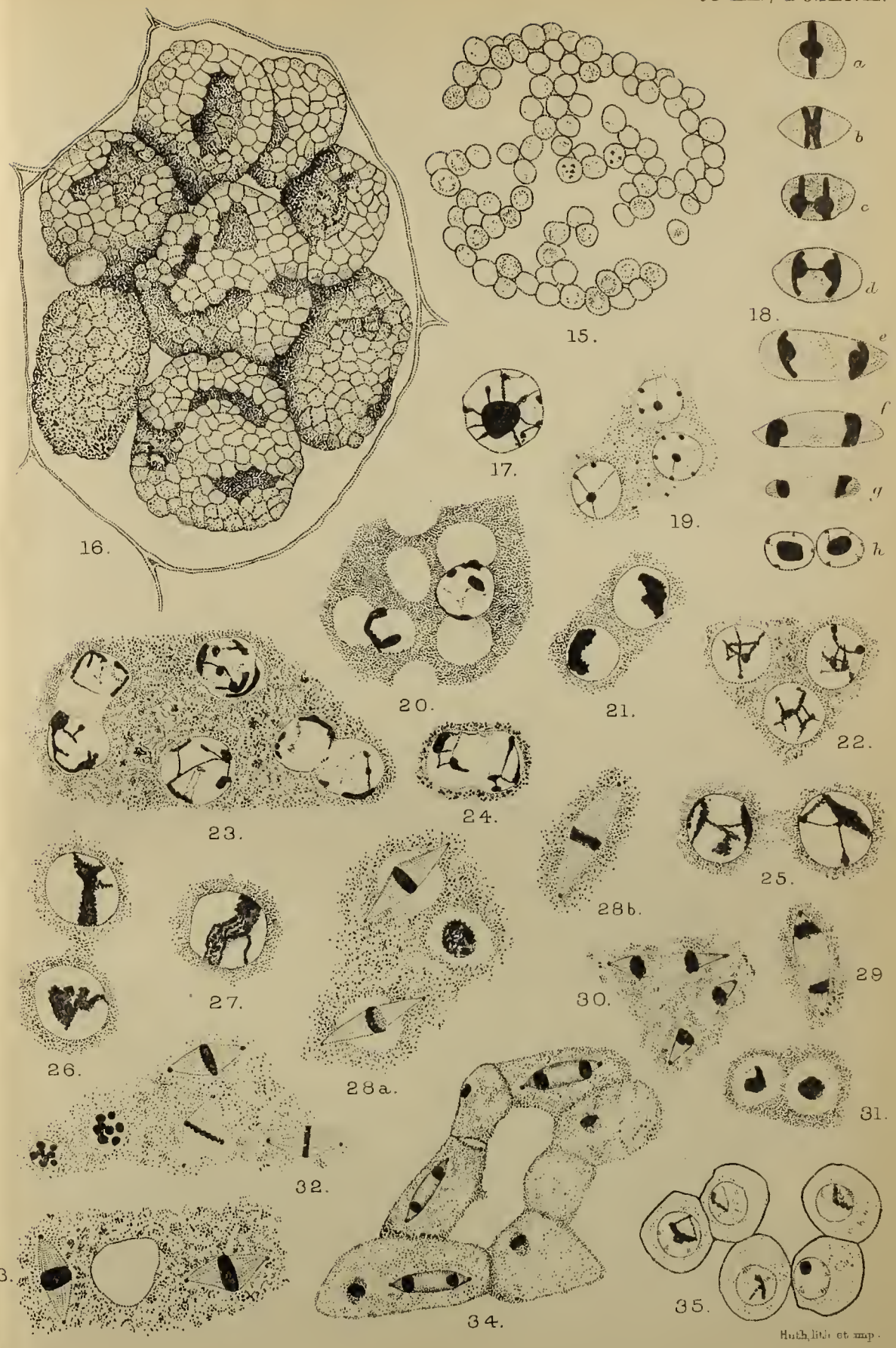

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OSBORN — SPONGOSPORA SUBTERRANEA.

The Panama Disease.

BY

ED. ESSED, B.Sc. (Edin.).

With Plate XXVIII.

I

THIS disease broke out among the banana plantations in Panama about five years ago. Considerable damage to the crops was only done in a certain district, where the disease appeared when the fields were five to six years old. From certain spots it spread all around, affecting at last a considerable area. The fields, when ten years old, were absolutely worthless. Thirty miles from this district, however, the plague makes its appearance, but sporadically.

In Costa Rica it badly rages only in spots; there are still magnificent plantations covering an area of ± 60,000 acres, where the disease does not spread and no real damage is done.

In neither of these countries were such serious consequences experienced as in Surinam, where it appeared on fields but one year old, and the loss amounted to 25–75 % of the second and third crop. On some plantations even entire fields were annihilated.

Different varieties of banana and plantain have been cultivated in this country for a century or more, but not so extensively as now; and as they were grown for shade of other crops—the plantain for food at the same time—they were not allowed to remain any longer on the same field than two years. This is the main reason why the disease never attracted the attention of the planters, and it will explain why the attack became such a serious and general one as soon as the same areas were covered by extensive fields of one of the most susceptible varieties, the Gros Michel.

As I was informed, the following varieties also are more or less susceptible: the Indian or Wine-coloured Banana, the Silverskin, the Apple, and the Horse Banana, the dwarf, and some varieties, unnamed, of plantain; resistant were the Ladies'-finger, the Congo variety, and the ordinary plantain.

The disease manifests itself by a peculiar withering of the leaves along the margin; along a mid-dorsal line on the midrib discoloration may be observed. Sometimes only the bud (the youngest convolute leaf) withers, not being able to unfold, whereas the older leaves are healthy; sometimes

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the older leaves show signs of decay, whilst the buds develop for a certain length of time, depending of course on the direction followed by the germ in propagating through the rhizome: this may be called the first stage. In the second stage development stops, the leaves droop, the plant looks water-starved, wrinkles appear on sheath and midrib of the leaves, which gradually dry up, and finally the pseudo-stem bends down along a line of least resistance.

As soon as the disease becomes evident, one is sure to find the tuberous rhizome, when cut asunder, showing on the exposed surfaces more or less signs of putrefaction. The healthy whitish colour is replaced by a yellowish hue with reddish brown spots or streaks scattered through the infested parts, and yellowish or brownish mucilage exuding from the slime canals. The roots do not participate in the process of decay before the tissue at their base is affected, proving that the germ does not enter by them, and since the starting-point of disintegration could be traced to an old wound-surface there is reason to assume that the fungus at the start behaves as a wound parasite or saprophyte, living at first on the exudation and by degrees preparing its way up into the damaged vessels.

Sections made from the rhizome showed at once that a fungus was the probable *causa morbi*. The mycelium is mainly massed in the wood vessels and the immediately surrounding tissue (see Pl. XXVIII, Fig. 1). The water-starved appearance of the affected plant is readily explained by the enormous amount of hyphae and spores in the vessels, which must seriously interfere with the passage of water. But a more effective impediment to the transpiration current and assimilation in general are the at first mucilaginous bodies plugging the wood vessels and sieve tubes and filling up cells and intercellular spaces of the parenchyma. These cartilaginous bodies I shall henceforth term sclerotia,[1] which I shall prove them to be.

The damage done by the fungus is not only of a mechanical but mainly of a physiological nature, as may be concluded from the wood vessels being discoloured and the contents of the sieve tubes, &c., resorbed by the hyphae running through them. The changes in the parenchyma seem at first to be slight, and manifest themselves by an unusual turbidity of the protoplasm, apparently caused by the action of an enzyme secreted by the fungus, to which enzyme also the brown discoloration and slimy degeneration of the cell-walls may be due. By degrees the contents of the cells are absorbed and replaced by the cartilaginous sclerotia. Some details of the formation of the resting mycelia may be mentioned here; on hyphae passing from cell to cell comparatively large, bladder-like structures arise, which, sometimes wrinkled and lobed, then again split in tufts and divided, seem to exercise a haustorial function. The enzyme alluded to before is probably secreted by these structures, as may be inferred from the

[1] Another name for these structures is perhaps desirable.

fact that the turbidity of the protoplasm is only noticed in cells where they are found, and that the sclerotia formed in these cells often show a marked resemblance in outlines to the said structures (see Pl. XXVIII, Fig. 2).

Transverse sections through the leaf-sheath showed that the hyphae entering the wood vessels send out branches in a direction vertical to the surface. They terminate in the intercellular spaces between the sub-epidermal layers, where oblong sclerotia are formed or irregularly shaped ones, which entirely fill up the sinuosities of the spaces (see Pl. XXVIII, Fig. 3). The spore-clusters arising from these sclerotia again give the impression of *Sorosporium* among the Ustilagineae. Some, very small and breaking up into minute spores, made me think of *Physoderma* among the Chytridiaceae.

Transverse sections through the midrib displayed a peculiar formation of the sclerotia in the stellate cells of the parenchyma composing the partition walls between the large air-spaces. The hyphae entering the cell send branches into the lobes, where they turn to sclerotia which give rise to minute spores at last (see Pl. XXVIII, Fig. 4). From these tiny sclerotia results the brown dotted appearance of the septa in an affected leaf.

The conditions met with in transverse sections of the lamina are essentially the same as in the sections of the sheath, but the vertical course of the fertile hyphae is more obvious. They push through or between the cells of the palisade parenchyma, some not beyond the back-wall of the inner layer of the subepidermal cells, gradually increasing in thickness and turning into a slimy tube or rod, which hardening into sclerotia may after a time of rest give rise to spores, as will be seen in Pl. XXVIII, Fig. 5. The spores may retain the position of the sclerotium or they may, in consequence of the tension in the surrounding tissue, be squirted into the overlying cells, from whence they are liberated after the decay of the leaf. Again, the hyphae may at once push through the subepidermal layers, filling the cells with sclerotia, or they may run out at the surface of the leaf as tiny brown gall-like swellings (mycocecidia). Then again traversing and rupturing the epidermal layer or entering the stomata, the hyphae reach the surface of the leaf, and branching in all directions give rise to numerous sickle-shaped conidia.

The slime canals are apparently attacked by the fungus; that means that hyphae may be running along or through them, and the possibility of the fungus deriving some benefit from this intimate contact may be inferred from the fact that the colour changes into some shade between yellow and brown—explaining why the mucilage exuding from the slime canals of an infected rhizome shows a corresponding hue—whilst spores may be seen to arise on or in the mass.

The process of chlamydospore-formation out of the sclerotia is of a marked Ustilaginoid character: the spores loosely adhere to each other,

forming clusters as in *Sorosporium.* Very frequently globular transparent bodies are found in the sclerotia, reminding one of the water-clear bubbles which Meyen discovered between the spores of *Ustilago longissima.* Their nature is difficult to explain; in some cases, however, I found them containing crystals of calcium oxalate. Besides the ordinary chlamydospores, large spore-shaped bodies of a smoky or shining black colour are seen to arise within the sclerotia. I shall later on try to give an explanation of their origin and nature (see Pl. XXVIII, Fig. 3).

During the final stage of the development of the disease all parts of the host plant are attacked by the fungus, which, enclosing the tissue-remnants in sclerotia, adapts itself to a saprophytic mode of life. Numerous spores—chlamydospores and conidia—are formed and crowded in pycnidia-like cavities. The pycnidia containing chlamydospores mainly form within the more compact inner portions of the mass, whereas those containing conidia arise on the subaerial parts, now and then with chlamydospores scattered between them. In the leaves also I found similar pycnidia between the subepidermal cells, containing chlamydospores, with no visible aperture for the escape of the spores; the walls in both cases were formed symphyogenetically.

All through the plant multicellular chlamydospores are met with. It will be shown later on that they are chlamydospores by their mode of origin and germination.

Pure Cultures.

The little-discoloured parts of the rhizome, abutting on those where putrefaction had gone to a great length, appeared to be most suitable for the preparation of the inoculation fluid, since the bacterial colonies did not appear in such an overwhelming number as to overrun the mycelia; moreover, the inoculation was carried out on large Drigalsky plates (Agar). Using a favourable dilution, what appeared to be two distinct kinds of mycelia and two kinds of bacterial colonies were obtained. The difference between the two kinds of mycelia was pronounced, in the colour and the mode of growth; they were transferred to separate Petri dishes.

The one kind of mycelium was hyaline and silvery shining; the hyphae were thin and the septa wide apart. On the fifth day a thickening of special hyphae had taken place, and a greater amount of transverse walls had divided them into a large number of isodiametric cells. Many branches were given off in monopodial fashion, and more or less vertical to the main axes. On the seventh day tiny protuberances arose, which the next day had cut off single multicellular Fusarium conidia and smaller bicellular conidia, which were slightly sickle-shaped or straight and always grouped in yeast-like fashion or in heads or bundles of 8–20. The following day the Fusarium conidia were seen to arise in heads of 10–50 on an incon-

spicuous, unbranched conidiophore as in *Gibberella cyanogena*. At the same time thin curling branches appeared, at the apices of which arose round cup-shaped chlamydospores with granular, richly vacuolated protoplasm. On the tenth day some thick hyphae appeared, giving rise to the same chlamydospores, intercalary as in *Chlamydomucor*. In one case they were wide apart, in another in immediate contact with each other, so that fusion took place, producing multicellular chlamydospores as alluded to before. On another culture they were seen to arise on the apex of short thick branches, as in *Hypomyces ochraceus* (see Fig. 7).

Anticipating this kind of chlamydospore formation, the protoplasm of the hyphae was drawn to favoured sites, where the spores would arise, and the interspaces were left entirely empty and finally collapsed, liberating the chlamydospores. Their colour when mature is dark-brown or violet; the exosporium is generally smooth, but now and then adorned with numerous warts. In some instances the hyphae did not produce resting spores, but broke up into their component cells, giving rise to oidia-like reproductive organs.

The sclerotia arose on extremely thin hyphae as transparent yellowish saucer-shaped bodies, measuring $\pm 100\,\mu$ across. The cultures were then nearly five weeks old. Sometimes they stood solitary, then again they were crowded together, and finally fused into a massive gelatinous body, in which no separating membranes, but often the above-mentioned globular bodies, were visible. Maturing, they assumed a darker colour and hardened into the identical gristly structures met with in the histological preparations. In the same way they broke up into identical chlamydospores. In the absence of fusion some of the single scyphoid sclerotia matured into large, dark-coloured, spore-like bodies, as mentioned before on p. 346; I shall term them giant chlamydospores. They may be expected to have the potentialities of a single scypho-sclerotium or of a group of chlamydospores that could arise out of such a sclerotium. The other mode of sclerotium formation hinted at in the description of the sections is readily explained by Figs. 1 and 6.

The process of chlamydospore-formation is initiated by pellucid rings within the sclerotia. The mass then splits in polygonal plates, each containing one or more spores; at last they are completed, still surrounded by a thin layer of mucilage, which is finally resorbed. Indeed, the whole process is identical with that of the Ustilagineae.

The second kind of mycelium was marked off from the first one by the dusky colour of the central underlying part and the conspicuous hemispherical shape. The dark colour was due to the basal growth, consisting of thick hyphae of a smoky hue. They terminated, however, in hyaline slender apical portions, and the secondary mycelium arising on them was also perfectly hyaline. On the seventh day chlamydospores arose, cut off

at the summit of thick, short side branches, or intercalary as in *Entyloma*. On the eighth day the conidia made their appearance on the hyaline parts of the mycelium; but few sclerotia were formed. From the tenth day onwards no real change was perceptible until, in the fourth week, the mycelium broke up into a dense mass of chlamydospores. At all events, it was shown that the difference of colour of what appeared to be two distinct kinds of mycelia was only due to a slight modification of the nature of the spores from which they arose.

Cultures on gelatine medium. It was not easy to produce a solid gelatine medium on account of the high temperature reigning in the tropics. Still, the mycelia raised in the semi-fluid medium excellently established the facts gathered from the agar cultures. One special feature of the gelatine cultures, however, was the appearance of the hyaline, bladder-like structures already described as haustoria. They arose and remained submerged in the semi-fluid medium, performing their haustorial function. Comparing the results of the histological research with the facts gathered from the pure cultures, one cannot fail to see the identity of the structures found in the plant tissues and those developed on the Petri dishes. The figures will fully explain the matter.

Cultures in Life-box.

From one of the most flourishing cultures a sufficient quantity of mycelium was transferred with the aid of a sterile, spatulate platinum wire to a tube containing ± 10 grains of sterilized water and divided by agitating moderately. A drop was brought on a thoroughly clean cover-slip, a drop of sterilized water placed on the bottom of the life-box, and the cover-slip then fixed on by means of some vaseline. Three other life-boxes were prepared for the sake of comparison. As a matter of convenience in describing the different modes of germination, I shall take the liberty of making some distinction between chlamydospores, gemmae, and oidia. By chlamydospores I designate the spores arising from the sclerotia; by gemmae, those formed in the course or at the apices of fertile hyphae; and by oidia, the spores which are set free by the breaking up of the fertile hyphae into the component, isodiametric cells.

The gemmae were the first to germinate; some gave rise to short, club-shaped tubes, at the apices of which one or two small conidia were cut off; another group grew tubes of 3–7 cells, small bicellular conidia arising apically and laterally. These conidia were often loosely joined together in pairs or in bundles of three or more, the adherence probably being caused by a thin layer of mucilage enveloping them. In another case the conidia were thrown off by the gemmae at once, without the interpolation of a promycelium, reminding one of the germination of *Entyloma*, which very often takes place on the host plant, as could be seen in this case

also. In yet another case a yeast-like budding took place, giving rise to a promycelium, which may laterally and apically throw off conidia or grow into a dark-coloured mycelium as met with before (see Pl. XXVIII, Fig. 7).

The chlamydospores may arrange themselves in beadlike fashion; some of the spores then emit germ tubes, which grow at once into a mycelium; others produce promycelia with conidia or conidia alone. Finally they give rise to branched promycelia, on which chlamydospores and conidia, arising on the extreme tips of the branches, put a stop to their further development (see Fig. 8).

The oidia did not differ in their mode of germination from the first-mentioned kind of gemmae, with which they are, in fact, identical, as could indeed be inferred from their mode of origin (see Fig. 8).

The bicellular conidia produced branched mycelia, which grew out at one or both ends; but in some cases fusion was seen to occur between two of these conidia; one of them assumed the nature of a chlamydospore, giving rise to a branched promycelium with conidia constricted off at the apices of the hyphae.

The Fusarium conidia, germinating under ordinary conditions, did not present any essential difference. The mycelia mostly arose on the apical cells, each giving off sometimes two germ tubes. But in many cases the protoplasm was drawn to one or two of the cells, which rounded off and then germinated exactly as the gemmae. The same transformation of Fusarium conidia into gemmae was noticed on an old culture, where, after two months, these conidia were replaced by gemmae; this surely throws some light on the occurrence of gemmae in the pycnidia and the prominence of the chlamydospore fructification in general.

The multicellular chlamydospores or gemmae presented the same peculiarities in germinating as mentioned in the case of the single cells.

Bits of sclerotium were seen showing a differentiation into spores; after a few days they were formed, still enveloped in mucilage. As soon as the mucilage was resorbed they started germinating in the same way as mentioned under chlamydospores. In another bit, germ tubes arose in the still undifferentiated mass as stout, highly refractive rods, which gradually emerged from the sclerotium, producing promycelia as before (see Pl. XXVIII, Fig. 8). This goes to show that the sclerotium is able to propagate the fungus in the same way as the chlamydospores. Another function of the sclerotium is made plain in the following account of the formation of what I think I am justified in considering a fruit body; for although it was not obtained under sterile conditions, and its premature withering prevented me from controlling my conclusion by raising new mycelia from the ascospores, it is plain that the identical Fusarium conidia and gemmae could not arise except on a part of the fungus under investigation.

A sucker of the banana in the second stage of the disease, from which

the upper part was cut off so as to expose a comparatively large surface, was left on my table. Within a few days I noticed that the process of putrefaction was rapidly advancing, turning the upper surface to a black corrugated mass. Shortly after that it was covered with silvery shining mycelium, upon which the already known gemmae were found when some small bits were brought under microscope. Later on I discovered two branched, antler-like, hairy, orange-coloured bodies arising on little protrusions bare of hyphae. One lobe of each was cut off and examined under the microscope; it consisted of closely packed hyphae, of which some were fertile, bearing conidia and gemmae—the identical Fusarium conidia and gemmae treated of before—whereas the remainder were sterile, the free ends sticking out and producing the hairy appearance of the upper part of the lobes.

The bodies were allowed to develop; they grew in a fortnight to double the size, when they were seen to wither and crumple, probably for want of moisture. Taken off with a portion of the underlying tissue and brought under low power, the basal part proved to be a bit of sclerotium. Sections were then made of the entire bodies, in the one case longitudinal, in the other transverse sections. Remarkable developmental changes had taken place; across the apices of the lobes a pseudo-parenchyma of a pink colour had arisen; the conidia had disappeared, and only a few gemmae were still to be found in the sinus between two lobes.

The stalk, $\pm \frac{1}{2}$ cm. high, consisted of a bright, yellowish brown pseudo-parenchyma, and was perfectly sterile. The broader upper half was enclosed in a sheath of closely packed paraphyses with free terminations, supporting, as it were, the pink-coloured stroma; they themselves were of a bright orange-red colour. The transverse as well as the longitudinal sections showed that the fruit body was not solid all over, but that in the median part a large air-space was partly filled up by strands of pseudo-parenchyma connecting the opposite walls of the cavity and producing a kind of spongy parenchyma.

I gather from the foregoing facts that I am justified in considering the structures in question as prematurely withered, ascigerous fruit bodies, which would have produced the asci in or on the pseudo-parenchymatous terminal discs (stromata), if the conditions had been favourable to their development. It has, of course, yet to be proved, and fully occupies my attention still; but since the raising of fruit bodies of Ascomycetes is not always possible in the laboratory, and the systematic position of the fungus could be determined from the pronounced pleomorphism, the development of a brightly coloured stroma, and in general from the great resemblance to many members of the well-defined homogeneous order of the Hypocreaceae, I think it right to classify it with this order; and on account of the formation of chlamydospores out of sclerotia, the mode of germination of these

spores, and the morphological peculiarities of the stroma, I propose to give it a place beside *Ustilaginoidea*. As far as I could find out we are dealing with a fungus not yet described, for which, on account of its great resemblance to *Ustilaginoidea* and its harmfulness to the banana, I propose the name *Ustilaginoidella musaeperda*.

Bacteria.

On account of the assertion of the expert of the United Fruit Co., that Bacteria were the cause of the Panama disease, I thought it necessary to ascertain—although the *Ustilaginoidella musaeperda* was shown to be undoubtedly connected with the disease—whether Bacteria also had anything to do with the plague, as a primary or secondary cause, besides the fungus. The bacterial colonies were isolated in the ordinary way; for the sake of convenience I shall indicate them by *a* and *b*.

The *a* colonies appeared on one of the plates only, so that it proved to me at once that this bacterium could not be the *Krankheitserreger*. Pure cultures, however, were raised on banana agar with the special purpose of identifying the micro-organism. The colonies grew into roundish elevations of a citrine colour measuring 1–1½ mm. in diameter. On the fifth day they flattened down, showing a slight indication of sliminess on the top, and the original granular consistency was only discernible along the somewhat crenate margin.

Stab cultures. Granular, yellow at the margin, merging into citrine in the centre, margin a little elevated.

Bouillon cultures. Slightly turbid, with slimy sediment.

Milk culture. Clotting after twelve days; acid reaction.

Potato culture. Citrine colour; sinuous margin; flat.

Glucose culture. No gas formed.

Staining. Gram-positive.

Microscopic appearance. Round cocci of $\pm 1\,\mu$ diam., in some cases joined in packets of two or four. It is clear that we are here dealing with one of the forms of *Micrococcus sulfureus*, a common micro-organism in air and soil.

The *b* colonies attained their maximum development after five days, presenting a great diversity of shape and size. Some were roundish, others irregular and lobed; in the one case the margins were crenate, in the other case moruloid. The longest axis of a colony was about 2 mm. The colour was greyish white, with faint, bluish-green fluorescence on the agar.

Stab culture. Thread-like, with a slight funnel-shaped dilatation near the surface; granulation scarcely perceptible. After twenty-four hours a circular, shining, thin, slimy layer around the inoculation spot, upon which arose (forty-eight hours) the greyish-white colony, evenly spread; agar fluorescing faintly blue-green.

Bouillon culture. Yellowish-green fluorescence; sediment in condensation liquid abundant, slimy; can only be divided by agitating well. Skin on the surface, yellow-green colour.

Milk culture. Clotting after nine days; acid reaction.

Potato culture. Flat, dull grey; margins sinuous. No gas and no indol formed.

Microscopic appearance. In hanging drop, short but slender rods, sometimes long threads and stout forms, all of which displayed great motility.

Staining. Gram-negative. One terminal cilium found by using Bunge's mordant and staining with fuchsin.

It is plain that this micro-organism is nothing but a form of *Bacterium fluorescens.* Reasoning from facts gathered during my research, it looks to me at least improbable that Bacteria have any share in the ruinous war waged on the banana plantations in Surinam.

To satisfy all conditions, however, I inoculated four suckers with the fungus-spores, four with Bacteria, and four with fungus-spores and Bacteria combined. Which will prove to be the mischief-maker time will tell.

EXPLANATION OF FIGURES IN PLATE XXVIII.

Illustrating Mr. Ed. Essed's paper on the Panama Disease. Part I.

Fig. 1. (*w.*) wood vessels full of hyphae spores, and sclerotium forming; (*s.*) sieve tubes plugged by sclerotium, spores forming; (*sc.*) scyphoid sclerotium.

Fig. 2. Haustoria in parenchyma. × 105.

Fig. 3. (*a*) spore-clusters; (*b*) sclerotium in intercellular space broken up into chlamydospores; (*c*) giant chlamydospores.

Fig. 4. Stellate cell in which sclerotia and spores are formed.

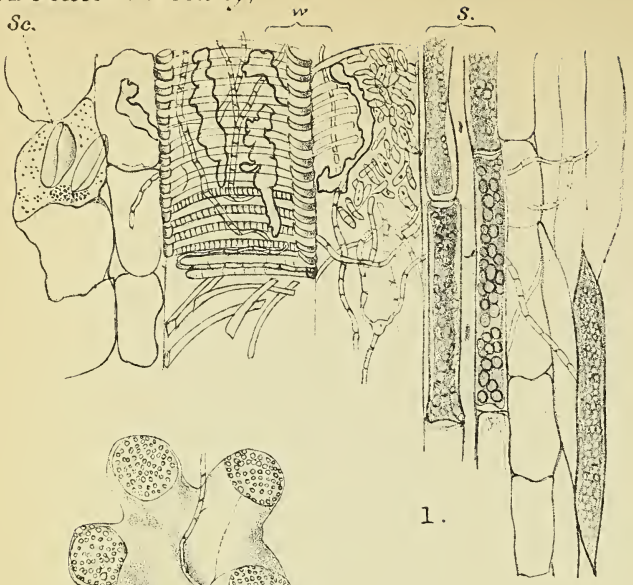
Fig. 5. Section through leaf, showing sclerotia and spores.

Fig. 6. Explained in text.

Fig. 7. Explained in text.

Fig. 8. Explained in text. × 255.

Fig. 9. (1) Fruit body on sclerotium. × 10. (2) Young stroma with spores and paraphyses. (3) Nearly mature stroma.



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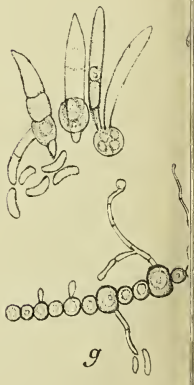

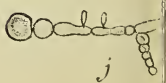

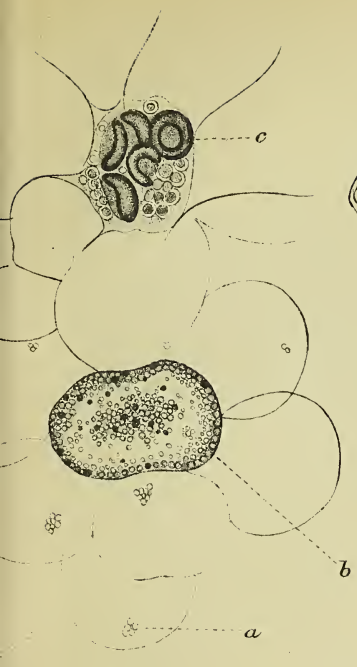

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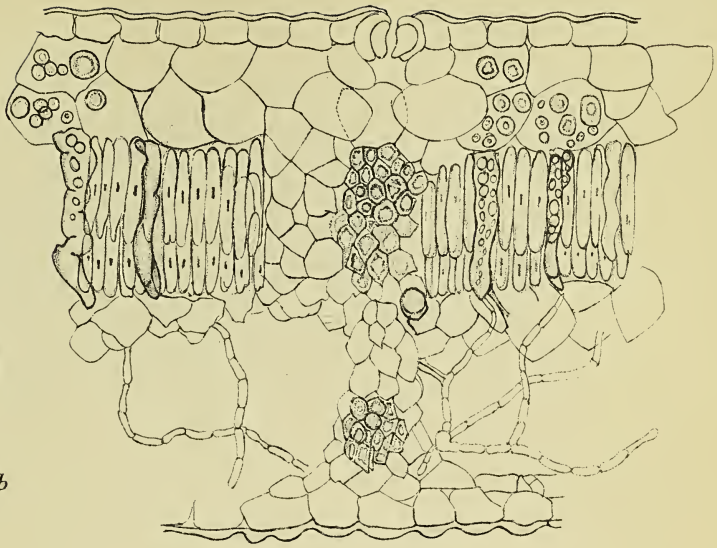

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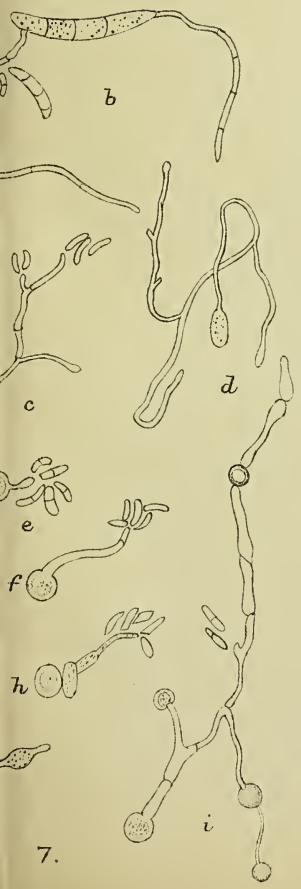

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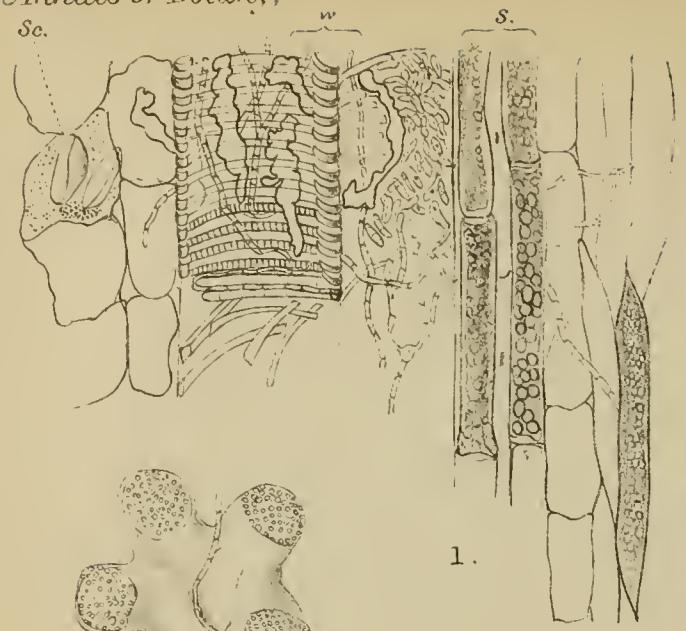

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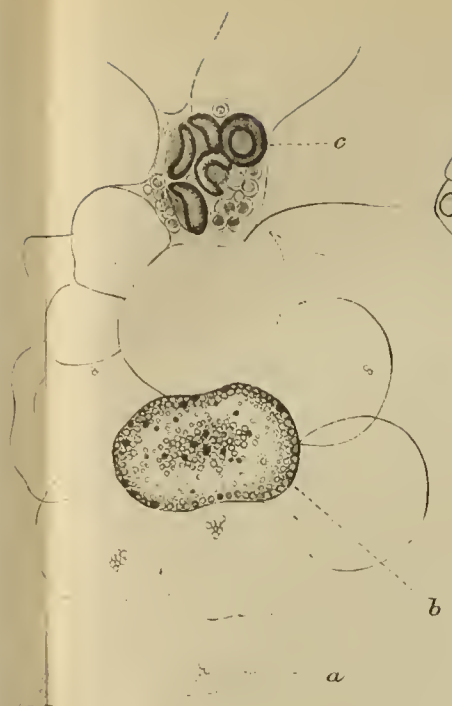

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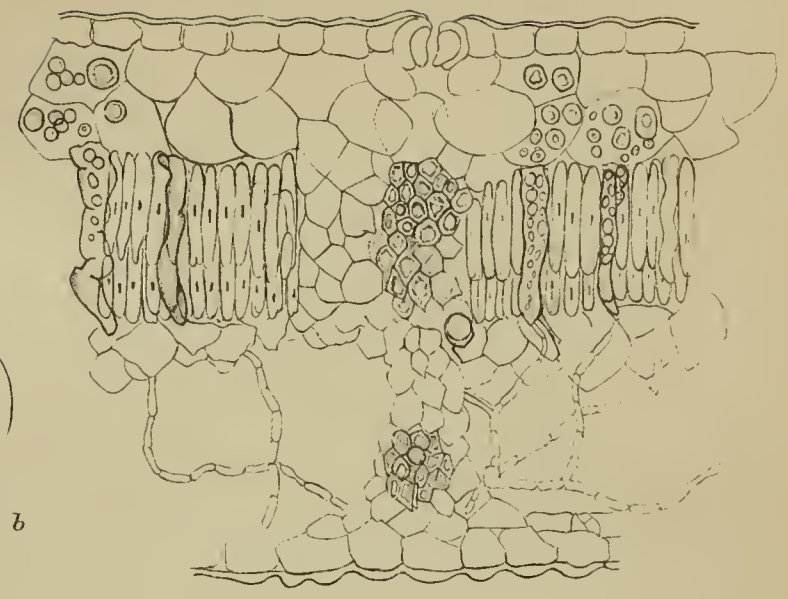

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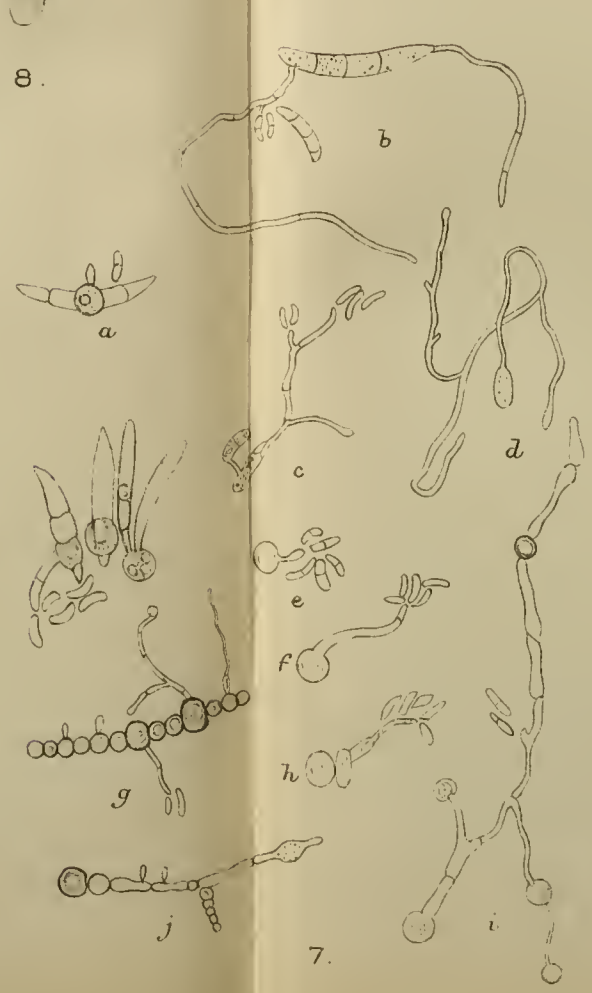

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ESSED — PANAMA DISEASE. I.

Huth Lith^r London.

The Panama Disease.

BY

ED. ESSED, B.Sc. (Edin.).

With Plate XXIX.

II

THE research was continued in order to control the results obtained at first and to study the development of the fungus in detail. The results are such as to enable me to add more particulars concerning the at first puzzling pleomorphism, and to elucidate and slightly correct some statements made in my first paper.

Sclerotia. As such were described structures arising from single hyphae, or even parts of them, whereas a true sclerotium is a structure formed by the interlocking of a number of hyphae, which give rise to a pseudo-parenchyma, often with distinct cortical and medullary parts. Resting mycelia is a useful general term ; but it appears to me preferable to use a special term for a definite structure: in this case I propose to use the term *pegmatium* to indicate structures arising from well-nourished hyphae, or even portions of them, which passing or not through a stage of slimy dissolution of their walls, harden into gristly or gummy bodies having the power to regenerate the fungus mediately by chlamydospores —into which they break up under favourable conditions—or immediately by mycelia arising from them without the interposition of a spore stage.

These pegmatia are found to consist of a sterile and a fertile part ; the first one, hard and gummy, is derived from the hyphal walls ; the latter, more or less gelatinous, from the protoplasmic contents of the hyphal cells. For the sterile part I wish to introduce the term *mycoporoma*, and for the fertile part the term *myclomyxa*.

Pegmatia. In hyphae from which some pegmatia arise, the protoplasmic contents are seen to break up into numerous globules, spore-initials apparent by their greenish opacity. In the one case the cell-walls then begin to thicken and gradually pass to a gelatinous substance, in which the spore-initials are hardly perceptible ; they become quite indistinct as soon as the mass begins to harden and to assume a yellow to brown hue. In

[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.]

another case the spore-initials are seen to prepare themselves a way through the hyphal walls, so that at last they are found densely massed on the surface of the hyphae, more or less deeply embedded (see Pl. XXIX, Fig. 1). During the migration of the spores, the hyphae swell to twice or thrice their original thickness and then harden into pegmatia. In a third case the hyphae become somewhat toruloid, some parts being largely distended. Here and there spore-initials are seen lying within or embedded in the cell-walls. Before pegmatium-formation sets in, the hyphae assume a smoky hue, the lumina become very much narrowed, and the outlines quaintly altered. Then the dissolution of the cell-walls ensues, giving rise to a gelatinous mass, out of which the pegmatia arise as yellowish, greenish, or dark-brown bodies. In a fourth case, gelatinous opaque masses exude through invisible openings in the cell-walls (see Fig. 2, *a*) ; these masses consist of the entire or partial contents of large hyphal cells, which arose from different adjacent cells by the absorption of the septa ; they harden in the end into pegmatia, of which the mycoporomatic coat is formed out of the gelatinous substance, and not from the hyphal walls. This mycoporoma may be compared with the hypothallus of some Myxogasteres. In a fifth case, terminal or interstitial cells of special hyphae emit little knob-like outgrowths, which gradually assume a cup shape. They are at first transparent, but gradually a thick mycoporomatic coat is formed, enclosing a glairy gelatinous inner part ; this is best seen when viewed through the upper surface, where the coat seems to be thinner and somewhat translucent. These bodies may arise in large numbers beside each other, and may not or do closely abut on each other ; complete fusion seems to be rare (see Fig. 2, *a*). They may be compared to the chlamydosporangia of *Sorosporium* and some other Ustilagineae, in which, however, the wall is formed out of infertile hyphae instead of the walls of fertile hyphae. In some cases, when originating on the apices of hyphae, they assume a globular shape ; the wall may then be a thin mycoporomatic coat, and the bodies may be fertile or not ; in the latter case large spore-like bodies, which are empty and therefore sterile, are seen to form within. Finally the bulk of the mycelium in the decaying plant may turn at the end of its vegetative development to the resting condition, enclosing the tissue-remnants within pegmatia. When plenty of moisture is present the pegmatia give rise to chlamydospores in huge numbers, which are at first polyhedric lumps, but gradually assume their definite shape. The exosporium appears to be formed out of the mycoporoma ; at any rate it always has the same colour, a reason why the chlamydospores arising from the pegmatia in the plant tissues show a great variety of colour, which may be some shade between yellow and dark brown. When a limited quantity of moisture is present the pegmatia at once germinate into new mycelia or they may give rise to fruit bodies, as was shown in my first paper.

Spores. The reproductive organs may be divided into ascospores—of which nothing definite can be said as yet—conidia, and chlamydospores, including oidia. The conidia are all more or less sickle-shaped, and may be unicellular or compound (2–5 celled). The most frequently occurring are the bicellular and the 4-celled conidia, which may be considered typical for this fungus. In my first paper the conidia were described as being cut off at the apices of short conidiophores, but I found them later and most frequently borne on long conidiophores, either unbranched, or branched in different ways. The branching is of the racemose type, showing a tendency to become verticillate. The single conidiophores are tapering and pointed at the tips, often bearing a not fully abstricted conidium. The conidia are loosely kept together in heads, consisting only of bicellular or 4-cellular conidia, but sometimes also of a mixture of 1–5 celled conidia (see Fig. 2, *a*). As the most specialized type of conidium fructification may be considered the Stilboid bodies, which, as described in the first paper, anticipate the ascigerous fruit body. In fact, the hyphae remaining after the conidia are shed constitute the para-, or rather periphysial sheath, macroscopically appearing as orange-red, hair-like outgrowths surrounding the golden yellow fruit body with a pink-coloured stroma. All the conidia show a great tendency to turn to chlamydospores; the transformation seems to take place mainly under the influence of moisture. In assuming the character of chlamydospores the colour becomes dark—some shade of brown—and the exosporium very much thickened. The chlamydospores have, as is said before, different modes of origin. They may be formed intercalarily as in *Chlamydomucor* or *Entyloma*; at the apices of special hyphae as in *Hypomyces*; out of the myclomyxa resulting from the dissolution of the walls of fertile hyphae or out of pegmatia, the resting stage of fertile hyphae. The process of chlamydospore-formation in this fungus may be looked upon as a specialization of the conditions encountered in the group of Hemibasidii: the chlamydospore-fructification anticipated by the slimy dissolution of the hyphal walls as in *Ustilago*; the chlamydospores formed in the course of the hyphae as in *Entyloma*, the spores enclosed in sporangium-like bodies as in *Sorosporium, Doassansia, Uleiella*; moreover, the germination of the chlamydospores in *Ustilaginoidella* alone shows the same variations as is found in the Hemibasidii as a group: as in the latter we find them in the one case producing promycelia with numerous small conidia (sporidia) cut off apically and laterally; in the other, germinating vegetatively, which need not be wondered at in the case of Ascomycetes, the chlamydospores of which very often lose their most typical character. I may here mention another mode of chlamydospore-formation, which was found on liquid nutritive media. The hyphae become chain- or oidium-like, with round bodies in the chain or on the apices of side branches. These bodies attract a large quantity of protoplasm from adjacent cells, in

which one or more plasmic globules, described before as spore-initials, are seen to form. In maturing they assume a dark violet colour and are liberated as large chlamydospores, or rather chlamydosporangia, measuring 80–120 feet (see Pl. XXIX, Fig. 3). They do not differ at all from the bodies which were mentioned in my first paper under the name of giant chlamydospores. In germinating the spore coat was seen to burst, allowing the germ tube to emerge. This germ tube consisted of one or more club-shaped cells; from the peculiar way in which they adhere to each other I infer that each cell is a separate germ tube derived from a special spore-initial, and that the adherence is only due to compression caused by growth in a limited space (see Fig. 4). The colour of the germ tube is dark, producing a mycelium which at first is also dark, but gradually becomes hyaline (see under '*Pure cultures*' in first paper). Besides the mode of oidium-formation mentioned in my first paper, I found some hyphae of the mycelium treated of above forming chains of club-shaped bodies (i. e. oidia). Some of these oidia passed into chlamydospores. Sickle-shaped conidia on parts of the same mycelium also underwent the same transformation (see Fig. 3). The chlamydospore-fructification is undoubtedly the most prominent feature of the reproductive habit of this fungus.

Haustoria. They arise as little knob-like excrescences, lateral or terminal, with opaque contents protected by a thin membrane. They grow out into flat saucer-like structures, or assume a funnel or a spoon shape, or become polypoid or tassel-like (see Fig. 5); they appear to assimilate food with the aid of their secretion, the turbidity of the protoplasm and the gummy degeneration of the walls of the cells, in which they arise, giving support to this assumption. The hyphae from which they arise become irregularly distended and turn in the end to pegmatia. The haustoria themselves may be wholly or partially transformed into mycoporoma. This takes place at the close of the parasitic mode of life of the fungus.

Pycnidia. Under this name were mentioned spore-masses found in the disintegrated tissues of the decaying rhizome and leaves. In fact they are better termed pseudo-pycnidia, since they arise in pre-existing cavities due to rupturing in consequence of contraction in the putrifying tissues. The shape is spheroidal or irregular. From all directions fertile hyphae enter the cavities and mainly crowd together in the lining cells. From this crowding together a felted mass ensues, out of which arise the mycoporomatic lining of the cavities and the huge number of chlamydospores filling them. Sometimes multicellular conidia are found among the chlamydospores; they are probably abstricted from hyphae, traversing the cavities before the chlamydospore-formation began (see Fig. 6). Considering the fact that some of these pseudo-pycnidia were mainly filled with conidia, and some conidia were found in a state of transformation into chlamydospores,

one may safely take for granted that the chlamydospores filling some pseudo-pycnidia are partly or even entirely derived from conidia.

Mycocecidia. Under this name mention was made of structures which on careful examination proved to be effluxes of pegmatia to the surface of the leaves, where the epidermis and subepidermal layers are absorbed. As will be seen in Fig. 7, remnants of absorbed tissue are enclosed in these mostly dome-shaped bodies, in which air-spaces are found, some of which contain chlamydospores in making or fully formed.

Enzymes. Judging from the histological preparations, there was good reason to think that the changes in the protoplasm and the cell-walls were caused by the action of an enzymic secretion of the fungus. To make sure, some cultures were raised on liquid sterilized banana extract in wide tubes. At the end of three weeks they were poured out on a filter and the liquid collected. To this absolute alcohol to four times the volume was added, when a yellowish precipitate was thrown down, which, collected on a filter and dried in the stove at a temperature of 37° C., had a weight of 110 mg. Dissolved in 22 grams of water (sterilized), and slices of a healthy banana sucker—cut off with the utmost precautions so as to secure sterility—being dropped in the solution contained in a large tube, which was then shut off with a lump of sterilized cotton-wool, it was noticed after three days that the solution became opaque and thickish, while the slices were very much swollen. After three more days the liquid became slimy, and the slices brought under the microscope (low power) showed that parts of the cell-walls were dissolved ; the spiral thickenings of the vessels were lying loose, and the hyaline transparency of the protoplasm was seriously disturbed. The aspect was the same as met with in the tissues of the diseased plant. The same experiment was repeated with the precipitate obtained from a watery extract of the mycelium ground down with sterilized sand. The results were identical. From the above-mentioned facts one might safely infer that the changes brought about by the secretion of the fungus are due most probably to at least two different enzymes, of which the one has properties approximating to cytase, if not actually cytase, and the other proteolytic qualities (vegetable trypsin). The first-mentioned enzyme, in fact, is one displaying the same qualities as the cytase of *Peziza sclerotiorum* described by de Bary—here also mention is made of ' curious organs of attachment in the shape of a kind of tassel ', which seem to be identical with the haustoria of the fungus described above. There was surely good reason to assume the presence of a second enzyme with proteolytic qualities ; for an enzyme with such a wide range of action, decomposing and gelatinizing the cellulose and pectose of the cell-walls and at the same time disintegrating the protoplasmic contents of the cells, was hardly conceivable. On the other hand it appeared to me very probable that the enzyme causing the disintegration of one or more of

the proteids in the protoplasm—the nucleus is not attacked, at any rate not primarily—was also the cause of the dissolution of the hyphal walls, which mainly consist of chitin, an albuminoid. In trying to prove this, I found that the action on the hyphal walls was very much enhanced when a few drops of ½ % HCl were added to the solution of the enzyme. I have satisfied myself as to the absence of any action of ½ % HCl alone. The change, however, was very slow in manifesting itself, and was not perceived sooner than on the eighth day, when the walls were found to gradually change into a highly refractive gelatinous mass. Along with this a large number of crystals of calcium oxalate were seen to be secreted, throwing light on the presence of the same in the tissues of the banana attacked by the fungus.

The coincidence of the softening of the hyphal walls, i. e. the secretion of enzyme, with the chlamydospore and pegmatium formation, gives a plausible explanation of the outbreak of the disease at the time of prominent seasonal changes, from drought to wet weather and *vice versa*, since chlamydospore-formation takes place when plenty of moisture is present and pegmatia arise when the water supply is limited, as was alluded to before.

The hyphal walls are composed nearly entirely of chitin, which was found in the following way: Pure cultures were raised on sterilized liquid banana extract in tubes of 3 cm. diameter. At the close of a fortnight a mycelium covering the surface of the liquid was removed to a dish of distilled water and well washed out so as to remove the adhering extract, and then transferred to a tube with Schweizer's reagent. It was left for four days, agitated from time to time. Removed from the solution, it was again thoroughly rinsed and left for four days in 2½ % NH_4OH, which was changed every day; washed out and dried in the stove at a temperature of 40° C. Brought under the microscope, not the least change could be detected, giving reason to infer the absence of cellulose. A fragment of 200 mg. was heated with KOH solution and subsequently treated with dilute H_2SO_4, 95 % alcohol and ether. A transparent horny substance was obtained of the same shape as the original material. When heated it was carbonized without preceding fusion. Other fragments proved to be only soluble in concentrated mineral acids and eau de Javelle, quite easily when heated; in this case the mycelium was carbonized before dissolution in H_2SO_4. The solution in hot HCl was evaporated till dry, when hygroscopic needles of glucosammonium chloride were seen to arise, which, dissolved in water and treated with KOH, gave rise to a precipitate of glucosamine. Another fragment was heated with alcoholic KOH solution and the mixture allowed to cool. The supernatant liquid was decanted and treated with dilute HCl in slight excess, when a comparatively small quantity of a gelatinous substance was thrown down, which, with the aid of staining methods recommended by Strasburger, proved to be pectine.

A very small percentage of carbohydrate was detected by heating 1 grm. of dried mycelium with 25 % H_2SO_4 for about two hours. The liquid was allowed to cool and filtered; the filtrate neutralized with KOH and the neutral solution treated with Fehling's solution, when a small amount of reducing carbohydrate was clearly demonstrated (the validity of the Fehling's solution was tested before). More elaborate investigation might throw more light on this matter. One thing may be now said: repeating this experiment several times, I was not always able to find carbohydrate on the one hand, or pectine on the other, from which I provisionally conclude that the amount of these constituents is rather varying.

Inoculation experiments. These experiments were carried out as follows :—

Two small beds in the kitchen garden of the Military Hospital were prepared by producing a fine tilth, digging the requisite number of plant-holes, and spraying the soil with a 20 % solution of formalin three times on three successive days. They were then left exposed to the sun for three days, when no vapours of formalin could be any longer detected. The suckers were all carefully examined as to their being healthy and the adhering soil removed from the spots to be inoculated. From each sucker a somewhat pyramidal fragment of the rhizome was cut out with the aid of a sharp knife, which was strongly heated every time before a stab was made; from this fragment the under part was cut off, so that the remaining upper part could be used as a lid on the opening produced. The inoculation liquid, previously prepared by shaking a portion of a pure culture in a small tube of sterilized water, was poured out on a bit of compressed cotton-wool, slightly dimpled in the middle to prevent the overflow of the fluid, and the cotton-wool pushed down the hollow with the inoculated surface downwards. At last the lid was tightly fitted in its place by the aid of another bit of cotton-wool spread over the opening. So, as was mentioned in my first paper, four suckers were inoculated with the fungus, four with fungus + Bacteria, four with Bacteria, whereas four were not inoculated and used as a check on the experiment. For convenience' sake I shall indicate the four groups by *f* = fungus, *fb* = fungus + Bacteria, *b* = Bacteria, and *c* = check. Two months after the inoculation I noticed on the leaves of one of *f*, tiny dark brown bodies, which, examined under the microscope, proved to be the mycocecidia described above. In the different sections made, hyphae were found, which at once disclosed their identity with the *Ustilaginoidella musaeperda*. All the outer leaf-sheaths of *f* and *fb* were ruptured longitudinally, but all the plants were still vigorous and healthy looking, and remained so until the middle of the fourth month, when the leaves of *f* and *fb* began to show signs of discoloration and marginal withering. A week after that, I found the outer leaves fallen back against the stems and the withering rapidly progressing and involving some of the

healthy-looking inner leaves, and at the close of the fifth month they were all dying ; *b* and *c* were still healthy-looking. All the plants were then dug out and examined ; the rhizomes of *f* and *fb* showed the typical brown streaks and dots of the Panama disease, and moreover it was clear that the infection started from the inoculation spot ; the rhizomes of *b* and *c* were perfectly healthy. Microscopic scrutiny corroborated the macroscopic examination.

To meet objections as to the suitability of an open field for an experiment such as this, it was repeated with small but vigorous suckers carefully washed out under the tap, so as to remove all the adherent soil particles, facilitated by the removal of all rootlets. The suckers after inoculation were laid out in troughs, which were filled with soil sterilized in the oven at a temperature of 120° C., the first day during three hours, and the second day during two hours. The results were even more striking. At the end of four weeks it was found that the suckers were intensely infested by the fungus, showing the typical characteristics of the Panama disease. I believe I have convincingly proved that this disease, *as it occurs in Surinam*, is caused by the *Ustilaginoidella musaeperda*. I may here mention that the inoculation and the disclosure of the results took place in the presence of the majority of the staff of the Military Hospital, while the manager of the United Fruit Company, whom I wish to thank for much valuable information, had the opportunity of seeing the results of the last-mentioned experiment.

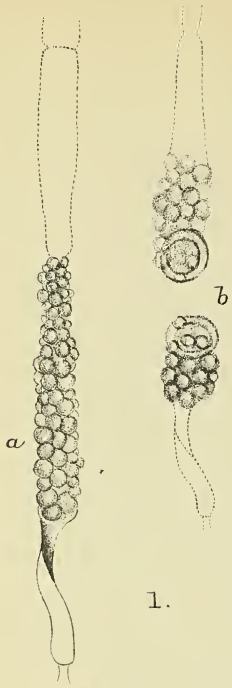
Final remarks. In the first paper I spoke of the Congo as a resistant variety, but since then it has proved not to be so resistant as was generally expected and believed at first. All efforts to discover a remedy against the plague were vain up till now ; I myself tried steeping of the suckers in $CuSO_4$ solution and frequent spraying with $CuSO_4 + (NH_4)_2SO_4$ with no results. My experiment, however, was on a very small scale and was carried out under conditions which could not be looked upon as securing all chances of success. I do not mean to say, of course, that the application of the above-mentioned mixture should lead to success, but neither is the contrary proved, since no certainty could be obtained of the perfect sterility of the suckers used. At all events, I am not inclined to admit the incurability of the disease, as is generally thought here. It is very probable that no success can be secured unless with stringent, most expensive measures, but if they could lead to success, and the expenses could be divided over a certain number of years of a permanent crop, then I do not see why all attempts to fight even a most dangerous enemy should be abandoned so soon. One can hardly give any advice as to the way to follow in experimenting, when the opportunity for one's own experiments is so unfavourable, but I hope that experts of the different experimental stations interested in the West Indies and Guiana will be at one with me that some serious fighting

is indispensable, if scientific men do not wish to lose the confidence of the planters, who would so willingly put themselves under the protecting wings of science.

EXPLANATION OF FIGURES IN PLATE XXIX.

Illustrating Mr. Ed. Essed's paper on the Panama Disease. Part II.

Fig. 1. Migration of spores; (*a*) surface view of hypha; (*b*) transverse section of same. × 350.
Fig. 2. (*a*) Hypha with conidia and pegmatia. × 255. (*b*) Modes of branching of conidiophores.
Fig. 3. (*a*) Chlamydosporangia; (*b*) chlamydospores; (*c*) oidia. × 350.
Fig. 4. Germinating giant chlamydospores. × 255.
Fig. 5. Haustoria (see text). × 255.
Fig. 6. Pseudo-pycnidium in decaying rhizome. × 255.
Fig. 7. Mycocecidium; (*a*) tissue-remnants; (*b*) air-spaces; (*c*) chlamydospores. × 450.



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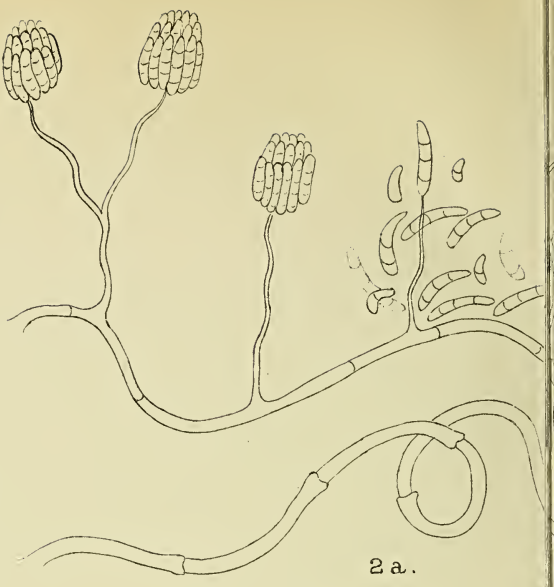

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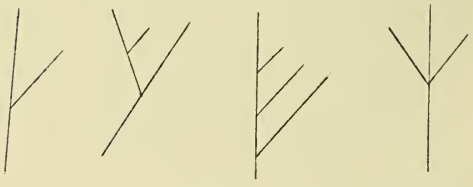

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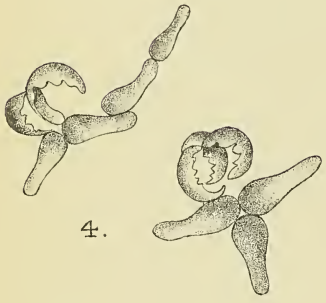

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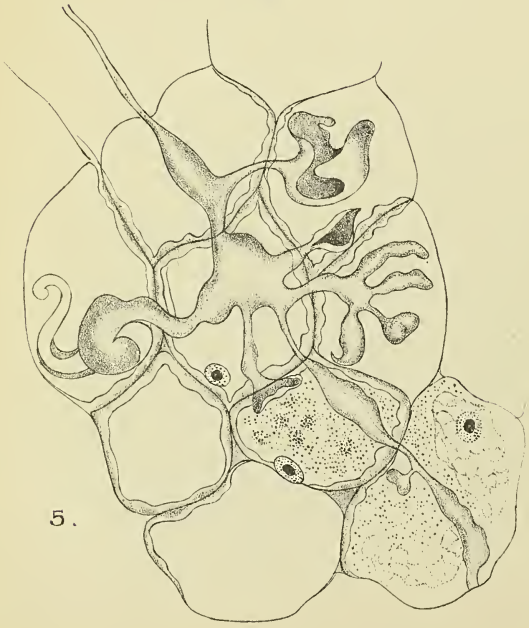

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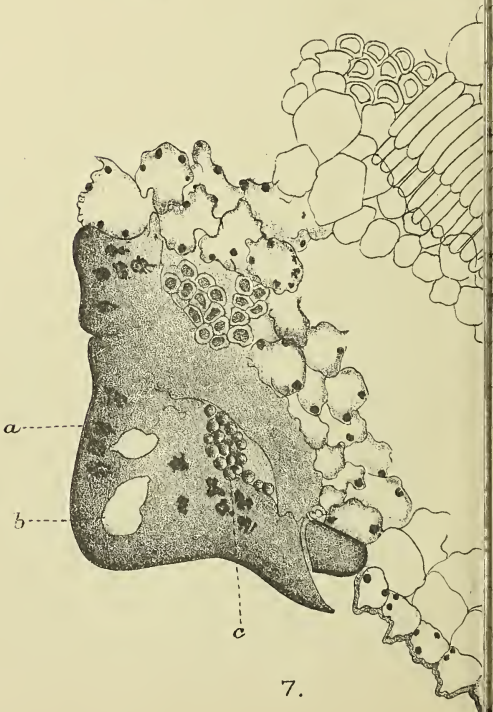
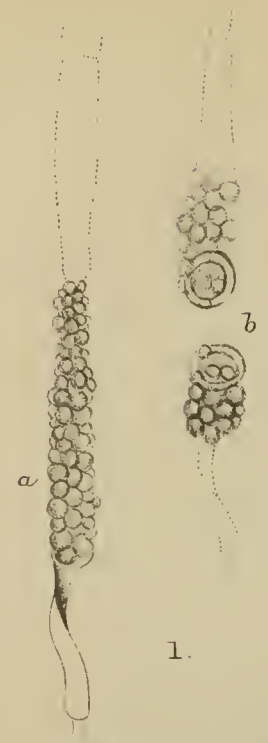


7.

Huth, Lith^r London



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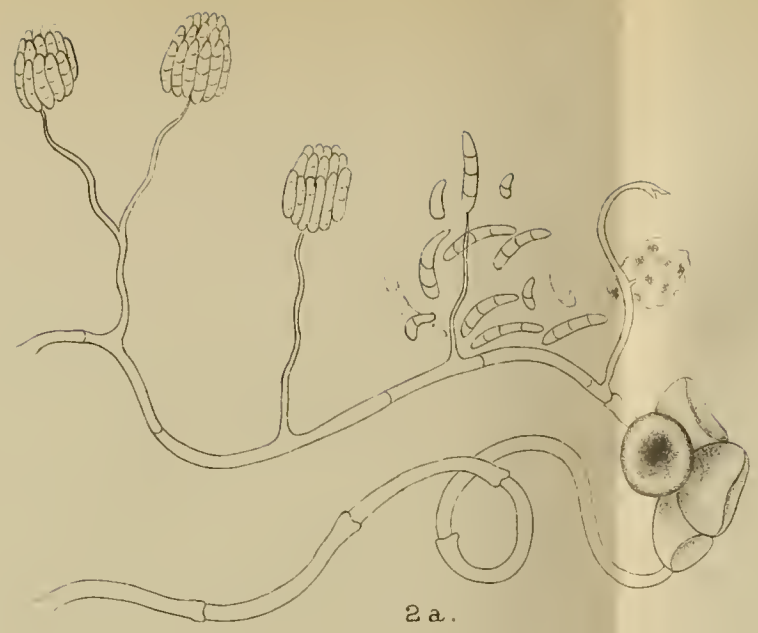

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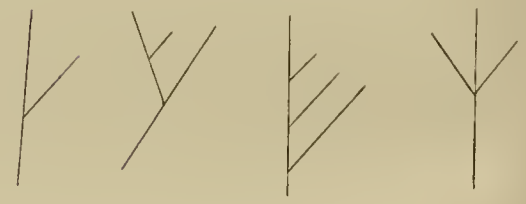

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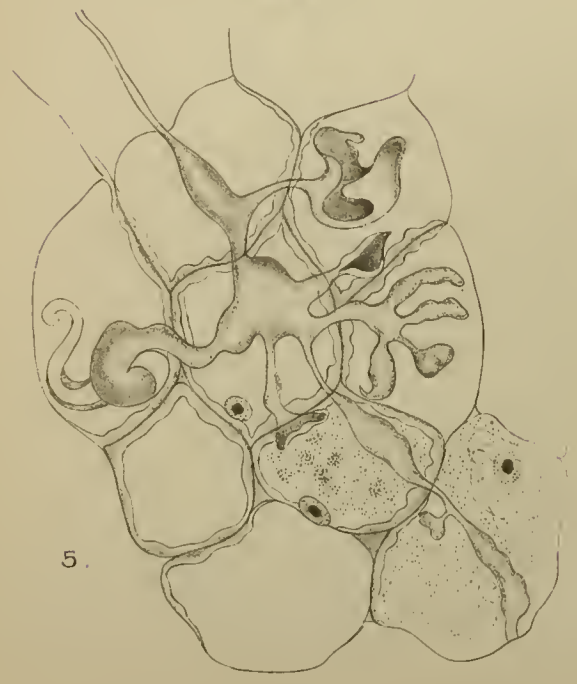

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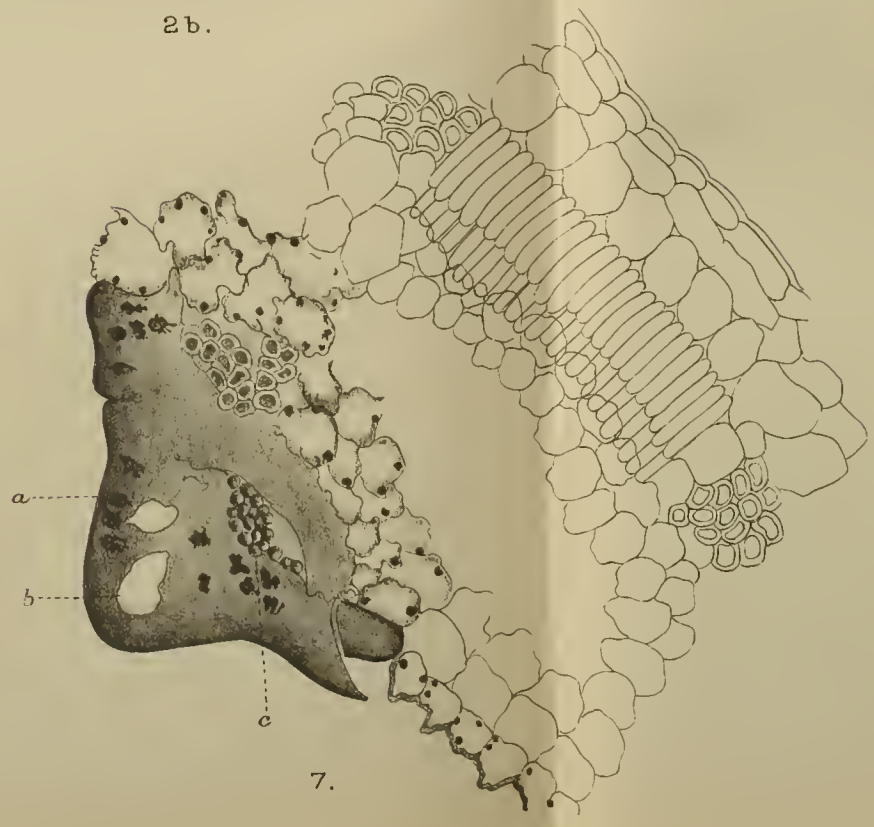

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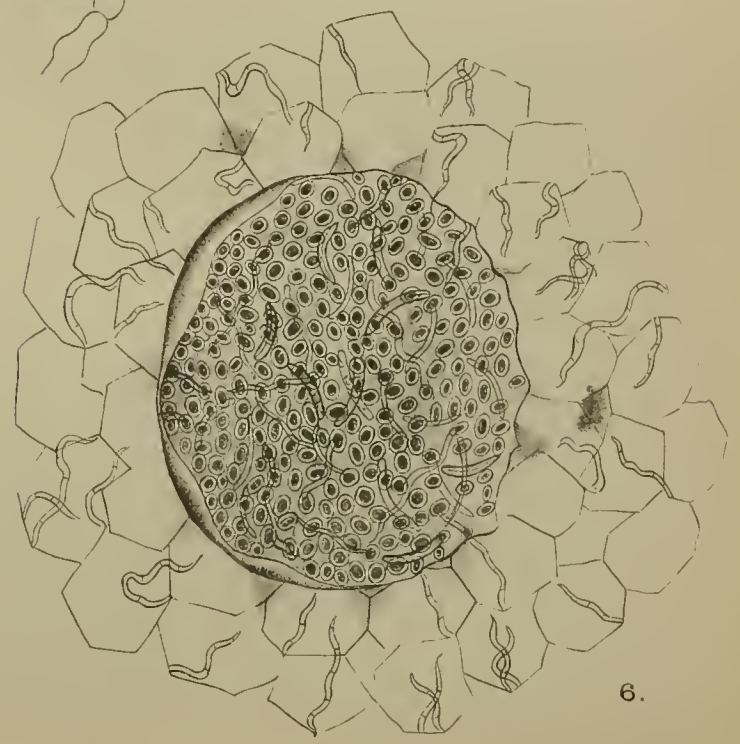

6.

ESSED — PANAMA DISEASE. II.

Huth Lith^r London

The Surinam Disease. A Condition of Elephantiasis of the Banana caused by Ustilaginoidella oedipigera.

BY

ED. ESSED, B.Sc. (Edin.).

With Plate XXX.

ALONG with the Panama disease, another plague was for a long time known to occur, though rarely, on banana fields in Surinam, but, as in the case of the Panama disease, it only attracted attention when large areas were stocked with banana, and the damage done, although less serious than that caused by the *Ustilaginoidella musaeperda*, was found to interfere with the output of the banana plantations. According to the manager of the United Fruit Co., Mr. Goldsmith Williams, this disease also made its appearance in Columbia, but, as here, it does not alarm the planters very much.

The disease manifests itself by a very often enormous distension of the base of the stem—this is why it was called 'bigie footoe' or *Elephantiasis*. In some cases it may not be apparent, but generally a kind of sloughing takes place, caused by the transverse rupturing of the leaf-bases along the line of insertion. The leaves then wither; the withering has nothing striking about it; it is the ordinary fading away of dying leaves. In scrutinizing them, small pegmatia (mycocecidia) may be found to have run out at the surface along the margins of injuries on the midrib and the blade, some having made their own way through the epidermis. The apex of the rhizome may remain growing for some time after the outer leaves are destroyed, but the young leaves do not fully develop, and become highly chlorotic. In this stage the entire stem-portion of the plant can be severed from the underground part by simply pushing it down; it ruptures transversely along the bases of the leaves. When an attempt is made to push down a banana stem killed by Panama disease, it can only be caused to bend, but never to break down along the leaf-bases. This is an important difference in the external symptoms of the two diseases.

Sections through the rhizome showed that the fungus attacks at first the parenchyma and the prosenchymatous cells of the peripheral upper region (see Pl. XXX, Fig. 1). So it was seen that the sloughing of the basal part was

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probably due to tension in the tissues caused by the huge amount of hyphae pushing through to the basal air-spaces and sheaths of the leaves, and to the slow disintegration under the influence of the enzymic secretion before or during the forming of pegmatia. Under ordinary circumstances, the lining of the basal air-spaces of the leaves show a corrugated whitish appearance, due to the desiccation and throwing off of the lining cells; but when the fungus is feeding on the banana, and hyphae are running to the air-spaces, the process is accelerated and intensified, and the lining becomes a yellowish, granulo-caseous substance, due to the breaking away of a large amount of hardened yellow slime cells, stimulated by the fungus, in some of which spores may be seen to arise. The swelling of the base of the stem proved to be brought on by metaplastic changes in the tissues.

Although the germ tubes of spores were not found actually penetrating the rhizome anywhere, it was seen that hyphae were running horizontally and moving centripetally not very much below the base of the outermost leaves. So it looks very probable that the infection takes place here, where the young cells with comparatively thin walls facilitate the entrance of the fungus; then, if the mycelium spreads through the upper peripheral parts of the rhizome at first, and if we consider that new buds on the rhizome in the most cases arise in the lower underground portion, it is readily explained why the propagation of the disease within a 'hill' may be checked by carefully removing a diseased member. Moreover, if the mycelium can only penetrate the base of the outermost leaves in a comparatively young condition, then it will be plain that the chances of infection are very much restricted, and it will at once explain why the disease is so slow in spreading.

Pure cultures were raised in the way followed in isolating the *Ust. musaeperda*. Here, again, the same bacterium appeared on the cultures, showing convincingly that this bacterium is an ordinary soil bacterium and not pathogenic at all. The young mycelium most strikingly resembled that of the *Ust. musaeperda*, but later on, when the reproductive development sets in, the differences become apparent (see Fig. 2).

Pegmatia. The only difference, but surely not essential, is the rather conical shape of the chlamydosporangioid pegmatia. All the other kinds of pegmatia are identical with those of the foregoing fungus.

Spores. The chlamydospores do not differ in their mode of origin and their outlines, but they are somewhat smaller, nearly always greenish brown. The oidia are about the same. The only essential difference, by which also the relation to the *Ust. musaeperda* is defined, lies in the conidia. In the *Ust. oedipigera* we meet with 1- and 2-cellular, very seldom 3-cellular, conidia; they are plumper and rather sausage-shaped, grouped in heads, which are kept together by a drop of hyaline mucilage. The branching is the same, but here we find an even greater tendency to become verticillate (see Fig. 3).

Haustoria. I only met with the most simple type found in *Ust. musaeperda*, viz. the flat, saucer-shaped haustoria.

Pseudo-pycnidia. Those found in the *Ust. oedipigera* are not so circumscript as those of the *Ust. musaeperda.* They contained, as in the latter, mainly chlamydospores.

Mycocecidia. These structures did not present any difference.

Enzymes. Here, again, we are faced by two enzymes of similar action. I must call attention to the fact that the action of the proteolytic enzyme is far less apparent than in the former fungus; it did not prove capable of softening the hyphal walls, as was seen in the first case; but since the same changes do take place in the one case as in the other, it is plain that the conditions under which the enzyme is acting in the plant are different from those under which the experiment is carried out.

Hyphal walls. These were found to be composed of chitin, with a small percentage of reducing carbohydrate. No pectin could be demonstrated.

The results of the inoculation experiments will be communicated later on.

Final remarks. At the start I said that the disease is not a serious one, because it does not spread to any extent, but it becomes even less so when it proves to be easily coped with. At least, the manager of the United Fruit Co., who had been experimenting for some time with $CuSO_4$ solution, found it capable of perfectly checking the spreading of the disease. And that is what might be expected in the light of what was said before as to the way in which infection takes place. For if it occurs at the base of the outer leaves, i. e. epigeally, then it is easily understood how the spores, &c., lying on or little below the surface of the soil, may be easily destroyed with a $CuSO_4$ solution of sufficient strength.

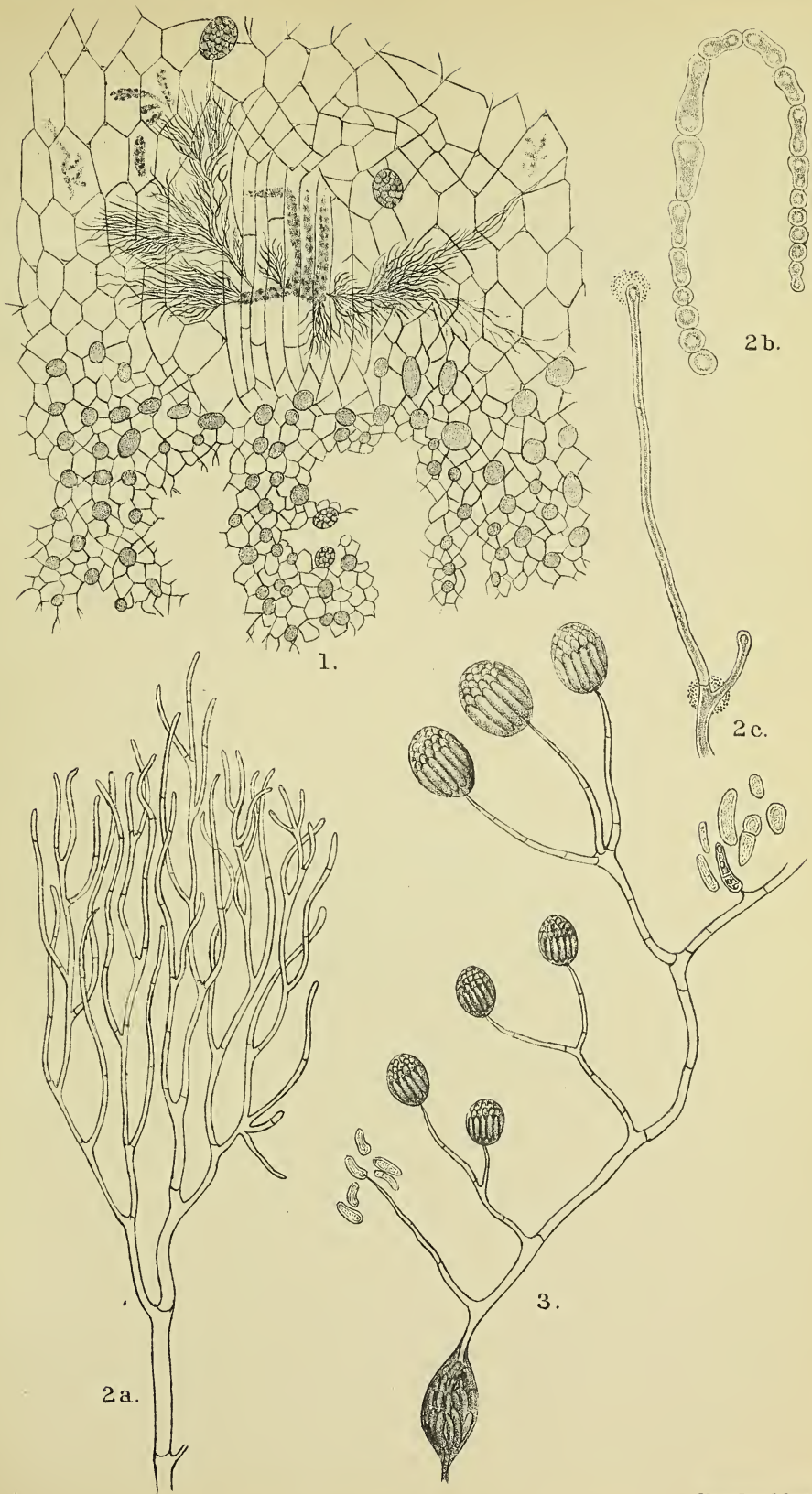
EXPLANATION OF FIGURES IN PLATE XXX.

Illustrating Mr. E. Essed's paper on the Surinam Disease, a condition of Elephantiasis of the Banana.

Fig. 1. Section through base of leaf, explained in text. × 255.

Fig. 2. (*a*) Branching of mycelium; (*b*) slimy exudation from tips of hyphae; (*c*) chain of chlamydospores and oidia.

Fig. 3. Conidial fructification.



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ESSED—SURINAM DISEASE

Rice Disease caused by Ustilaginoidella graminicola.

BY

ED. ESSED, B.Sc. (Edin.).

With Plate XXXI.

RICE has for a long time been cultivated in Guiana, but it seems that nothing was known before of any disease destroying rice crops, until about a year ago, when a rather serious disease broke out among the rice fields. Care was bestowed on it by the staff of the experimental station, where it was investigated without the cause being found; at least in the annual report, 1909, I read that neither the Government botanist nor the Agricultural instructor succeeded in finding the cause of the disease. I am well pleased to be able to communicate the positive results of my research.

The plants are often attacked in their most tender age, and then the whole crop may come to nothing, the haulms drying up long before the time of flowering; or they may be taken hold of shortly before flowering or at the time of flowering, and then only a small percentage of the flowers may escape destruction. Many apparent fruits are found to be empty husks. The disease first manifests itself by dark-brown intercostal spots with yellowish margins appearing on the leaves and sometimes on the sheaths also (see Pl. XXXI, Fig. 1). The general aspect is that of 'Rust'. Besides the rice, another grass, a species of *Panicum*, was found to be suffering from the same disease.

If a fragment of a leaf, having been stained with eosin and passed through alcohol of progressive strengths, carbol-xylol, and xylol, and at last enclosed in balsam, be brought under a low power, the dark spots will be seen to consist of a dark-brown centre—where the epidermis may be absorbed—gradually passing to a yellow periphery with indistinct lines of demarcation. Hyphae are seen to run through and between the epidermal cells, branching in all directions, entering and emerging from the stomata; spores of the same types as met with in the two foregoing species are found lying on the leaf, and crowded in and between the epidermal cells and in the emergences. The brown spots are nothing but pegmatia, cementing, as it were, the cells attacked and so rendering the cell-walls quite indistinct (see

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Fig. 2). The prosenchyma and the wood vessels are only seized upon in a far advanced stage of the disease, when pegmatia and small chlamydospores of irregular shape and greenish brown colour may be seen to form within the vessels and sclereids.

Pure cultures were not easily obtained, on account of the overwhelming number of bacterial colonies overrunning the mycelia. But when the leaves were first rinsed under the tap for a long time, steeped in 90 per cent. alcohol for two minutes, and washed out again under the tap for some time so as to remove the alcohol, and at last dried in the stove at a temperature of 40° C. for two days, the leaf fragments could be easily pounded into a coarse powder, which could be shaken with a sufficient quantity of sterilized water so as to obtain a suitable inoculation fluid. A few drops of the liquid, strained through a linen cloth previously steeped in boiling water, were poured on four Drigalsky plates and spread. The results were gratifying; two days after inoculation I was able to transfer nine pure growing mycelia.

Since the differences between this and the two foregoing Fungi are not very great, it will suffice to cite these differences, so avoiding unnecessary prolixity.

Pegmatia. Not differing; the chlamydosporangioid form smaller.

Spores. Chlamydospores smaller than in the two other species; colour greenish brown. Chlamydosporangia smaller, but of the same hue as in the two other species. Conidia 1–5 celled, sickle shaped, rather larger than in the two other species. As typical for this fungus may be considered the 1-, 3-, and 5-celled conidia, the 2- and 4-celled ones being scarce. Conidiophores as in *Ust. oedipigera.*

Haustoria, as in the last-named fungus.

Enzyme. The enzyme obtained in the ordinary way was only capable of gelatinizing the cell-walls; no proteolytic action was discovered.

Hyphal walls, as in *Ust. oedipigera.*

The results of the inoculation experiments will be mentioned later on.

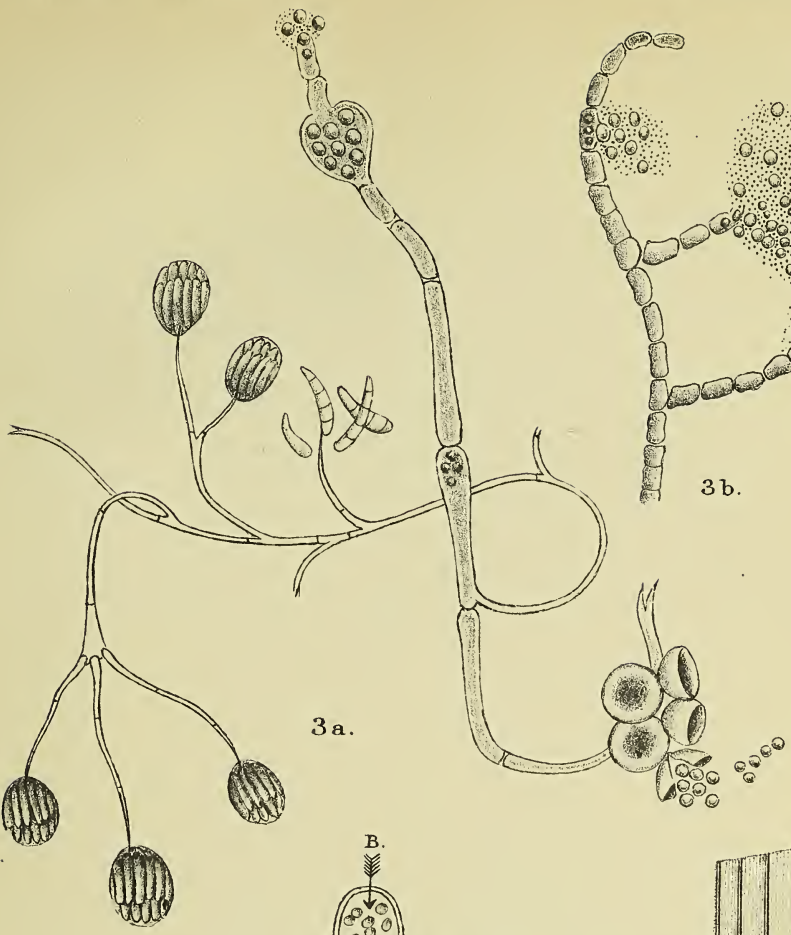
EXPLANATION OF FIGURES IN PLATE XXXI.

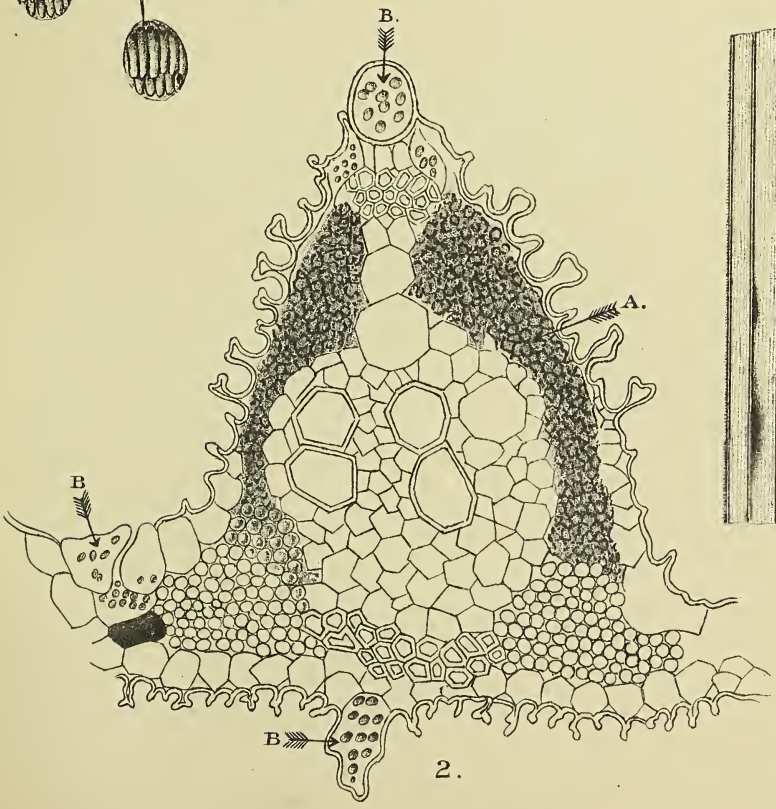
Illustrating Mr. Ed. Essed's paper on Rice Disease.

Fig. 1. Diseased spots in leaf. × 6.

Fig. 2. Transverse section of leaf. (*A*) pegmatia in parenchyma; (*B*) spores in emergences and stoma. × 255.

Fig. 3. (*a*) Fertile hyphae with conidia, chlamydospores, and pegmatia; (*b*) chain of oidia and chlamydospores.





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ESSED — RICE DISEASE

Some Observations on the Life-history of Anabaena Cycadeae.

BY

ETHEL ROSE SPRATT, B.Sc., A.K.C.

Demonstrator in Botany at King's College, London.

With Plate XXXII.

AMONG the members of the Cyanophyceae perhaps none are more interesting than those which are found endophytic in certain other plant structures. The best known examples of these are *Anabaena Azollae*, inhabiting the leaves of *Azolla*; *Anabaena Cycadeae*, living in the modified roots of *Cycas*; and the *Nostoc* sp. associated with the thallus of *Anthoceros* and *Blasia*. In each of these cases the region occupied by the Alga is an intercellular space. In *Cycas* this space is in the form of a zone between the cortical cells, just below the epidermis, known as the algal zone, which Bottomley has shown always contains living in it two kinds of nitrogen-fixing Bacteria, *Pseudomonas radicicola* and *Azotobacter*, in addition to the *Anabaena*. Considering, therefore, the highly specialized habitat in which the organism is found, and the probable symbiotic relationship existing between the *Anabaena* and the two species of Bacteria, the life-history of this Alga provides a very interesting subject for investigation.

Material and Methods.

The root tubercles of *Cycas* are found to retain their vitality for weeks, and even months, if they are wrapped in moist blotting-paper and kept in a tin box. This keeps them in a healthy condition for a surprising length of time, and by examining portions of the algal zone from time to time, the various stages constituting the life cycle of the *Anabaena* may be observed.

Several methods were employed to induce the organism to grow in an artificial medium, but with very varied success. The most promising medium was water containing some sap, obtained by crushing a few *Cycas* roots and tubercles. Solutions containing inorganic salts were not at all successful. To some of the former medium 1 % agar-agar was added, the solution boiled and subsequently cooled, so that a solid medium was

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produced such as Klebs and others have used in cultivating various Algae. Here, however, exceedingly slight growth of the *Anabaena* was obtained, although bacterial colonies appeared. Blotting-paper soaked in dilute sap from the tubercles, and kept moist in a Petri dish, was found to provide a suitable medium for the artificial culture of the Alga, small green patches of *Anabaena* appearing after a time in various parts of the blotting-paper. They appeared to lodge themselves in the pores of the substratum and there grow and divide. In the case of all the above media, several positions both with regard to light and temperature were tried, and the most favourable for the development of the organism appeared to be one illuminated by diffuse light, with a temperature of about 35° C.

In view of the fact that during some period of its life-history this *Anabaena* must continue its existence in the soil around the *Cycas* roots in order that it may infect the newly formed tubercles, a kind of artificial soil, placed in a Petri dish and kept damp, was inoculated with some of the Alga. Here, however, arose a new difficulty, namely, the manipulation of such a medium for the examination of the growth therein. Further, as will be shown later, it is exceedingly improbable that the organism exists in the soil in its easily recognizable vegetative condition.

The various stages in the life cycle were repeatedly obtained directly from the nodules, kept for varying periods, as described above. In order to determine the connexion between the stages thus observed, a number of hanging-drop preparations were employed.

When studying the action of various stains and reagents on the organism, it was found most successful and convenient to use albuminized slides, and then allow the drop containing the Alga to dry on the slide slowly at the atmospheric temperature. With this method very little contraction of cells or their contents took place, and when dry, the organisms were adhering to the slide, so that any reagent could be readily applied for any length of time. When it was desired to use a fixative, a small quantity of 2 % osmic acid was added to the drop containing the Alga. Flemming's fluid and Bouin's fixative have also been used with considerable success. The various stains and chemicals used, together with their reactions, will be described later, as they emphasize particular portions of the life cycle.

Vegetative Structure.

The species of *Anabaena* found in the *Cycas* tubercle (Pl.XXXII, Fig. 1) is a typical member of that group, each filament having around it a sheath of highly transparent mucilage. The trichomes are more or less straight or circinnate, and heterocysts are very abundant. The vegetative cells are small spherical cells, slightly flattened at each end. Their contents are blue-green in colour, usually very granular, but they may assume a homogeneous appearance.

The cellular investments of the cyanophyceous cell are quite unique. They are, however, difficult to recognize in the unstained condition, hence Kützing and Borzi thought that the protoplast was bounded merely by a plasmic membrane inside the mucilaginous sheath. Gomont and Kirchner demonstrated the presence of a definite membrane in all cases, and Fritsch more recently gives a detailed account of the cellular investments, with which the following observations on *Anabaena Cycadeae* are in accordance.

The external mucilaginous sheath (Fig. 3, *m.s.*) is comparatively narrow, and remains unaffected by any stain used, except vesuvin, which renders it brown. It is, however, distinctly visible both in stained and unstained preparations as a highly transparent, refractive zone surrounding the cell. This mucilage ceases to be secreted by the heterocysts, consequently it is only found enveloping these in their early stages of development (Fig. 3).

Each protoplast, when fully formed, is typically provided with two investments. In very young filaments, however, there is only a colourless investment present, which becomes evident when the external mucilaginous sheath is stained brown by vesuvin (Fig. 4). It is convenient to call this membrane the inner investment, and in older cells between it and the mucilage there is a small cylindrical sheath known as the outer investment (Fig. 3, *o.i.*). The adjacent cells of a filament are separated by a transparent colourless band, the transverse septum (Fig. 3, *t.s.*), which has slightly concave lateral limits, and distinctly separates the outer investments of the two cells; the latter have well-defined lateral portions, but these are joined by much less readily discernible transverse membranes.

Gomont found that a 33 % solution of chromic acid dissolved away the greater portion of the protoplasmic contents, leaving the cell membranes intact. This was indeed found to occur except in some very young vegetative cells where the definition had been somewhat lost. The cyanophyceous cell membrane is quite unlike that of any other Algae, apparently being of a very rudimentary type of development, and of a viscous mucilaginous nature, while it is capable of offering great resistance to oxidizing agents, and at the same time possessing remarkable elasticity, as shown by Brand's plasmolysis experiments. Kohl has demonstrated the presence of chitin, cellulose, and pectin in the membrane. The presence of the latter is clearly indicated by its absorbing Ehrlich's haematoxylin and remaining unaffected by chloriodide of zinc, and also iodine followed by dilute sulphuric acid. Iodine renders the outer investment dark brown, but the inner one remains clear and transparent.

In *Anabaena Cycadeae* the cytoplasm extends from the cell-wall to the central body, and although the peripheral layers contain the pigment and are undoubtedly the centre of assimilative activity, there does not appear to be any definite chromatophore present.

Many observers, amongst whom are Stockmeyer, Zukal, Zacharias,

Marx, Massart, Fischer, and Palla, have failed to find any nuclear structure in the central body present in the cyanophyceous cell; but Wille, Scott, and Zacharias in his later work, have shown it to be of a nuclear character. Hegler says that it consists of a faintly stainable ground substance in which is embedded a small quantity of chromatin, but it possesses no nuclear membrane or nucleolus. Wager and Kohl have confirmed his observations, and in *Anabaena Cycadeae* also there does not appear to be any true nuclear membrane or nucleolus. Occasionally, however, such forms as Figs. 5 and 6 (Pl. XXXII) have been observed where a body somewhat resembling a nucleolus is present, and in Fig. 5 there almost appears to be a rudimentary membrane. Irregular forms similar to those described by Kohl have also been noticed (Figs. 6 and 7). The central body contains albuminous material, which takes up methyl green, gentian violet, carbol fuchsin, and to some extent haematoxylin. It is present as granules, which vary both in size and number (Figs. 5, 6, and 7). The vegetative cells contain from one to five, whilst in the spores they are typically more abundant (Fig. 7), but may apparently be collected into one large granule (Figs. 5 and 6). Kohl found pectin substances, and also some which assumed a blue-black colour when treated with chloriodide of zinc, present. In *Anabaena Cycadeae* the former are indicated by the blue colour produced with haematoxylin, but no visible effect was obtained with chloriodide of zinc, even after forty-eight hours' immersion in this fluid.

The chief product of photosynthesis is glycogen, which is indicated by the reddish brown colour produced in the peripheral cytoplasm by treatment with iodine in potassium iodide; the colour being removable by the solution of the glycogen in water, unless the material has been previously treated with alcohol. Oil is frequently present in the form of very minute drops in the heterocysts and spores, as shown by the black colour produced in the presence of osmic acid.

A number of very distinct granules are present in the cytoplasm, which represent reserve albuminous material, and have been called cyanophycin granules. Zukal described them as distinctly differentiated portions of the protoplasm, and Hieronymus found them to contain nitrogen and phosphorus. They certainly appear to grow during the life of the cell, being larger in old cells and spores. This, with their disappearance in nodules kept in somewhat unfavourable conditions, is quite in agreement with their being reserve food material. They are most effectively stained by prolonged treatment with an alcoholic solution of eosin, when they become deep red. They swell and eventually disappear in dilute acids or caustic potash, become deep brown with iodine in potassium iodide followed by sulphuric acid, and are unaffected by alcohol, xylol, ether, Millon's reagent, or chloriodide of zinc. These granules are very abundant in the vegetative cells and spores (Fig. 8). In young heterocysts the terminal granules, and

in some cases a central granule, respond similarly, but the old heterocysts are completely unaffected.

Vegetative Division.

The first indication of a division in a vegetative cell is the appearance of an indentation near the middle of the lateral walls (Fig. 9, *b*). This constriction commences in the cell sheath, and extends to the inner investment. A very thin colourless area in the cell contents becomes visible, near the centre of the mother-cell (Fig. 9, *c*). Wager says the division of a cyanophyceous cell is brought about by the formation of a transverse septum, which grows inwards from the lateral walls, dividing the cytoplasm and nucleus into comparatively equal parts. Kohl describes a distinct polar separation of chromatic substances accompanied by the formation of a definite chromatic figure. Gardner, more recently, says that in the blue-green Algae are exhibited a series of nuclear structures passing gradually from a scarcely differentiated form of nucleus in which direct division occurs to a complex form showing a primitive type of mitosis. The division of the chromatin may precede or accompany the ingrowth of the cell-wall. In *Anabaena Cycadeae* the central body is a simple structure which divides directly after the new wall has begun to form; then a colourless strip appears between the two central bodies, and gradually extends across the cell contents to meet the investment, thus developing into the intercellular colourless area (Fig. 9, *d*). The new cells separate, and the individual cylindrical sheath of each becomes evident (Fig. 9, *e*).

Heterocysts.

Heterocysts are always abundant, but particularly in old material and fully developed tubercles gathered in autumn. They vary somewhat in size, but are usually a little larger than the vegetative cells (Figs. 3, 10).

They are formed from vegetative cells, their contents gradually becoming paler and more homogeneous. A bright highly refractive granule then appears near one, and later both, of the end walls of the cell (Fig. 10). These are cyanophycin granules, and frequently an exactly similar granule appears in the adjacent cell, on the side nearest the heterocyst (Fig. 10). Thickening of the cell-wall begins in one or both of the end walls, and extends laterally until the whole wall is thickened (Fig. 11); at the same time there is a chemical change, and the heterocyst walls exhibit definite cellulose reaction. The walls adjoining the vegetative cells develop two little lip-like prominences internally, one on either side of the pore (Fig. 12), which in old heterocysts may meet and form a plate across the pore. The granules disappear, the contents becoming colourless and contracting, but they remain attached to the wall in connexion with a vegetative cell until the

heterocysts are detached (Pl. XXXII, Fig. 13). This development is in harmony with that described by Fritsch for some other *Anabaena* sp., but differs somewhat from Brand's account of *Nostoc commune* and *Tolypothrix* sp., since he only mentions granules in connexion with fully formed heterocysts, and does not describe the transition from the lip-like thickenings to a plate.

Intercalary heterocysts usually occur singly, but in old filaments the vegetative cell on either side of a heterocyst may be thus differentiated, in which case the filament breaks and liberates the old one (Fig. 14). A dark green intercellular substance is sometimes excreted between the two adjacent cells (Fig. 15, *a*), which assumes a flat, bi-concave shape, and loses its colour, while the two cells are transformed into heterocysts (Fig. 15, *b*); then the filament breaks at that point (Fig. 15, *c*). This phenomenon was observed in *Anabaena* obtained from old nodules, and also in hanging-drop cultures about a fortnight old. Brand describes a similar formation in connexion with the production of pseudo-branches and heterocysts in *Tolypothrix* and in *Nostoc commune* during the disjointing of heterocysts in old cultures. The heterocysts are stained bright green by methyl green, while only the central granules of the vegetative cells are thus affected. Their contents are also stained by aniline gentian violet, haematoxylin, fuchsin, and orange G.

Many suggestions have been put forward with regard to the functions of the heterocysts. Some of the earlier observers, amongst whom are Borzi, Hansgirg, and Kirchner, regarded them as subserving vegetative reproduction, and in some cases, as described above, their formation is connected with the breaking up of a filament, and the consequent formation of hormogonia. Hieronymus and Hegler interpreted them as receptacles for the storage of reserve substances. Brand described the liberation of their contents as gonidia, capable of producing new filaments in *Nostoc commune* and *microscopicum*; he also ascribed to them a storage function, and considered their contents as replenishing the exhausted adjoining cells. Fritsch points out that their differentiation into terminal and intercalary, as well as their occurrence in numbers side by side, compel us to attribute to them some function other than that of limiting the filaments, although this may be part of their work. He also regards them as recipients of reserve food material, serving for its storage under certain conditions. The fact that in *Anabaena* they only occur after some vegetative cells, capable of assimilating food material, and producing structures suitable for storing the surplus organic material manufactured, have been formed, together with the variation in their cell contents at different periods, suggests that this is a probable function. If, however, these are the only functions, what is the meaning of detached heterocysts, which certainly occur under both natural and artificial conditions? Brand's observations on the germination of their contents hitherto stand isolated, but this would account for their occurrence detached, and would also be an important stage in the life-history of

Anabaena. Examples of this have been seen repeatedly in heterocysts of *Anabaena Cycadeae*, and will be described in detail in a later paragraph.

Spores—their Formation and Germination.

Spore formation in several members of the Cyanophyceae has been described by Borzi and Fritsch. This method of reproduction occurs abundantly in *Anabaena Cycadeae* in the algal zone of the nodule, and also in hanging-drop cultures under favourable conditions.

In this species the sporogenous cells are distinguishable by their well-marked lateral walls. They usually appear first in portions of the filament furthest from the heterocysts. Each protoplast is surrounded by a thin strip of colourless substance which assumes a faint brown coloration with iodine, and is continuous with the similarly staining intercellular substance separating adjacent cells. This constitutes the actual cell membrane, the inner investment (Fig. 16, *i. i.*), and is bounded laterally by the cell sheath (Fig. 16, *o. i.*), which may be a specialized inner portion of the mucilaginous envelope (Fig. 16, *m. s.*), and becomes more markedly defined as the spores develop and the necessity for a firm outer covering arises. The transverse limits of the cell sheath gradually become more distinct, and the cells move further apart. Eventually the sheath closes round the open ends, enveloping as it does so a portion of the intercellular septum (Fig. 16). The mature spore thus has a complete exospore and endospore (Fig. 16, *ex. en.*). The remaining portion of the intercellular septum swells and separates the spores, which in this species are very similar in size and shape to the heterocysts.

The mature spores may rest, but they are also capable of germinating immediately under suitable conditions. A large number of germinating spores were examined in hanging-drop cultures, where the process could be watched *in situ* in the filaments for any length of time.

There appear to be four main types of germination. The most common is that in which the contents are slowly protruded through a pore in the spore wall (Fig. 17). The contents contract, and a colourless papilla appears at one side (Fig. 17, *b*), which advances to the inner investment (Fig. 17, *c*) and then pushes this membrane out in front of the escaping protoplast, probably to protect the latter as it passes through the exospore, during which passage it is distinctly compressed (Fig. 17, *d* and *e*). The liberated protoplast is immediately followed by mucilage, which caused its liberation, and envelops it before a distinctly differentiated membrane appears (Fig. 17, *f*).

The second type of germination is that in which a portion of the spore membrane is split off (Figs. 18 and 19). The lid so formed may be pushed out in front of the exuding mucilage (Fig. 18, *a* and *b*), or it may remain

attached at one side to the rest of the spore wall (Pl. XXXII, Fig. 19). Another variation is caused by the contents germinating *in situ* in the portion of the spore wall (Fig. 19).

The third type is exhibited in those cases in which the exospore and possibly also the endospore become mucilaginous (Fig. 20), and the protoplast divides in its original position in the filament.

These three types agree with those described by Fritsch for *Anabaena* sp., whilst the fourth is in accordance with Borzi's observations. In this case the contents divide by a delicate wall into two initial cells of the filament (Fig. 21, *a*). After this the membrane splits (Fig. 21, *b*), and then the cells can expand and continue their growth (Fig. 21, *c*).

Formation of Gonidia.

Reproductive bodies termed gonidia have long been known to occur in the Chamaesiphoneae, where they are well differentiated, and a number of observations suggest their formation in the Cyanophyceae. Brand describes their occurrence in *Phormidium uncinatum*, where they arise singly by the rejuvenescence of the whole or greater part of the cell contents. He also observed the liberation of these bodies from the heterocysts of *Nostoc* sp., and adds that similar structures may be developed from the vegetative cells in these species. Fritsch found in some of his old material some rather abnormally shaped cells amongst heterocysts and sporogenous filaments, which he says had their contents rounded off and contracted, and they had acquired a new membrane of their own inside that of the mother-cell. He also observed the liberation of these contents as a spherical gonidium, but saw no indication of division either before or after, and concluded that they were caused by the arrest of spore formation.

The formation of gonidia appears to play an important part in the life cycle of *Anabaena Cycadeae*, as was suggested above. It is interesting that it should be in this Alga, living in such a unique position, in symbiosis with a plant which has existed and flourished through a very long geological period, that we should have a confirmation of the phenomenon hitherto only observed by Brand, namely, the germination of the contents of the heterocysts for purposes of reproduction. This may be observed in material from old nodules and hanging-drop cultures, particularly in such as have been allowed to undergo partial desiccation, and then favourable conditions for further development have been restored.

Material obtained in this way is composed mainly of heterocysts and spores. The former have a third investment inside, round the contracted cell contents (Fig. 22), which are no longer homogeneous, but have resumed their granular appearance. The contents undergo direct division, so that two small granular bodies are apparent in the newly-formed membrane (Fig. 23), each of which divides directly again, and eventually a number of

small spherical protoplasts are formed inside the original heterocyst walls (Fig. 25). These under certain conditions are liberated by the opening of one of the pores in the wall (Figs. 24, 25). The gonidia, when set free, have pale green contents, surrounded by an exceedingly delicate membrane, which soon becomes more definite, and the outward investment appears, also a very distinct granule lying near the centre of the cell, which no doubt represents the visible portion of the central body. Each of these small bodies is capable of resting for a time or germinating to form a small but typical *Anabaena* filament (Fig. 26), by division comparable to the process in a vegetative cell. Increase in size occurs both before and after division.

In view of the fact that the old material contained many spores, it is noteworthy that their contents on liberation are capable of differentiating directly into a heterocyst, which can then produce gonidia.

The formation of gonidia in *Anabaena Cycadeae*, besides supporting the proposition that heterocysts are the abortive relics of a method of reproduction once prevalent among the Cyanophyceae, also offers some solution to the problem: How does the *Anabaena* get into the *Cycas* tubercle? Since the Alga is not present in very young tubercles, it must at some time find an entrance, and must also be living in the medium around the roots, the soil. Means of entry appear to be afforded by the lenticels, which are present on the tubercles, through which the organism could readily enter as a small gonidium, and it is difficult to conceive how it could get through such a small opening in any other form. *Anabaena Cycadeae* is undoubtedly set free in the soil by the decay of old tubercles, in the form of spores and heterocysts, which under certain conditions give rise to gonidia, capable of forcing their way through the lenticels into the *Cycas* tubercle, where they encounter conditions favourable to their growth, and consequently there they flourish, producing vegetative cells, spores, and heterocysts.

Having entered the nodule, they confine their growth to a particular zone about four layers deep, and here, after their entrance and growth, the intercellular space, known as the algal zone, arises. It is very probable that conditions of aeration and illumination play an important part in the selection of this portion of the nodule, because in culture experiments where sections were employed the *Anabaena* spread to other regions besides the zone; here, however, conditions of illumination and aeration would be practically uniform throughout the section. The *Anabaena*, having chosen their home in the nodule, are very soon surrounded by the nitrogen-fixing bacteria, one of which, *Azotobacter*, entered the lenticels, like the *Anabaena*, whilst the other, *Pseudomonas radicicola*, was already established, and was indeed the primary cause of the tubercle formation. There is thus in the algal zone a wonderful symbiotic community consisting of an organism

capable of obtaining its energy from the sun and manufacturing carbohydrate food material, not only for its own wants, but also sufficient to supply the source of energy to the Bacteria, which in their turn supply the nitrogenous material available to the Alga ; and all three find a habitat in the Gymnospermous plant, which undoubtedly benefits in its turn from the products of the metabolic activities of these organisms.

In conclusion, my most sincere thanks and gratitude are due to Professor W. B. Bottomley for his kindness and advice during the progress of the work, and to Dr. Fritsch for many helpful suggestions.

Summary.

1. *Anabaena Cycadeae* is a typical *Anabaena.*

2. Each mature cell has two investments, an inner and outer, in addition to the external mucilaginous sheath.

3. The chlorophyll and phycocyanin are lodged in the peripheral cytoplasm, no definitely organized chromatophore being present.

4. The central body is a simple structure only capable of direct division.

5. The chief product of assimilation is glycogen.

6. Cyanophycin granules are very abundant.

7. In the vegetative division the ingrowth of the lateral walls is accompanied by direct division.

8. Terminal and intercalary heterocysts are formed from vegetative cells. They may become detached, and appear to have three functions :—

a. To limit the filaments—vegetative reproduction.

b. For storage of reserve food material.

c. Reproduction by formation of gonidia.

9. Spores are formed. The exospore and endospore are the fully developed cell sheath and inner investment respectively.

10. There are four types of spore germination :—

a. The contents are protruded through a pore in the spore membrane.

b. The spore membrane is ruptured.

c. The spore membrane becomes mucilaginous.

d. The contents divide before escaping from the spore wall.

11. Gonidia are formed by the rejuvenescence and subsequent division of the contents of the heterocysts. A distinct membrane appears inside the walls of the heterocyst. A number of spherical gonidia are formed, each of which is capable of dividing to form a new *Anabaena* filament.

12. *Anabaena Cycadeae* maintains its existence in the soil in the form of heterocysts and spores, which develop into gonidia, and these enter the *Cycas* tubercles through the lenticels.

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EXPLANATION OF FIGURES IN PLATE XXXII.

Illustrating Miss Spratt's paper on *Anabaena Cycadeae*.

Fig. 1. Nodules of *Cycas*. Natural size.

Fig. 2. Portion of transverse section of nodule, showing algal zone; *c.*, cork; *o.c.*, outer cortex; *A.Z.*, algal zone; *i.c.*, inner cortex. × 500.

Figs. 3–26 are magnified 2,200 times; *m.s.* denotes mucilaginous sheath; *o.i.*, outer investment; *i.i.*, inner investment; *t.s.*, transverse septum; *H.*, heterocyst; *yg.H.*, young heterocyst; *C.B.*, central body; *g.*, granules; *c.g.*, cyanophycin granules; *v.c.*, vegetative cell; *T.H.*, terminal heterocyst; *I.H.*, intercalary heterocyst; *ex.*, exospore; *en.*, endospore; *p.*, papilla; *m.*, mucilage; *sp.c.*, spore contents; *sp.w.*, spore wall.

Fig. 3. Vegetative filament of *Anabaena*, showing the cellular investments.

Fig. 4. Very young vegetative filament.

Fig. 5. Vegetative cell, showing the central body with a semblance of a nucleolus and nuclear membrane.

Fig. 6. Typical vegetative cell, showing central body.

Fig. 7. Vegetative filament, showing several different stages in the central body.

Fig. 8. Vegetative filament, showing the cyanophycin granules.

Fig. 9. Vegetative cells in the process of division; *a* = typical cell; *b* = constriction in investment just appearing; *c* = division of central body; *d* = complete division of contents; *e* = membranes completed and two cells formed.

Fig. 10. Filament in which heterocysts are developing.

Fig. 11. Filament containing terminal and intercalary heterocysts in which the thickening of the membranes has commenced.

Fig. 12. Filament in which the heterocysts have developed the lip-like prominences (*l.*), and the contents (*c.c.*) have contracted.

Fig. 13. Detached heterocyst.

Fig. 14. Development of a new heterocyst on either side of an old one which is consequently liberated.

Fig. 15. Development of new heterocysts following the excretion of intercellular substance (*s.*).

Fig. 16. Filament showing the development of spores (*sp.*).

Fig. 17, *a–f.* Series of stages illustrating the first type of spore germination.

Fig. 18, *a–c.* Series illustrating the second type of germination; *l.*, piece of spore wall split off.

Fig. 19. Spore germinating. Piece of wall remains attached.

Fig. 20. Third type of germination.

Fig. 21, *a–c.* Fourth type; *w.*, new membrane dividing spore contents; *p.*, pore.

Fig. 22. Heterocyst in first stage of gonidia formation; *i.m.*, new internal membrane.

Fig. 23. Contents of heterocysts divided into two in (*a*) and three in (*b*).

Fig. 24. Three gonidia being liberated.

Fig. 25. Formation of number of gonidia (*g*); *a* = beginning of their liberation; *b* = liberation continued; *c* = structure of gonidia visible.

Fig. 26. Gonidium germinated to form a small filament.

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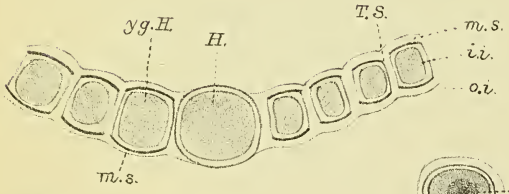

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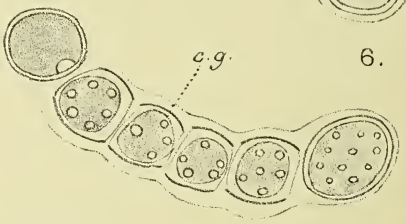

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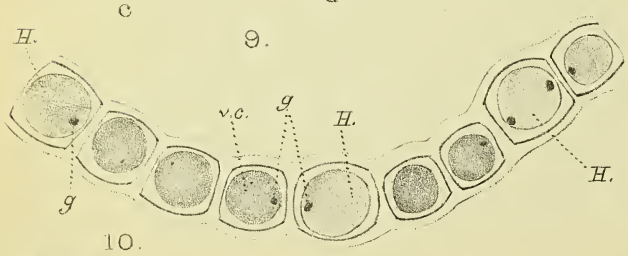

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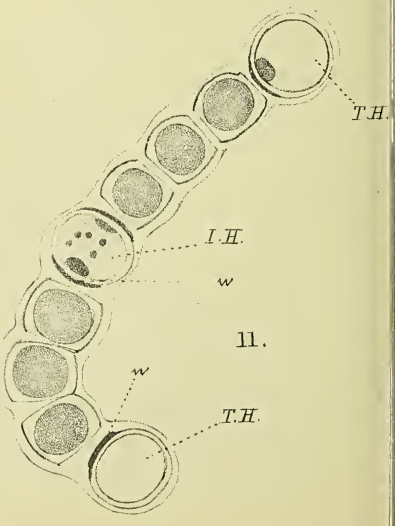

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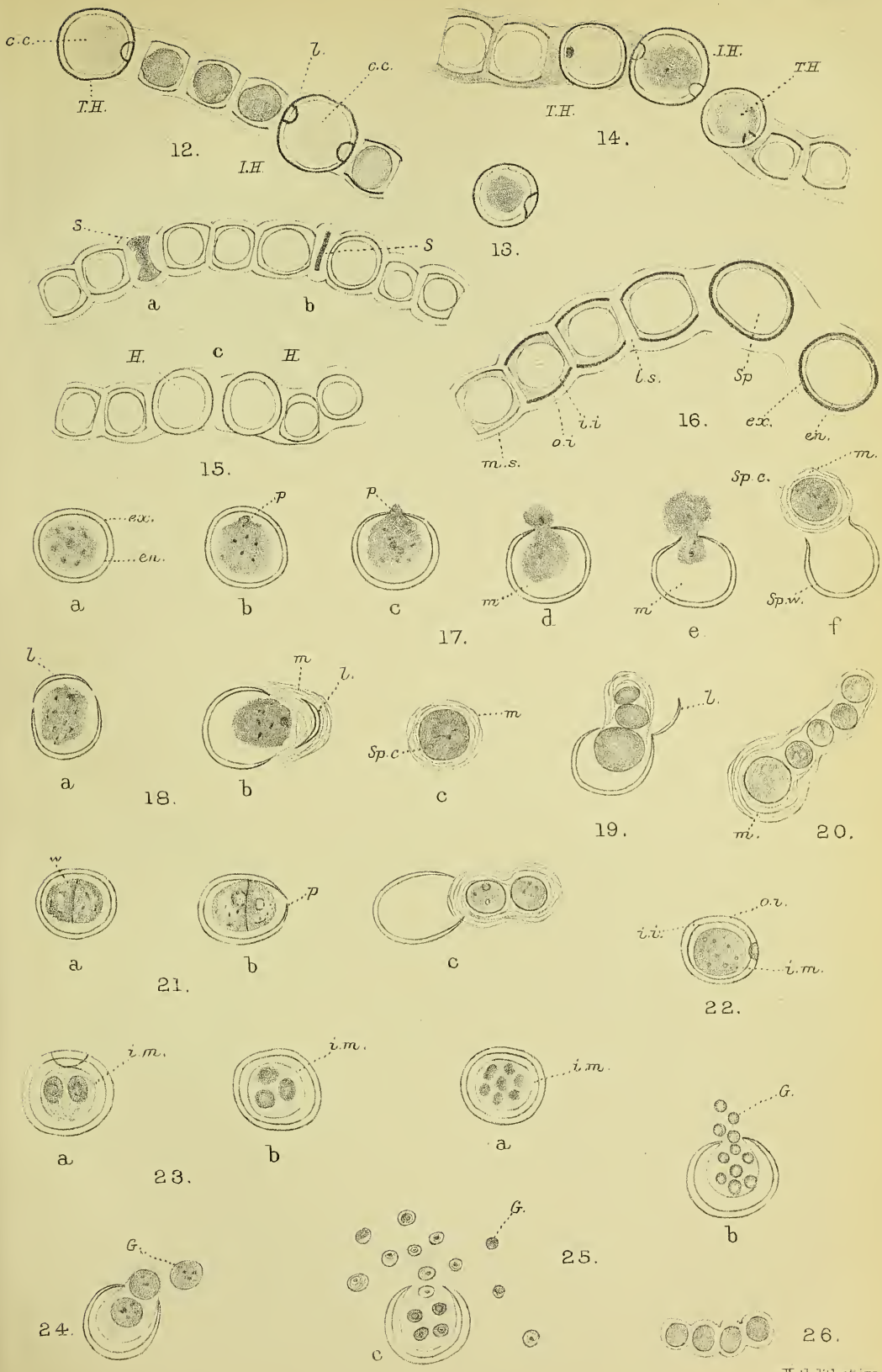

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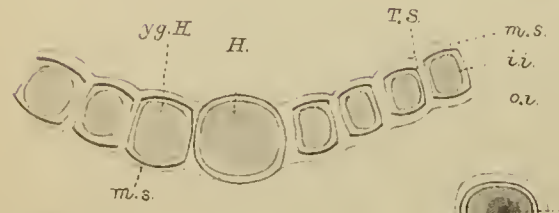

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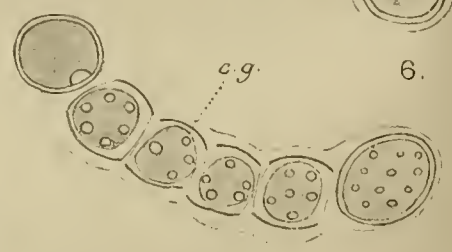

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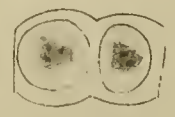

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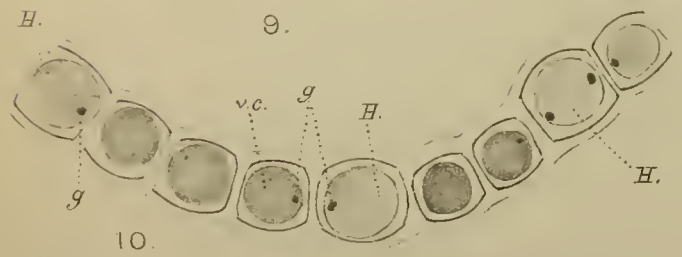

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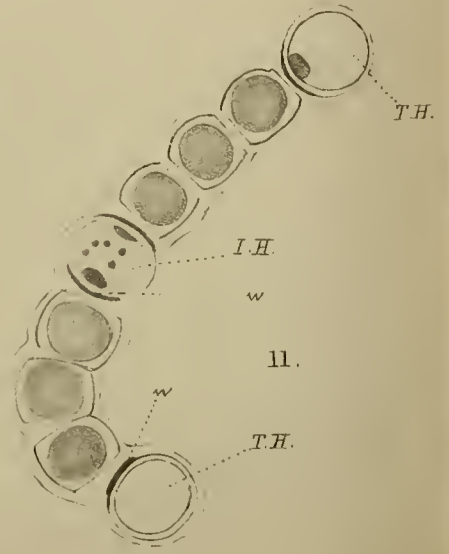

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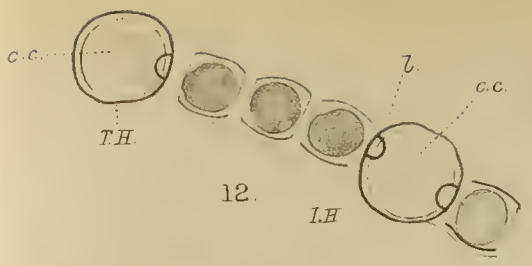

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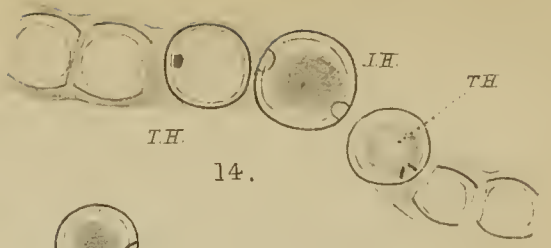

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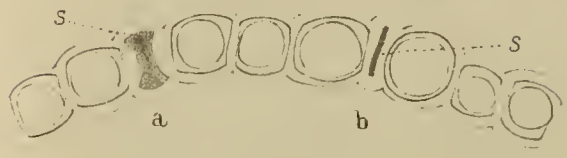

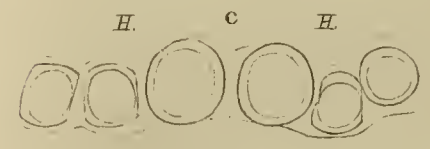

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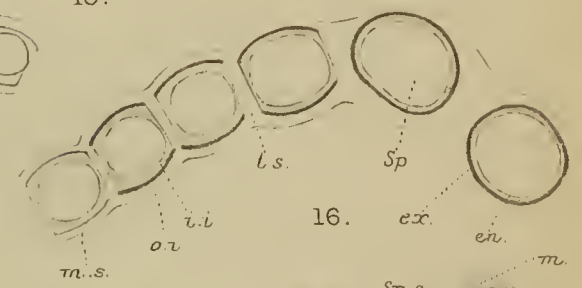

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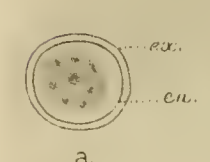

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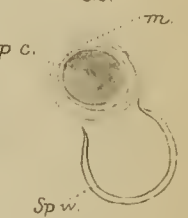

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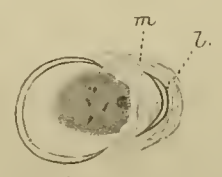

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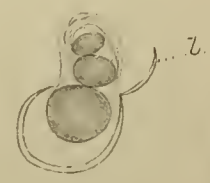

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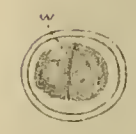

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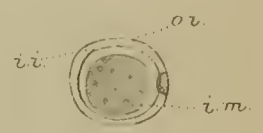

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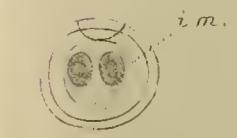

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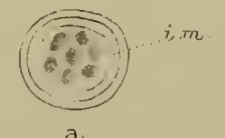

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SPRATT — ANABAENA CYCADEAE

The Structure of Mesoxylon Sutcliffii (Scott).

BY

ARTHUR J. MASLEN, F.L.S., F.G.S.

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With Plates XXXIII–XXXVI.

CONTENTS.

I. INTRODUCTION.

LAST year Dr. D. H. Scott, F.R.S., and I published a preliminary note: 'On *Mesoxylon*, a new genus of Cordaitales,'[1] the object of which was to establish the new genus and to give brief diagnoses of the five species which had been recognized in preparations obtained from the calcareous nodules of the Lower Coal Measures of Lancashire.

Of the five species—*Mesoxylon Sutcliffii*, *M. poroxyloides*, *M. multirame*, *M. Lomaxii*, and *M. platypodium*—one form, *M. Sutcliffii*, had been already shortly described by Dr. D. H. Scott under the name *Poroxylon Sutcliffii*,[2] with the qualification that 'though the plant is certainly related to the French Poroxylons, its place in the same genus must be regarded as provisional, until the investigations now in progress are completed.' In the same work some of the other species of *Mesoxylon* were referred to under *Cordaites*.[3]

[1] Annals of Botany, vol. xxiv, 1910, p. 236.

[2] Studies in Fossil Botany, 2nd Edition, 1909, p. 511, Fig. 184.

[3] loc. cit., pp. 526, 551, 651.

The object of the present paper is to give a full account of the structure of the stem and part of the leaves of *Mesoxylon Sutcliffii*, the only portions of the plant which are at present known, and to discuss the probable affinities of this form. The generic name *Mesoxylon* has been chosen to express the intermediate position of the genus between *Poroxylon* and *Cordaites*, and its special interest lies in the fact that the different species now known appear to almost completely bridge the gap, so far as the anatomy of the stem is concerned, between the Poroxyleae and the Cordaiteae, and thus form valuable links in the chain of forms connecting the Pteridosperms and Cycadofilices, with which *Poroxylon* and *Mesoxylon* have much in common, with the more typically Gymnospermous family of the Cordaiteae.

The family Poroxyleae was founded on specimens which were originally discovered in the Permo-Carboniferous deposits of Grand'Croix and Autun, and which were first described by M. Renault in 1879.[1] In 1886 appeared the 'Recherches sur les Poroxylons' by MM. Bertrand and Renault,[2] in which a very detailed account of the anatomy is given, and the extraordinarily perfect manner in which the tissues of the vegetative organs are preserved is illustrated. In this important memoir a full account is given of the structure of the stem, leaf, and root, and to this day it probably remains the most minutely detailed account which we possess of the anatomy of the vegetative organs of any fossil plant. Several species are described, *Poroxylon Boysseti* (which includes the specimens first discovered, and described by M. Renault in 1879), *P. Duchartrei*, *P. Edwardsii*, *P. Stephanense*, &c., of which *P. Edwardsii* and *P. Boysseti* are the two best known French forms.

The most important characters of the stem of *Poroxylon* are briefly as follows. The comparatively slender stems, less than 2 cm. in diameter, bore relatively large simple leaves arranged in a spiral manner and separated by rather long internodes, which in the middle region of strongly developed branches attained a length of 2 or 3 cm. The leaves themselves were simple, broad, and thick, and the lamina was traversed by numerous parallel veins. In the axils of many of the leaves the stem bore axillary branches. In structure the stem presents a fairly large continuous pith surrounded by a distinct ring of collateral leaf-trace bundles, and the development of the primary xylem of these bundles is described as being entirely centripetal. Each leaf-trace lying on the margin of the pith consists of two bundles which remain distinct for some distance below their entry into the interior of the stele, finally, however, fusing together, and, after running down through

[1] B. Renault, (*a*) Sur un nouveau genre de tiges silicifiées de l'époque houillère. Comptes rendus de l'Académie des Sciences, t. lxxxviii, Séance du 6 janvier 1879. (*b*) Structure comparée de quelques tiges de la Flore carbonifère. Paris, 1879, pp. 270–8. Nouvelles Archives du Muséum, ii, 2e série.

[2] Bertrand and Renault, Archives Bot. du Nord de la France, 1886, pp. 243–389.

thirteen internodes, joining the trace of a leaf vertically below. The continuous zone of centrifugal secondary wood is composed of rather large pitted elements separated by narrow medullary rays. The secondary phloem is excellently preserved in some forms, and in *P. Edwardsii* it consists, between the medullary rays, of alternate tangential bands of large-celled parenchyma and of sieve tubes with numerous compound sieve plates on their radial walls. The cortex is parenchymatous and contains in its outer part a strengthening system of hypodermal strands of sclerenchyma of the ordinary Dictyoxylon type, which is so commonly found in the stems and petioles of Palaeozoic plants. Periderm formation commenced at an early stage, and in older stems in which the phellogen cut right down into the phloem the whole of the cortex was thrown off as bark. Each leaf received from the stem a single large bilobed bundle (i. e. a double leaf-trace) at the base of the petiole, and division of this did not take place until the bundles had entered the leaf.

Since the publication of Bertrand and Renault's memoir on *Poroxylon* in 1886, Renault has added to our knowledge of the genus,[1] and has given an account of the phloem of *P. Boysseti* which shows that the regular structure of the phloem of *P. Edwardsii* mentioned above does not hold for the genus as a whole, and so is only a specific character in *Poroxylon*.[2]

More recently still M. Grand'Eury has identified the leaves of *Poroxylon* in the form of carbonaceous impressions, and finds that they are of great size, reaching a length of as much as a metre, with a breadth of 0·15–0·20 metre, and gradually narrowing at the base into the petiole.[3] M. Grand'Eury also attributes the platyspermic seed known as *Rhabdocarpus*, Brongniart, to *Poroxylon*:[4] the attribution is on the ground of association, but if it is confirmed the evidence of affinity between the Poroxyleae and the Cordaiteae, which has hitherto been based on a study of the vegetative structure, will be very considerably strengthened.

With regard to the affinity of *Poroxylon* MM. Bertrand and Renault regarded the form as related, on the one hand, to the Cordaiteae, and on the other to the Sigillarieae. The relation to the Lycopods was supported by the case of *Sigillariopsis* (probably a form of *Sigillaria*), which possesses double foliar bundles and occasionally pitted tracheides. Bertrand and Renault, however, also indicated some of the points of resemblance between *Poroxylon*, *Lyginodendron*, and *Heterangium*, in the twin leaf-traces and pitted tracheides.[5] In their 'conclusions' they state that: 'Les Poroxylons sont donc voisins des Sigillariées, des *Sigillariopsis*, des Lyginodendrons et des *Heterangium*, mais comme les rapports de ces types fossiles avec nos

[1] Renault, Bassin houiller et permien d'Autun et d'Épinac. Flore Fossile, 1896, Étude des gîtes minéraux de la France.

[2] loc. cit., p. 284.

[3] Sur les *Rhabdocarpus*, les graines et l'évolution des Cordaïtées. Comptes rendus, t. cxl, 1905, p. 995.

[4] loc. cit.

[5] Sur les Poroxylons, loc. cit., pp. 243, 378.

plantes actuelles ne sont pas connus, ce rapprochement ne fixe pas la place des Poroxylons dans la classification.'

In Renault's later work he sums up the affinities of *Poroxylon* and states that: 'Les Poroxylons sont un type fossile sans représentants dans la nature actuelle; ce sont des Phanérogames gymnospermes inférieures, plus voisines des Cryptogames vasculaires à structure radiée que nos Cycadées, mais supérieures aux Sigillaires, aux *Sigillariopsis*, aux *Lyginodendron* et aux *Heterangium*. Ils n'ont aucun rapport avec les Filicinées.'[1] In the light of our present knowledge the affinity of *Poroxylon* with the gymnospermous Cordaiteae on the one hand, and with the pteridospermic Lyginodendreae on the other, and so with the Filicineae, appears to be indisputable, while that with *Sillgiaria* and *Sigillariopsis* is certainly much more remote.

Our very complete knowledge of the vegetative and reproductive organs of *Cordaites* is principally due to the classic researches of MM. Grand'Eury and Renault,[2] an epitome of which is given in all the textbooks.[3]

The principal anatomical distinctions between the stem of *Cordaites* and that of *Poroxylon* are the usually discoid pith of *Cordaites*, that of *Poroxylon* being continuous; the denser, more coniferous, wood of *Cordaites*; the structure of the phloem in *Cordaites*, different from that of *Poroxylon*; the division of the double leaf-trace in the pericycle in *Cordaites*, whereas in *Poroxylon* division is deferred until the leaf has been reached; and the absence in *Cordaites* of centripetal wood in the stele, the leaf-traces only acquiring it on entering the leaf. As Renault states, the stem of *Cordaites* is 'absolutely deprived of centripetal wood'.[4]

As already mentioned, the species of the new genus *Mesoxylon* appear to completely bridge the gap between the Poroxyleae and Cordaiteae. They may be said to combine the anatomical habit of a *Cordaites* with the centripetal xylem of a *Poroxylon*.

Before passing to the detailed description of *Mesoxylon Sutcliffii* it will be well to repeat here the generic diagnosis of *Mesoxylon* given in our preliminary note.

MESOXYLON (Scott and Maslen. Annals of Botany, vol. xxiv, 1910, p. 237).

Pith relatively large, discoid.[5]

Wood dense, with narrow, usually uniseriate medullary rays, and relatively small tracheides.

[1] Renault, Bassin houiller et permien d'Autun, &c., loc. cit., p. 292.

[2] Grand'Eury, Flore carbonifère du Département de la Loire, 1877. Renault, loc. cit., Structure comparée, &c., 1879; Bassin houiller et permien d'Autun, &c., loc. cit., 1896.

[3] e. g. Scott, Studies, 2nd Edition, pp. 518–54.

[4] Renault, Bassin houiller et permien d'Autun, &c., loc. cit., p. 332.

[5] This point is not yet demonstrated in the case of *M. platypodium* in which the interior of the pith is not preserved (see Prel. Note, p. 239).

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.

The tracheides of the leaf-traces, so far as observed, are spiral or scalariform, and in some species this is also the case in the inner part of the intermediate secondary wood.

Throughout the genus the wood is of the kind usual in Cordaitales, the bulk of the secondary tracheides having multiseriate bordered pits on the radial walls.

Outer cortex strengthened by a system of sclerenchymatous bands of the Dictyoxylon or Sparganum type.

Mesoxylon Sutcliffii.

(*Poroxylon Sutcliffii*, Scott, Studies in Fossil Botany, 2nd ed., 1909, p. 511, Fig. 184).

II. General Characters.

The general characters of the genus *Mesoxylon* have already been described (p. 384); it is proposed now to give those of our most fully known species, *Mesoxylon Sutcliffii*. The specific name, in honour of the owner of the colliery at Shore (reopened on account of its richness in fossil remains, and whence all the species have been obtained), was originally suggested by Mr. J. Lomax, who sent out the sections under the name *Cordaites Sutcliffii*.

At present only the stem, the leaf-bases, and the lower part of the petiole are known, and this is the only species of *Mesoxylon* in which anything is known of any part of the leaf other than the adherent leaf-bases. The stem of *Mesoxylon Sutcliffii* as represented by the numerous series of sections which have been examined in the preparation of this paper—belonging to at least nine distinct specimens—shows remarkably little variation in size. All the specimens have been somewhat flattened by pressure, but the average diameter, including the leaf-bases, is about 3 cm., with a variation of only about 0·2 cm. around this size. The stem is somewhat larger than that of any of the other species of *Mesoxylon* described in our preliminary note, excepting *M. Lomaxii*, which is of considerably larger size, being about 5 cm. in diameter. Several of these species were, however, described from single specimens, and the discovery of others will doubtless show that all the species vary considerably in size.

Comparing *Mesoxylon Sutcliffii* with the French forms of *Poroxylon*,

it is seen that the stem of the English form is larger than that of *Poroxylon*, which had comparatively slender stems less than 2 cm. in diameter.

The outer surface of the stem has a very irregular contour, well seen in the transverse sections (Pl. XXXIII, Fig. 1), which is due to the numerous crowded leaf-bases which completely cover the surface of the stem. Usually sections of some six or seven adherent leaf-bases are shown in the transverse sections (Pl. XXXIII, Fig. 1, *l. b.*), and evidently the leaves covered the surface of the stem in a close spiral. Crowding of the leaves occurs in several of our species of *Mesoxylon* (*M. Sutcliffii*, *M. poroxyloides*, *M. multirame*), while in others (*M. Lomaxii*, *M. platypodium*) the leaf-bases are more scattered, but in no other known form are the leaves so densely crowded as they are in *M. Sutcliffii*.

In the crowding of the leaves *Mesoxylon Sutcliffii* is in strong contrast to *Poroxylon*, in which the leaves were separated by long internodes, sometimes as much as 2 or 3 cm. in length. On the other hand, in some forms of *Cordaites* the leaves appear to have been crowded on the stem, although in the species hitherto described there was probably always a free internodal surface between the leaf insertions, and the leaves were therefore less crowded than in *Mesoxylon Sutcliffii*.

The stem probably bore its leaves in seven or eight vertical rows, or orthostichies, but some difficulty has been experienced in determining the exact phyllotaxis of *M. Sutcliffii*, owing partly to the flattening by pressure of most of the specimens, and partly also to the more or less patchy preservation of the internal tissues, including the leaf-traces, which is characteristic of the plants preserved, as these are, in the roof nodules. Pl. XXXIII, Fig. 1, shows a transverse section of a stem which is only slightly flattened, and around it there are seven leaf-bases, which, counting also the petiole which would have been opposite to the bud shown on the top of the photograph, would indicate eight orthostichies. The angular divergence between two successive leaf-traces (Pl. XXXIII, Fig. 1, *l.t.*, *l.t.*) appears to be about 140°, and the phyllotaxis is probably $\frac{8}{21}$, in which case the angular divergence would be 137°. The phyllotaxis is thus of a higher order than that of *Poroxylon*, which was determined by MM. Renault and Bertrand to be $\frac{5}{13}$.

Although the leaves of *Mesoxylon Sutcliffii* are at present known only by their adherent bases and by a small portion of the petiole (Pl. XXXV, Fig. 18), these show that they were probably broad strap-shaped leaves essentially similar to those of *Cordaites* (indeed some of the familiar impressions of Cordaitean leaves in the English Coal Measures may really belong to *Mesoxylon*), and of *Poroxylon*, the leaves of which have recently been identified by M. Grand'Eury in the form of impressions (see p. 383). That the leaves of *Mesoxylon Sutcliffii* were deciduous appears to be shown by the presence of a well-marked meristematic layer at their base (Pl. XXXIV, Fig. 13, *a*).

Passing to the internal structure of *Mesoxylon Sutcliffii*, we find that it presents, of course, all the general characters of the genus which have been already briefly described (p. 384). The stem is characterized by the possession of a relatively large pith (Pl. XXXIII, Fig. 1), which in our specimens averages about 1·4 cm. in diameter, or nearly one-half of that of the stem as a whole, including the bases of the leaves.

In the relative dimensions of the pith *M. Sutcliffii* resembles *Cordaites* and differs from *Poroxylon*, in which the pith is actually and relatively considerably smaller.

Transverse sections (Pl. XXXIII, Fig. 1) show that the pith presents a well-marked division into two regions: an outer one, *p.p.*, consisting of a narrow continuous zone of parenchymatous cells, many of which possess dark contents, and a much larger central region, *c.p.*, in which the medulla has a disorganized appearance. Longitudinal sections, however, show that the inner pith has a marked discoid structure (Pl. XXXIII, Fig. 2, *c.p.*) somewhat resembling the well-known discoid pith found in most species of *Cordaites*.

The discoid pith is an important character which is probably common to all our species of *Mesoxylon*[1] and to most forms of *Cordaites*, and it is one of the characters in which the new genus differs from *Poroxylon*, in which the pith is throughout a continuous mass of parenchymatous cells. Surrounding the pith, and in contact with the zone of secondary wood, are a considerable number of 'bundles' arranged very distinctly in pairs (Pl. XXXIII, Fig. 1, *l.t.*, *l.t.*, Figs. 3, 4). All these bundles represent the downward prolongation of the traces which have come in from the leaves, and which are also frequently seen traversing the cortex, pericycle, phloem, and secondary wood on their way to the inner part of the stele. In the transverse section from which Pl. XXXIII, Fig. 1, has been made, twelve or more of these leaf-traces can be seen traversing various regions of the stem, and some of these are visible in the photograph at *l.t.*, *l.t.*, *l'.t'.*, *l'.t'.*, &c. The great number of these traces is correlated with the complex phyllotaxis and crowding of the leaves in this species.

Each leaf-trace, as it lies on the margin of the pith, usually consists of two distinct bundles which are widely separated in the upper part of its course, and gradually approximate and ultimately fuse laterally into one bundle as the trace is followed down the stem (compare Pl. XXXIII, Figs. 3, 4, 5, which show a perimedullary leaf-trace at successively lower levels).

Each leaf-trace bundle consists distinctly of two portions, an outer part consisting of centrifugally-developed wood, and an inner part composed of centripetal xylem. These two portions are clearly shown on

[1] This point is not yet demonstrated in the case of *M. platypodium* in which the inner pith is not preserved in our specimens.

Pl. XXXIII, Figs. 3 and 4. The centrifugal wood, *s.b.*, which constitutes by far the larger part of the bundles, consists of rows of radially arranged spiral or scalariform tracheides (Pl. XXXV, Fig. 14) which pass outwards, at the external limit of the bundle, by intermediate forms, into the ordinary pitted tracheides which form the continuous zone of secondary wood. The centripetal primary xylem (Pl. XXXIII, Figs. 3, 4, *p.b.*) of a trace bundle is much smaller in amount than the centrifugal wood, and consists of non-radially arranged elements, with spiral and scalariform thickenings, forming an arc. As far as we have been able to determine, the development of the inner arc of wood in *Mesoxylon Sutcliffii* was entirely in the centripetal direction, for we have been unable to demonstrate the presence of any centrifugal primary xylem elements, although it is possible that such may exist (see p. 393).

In the possession of paired leaf-trace bundles at the margin of the pith, *Mesoxylon Sutcliffii* agrees with the other species of *Mesoxylon* briefly described in our preliminary note, although there is much difference in the five species in the rapidity of convergence of the twin-bundles after their entry into the inner part of the stele. Twin leaf-trace bundles are also a feature of *Poroxylon*, and here, as in *Mesoxylon*, the two strands of a trace remain distinct for a considerable distance down the stem. Another Carboniferous plant in which the leaf-traces are given off in pairs from the margin of the pith is *Dadoxylon Spenceri*,[1] a form which may prove to be closely related to *Mesoxylon*, and the same thing is shown in the living *Ginkgo*,[2] relatives of which probably existed as far back as the Carboniferous Period.

Surrounding the pith and the leaf-trace bundles in all the specimens occurs a zone of secondary wood which has a thickness in *Mesoxylon Sutcliffii* of about 0·3 cm. (Pl. XXXIII, Fig. 1, *s.x.*). As is commonly the case in fossil plants which are preserved in the roof nodules, the preservation is somewhat patchy, and in all the specimens of *M. Sutcliffii* this is especially the case in the secondary xylem zone, the middle portion of which is often entirely destroyed (see Pl. XXXIII, Fig. 1, *s.*).

The secondary xylem consists entirely of radially-arranged tracheides of small size (usually about 0·025 mm. in diameter) and narrow uniseriate medullary rays, usually from 1–6 cells in height. With the exception of those which form part of the leaf-trace bundles, nearly all the secondary tracheides possess rows of bordered pits on their radial walls. The appearance of the tracheides in radial section is shown on Pl. XXXV, Fig. 15, and in tangential section on Pl. XXXV, Fig. 16. The wood is thus of the dense type with (usually) uniseriate rays and relatively narrow pitted tracheides which is common to all the species of *Mesoxylon*, and which is

[1] Scott, Primary Structure of certain Palaeozoic Stems, &c. Trans. Roy. Soc. Edinburgh, vol. xl, pt. ii, 1902.

[2] Seward and Gowan, Ann. Bot., vol. xiv, 1900.

known to be characteristic of *Cordaites* and of those specimens of wood which have been described under the names of *Dadoxylon* and *Araucarioxylon*, some of which have been identified as belonging to the Cordaiteae. Moreover, the secondary wood of *Mesoxylon* is practically indistinguishable from that of a Conifer of the family Araucarieae. Pairs of leaf-trace bundles are sometimes seen passing out through the zone of secondary wood, although, owing to the patchy preservation of this tissue and to the nearly horizontal course taken by the bundles, they are not as commonly met with in the transverse sections as might have been expected.

Outside the secondary xylem a cambium occurs, and the stem thus had secondary growth in thickness of a normal character.

The cambium is followed by a continuous zone of phloem, which is shown on Pl. XXXIII, Fig. 7, *p.*, and Pl. XXXV, Fig. 17, *p.* The secondary phloem has a thickness of about 0·6–0·8 mm., and consists of elements arranged in radial rows corresponding to those of the secondary wood separated by narrow medullary rays. In addition to long empty-looking cells (? sieve tubes) the phloem includes many long tubular elements containing dark contents. The structure of the phloem is much more like that of *Cordaites* than that of *Poroxylon Edwardsii* (see p. 383).

Surrounding the phloem there is a fairly thick band of tissue which may be interpreted as the pericycle (see Pl. XXXIII, Fig. 7, *pe.*, and Pl. XXXV, Fig. 17, *pe.*), although it does not appear to be sharply marked off from the cortical tissue outside. Longitudinal sections show that this zone consists mainly of rather large short parenchymatous cells, most of which possess brown contents (Pl. XXXIV, Fig. 13, *pe.*), and which are essentially similar to those which form the greater part of the outer persistent pith.

The paired leaf-trace bundles, after their escape from the zone of secondary xylem (which they traverse in a nearly horizontal direction), ascend steeply (almost vertically) through the phloem and pericycle, so that in transverse sections of the stem the bundles are cut across nearly transversely, as is shown on Pl. XXXIV, Figs. 8 and 9. When once free from the xylem cylinder the leaf-trace bundles possess a distinct collateral structure with external phloem, and this collateral structure is preserved as far out as the bundles can be traced into the leaves.

On reaching the phloem and pericycle the leaf-trace bundles, which still possess centrifugal and centripetal wood, experience a marked tangential dilatation which is preparatory to the division of each bundle into two which takes place in the inner part of the pericycle (Pl. XXXIV, Fig. 8). A similar division to that described in *Mesoxylon Sutcliffii* takes place in the other species of *Mesoxylon*; in one species, however—*M. platypodium*—each of the bundles had already divided, as regards its primary xylem, even before leaving the wood.[1]

[1] Scott and Maslen, *Mesoxylon*. Preliminary Note. Ann. Bot., vol. xxiv, p. 239.

A similar division of each leaf-trace bundle into two in the pericycle also takes place in many (or all ?) species of *Cordaites*, in which genus the leaf-trace is also a double one, while in *Poroxylon*, which agrees with *Mesoxylon* and differs from *Cordaites* in the possession of paired leaf-trace bundles at the margin of the pith, division of the bundles did not take place until the trace had entered the leaf.

The primary cortex of *Mesoxylon Sutcliffii*, as well as the leaf-bases by which the stem is covered, consists of parenchyma traversed in the outer part by a system of strengthening bands of the Dictyoxylon or Sparganum type similar to that found in so many other Palaeozoic plants (see Pl. XXXIII, Fig. 1, *d.*). Secondary cortical tissues were formed in abundance, and a number of successive periderms are seen (Pl. XXXIV, Fig. 9, *p'.*, *p'.*, and Fig. 10, *p'.*), which eventually cut right down to the pericycle, or even deeper still. The leaf-trace bundles which, as we have seen, divide in passing through the pericycle, continue to do so in traversing the cortex before entering the leaf (see Pl. XXXIV, Figs. 9 and 10). In some sections as many as eight separate bundles belonging to one trace are seen in the cortex near the junction of the cortex and an adherent leaf-base, and ten or more in the leaf-base itself while it is still adherent to the stem.

In *Mesoxylon Sutcliffii* the lower part of the free petiole is sometimes preserved, and one of these is shown on Pl. XXXV, Fig. 18; it is of flattened form, and shows ten bundles. As many as sixteen bundles are sometimes found in the petiole within a very short distance of its insertion, showing that division of the trace bundles continues to take place after they enter the leaf.

Throughout their course, as far as they can be traced in our specimens, the leaf-trace bundles retain their collateral structure, and in the petiole the inner wood of the bundles appears to attain a more distinctly mesarch structure. In the possession of mesarch collateral leaf bundles our plant agrees with some species of *Cordaites* (see p. 406) as well as with modern Cycads.

An interesting feature in the morphology of *Mesoxylon Sutcliffii* is the abundance of axillary buds; indeed, a bud appears to occur in the axil of every leaf in our specimens. Axillary branching is a feature of several of the species of *Mesoxylon* (*M. multirame*, *M. platypodium*, see preliminary note), as well as of *Poroxylon*. In *Mesoxylon Sutcliffii*, owing to the crowding of the leaves on the stem, axillary buds are more frequently seen than in any other known Carboniferous plant; a single transverse section such as that shown on Pl. XXXIII, Fig. 1, frequently shows two or three buds, *a.b.*, *a.b.*, and in addition to these, other bud-steles, *a.b.s.*, embedded in the cortex, which pass out to buds which arise in the axils of leaves at a higher level. Pl. XXXIV, Fig. 10, shows one of these axillary bud-steles in the cortex with its subtending leaf-base and a row of six leaf-trace bundles. In

several of the sections *two* distinct bud-steles corresponding to a single leaf are seen embedded in the pericycle (see Pl. XXXIV, Fig. 12): each of these appears to be a complete closed stele, and they doubtless result from the division of the single larger stele which is seen in slides cut from the same specimen at a somewhat higher level.

A transverse section of an axillary bud is shown on Pl. XXXVI, Fig. 20, and it will be seen that the stele possesses a relatively large pith surrounded by five or six distinct bundles separated by broad medullary rays. Fig. 20 also shows some of the closely packed leaves of the bud, while on Pl. XXXVI, Fig. 21, a better preserved bud-scale is shown with two very small bundles, *b.*, *b.*, close together near the centre of the leaf.

In all the sections of *Mesoxylon Sutcliffii* which have been examined, the buds appear to be in almost exactly the same stage in development; in no case has a bud grown out into a distinct branch. Presumably they were resting buds of some kind, though whether vegetative or reproductive has not been determined.

Having described the general structure of *Mesoxylon Sutcliffii*, we proceed now to a more detailed description of the various tissues, taking them in order from within outwards.

III. The Pith.

The stem of *Mesoxylon Sutcliffii* possesses a relatively very large pith, as shown on Pl. XXXIII, Fig. 1, the average diameter in our specimens being about 1·4 cm., or nearly one-half that of the stem as a whole, including the leaf-bases. The large size of the pith appears to be characteristic of the new genus as a whole, since in four out of the five species yet discovered it has a diameter nearly half of that of the whole stem. There is a well-marked division of the pith into two regions: an outer narrow continuous zone of parenchymatous cells, *p.p.*, and a much larger central region, *c.p.*, which has a discoid structure as seen in longitudinal sections (see Pl. XXXIII, Fig. 2, *c.p.*) resembling the well-known discoid pith found in most species of *Cordaites*. The discoid structure was no doubt due to the fact that the more central part of the pith was not able to follow the growth of the stem in length, and consequently split across at short intervals, leaving gaps between the persistent diaphragms; at the outer edge, next the wood, the medullary tissue remained continuous.

The central discoid pith has a diameter of about 1·1 cm., that of the whole pith being about 1·4 cm. In most of the sections the inner pith has contracted away from the persistent outer zone with which it was originally continuous. Thus, in the transverse section shown on Pl. XXXIII, Fig. 1, the outer edge of the discoid pith, *c.p.*, has contracted away from the outer pith, *p.p.*, at the sides of the section, leaving a space between, while at the top and bottom the two tissues are still continuous. Again, in the longitudinal

section shown on Pl. XXXIII, Fig. 2, a wide space occurs between the two regions of the pith on the left, while on the right they are perfectly continuous.

The *central pith*, as shown on Pl. XXXIII, Fig. 2, *c.p.*, consists of somewhat irregular, but on the whole transversely arranged, diaphragms with intervening spaces. Between the horizontal plates of tissue there are numerous fine connecting strands, and the discoid structure is clearly the result of longitudinal tension and contraction during the growth of the plant. In the transverse sections (Pl. XXXIII, Fig. 1) the discoid pith presents quite an irregular appearance, owing to the fact that the transverse diaphragms are rarely strictly horizontal (see Fig. 2) in the somewhat contracted condition in which they are preserved. In transverse sections, the cells of which the diaphragms are composed appear nearly round in form, with a diameter in the central region of 0·15 mm. to 0·20 mm. In the longitudinal sections the cells appear somewhat flattened, and are seen to be arranged in more or less horizontal rows. All the cells have a somewhat disorganized appearance. The cells in the central parts of the diaphragms are larger than any of those in the outer persistent pith.

The *outer continuous zone of the pith* consists entirely of more or less isodiametric parenchymatous cells, many of which possess dark brown contents. This zone is well shown in transverse section on Pl. XXXIII, Figs. 3 and 4, and in longitudinal section on Pl. XXXIII, Fig. 6. As is shown in the latter figure, the cells of the outer pith are arranged in vertical rows when seen in longitudinal sections. The cells frequently present the appearance of not being in contact, an appearance which is probably due to contraction of the contents prior to fossilization. The sections (Pl. XXXIII, Figs. 3, 4, 5) usually show some differentiation of the cells of the outer pith in concentric regions or zones, which pass, however, gradually into one another. Immediately adjacent to the outer boundary of the discoid pith, and passing gradually into it, occurs a zone of empty-looking cells with thin walls (Pl. XXXIII, Fig. 3, *p'*., Fig. 4, *p'*., Fig. 6, *p'*.) ; next follows a zone in which the cells are usually filled with brown contents (Pl. XXXIII, Fig. 3, *p''*., Fig. 6, *p''*.) ; outside this zone of cells with dark contents a number of smaller empty cells are sometimes seen next to the xylem (Pl. XXXIII, Fig. 4, *p''''*.), and these in longitudinal sections are found to be vertically elongated cells with rather thick walls, and quite different in shape from the somewhat tabular (short vertically) dark cells within (see Pl. XXXIII, Fig. 6, *p''*.)

The zone of contents-filled cells itself usually consists of elements of two kinds, some of the cells containing pale-brown contents, while the others are filled with very dark-brown, sometimes almost black, material. The very dark cells frequently occur as a kind of subzone outside the paler-coloured ones (Pl. XXXIII, Figs. 4 and 5, *p'''*.). The subzone of cells with very dark contents is not always clearly differentiated in the transverse sections, and

when present it is not equally developed all round the periphery of the pith, being usually absent, or nearly so, opposite to the outgoing leaf-traces, especially where the two bundles are widely separated preparatory to their exit as on Pl. XXXIII, Fig. 3. Where, as shown on Pl. XXXIII, Figs. 4 and 5, the bundles of the trace are more closely approximated (i. e. when the trace is cut through at some distance below its entrance into the central part of the stele), the zone of cells with very dark contents, *p'''*., again appears. Somewhat similar cells with dark contents ('secretory sacs') occur in the parenchymatous tissues of *Pitys* and *Cordaites*.

IV. The Leaf-traces surrounding the Pith.

As already briefly described, a considerable number of leaf-trace bundles occur around the pith in contact with the zone of secondary wood. These bundles occur in pairs, a pair ultimately passing out to each leaf. If a leaf-trace is cut through soon after its entry into the interior of the stele the twin-bundles are widely separated (Pl. XXXIII, Fig. 3), but when traced down through the stem the two bundles gradually approximate to one another (Pl. XXXIII, Fig. 4), and fuse laterally into one bundle (Pl. XXXIII, Fig. 5), and, finally, when traced still lower, the identity of this as a distinct bundle is lost.

Each leaf-trace bundle on the margin of the pith consists of both centrifugally and centripetally developed xylem, the former constituting by far the greater part of the bundle and passing gradually outwards into the tracheides which form the continuous ring of secondary wood.

The centripetal primary xylem of a trace bundle is much smaller in amount than the centrifugal wood, and consists of a number of non-radially arranged elements with spiral and scalariform thickenings, forming an arc, convex inwards, abutting at its ends on the rows of centrifugally developed tracheides of the bundle (Pl. XXXIII, Fig. 3, *p.b.*). Between the centrifugal outer xylem and the primary centripetal xylem there is a small mass of tissue consisting of delicate parenchymatous cells (Pl. XXXIII, Figs. 3 and 4, *px.*). This doubtless consists of conjunctive parenchyma accompanying the protoxylem elements, and it thus serves to mark the position of the protoxylem. Each bundle is surrounded laterally and internally by an empty space, which is shown on Pl. XXXIII, Figs. 3 and 4, *s.*; it represents the position of a very delicate tissue, which is rarely preserved even in part. This conjunctive tissue accompanies the bundles out through the wood, and is preserved around the bundles in the phloem and pericycle (Pl. XXXIV, Fig. 8, *b.*).

As far as it has been possible to determine, the development of the primary wood in the stem of *Mesoxylon Sutcliffii* was entirely in the centripetal direction. As can be seen on Pl. XXXIII, Figs. 3 and 4, the smallest elements of the inner mass of xylem, *p.b.*, occur at or near its outer limit

and in contact with the conjunctive tissue, while the elements outside this are radially arranged and presumably partly or entirely of secondary origin. Unfortunately the longitudinal sections do not suffice to decide the question as to the existence of some centrifugally developed primary xylem. An analogous case is found in *Poroxylon*, where, according to the observations of MM. Bertrand and Renault, there is no centrifugal primary xylem, but in this case also this is a point which might repay further investigation.[1] In the Cordaiteae of the type described by Renault, to which *Mesoxylon* is evidently in many respects closely related, the elements of the wood are radially arranged throughout, so that in transverse sections there is no distinction between primary and secondary xylem. In the radial sections of *Cordaites*, however, we find a marked change, from the pith outwards, in the structure of the walls of the tracheides.[2] The narrow spiral elements of the protoxylem are succeeded by wider spiral tracheides, and these again by scalariform elements. It is not until many rows have been passed that we come to the pitted tracheides, which form the bulk of the wood. The transitional region between primary and secondary xylem in *Cordaites* is thus an extensive one, and it is impossible either in transverse or in longitudinal sections to draw a sharp line between the two tissues. The protoxylem elements in *Cordaites* are localized in groups, often projecting somewhat into the pith, and marking the position of the primary bundles. Comparing the structure of *Cordaites* with that of *Mesoxylon Sutcliffii*, it is seen that the radially-arranged xylem of a leaf-trace bundle of *M. Sutcliffii* agrees in a general way with the inner wood of *Cordaites* in consisting of spiral and scalariform tracheides, passing outwards, at the limit of the bundle, and by intermediate forms, into the pitted tracheides which form the bulk of the wood, but it has been impossible to identify any definite protoxylem elements in the radially-arranged portion of the bundle, either by their smaller size in transverse section or by their special thickening in longitudinal ones. In *Mesoxylon Sutcliffii* the smallest xylem elements always occur at the outer limit of the *inner*, centripetal wood. On analogy, then, with *Cordaites*, we may perhaps regard the radially-arranged xylem of the leaf-trace bundles of *Mesoxylon Sutcliffii* as representing part of the primary wood and also the transition region between the primary and secondary xylem. Passing to the mass of irregularly (i. e. non-radially) arranged tracheides which constitutes the inner wood of the leaf-trace bundles, it is found, as already mentioned, that the smallest elements occur on its outer side, so that the structure of this part of the wood is apparently exarch, as is the case in *Poroxylon*, according to MM. Bertrand and Renault.

[1] Scott, Studies, 2nd Ed., p. 505.

[2] See figure of *Cordaites* (*Araucarioxylon*) *Brandlingii*, in Scott, Studies, 2nd Ed., Fig. 190, p. 528.

In connexion with the subject of the inner wood of the leaf-traces of *Mesoxylon Sutcliffii*, mention may also be made of *Pitys antiqua* and *Dadoxylon Spenceri*, two plants which are probably closely related to the Cordaiteae, and of *Calamopitys fascicularis*, which probably also belongs to the same plexus of intermediate forms between the Pteridosperms on the one hand and the Cordaiteae on the other. *Pitys antiqua*[1] is a tree found in the Lower Carboniferous of Southern Scotland, and its wood agrees with that of the Cordaiteae and *Mesoxylon*, except for the greater width of the medullary rays. Around the large pith as many as forty or fifty xylem strands are found, most of which are embedded in the pith at some little distance from the inner edge of the woody zone, with which they only come into contact when about to make their exit as leaf-traces. Here the leaf-trace as a whole consists of but one bundle, and this appears to correspond with the inner wood only of the trace bundle of *Mesoxylon Sutcliffii*, and from this it differs in being definitely mesarch in structure and in being usually separated from the main zone of wood. In *Dadoxylon Spenceri*,[2] a Coal-Measure form, the leaf-traces are given off in pairs as in *Mesoxylon*, and here they are also in contact with the woody zone. Traced downwards, the strands of each pair fuse as in *Mesoxylon*; they are mesarch in the upper part of their course, but at a lower level the centripetal wood appears to die out, a change which also occurs, as we shall see, in *Mesoxylon Sutcliffii*. *Dadoxylon Spenceri* possesses dense secondary wood with narrow tracheides and uniseriate medullary rays, i. e. wood of the Cordaitean type, and in all probability close relationship will ultimately be found to exist between this form and the genus *Mesoxylon*.

In the stem of *Calamopitys fascicularis*,[3] again, which stands probably nearer to the Pteridosperms than *Pitys* and *Dadoxylon*, we find the pith surrounded by a circle of primary xylem strands, eight or nine in number. Each of the traces consists of a single very large bundle in which no radially arranged elements are found, and in which the structure is distinctly mesarch. In the large strands near their exit the protoxylem is central; lower down, as the strand diminishes in diameter, the centripetal wood becomes relatively reduced in amount.

Returning to *Mesoxylon Sutcliffii*, there may be just a suggestion in some of our slides that the inner wood is not entirely centripetal in its development, i. e. that there may be a few non-radially arranged elements *outside* the mass of thin-walled tissue which marks the position of the protoxylem. Although it has been impossible to prove that this is the case, the analogy of *Dadoxylon Spenceri* and the other forms just described appears to make it not improbable that the inner wood of the perimedullary

[1] Scott, Primary Structure of certain Paleozoic Stems, &c. Trans. Roy. Soc. Edinburgh, vol. xl, pt. ii, 1902.

[2] Scott, loc. cit., p. 357. [3] Scott, loc. cit., p. 332.

traces in *Mesoxylon Sutcliffii* is mesarch also. As the whole of the elements of the trace, centrifugal as well as centripetal, consists of tracheides with similar spiral and scalariform thickenings, the longitudinal section affords little help in determining this point.

Tracing the twin-bundles of a leaf-trace of *Mesoxylon Sutcliffii* downwards from the level at which they have just reached the margin of the pith, after coming in from a leaf, the following changes are seen to take place.

Pl. XXXIII, Fig. 3, shows a leaf-trace shortly after it has entered from a leaf, magnified about 40 diameters. The two bundles of the trace are, at this level, separated by tissue, *s.x.*, about equal in width to that of one of the bundles, and the distance between the bundles (measured from the centre of each) is about 0·9 mm. The intermediate tissue consists partly of ordinary secondary xylem elements, *s.x.*, arranged in radial rows, and partly of larger cells which belong to the pith.

There is considerable difference between the five species of *Mesoxylon* in the distance between the bundles of a trace, and in the rapidity with which they fuse together when followed down the stem:[1] in *M. Lomaxii* the twin-bundles fuse immediately on reaching the pith, while *M. platypodium* is characterized by the extreme separation (as much as 2 mm.) of the bundles. *Mesoxylon Sutcliffii* occupies an intermediate position in this respect, the separation being about 0·9 mm.

At this level (Pl. XXXIII, Fig. 3) the centripetal wood, *p.b.*, is quite well developed, and the arc-like form is more pronounced than in sections of the trace at lower levels. Each bundle is partly surrounded by the space, *s.*, above mentioned.

On Pl. XXXIII, Fig. 4, a leaf-trace is shown after it has passed down through, probably, several internodes. Here the bundles have become approximated to one another, and the distance between the two bundles is now about 0·55 mm. Otherwise there is but little difference between the trace at the two levels represented in Figs. 3 and 4, excepting that at the lower level the centripetal wood is perhaps slightly smaller in amount, and its arc-like form is less evident.

Pl. XXXIII, Fig. 5, shows a leaf-trace at a still lower level. Here the two original bundles have fused laterally into one broad bundle, although the two masses of centripetal xylem, *p.b.*, are still distinct. The distance between the centres of the individual bundles is now only 0·4 mm. The amount of centripetal wood is distinctly less and the arc form is lost. Examination of the traces at even lower levels shows that the previously distinct inner wood of the two bundles fuses into one straight line and finally disappears altogether, leaving only the radially arranged centrifugal elements abutting on the pith, and these gradually lose their identity as distinct bundles altogether.

[1] Scott and Maslen, Preliminary Note, loc. cit.

On Pl. XXXIII, Fig. 6, a longitudinal section passing through the two bundles of a leaf-trace at the margin of the pith is shown. The cells of the outer pith are shown at *p''*., and the inner part of the secondary xylem-ring at *s.x.* The latter consists mainly of closely packed, straight, pitted tracheides with narrow medullary rays shown at *a*, but on its inner margin, *b*, the tracheides are spiral or scalariform, and follow a more undulating course and have broader medullary rays: the latter part, in fact, presents a similar appearance to that of the centrifugal xylem of the leaf-traces described below. In this more or less tangential section (Pl. XXXIII, Fig. 6) the leaf-trace bundles, *l.t.*, *l.t.*, are seen in the outer part of the pith. In the upper part of the photograph (Pl. XXXIII, Fig. 6) two distinct bundles are shown with medullary elements between them, while below they have fused into one broad bundle exactly as we have seen to take place from a study of the transverse sections.

The long straight course of the bundles is well seen in this slide, where the same two bundles can be traced for a distance of 0·6 cm., and they are of course only intercepted by the plane of section for a short part of their course. The very slow convergence of the bundles of a trace is also well illustrated by this slide, since there is but little diminution in the distance between the two bundles in the distance (0·6 cm.) shown in the photograph.

The centrifugal tracheides of the bundles follow a somewhat undulating course, as is seen in the photograph (Pl. XXXIII, Fig. 6, *s.b.*), and between them are relatively broad medullary rays. The appearance of this tissue in longitudinal sections is quite different from that of the secondary xylem zone with its closely packed, straight tracheides and narrow medullary rays. Some of the centrifugal xylem elements from one of the bundles shown on Pl. XXXIII, Fig. 6, are also shown on a larger scale on Pl. XXXV, Fig. 14. Here the thickening of the walls of the tracheides appears to be of the spiral kind, and between the tracheides the relatively broad medullary rays are shown. The scalariform centrifugal tracheides of the bundle pass gradually, by transitional forms, into the pitted tracheides which form the ring of secondary wood. In the centrifugal xylem of the bundle the spiral or scalariform tracheides are thickened on all sides, while in the ring of secondary xylem outside the bundles the pitting is confined to the radial walls, as is usually the case in secondary xylem elements. The centripetal wood of the bundles is shown on Pl. XXXIII, Fig. 6, *p.b.*, cut obliquely as the plane of the section passes into the pith. It forms a denser kind of wood than the centrifugal xylem, as it consists entirely of spiral or scalariform tracheides. Between the centrifugal and centripetal wood the slide shows some traces of narrow elements with loose spiral thickening which doubtless belong to the protoxylem. The fact that all the tracheides of the leaf-trace in *Mesoxylon Sutcliffii* (as well as in the other species of the

genus) have spiral or scalariform thickening is a difference between this form and *Poroxylon*, in which not only are the centrifugal elements provided with vertical rows of bordered pits, but even in the arcs of primary (centripetal) wood the more internal part of each bundle consists of pitted elements passing outwards into scalariform ones and these into the spiral tracheides of the protoxylem, exactly as is the case in *Lyginodendron* and *Heterangium*.

The sections show that not only does the centripetal xylem gradually diminish in quantity and finally die out altogether when the trace bundles are followed down the stem, but that the centrifugal wood also gradually decreases in amount. Owing to the difference in the nature of the tracheides of the centrifugal portion of a bundle and of the centrifugal secondary wood outside, it is easy to distinguish them in longitudinal sections.

V. The Xylem, Phloem, and Pericycle; and the Leaf-traces passing through them.

Surrounding the pith and the leaf-trace bundles occurs the zone of secondary wood, having a thickness in *Mesoxylon Sutcliffii* of about 0·3 cm. (Pl. XXXIII, Fig. 1, *s.x.*). The patchy preservation of this tissue has already been mentioned (p. 388), and it is illustrated by the sections shown on Pl. XXXIII, Figs. 1 and 2. As is shown in Fig. 1 the middle portion of the wood is often entirely destroyed, resulting in an irregular space, *s.*, while the outer and inner portions may be excellently preserved. The same thing is shown in the radial longitudinal section seen in Fig. 2: the secondary wood, *s.x.*, on the left of the section shows a middle empty space, while on the right nearly the whole of the xylem has been destroyed. Why the middle portion of the wood should be specially liable to destruction, while soft delicate tissues such as the phloem and pericycle are often well preserved, is not easy to determine.

The wood consists entirely of radially arranged tracheides of small size, and of narrow, generally uniseriate, medullary rays usually from 1–6 cells in height (see Pl. XXXV, Fig. 16). Two or three vertical rows of bordered pits are usually seen in radial sections on the radial walls of each tracheide (Pl. XXXV, Fig. 15), while in tangential sections no pits are visible (Pl. XXXV, Fig. 16) since they are confined to the radial walls of the elements. The tracheides and narrow medullary rays of the secondary xylem are shown in transverse section on Pl. XXXIII, Fig. 7, *s.x.*, and Pl. XXXV, Fig. 17, *s.x.*, &c.

The similarity in structure of the secondary xylem of *Mesoxylon* and that of other Cordaitales has already been pointed out (see p. 388). Comparison of the secondary xylem of *Mesoxylon Sutcliffii* with that of *Poroxylon* shows that in the latter form the tracheides are much larger, and that, owing to this

fact and to the greater thickness of the medullary rays, the wood of *Poroxylon* is of a softer, less compact type, comparable rather with that found in *Lyginodendron* and other Cycadofilices. Indeed, the dense character of the wood seems to afford a ready means of distinction between the Cordaiteae and the forms more closely related to them, such as *Mesoxylon*, and the more distant Poroxyleae, Cycadofilices and Pteridosperms, in which the secondary xylem is of a softer, more Cycadean type with larger tracheides and broader medullary rays.

The radially arranged wood which occurs immediately around the pith and between the perimedullary leaf-trace bundles (the 'intermediate secondary wood' of our preliminary note) presents a somewhat different appearance in transverse sections to that of the rest of the wood. In many places this wood doubtless represents the downward extension of the centrifugal portion of leaf-trace bundles which have nearly lost their individuality. In other places, however, where there is not the slightest trace of bundles, the innermost tracheides of the radial rows are smaller than those further out, while the number of radial rows is less and the width of the rays greater. In other words, the intermediate wood for a short distance in from the pith presents the less compact appearance which is characteristic of the outer wood of the traces.

Radial longitudinal sections show, however, that in some places there are only one or two scalariform, spiral or transitional elements between the ordinary tracheides with bordered pits and the cells of the pith. In other cases, where a greater thickness of such elements is shown, it is probable that the prolongation of a leaf-trace is cut through. In the more or less tangential section represented on Pl. XXXIII, Fig. 6, spiral elements are shown at the inner limit of the secondary wood at *b*; it appears probable that this is really part of a leaf-trace. It thus appears that, with the possible exception of a very few elements on its inner edge, the whole of the secondary xylem-ring, apart from the leaf-traces, consists of pitted tracheides.

Pairs of leaf-trace bundles are sometimes seen in the transverse sections passing out through the secondary wood. When cut across in the inner part of the secondary xylem the bundles are cut nearly transversely, but when in the outer part they appear in obliquely longitudinal section, from which it is inferred that their course through the wood is at first highly inclined (i. e. nearly vertical), and then, having fairly entered the secondary wood, they curve rapidly (owing to the growth in thickness of this zone) and assume a more nearly horizontal direction. After leaving the wood the inclination again increases, and in the phloem and pericycle the bundles are cut nearly transversely. One of the longitudinal sections,[1] a tangential one passing through the outer part of the secondary xylem, shows a pair of

[1] Slide No. 2666 (S).

leaf-trace bundles cut across nearly transversely, owing to their nearly horizontal course through the wood. Each bundle in traversing the wood has its centripetal wood directed upwards and projecting into a space, resembling somewhat the well-known rootlet bundles of a *Stigmaria* as seen in tangential sections of the wood. The space into which the centripetal wood projects clearly corresponds with that shown in the transverse sections around the bundles surrounding the pith (Pl. XXXIII, Figs. 3 and 4, *s.*), and it was doubtless occupied by delicate conjunctive parenchyma. Between this space (as seen in the tangential section) and the secondary tracheides of the xylem-ring there is a tissue which is probably continuous with the outer pith.

The transverse sections appear to indicate that in many cases the two bundles of a trace may have emerged from the wood at slightly different levels, so that when one bundle was well out in the phloem and is cut nearly transversely, the other was still embedded in the wood, and so is cut through in a more or less longitudinal direction. Thus in the trace at the right of the photograph shown on Pl. XXXIII, Fig. 1, one of the bundles is seen to be fully out while the other has not yet escaped from the secondary xylem. Slight obliquity of the section together with the very rapid curving upwards of the bundles when they leave the xylem may perhaps be sufficient reason to account for this appearance.

The secondary xylem is followed by the cambium. This tissue, which is rarely preserved, is shown on Pl. XXXV, Fig. 17, *c.*, and consists of thin-walled cells of tabular form, narrower in the radial direction than in the tangential one. Traversing the cambium are seen narrow medullary rays continuous with those of the secondary xylem.

Beyond the cambium occurs a continuous zone of secondary phloem having a width of about 0·6–0·8 mm. when measured at some distance from an emerging leaf-trace, near to which the phloem is usually much disturbed. The phloem zone is shown in transverse section on Pl. XXXIII, Fig. 7, *p.*, Pl. XXXV, Fig. 17, *p.*, and in longitudinal section on Pl. XXXIII, Fig. 2, *p.*, and Pl. XXXIV, Fig. 13, *p.* It consists mainly or entirely of elements which are arranged in radial rows corresponding to those of the secondary wood and with similar narrow medullary rays. In addition to long thin-walled elements (? sieve tubes) the phloem contains many long tubular cells or vessels with dark contents. These latter elements appear to be of two kinds. Some present a characteristic square form in transverse sections and have very dark contents (Pl. XXXIII, Fig. 7, *p'.*, and Pl. XXXV, Fig. 17, *a*), while in longitudinal sections they appear as very long straight tubes without apparent cross-walls. They may occur in any part of the secondary phloem, but are found more especially in the inner portion as seen on Pl. XXXIII, Fig. 7. They frequently occur in tangential rows as shown on Pl. XXXV, Fig. 17. The other tubular elements with contents

of the phloem have much paler contents and are more like the majority of the cells in the pericycle and the outer pith as seen in transverse sections. They are usually somewhat larger than the cells with darker contents, although smaller than the cells of the pericycle (see Pl. XXXIII, Fig. 7, *p″*., and Pl. XXXV, Fig. 17, *b*). These, too, appear in longitudinal sections as long tubes without obvious cross-walls, although they (or their contents) are broken across at irregular intervals.

Between the cells with contents, occur numerous long, narrow, thin-walled empty-looking cells (see Pl. XXXV, Fig. 17, *c*), which may be of the nature of sieve tubes, although no actual sieve plates or sieve areas can be seen. Other thin-walled empty elements appear to have cross-walls at fairly close intervals, and these may perhaps be regarded as phloem parenchyma.

The medullary rays in the phloem are shown on Pl. XXXV, Fig. 17, *m.r.*, in transverse section. They are not well shown in any of our radial sections of the phloem, but the cells appear to be narrow vertically and somewhat elongated radially.

At the outer limit of the phloem, or between that tissue and the pericycle, there sometimes occurs a more or less well-defined tissue consisting of thin-walled cells and cells with very dark contents. This tissue is shown on Pl. XXXIII, Fig. 7, *p‴*., and Pl. XXXV, Fig. 17, *d*. It occurs more especially opposite to a leaf-trace which is preparing to come out through the zone of secondary wood, and when the two bundles of a trace have escaped from the xylem and are passing outwards and upwards through the pericycle and cortex into the leaf, they are accompanied by two arcs of this tissue. This is clearly seen on Pl. XXXIV, Fig. 8, which shows a pair of leaf-trace bundles passing out through the pericycle and carrying with them the dark cells, *c*. From their position at the outer limit of the secondary phloem, and localization opposite to the primary xylem groups of the leaf-trace bundles, it seems probable that this tissue really represents the primary phloem groups of the original bundles.

The phloem, as a whole, appears to be much like that of *Cordaites*, which consists, according to Renault, of sieve tubes and phloem parenchyma with 'gum' tubes, and, in some forms, bast-fibres.[1] In *Poroxylon* the structure of the phloem is not the same in different species. In *P. Edwardsii* the secondary phloem is extraordinarily well preserved, and is made up of distinct alternate tangential bands of sieve tubes and of parenchyma, with broad medullary rays consisting of large cells. Nothing of this kind is found in *Mesoxylon Sutcliffii*. Indeed, the phloem is of quite a distinct type, as was at once recognized by Professor Bertrand (one of the authors of the original detailed description of *Poroxylon*), to whom a section of *Mesoxylon Sutcliffii* was submitted by Dr. D. H. Scott for examination.

[1] Renault, Bassin houiller et permien d'Autun, &c., p. 335.

In *Poroxylon Boysettii,* however (which includes the specimens first discovered, and described by M. Renault in 1879), the phloem has a different structure,[1] showing that the regular structure of the phloem described above is only a specific character in *Poroxylon.*

Surrounding the phloem there is a fairly thick band of tissue which may be interpreted as the pericycle. In transverse sections (see Pl. XXXIII, Fig. 7, *pe.*, and Pl. XXXV, Fig. 17, *pe.*) this zone is seen to consist mainly of rather large cells, most of which are filled with brown contents, and without any obvious arrangement in radial rows. Longitudinal sections (Pl. XXXIV, Fig. 13, *pe.*) show that this tissue consists chiefly of short parenchymatous cells without any very definite arrangement excepting that in some places the elements occur in vertical rows. There are also a number of tubular elements occupied by brown contents and probably formed from vertical rows of cells.

The pericycle is easily distinguished from the phloem in transverse sections by the larger size of its cells and the absence of radial arrangement; and also from the cortex outside it (Pl. XXXV, Fig. 17, *i.c.*) by the abundant contents of the cells. In longitudinal sections it is distinguished from the phloem by the shortness of most of its contents-filled cells as well as by the larger size of these elements. The pericycle tissue closely resembles the greater part of the persistent outer part of the pith (Pl. XXXIII, Fig. 6, *p″*.), from which it mainly differs in the presence of 'vessels' with brown contents which are absent from the pith, and in the frequent great disturbance of the tissues resulting from the emergence of the numerous leaf-traces.

The leaf-traces in the phloem and pericycle. As we have seen (p. 399), the leaf-trace bundles when traversing the zone of secondary xylem ascend very slowly, but on reaching the phloem the inclination again increases, so that in transverse sections of the stem they are cut nearly transversely. Before the exit of the xylem of the trace bundles from the secondary wood, and even before they have left the perimedullary position, considerable disturbance is visible in the phloem zone opposite to the emerging bundles, and the before-mentioned arcs of dark cells (p. 401) take up their position at the outer side of the phloem.

When once they are free from the xylem cylinder the leaf-trace bundles show a distinct collateral structure with external phloem, and this structure is preserved as far out as the bundles can be followed into the leaves. On reaching the phloem and pericycle, each bundle experiences a marked tangential dilatation, and the centrifugal xylem of the bundles becomes of a much less compact character than that of the bundles on the margin of the pith, or of the secondary xylem of the stele, and consists of a number of narrow bands of tracheides separated by broad medullary rays, as shown in the bundles seen on Pl. XXXIV, Fig. 8, *s.b.* At the same time the primary

[1] Renault, loc. cit., p. 284.

centripetal xylem of each original bundle divides into a number of small patches lying within, but usually separated by a small amount of parenchyma from, the wedges of centrifugal wood (Pl. XXXIV, Fig. 8, *p.b.*). The enlargement and tangential dilatation of the bundles is preparatory to their division into two, which takes place in the pericycle, a strictly collateral structure being preserved.

Pl. XXXIV, Fig. 8, shows a pair of leaf-trace bundles in the inner part of the pericycle. Each of the bundles shows the above-mentioned characteristics, although the actual division of the bundles has not yet taken place excepting in the xylem portion. Each bundle has a tangential width of about 0·9 mm., which is more than double that of a bundle at the margin of the pith. Outside the xylem of the bundles is the phloem, *ph.b.*, similar to and continuous with, that of the stem, and outside this again the arc of primary phloem, *c.*, before mentioned (p. 401).

Surrounding the xylem portion of the bundles there occurs a delicate conjunctive tissue, *b*, which is doubtless the same as that already mentioned as occurring around the trace bundles at the margin of the pith, and which is there usually represented by an empty space (Pl. XXXIII, Fig. 3, *s.*). Outside this there is a relatively broad sheath of cells with contents (Pl. XXXIV, Fig. 8, *s.*) surrounding the bundles, especially internally and laterally (at the level shown on Pl. XXXIV, Fig. 8, it is practically absent on the outer side of the bundle), and this sheath accompanies the bundles out into the cortex. The tissue composing this sheath is probably continuous along the course of the leaf-trace with the tissue forming the outer part of the pith, which thus becomes continuous with the very similar tissue of the pericycle. Confirmation of this is found in the tangential section of the wood of the stem described on p. 399 ; the slide shows two leaf-trace bundles cut transversely in the xylem, and each of these is accompanied by a tissue similar to that of the outer pith.

A similar division of each leaf-trace bundle into two in the pericycle, which is here described in *Mesoxylon Sutcliffii*, also takes place in many species of *Cordaites*, in which the leaf-trace is also a double one. A similar division also takes place in the other species of *Mesoxylon* described in our preliminary note ; in one species, however—*M. platypodium*—each of the bundles had already divided, as regards its primary (centripetal) xylem, even before leaving the wood.[1]

In *Lyginodendron*, and in *Calamopitys*, division also takes place in the pericycle, but in these forms there is only one trace bundle to divide, as the trace when passing through the secondary wood from the perimedullary position is single. In *Poroxylon*, however, which agrees with *Mesoxylon* in the possession of paired leaf-traces at the margin of the pith, according to MM. Bertrand and Renault, each leaf received from the stem a single large

[1] Preliminary Note, loc. cit., p. 239.

bilobed bundle (i.e. a double leaf-trace) at the base of the petiole, and division of this did not take place until after the bundles had entered the leaf. It thus appears that in the early division of the leaf-trace bundles, as in many other respects, *Mesoxylon* is much nearer to *Cordaites* than to any other form, while to a certain extent it combines characters found in *Poroxylon*, *Lyginodendron*, and *Calamopitys*.

VI. The Cortex and Leaf-bases.

The primary cortex and leaf-bases of *Mesoxylon Sutcliffii* consist of parenchyma traversed in the outer part by strengthening bands similar to those of many other Palaeozoic plants.

The thickness of the cortex between the leaf-bases can hardly be determined, as the surface of the stem is practically covered with bases of leaves.

The junction between the pericycle and the cortex is not a very sharp one, although it is as a rule fairly well defined by the difference between the contents-filled cells of the pericycle and the empty-looking ones of the cortex which are shown on Pl. XXXIII, Fig. 7, *i.c.*, and Pl. XXXV, Fig. 17, *i.c.* The cells of the inner part of the cortex are also usually somewhat smaller than those of the pericycle. Longitudinal sections show that the cells of the inner cortex are arranged in more or less vertical rows. The outer portion of the primary cortex and the leaf-bases consist of much larger cells reaching a diameter of 0·2 mm., and a rough arrangement in vertical rows is sometimes visible in this portion also. At the outer limit of the cortex there is some trace of a small-celled epidermal layer.

The Dictyoxylon outer cortex contains a number of strengthening bands arranged as more or less radial plates as seen in transverse sections (Pl. XXXIII, Fig. 1, *d.*, and Pl. XXXIV, Fig. 10, *l.b.*). Each plate or band consists of much smaller but thicker-walled elements than those forming the rest of the outer cortex. In longitudinal sections the plates are seen to be composed of much elongated fibres with oblique ends, very different from the empty, often radially elongated, cells in which the bands are enclosed.

Secondary cortical tissues were formed in abundance in *Mesoxylon Sutcliffii*, and a number of successive periderms are seen which ultimately cut right down to the pericycle, or even deeper still. Numerous wavy bands of periderm are seen on Pl. XXXIII, Fig. 1, *pm.*, and Pl. XXXIV, Fig. 9, *p'.*, *p'.*, Fig. 10, *p'*. Secondary cortical tissue was formed on both sides of the phellogen and often in about equal quantity. In some places there is a marked difference between the elements formed on the outer side and those developed on the inner side of the phellogen ; the former being radially flattened cells with thicker walls, and the latter often radially elongated cells with thinner walls.

In the deep-seated origin of the periderm *Mesoxylon Sutcliffii* resembles

both *Poroxylon* and *Lyginodendron*. In both these genera the phellogen arises in the pericycle, and in *Lyginodendron* periderm bounds the pericycle externally, forming an almost continuous layer, which arches out opposite to the leaf-traces just as is usually also the case in *Mesoxylon Sutcliffii* (Pl. XXXIV, Fig. 10). In the new form, however, the periderms are formed in a much more irregular manner, at varying depths, cutting across the cortex and leaf-bases in arcs.

The leaf-trace bundles continue to divide in traversing the cortex. Pl. XXXIV, Fig. 9, shows a leaf-trace in the inner part of the cortex ; each of the original bundles is now represented by three, so that the trace as a whole consists of six bundles. On Pl. XXXIV, Fig. 10, another trace is shown somewhat further out in the cortex, but still consisting of six bundles. Each of the bundles possesses the same characters as were described above for the bundles in the pericycle, excepting that the bundle sheath is now a complete one, that part of it on the outer side of the bundle consisting of tissue which has been carried out from the pericycle (cp. p. 403). In some slides a trace consisting of as many as eight bundles may be seen in the cortex, or near to the junction between the cortex and the leaf-base.

The leaf-bases and petioles. As already described, in *Mesoxylon Sutcliffii* seven or eight of the large leaf-bases serve to cover the stem, as seen in transverse sections (Pl. XXXIII, Fig. 1). The general structure of the leaf-bases has been already given, and it remains only to describe now the leaf-trace bundles in the adherent leaf-bases and in the free petioles. Division continues as the trace is followed outwards from the cortex, and ten or more bundles may be seen in the leaf-base while it is still adherent to the stem.

The lower portion of the free petiole is sometimes preserved in *Mesoxylon Sutcliffii*, and one of these is shown on Pl. XXXV, Fig. 18, cut in transverse section. The petiole shown in the figure is of flattened form and has a length of nearly 9 mm. and a width of nearly 2 mm. Its outer surface presents a more or less crenulated margin, while the inner side is torn and disorganized. The petiole itself appears to consist entirely of parenchymatous cells, many of which possess very dark contents. The petiole shown in Fig. 18 shows a row of about ten bundles, some of which present the appearance of being about to divide. As many as sixteen bundles have been seen in a petiole within a very short distance of its insertion on the stem, showing that the division of the bundles continues to take place after their entry into the leaf.

One of the bundles in the petiole shown on Pl. XXXV, Fig. 18, is also shown more enlarged on Pl. XXXV, Fig. 19. Only the xylem is preserved. The bundles still preserve their original collateral structure, and in this respect *Mesoxylon Sutcliffii* agrees with both *Poroxylon* and *Cordaites*. The bundles in the petiole still possess a considerable amount of radially arranged xylem, *x.*, but it is distinctly less in quantity than in the more deeply seated

portions of the bundles. On the other hand, the inner wood of the bundle has increased in relative size, and it now equals, or nearly equals, the radially arranged elements in amount. Moreover, evidence of mesarch structure in the inner wood is somewhat more clearly shown than is the case in other regions. Some of the elements which lie external to the thin-walled cells which mark the position of the protoxylem, *px.*, probably belong to the inner wood. Unfortunately, we have no longitudinal sections passing through the bundles in the petioles, and so we have been unable to confirm our observations on the transverse sections. In Fig. 19 the disorganized phloem tissue is represented at *p.*, and surrounding the bundle are some of the contents-filled cells of the leaf tissue. In the probable possession of mesarch leaf bundles our plant agrees with *Poroxylon*, some species of *Cordaites*, and *Lyginodendron* among fossil plants, as well as with the modern Cycads. Dr. M. C. Stopes has shown that in some species of *Cordaites* (*C. principalis*, Germ.) the centrifugal part of the xylem is absent, so that the leaf bundles are exarch, and she suggests that 'as a Cordaitean character possibly too much weight may have been attached to the presence of centrifugal xylem in the foliar strand'.[1] From an examination of the original figures of structure specimens of Cordaitean leaves given by Renault, Grand'Eury, and Felix, she concludes that 'the majority of known Cordaitean leaves appear to be without centrifugal xylem', and compares the leaf bundles of most species of *Cordaites* with the exarch petiolar bundles of *Medullosa*.[2] The bundles of *Cordaites* described by Dr. Stopes are far out in the flattened lamina of the leaf, while the leaf bundle of *Mesoxylon Sutcliffii* shown on Pl. XXXV, Fig. 19, is quite near to the base of the petiole; until sections of the lamina of *Mesoxylon Sutcliffii* have been identified, it will be impossible to determine to what extent the centrifugal portion of the bundles persists out into the leaves.

Exarch bundles are also found at the edges of the leaves of *Poroxylon*, while the more central main bundles are mesarch.[3]

VII. The Axillary Buds.

The characteristic axillary buds of *Mesoxylon Sutcliffii* have already been briefly described. The transverse section represented on Pl. XXXIII, Fig. 1, shows two buds, *a.b.*, *a.b.*, as well as bud-steles, *a.b.s.*, passing out through the cortex to buds which arise in the axils of leaves at a level above that of the plane of section. Pl. XXXIV, Fig. 10, shows part of a transverse section of a stem with a bud-stele, *a.b.s.*, in the cortex cut nearly transversely, outside which is a row of six leaf-trace bundles and the subtending leaf-base into which the bundles will pass. The same bud-stele is shown on

[1] Dr. M. C. Stopes, On the Leaf-structure of *Cordaites*. New Phytologist, vol. ii, 1903, p. 97.
[2] loc. cit. [3] Bertrand and Renault, Sur les Poroxylons, loc. cit., p. 354.

Pl. XXXIV, Fig. 11, more highly magnified, and it illustrates the general oval form (elongated tangentially) and the other general characters of the stele. The xylem portion of this stele measures about 0·70 mm. × 0·35 mm., and its flattened form is clearly natural and not due merely to the compression of the stem in which it is enclosed, since the latter shows but little compression, and that in a different plane from that of the bud-stele. The stele consists of a fairly large medulla, *p.*, forming a continuous tissue and surrounded by a zone of radially arranged xylem elements, *x.*, separated by relatively broad medullary rays, *m.r.* The xylem elements of the bud-steles are much smaller than those of the leaf-trace bundles or of the continuous ring of secondary xylem of the main axis, and this difference in size of the tracheides appears to furnish a ready means of distinguishing the bud-steles from the leaf-trace bundles when they are seen in longitudinal sections.

It has not been possible to distinguish any centripetally developed xylem elements in the bud-steles, although they are probably present, nor is any division into distinct bundles seen as long as the stele is embedded in the tissues of the main axis.

As already mentioned, twin bud-steles are not infrequently seen embedded in the pericycle or the inner cortex of *Mesoxylon Sutcliffii*, as shown on Pl. XXXIV, Fig. 12. Sections cut from the same specimen at a somewhat higher level show only one larger stele, and the two steles in the lower section doubtless result from the division of this one as it passes slowly downwards and inwards. Division of a bud-stele is also shown in one of the longitudinal sections.[1] In all probability the oval tangentially elongated form of the bud-stele shown on Pl. XXXIV, Fig. 11, is to be correlated with its approaching division into two steles.

In many transverse sections, however, only a *single* bud-stele is shown, even when quite close to the secondary wood, which seems to indicate that the division of the bud-stele, if it took place, often occurred while it was traversing the secondary wood of the main axis. This division must have taken place if the connexion of the vascular tissues of the branch with those of the main axis was similar to that of *Poroxylon*, in which the vascular system of the branch was inserted on the two bundles of the main axis, between which the trace of the subtending leaf passed out. Unfortunately, none of the longitudinal sections afford clear evidence of the actual mode of insertion of the bud-steles on the bundles of the main axis. In connexion with the twin axillary steles of *Mesoxylon Sutcliffii*, it is interesting to note that in another species—*M. platypodium*—a form with very broad leaf-bases, there are always two axillary steles corresponding to a single leaf.[2]

Following the bud-stele outwards into the axis of the branch (bud), some interesting modifications of structure are seen, and it is particularly interesting to be able to compare the structure of the extremely young

[1] Slide No. 2674 (S).

[2] Preliminary Note, p. 239.

stem of the bud with that of the ordinary mature stem. Pl. XXXVI, Fig. 20, shows a transverse section of an axillary bud magnified about 40 diameters. Surrounding a relatively large continuous pith, *p.*, is a ring of some five or six minute but distinct bundles, *b.*, separated by broad medullary rays, *m.r.* The xylem of the bundles consists mainly of rows of very small tracheides, and within these there is some evidence of minute patches of irregularly arranged elements, evidently corresponding to the centripetally developed wood of the bundles of the main axis. Outside the wood some rows of thin-walled phloem elements are visible.

Between the larger bundles, and in the medullary rays, are seen small bundles (Pl. XXXVI, Fig. 20), which are evidently leaf-trace bundles, passing out to the bud-scales, and similar ones are also seen traversing the pericycle and cortex (Fig. 20, *l.t.*, &c.). Each of these minute leaf-trace bundles appears to consist of about half a dozen very small tracheides without radial arrangement, and to be composed entirely of the centripetally developed xylem sometimes seen at the margin of the pith.

No arrangement of the leaf-trace bundles in pairs is visible, although opposite to each 'gap' between the main bundles two leaf-trace bundles are usually seen, one of these, however, being much further out than the other. The wood at this early stage in the development of the 'branch' is of a much less dense character, with broader medullary rays, than is characteristic of the ordinary secondary xylem of the mature stem of *Mesoxylon* and other Cordaiteae, while it more resembles the less dense centrifugal wood forming part of the leaf-trace bundles, which may be partly of primary rather than of secondary origin (*ante*, p. 394). Indeed, it seems probable that the whole of the xylem of the bundles in the section shown on Pl. XXXVI, Fig. 20, is best regarded as primary wood.

Outside the stele occurs a relatively broad band (Pl. XXXVI, Fig. 20, *pe.*) consisting of small cells, some of which possess dark contents. This tissue, which closely resembles the pith in appearance, probably corresponds with the tissue which has been distinguished as the pericycle in the main axis. Beyond this tissue appears a larger-celled cortex, *c.* Pl. XXXVI, Fig. 20, also shows some of the closely-packed leaves or scales, *s.*, *s.*, of the bud. Another somewhat better preserved section of a bud-scale is shown on Pl. XXXVI, Fig. 21. It shows a small-celled epidermal layer, *e.*; a distinct hypodermal tissue near the outer surface, consisting of small thick-walled cells, *h.*; a relatively large-celled mesophyll tissue, *m.*, and two very minute bundles, *b.*, *b.*, close together near the centre of the leaf, each consisting of about half a dozen or fewer exceedingly small tracheides about 0·007 mm. in diameter.

In all the sections of *Mesoxylon Sutcliffii* which have been examined, the buds appear to be in almost exactly the same stage in development; in no case has a bud grown into a distinct branch. Presumably they were

resting buds of some kind, but whether vegetative or reproductive it has been impossible to determine. In the only other species of *Mesoxylon* in which axillary members are present, viz. *M. multirame*, a leafless axillary branch, resembling a phylloclade, is present in most of the leaf axils[1]; these are very different from the little buds of *M. Sutcliffii*, and evidently the two organs had quite distinct functions.

An epitome of the generic characters of *Mesoxylon* has already been given (see pp. 384, 385), and the following is a brief diagnosis of the species described in the present paper. A full account of the other species of *Mesoxylon* will be given in a forthcoming paper by Dr. D. H. Scott, F.R.S.

VIII. *Mesoxylon Sutcliffii* (*Poroxylon Sutcliffii*, Scott, Studies in Fossil Botany, 2nd Edition, 1909, p. 511, Fig. 184).

Only the stem and bases of leaves known.

Leaf-bases crowded, completely covering the surface of the stem.

Pith large (diameter nearly half that of the stem as a whole), discoid, with a persistent outer zone.

Twin-bundles of the leaf-traces, when they reach the margin of the pith, separated by tissue about equal in width to that of one of the bundles, and remaining separate when traced downwards through several internodes before fusing; subdividing in the pericycle and cortex to form about eight bundles in all.

Centripetal xylem distinct, persisting below the point of fusion of the two leaf-trace bundles.

Tracheides of the leaf-traces (centripetal and centrifugal), spiral or scalariform; those of the intermediate secondary wood pitted, except perhaps at the extreme inner margin.

Medullary rays of the secondary xylem uniseriate, usually 1–6 cells in height.

Dictyoxylon zone of cortex somewhat narrow.

Petiole of leaf flattened, containing about sixteen bundles near its insertion on the stem.

An axillary bud present in the axil of every leaf.

Roof nodules; Shore, Littleborough.

IX. Conclusions.

Mesoxylon 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; indeed, it seems not improbable that many of the familiar Cordaitean leaves which occur in the English Coal Measures may really belong to the new genus.

[1] Scott and Maslen, Preliminary Note, p. 238.

A summary of the most important characters in which *Mesoxylon Sutcliffii* resembles, and differs from, *Poroxylon* and *Cordaites* will serve to make this clear.

Mesoxylon Sutcliffii RESEMBLES *Poroxylon* in the possession of centripetal xylem strands forming part of the leaf-trace bundles in the perimedullary position, as well as in the bundles passing out to the leaves; in the paired leaf-trace bundles, which remain separate for a considerable distance (several internodes) after their entry into the inner part of the stele; in the persistently collateral structure of the leaf-traces from the perimedullary position right out into the leaves; in the possession of rows of bordered pits on the radial walls of the secondary xylem elements; in the spiral arrangement of the leaves; in the mesarch bundles in the leaves; in the presence of numerous axillary buds, &c.

Mesoxylon Sutcliffii DIFFERS from *Poroxylon* in the discoid pith; in the fact that all the xylem elements of the leaf-traces, both centripetal and centrifugal, consist of spiral or scalariform elements, whereas in *Poroxylon* not only are the centrifugal elements of the leaf-traces provided with rows of bordered pits, but the more internal portion of the centripetal xylem is pitted also; in the denser character of the secondary xylem, which consists of smaller elements with narrower or shorter medullary rays; in the structure of the phloem; in the divisions of the leaf-trace bundles in the pericycle and cortex before they enter the leaf; in the crowding of the leaves on the stem, &c.

The points of RESEMBLANCE between *Mesoxylon Sutcliffii* and *Cordaites* are very numerous, and are generally those in which it differs from *Poroxylon*. They may be summarized as follows: the large size of the pith; the discoid structure of the inner pith, which is found in most species of *Cordaites* and probably in all the forms of *Mesoxylon* yet recognized; the secondary xylem is practically identical in both genera; the phloem is similar; the division of the paired leaf-trace bundles in the pericycle and cortex, so that a number of bundles entered the base of the leaf, &c.

The most important of the DIFFERENCES between *Mesoxylon Sutcliffii* and *Cordaites* is found in the presence of the strands of centripetal wood surrounding the pith in *Mesoxylon*, whereas in *Cordaites* the whole of the wood of the stele is described as being centrifugal in development.

All the species of *Mesoxylon* which were described in our preliminary note agree in the possession of similar strands of centripetal xylem, although there is some variation in its amount, in the rapidity with which it dies out when traced down the stem, as well as in the degree of separation of the twin-bundles when they reach the perimedullary position after coming in from the leaves (see p. 396). In *M. multirame* the centripetal xylem dies out rather rapidly after the leaf-trace has reached the pith, so making a nearer approach to the condition which we get in *Cordaites* itself, in which the

centripetal wood of the leaf-traces which is present in the outer part of their course is entirely lost before they reach the boundary of the pith. As Dr. Scott says: 'In the Cordaiteae the old "Cryptogamic" or centripetal wood appears to have been on the verge of extinction, and its presence or absence may here be of little taxonomic significance.'[1] This being the case, *Mesoxylon*, which resembles *Cordaites* in so many of its other characters, may best be included in the family Cordaiteae. The affinity of the French Poroxyleae with the Cordaiteae has been recognized ever since the description of the forms by MM. Bertrand and Renault. The discovery of the leaves of *Poroxylon*, and the attribution to this form of the platyspermic seed known as *Rhabdocarpus*, Br., by Grand'Eury, if the discovery is confirmed, considerably strengthen the affinity otherwise indicated between the Poroxyleae and Cordaiteae. Marks of similarity between the Poroxyleae and *Lyginodendron* and *Heterangium* were also indicated by the French observers, and at the present time it is well recognized that the agreement in the structure of the stem between *Poroxylon* and *Lyginodendron* is in many respects a close one, although, with a common type of stem structure, there existed in the one form a foliage type which in form and structure resembled that of *Cordaites* and recent megaphyllous Gymnosperms, while in the other the foliage was in every respect that of a fern. With *Calamopitys Saturni*, Unger, again, *Poroxylon* has much in common.

In consequence of the obvious affinities of *Poroxylon* in both directions, and of the very perfect manner in which the tissues have been preserved, and the very detailed account which we owe to MM. Bertrand and Renault, the Poroxylons have come to possess a crucial significance in the discussion of the relation of the Cordaiteae to the Pteridosperms, and so on that of the broader question of the derivation of the higher Gymnosperms.

The species of *Poroxylon* were described from deposits of Permo-Carboniferous Age, and thus can hardly be considered as forming an actual link between families which had been fully differentiated long before that time; it is therefore particularly interesting to find, at a much lower horizon—the Lower Coal Measures of Lancashire—forms which, while possessing centripetal wood as in *Poroxylon*, are in other respects much closer to *Cordaites*, and probably stand nearer to the direct line of Cordaitean descent. Another Coal-Measure plant, *Dadoxylon Spenceri*, Scott, already briefly described (p. 395), although differing from *Mesoxylon Sutcliffii* and *Cordaites* in the small size of the pith, resembles *Mesoxylon* in so many other respects that it may prove to be closely related. *Pitys antiqua*, again, a Lower Carboniferous form (see p. 395), agrees with *Mesoxylon* in the structure of its wood excepting for the greater width of the medullary rays and the definitely mesarch structure of its single xylem strands surrounding the pith; judging from the anatomical structure of the pith and wood (the

[1] Studies, 2nd Edition, p. 526.

only parts yet known), it probably stands somewhat nearer to the Pteridosperms than *Mesoxylon* does, although well on the way towards the typically Gymnospermous family of the Cordaiteae. A feature of the Cordaiteae and of the plexus of form connecting this family with the Pteridosperms is the double leaf-trace shared at the present day by *Ginkgo*. Recent work on the occurrence of the double leaf-trace in Gymnosperms and in angiospermous seedlings,[1] as well as its common occurrence in so many Palaeozoic plants, suggests that it may have considerable taxonomic importance.

The fusion of the twin-bundles of the leaf-trace which in *Lyginodendron* and *Calamopitys* takes place in the pericycle, is pushed further back in the higher forms which lead on to the Cordaiteae. In most of these forms (*Poroxylon*, *Dadoxylon Spenceri*, *Mesoxylon*) fusion does not take place until after the perimedullary position has been reached and until the trace has penetrated a considerable distance down the stem, the centripetal xylem persisting until after fusion has been effected. In one form of *Mesoxylon*, however, viz. *M. Lomaxii*, the strongly converging twin-bundles fuse into one immediately on reaching the pith.[2] As Dr. Scott has pointed out, the division of the trace extends, on the whole, lower down the stem in the later forms.[3]

By the study of *Mesoxylon* and the other more ancient Cordaitean stems we are able to trace some of the stages in the gradual extinction of the centripetal wood of their Cryptogamic ancestors, a process which appears to have been completed in the true *Cordaites*. The endarch structure thus reached in *Cordaites* has persisted as a characteristic feature in the anatomy of the stems of the higher plants.

In conclusion, I wish to tender my grateful thanks to Dr. D. H. Scott, M.A., F.R.S. After the preparation of our joint preliminary note on *Mesoxylon* it had been intended to publish a full description of all the species under our joint names. As this work could not have been completed for some considerable time, Dr. Scott very generously suggested that I should publish a detailed account of *M. Sutcliffii* first. Throughout the progress of the work I have had the invaluable help of Dr. Scott's suggestions and criticism. I also wish to thank Mr. L. A. Boodle, F.L.S., who has kindly allowed the work to be done at the Jodrell Laboratory, Kew, and who also supplied some of the photographs used in the plates. The other photographs are by Mr. W. Tams.

All the specimens were discovered by Mr. James Lomax in the Shore material, and the sections cut by him.

[1] Miss E. N. Thomas, A Theory of the Double Leaf-trace, founded on Seedling-structure. New Phytologist, vol. vi, 1907, p. 77.

[2] Scott and Maslen, loc. cit., p. 239.

[3] Studies, 2nd Edition, p. 652.

EXPLANATION OF PLATES XXXIII–XXXVI.

Illustrating Mr. A. J. Maslen's paper on *Mesoxylon Sutcliffii.*

PLATE XXXIII.

Fig. 1. Transverse section of stem, showing the large pith, the ring of wood and phloem, the cortex with attached leaf-bases, and two axillary buds. *c.p.*, central pith; *p.p.*, peripheral pith; *l.t.*, *l.t.*, &c., paired leaf-trace bundles at the margin of the pith (in some of these the two bundles are widely separated, in others they are closely approximated, and in yet others the two have fused laterally into one bundle); *s.x.*, ring of secondary xylem; *p.*, phloem; *pe.*, pericycle; *l'.t'.*, &c., leaf-traces in the phloem and pericycle; *d.*, Dictyoxylon outer cortex; *pm.*, periderms; *l.b.*, leaf-bases, of which seven are seen completely enclosing the stem; *a.b.*, *a.b.*, axillary buds, one of which is accompanied by the subtending leaf-base; *a.b.s.*, axillary bud-stele in the cortex; *s.*, space in secondary xylem. Slide No. 2621 (S).[1] Magnification just over 3 diameters.

Fig. 2. Radial longitudinal section of stem, showing discoid pith, &c. *c.p.*, central discoid pith; *p.p.*, *p.p.*, peripheral pith; *l.t.*, leaf-trace bundle at margin of pith; *s.x.*, secondary xylem with a central space due to disappearance of the tissue by disorganization (on the right-hand side of the section nearly the whole of the wood has been destroyed); *p.*, phloem; *pe.*, pericycle; *l'.t'.*, leaf-trace bundle passing into leaf-base; *d.*, strengthening bands of Dictyoxylon outer cortex; *l.b.*, *l.b.*, leaf-bases; *a.b.*, *a.b.*, leaves belonging to axillary buds; *a.b.s.*, bud-stele in the phloem or pericycle; *a.*, position of abscission layer at base of petiole. Slide No. 2668 (S). Magnification 4 diameters.

Fig. 3. Transverse section of a leaf-trace at the margin of the pith with the two bundles widely separated. *s.b.*, centrifugal xylem of the bundles; *p.b.*, centripetal xylem of the bundles; *px.*, position of protoxylem; *s.x.*, secondary xylem between the bundles; *s.*, space formerly occupied by delicate conjunctive parenchyma; *p'.*, empty thin-walled cells of outer pith; *p''.*, thicker walled cells of outer pith with contents. Slide No. 2634 (S). Magnification nearly 40 diameters.

Fig. 4. Transverse section of a leaf-trace at the margin of the pith with the two bundles closely approximated. Reference letters as in Fig. 3; also *pa.*, delicate parenchyma between centripetal and centrifugal xylem of bundles; *p'''.*, outer pith cells with very dark contents; *p''''.*, small empty-looking cells of outer pith next the xylem. Slide No. 2653 (S). Magnification about 40 diameters.

Fig. 5. Transverse section of a leaf-trace at the margin of the pith with the centrifugal xylem of the two bundles fused laterally into one, although the two masses of centripetal xylem are still separate. *s.b.*, centrifugal xylem; *p.b.*, centripetal xylem; *m.r.*, wide medullary rays in centrifugal xylem of bundles; *m'.r'.*, narrow medullary rays in secondary xylem beyond the bundles; *p'''.*, outer pith cells with very dark contents. Slide No. 2636 (S). Magnification about 40 diameters.

Fig. 6. Longitudinal section passing through the two bundles of a leaf-trace at the margin of the pith. *l.t.*, *l.t.*, leaf-trace bundles; *p.b.*, denser centripetal xylem of bundle; *s.b.*, less compact centrifugal xylem of bundle consisting of undulating tracheides separated by relatively broad medullary rays; *s.x.*, secondary xylem of stem consisting in the outer part, *a*, of closely packed straight tracheides with narrow medullary rays, and in the inner part, *b*, of more loosely arranged elements with broader medullary rays; *p'.*, empty cells of outer pith; *p''.*, cells with contents of outer pith. Slide No. 2667 (S). Magnification 20 diameters.

Fig. 7. Transverse section of outer part of secondary xylem, phloem, and pericycle. *s.x.*, secondary xylem; *p.*, phloem; *pe.*, pericycle; *p'.*, square cells of phloem with very dark contents; *p''.*, cells with paler contents; *p'''.*, dark cells at outer limit of phloem; *i.c.*, cells of inner part of cortex. Slide No. 2637 (S). Magnification about 40 diameters.

PLATE XXXIV.

Fig. 8. Transverse section, showing a leaf-trace in the inner part of the pericycle. The xylem portions of the bundles have already divided. *s.b.*, centrifugal xylem of leaf-trace bundles; *p.b.*, centripetal xylem of leaf-trace bundles; *ph.b.*, phloem of bundles still undivided; *c.*, cells with dark

[1] The letter (S) following the slide number signifies that the sections are in Dr. D. H. Scott's collection.

contents at outer limit of phloem of bundles; *b.*, delicate conjunctive tissue; *s.*, sheath of cells with contents surrounding the bundles: *s.x.*, secondary xylem of stele; *p.*, phloem of stele; *pe.*, pericycle; *i.c.*, cells of inner cortex. Slide No. 2615 (S). Magnification about 20 diameters.

Fig. 9. Transverse section, showing a leaf-trace in the inner part of the cortex. Each of the original bundles has divided into three. *l.t.*, leaf-trace bundles; *s.*, sheath surrounding bundles; *s.x.*, secondary xylem of stele; *i.c.*, cells of inner cortex; *p'.*, *p'.*, periderms; *a.b.s.*, axillary bud-stele just escaping from the secondary xylem of the stele. Slide No. 2625 (S). Magnification about 10 diameters.

Fig. 10. Transverse section, showing a bud-stele in the cortex with its subtending leaf-base and a row of six leaf-trace bundles passing into it. *a.b.s.*, axillary bud-stele; *l.t.*, *l.t.*, two of the leaf-trace bundles; *s.x.*, outer part of secondary xylem of stem; *l.b.*, leaf-base; *d.*, Dictyoxylon bands; *p'.*, periderm. Slide No. 2653 (S). Magnification 9 diameters.

Fig. 11. The bud-stele shown in Fig. 10 more highly magnified. *p.*, pith; *x.*, xylem of bud-stele; *m.r.*, medullary rays. Slide No. 2653 (S). Magnification about 140 diameters.

Fig. 12. Transverse section, showing twin bud-steles in the cortex. *b.*, bud-steles; *l.t.*, leaf-trace bundles; *s.x.*, secondary xylem. Slide No. 2657 (S). Magnification about 34 diameters.

Fig. 13. Longitudinal section of the outer part of the stem, showing part of a leaf-base with the bud-stele in its axil, &c. *a.b.s.*, bud-stele; *l'.t'.*, leaf-trace bundle; *s.*, sheath enclosing leaf-trace bundles; *s.x.*, secondary xylem of stem; *p.*, phloem of stem; *pe.*, pericycle; *c.*, cortex; *l.b.*, leaf-base; *a.*, position of abscission layer at base of petiole. Slide No. 2667 (S). Magnification about 8 diameters.

PLATE XXXV.

Fig. 14. Longitudinal section of one of the leaf-trace bundles also shown on Pl. XXXIII, Fig. 6, showing tracheides and medullary rays. *t.*, tracheides; *m.r.*, medullary rays. Slide No. 2667 (S). Magnification 370 diameters.

Fig. 15. Part of one of the tracheides of the secondary xylem in radial section. Slide No. 2671 (S). Magnification about 400 diameters.

Fig. 16. Tangential section of the secondary xylem, showing the tracheides and narrow medullary rays. *t.*, tracheides; *m.r.*, medullary rays. Slide No. 2665 (S). Magnification about 400 diameters.

Fig. 17. Transverse section of the phloem, pericycle, &c. *s.x.*, outer part of secondary xylem consisting of tracheides and narrow medullary rays; *c.*, cambium cells; *p.*, phloem consisting of thin-walled elements (? sieve tubes), and phloem parenchyma, *a*; square cells with very dark contents, *b*; cells with paler contents, *c*; and narrow medullary rays, *m.r.*; *d.*, cells with dark contents at outer limit of phloem; *pe.*, pericycle; *i.c.*, cells of inner cortex. Slide No. 2633 (S). Magnification about 75 diameters.

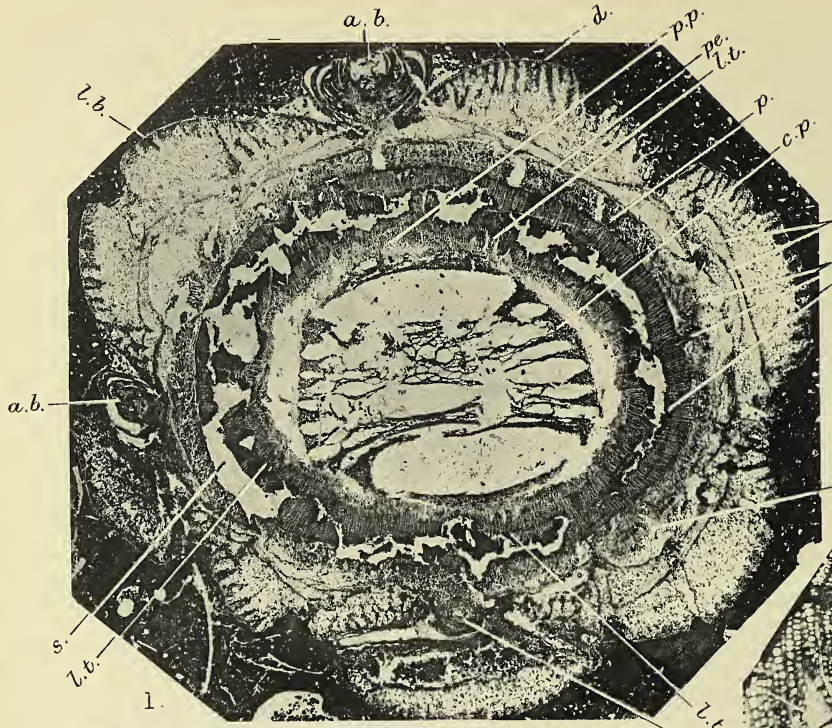
Fig. 18. Transverse section of petiole, showing a row of ten bundles each with centrifugal and centripetal xylem. *i.x.*, inner xylem of bundle; *o.x.*, outer xylem of bundle; *o.*, outer (lower) side of petiole. Slide No. 2631 (S). Magnification about 12 diameters.

Fig. 19. Transverse section of one of the bundles shown in Fig. 18 more highly magnified. Only the xylem is preserved. *s.x.*, outer xylem elements in radial rows; *px.*, protoxylem; *p'.x'.*, centrifugally developed primary xylem elements; *p''.x''.*, centripetally developed primary xylem elements; *p.*, disorganized phloem. Slide No. 2631 (S). Magnification about 230 diameters.

PLATE XXXVI.

Fig. 20. Transverse section of axillary bud. *p.*, pith; *b.*, bundles; *m.r.*, medullary rays; *.t.*, *l.t.*, &c., leaf-traces; *pe.*, pericycle; *c.*, cortex; *s.s.*, &c., scales of bud. Slide No. 2620 (S). Magnification 40 diameters.

Fig. 21. Transverse section of one of the leaves of an axillary bud. *e.*, epidermis; *h.*, hypodermal layer; *m.*, mesophyll; *b.*, *b.*, bundles. Slide No. 2632 (S). Magnification 80 diameters.



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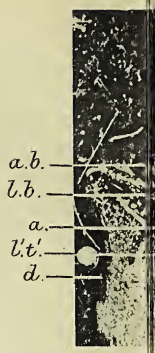

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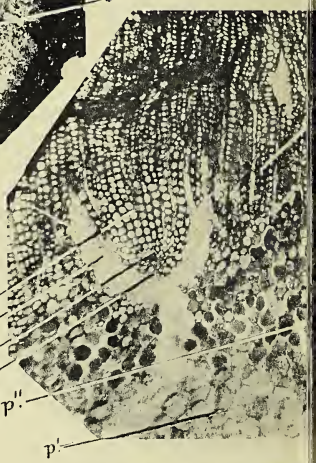

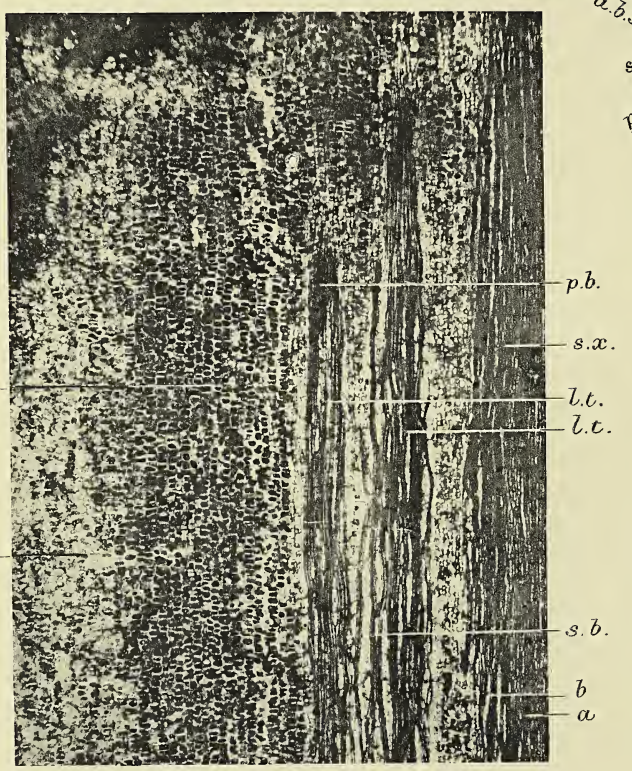

L.A.Boodle & W.Tams, Phot.

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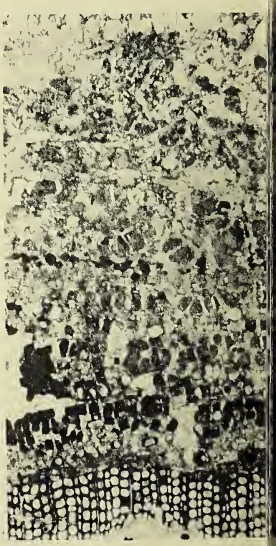

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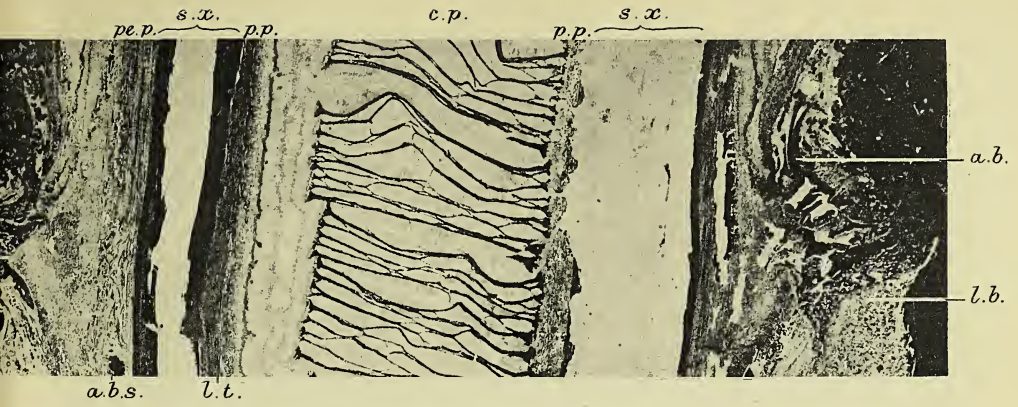

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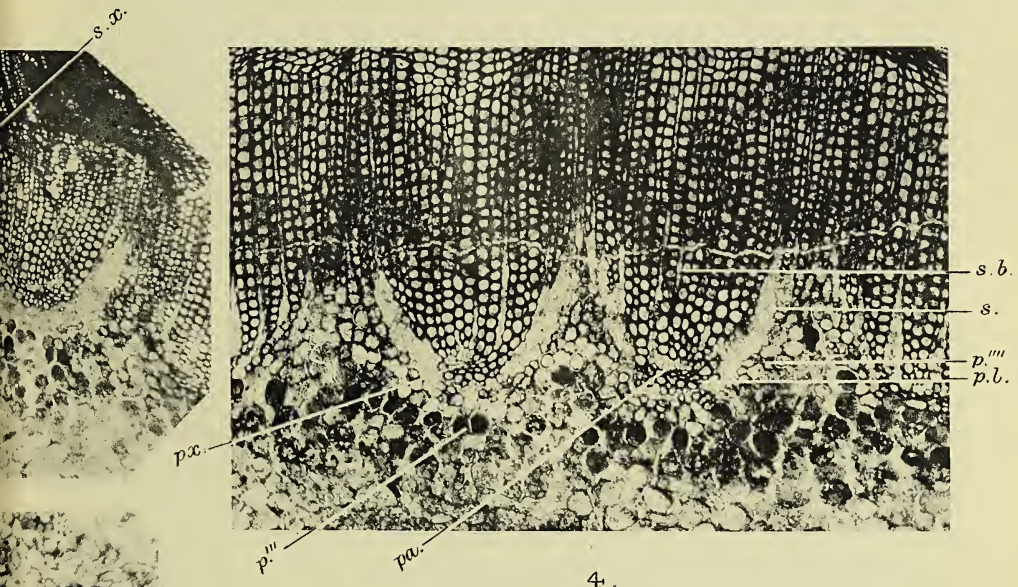

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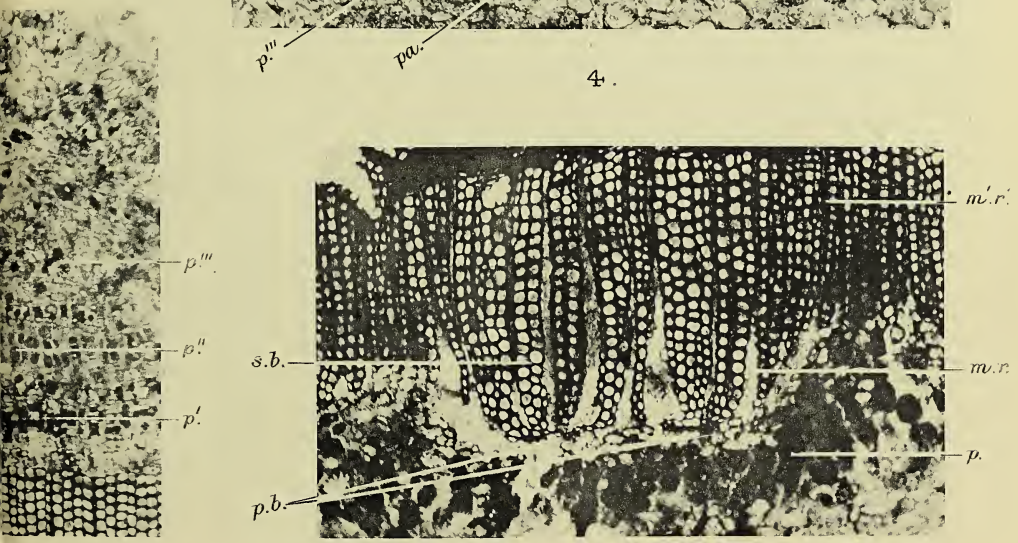

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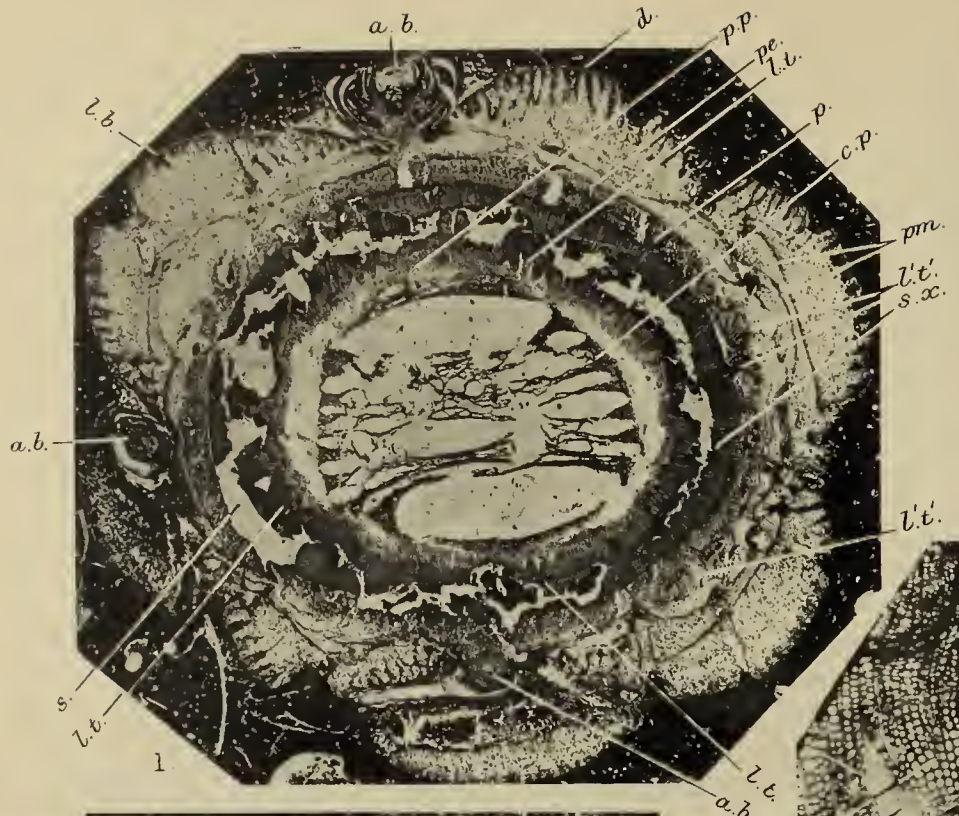

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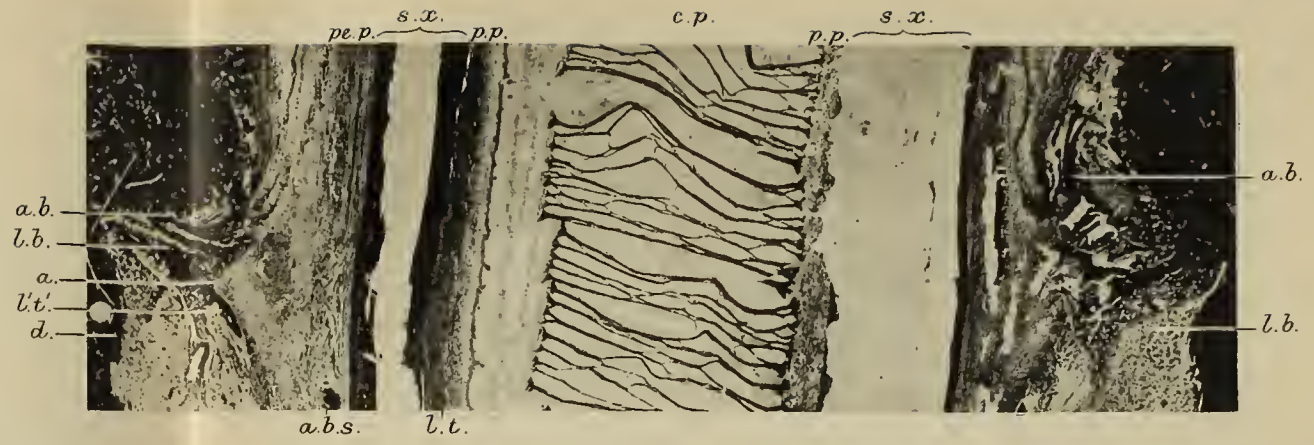

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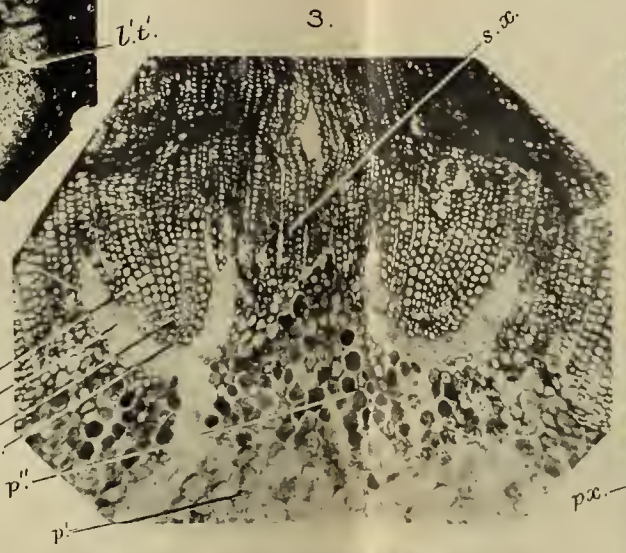

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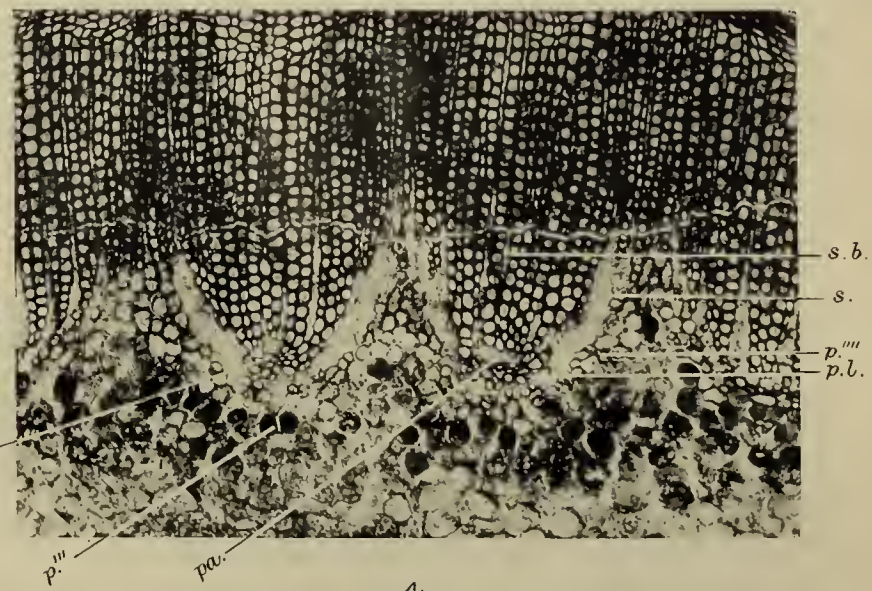

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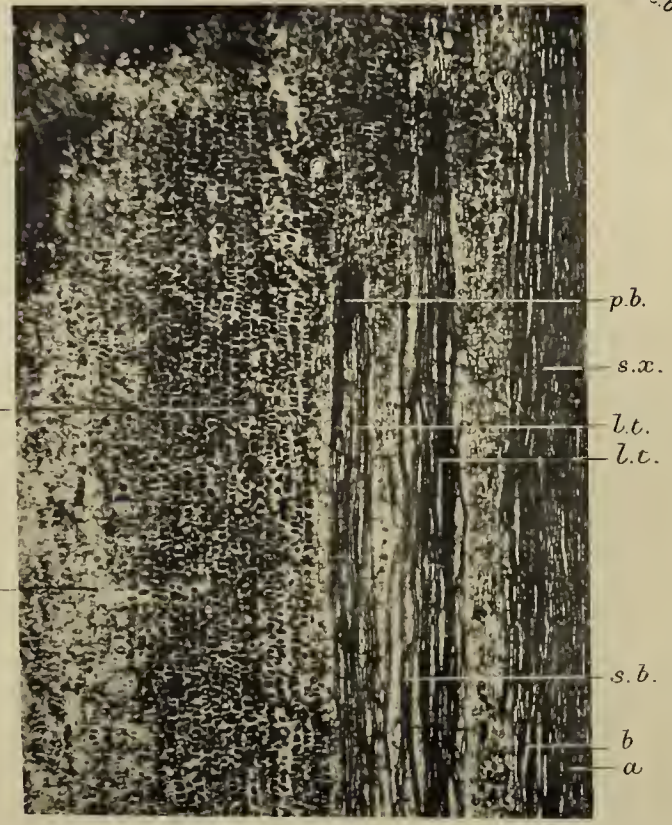

L. A. Boodle & W. Tams, Phot.

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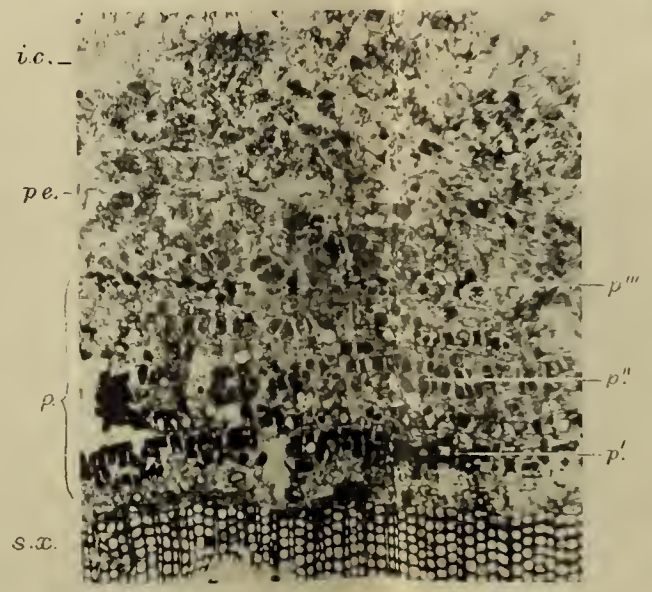

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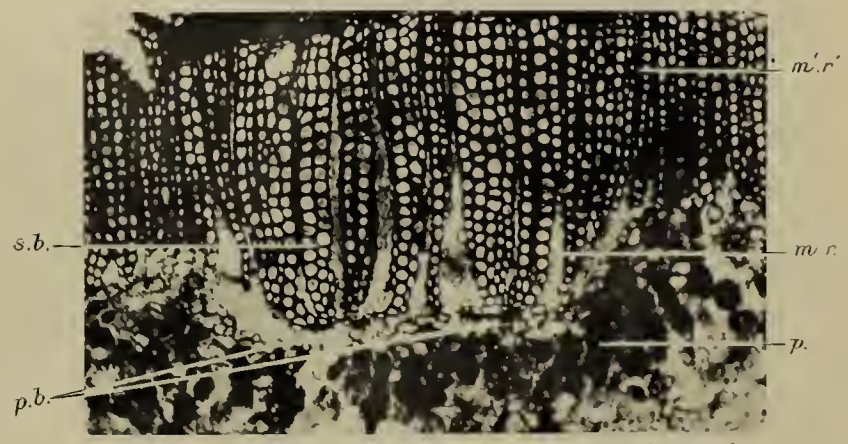

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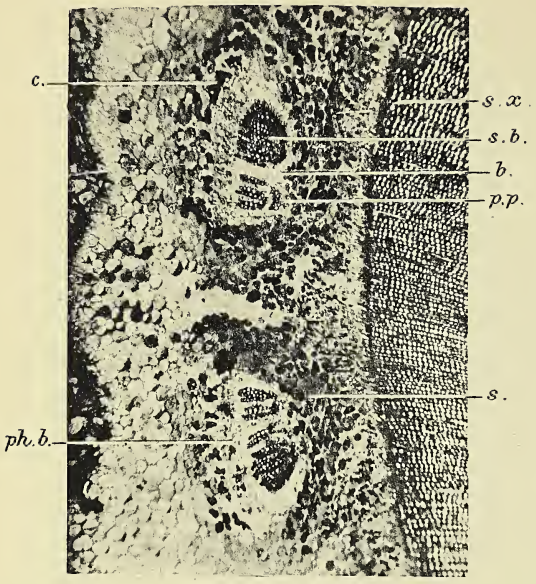

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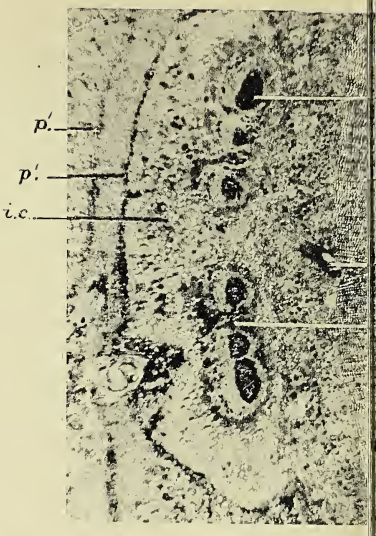

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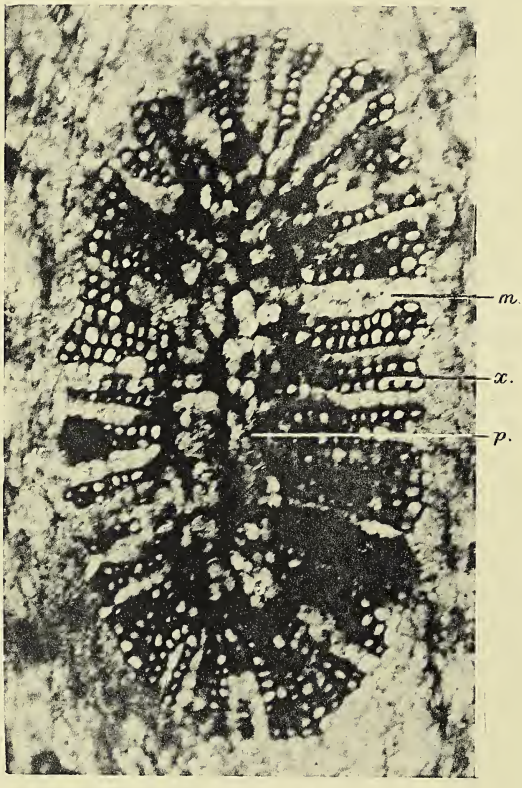

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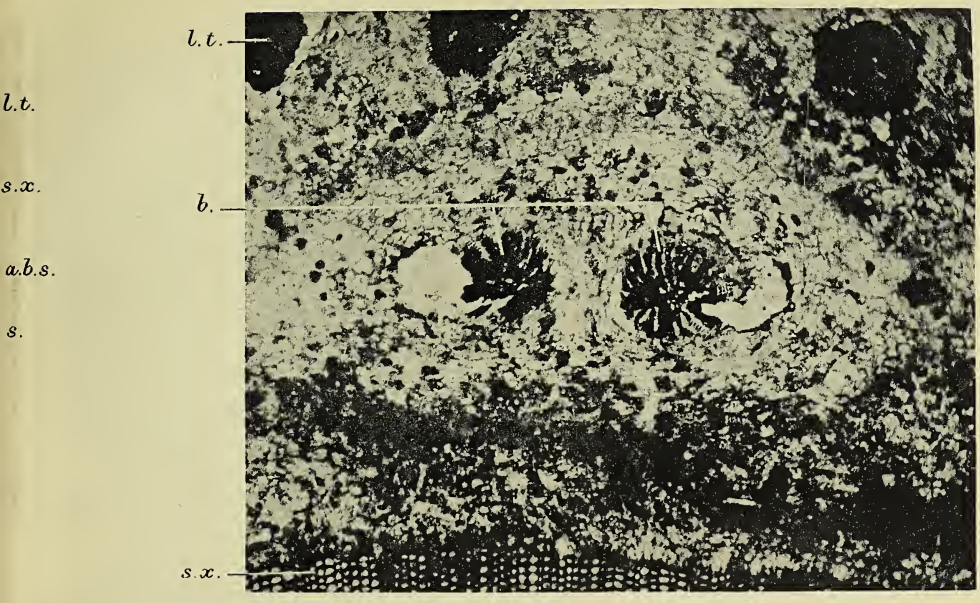

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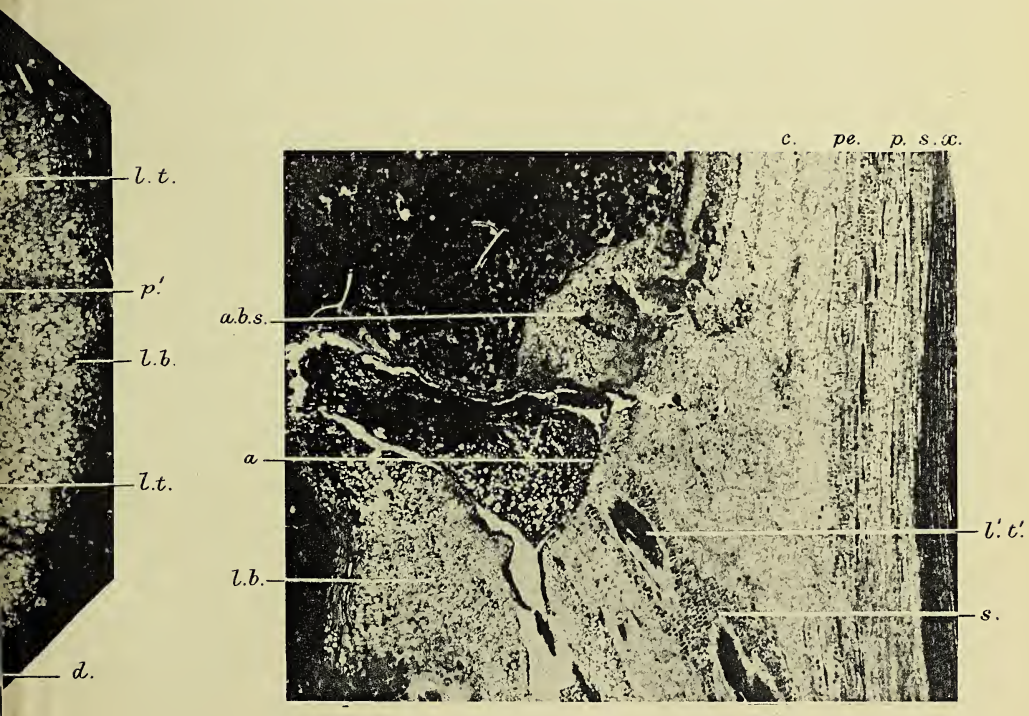

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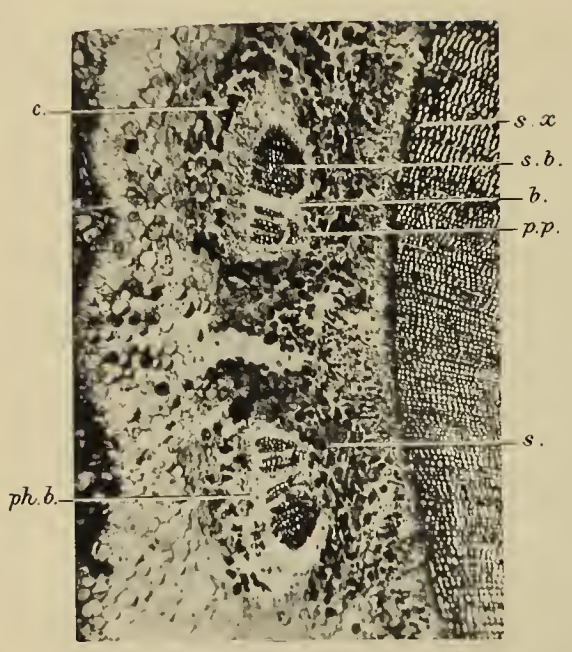

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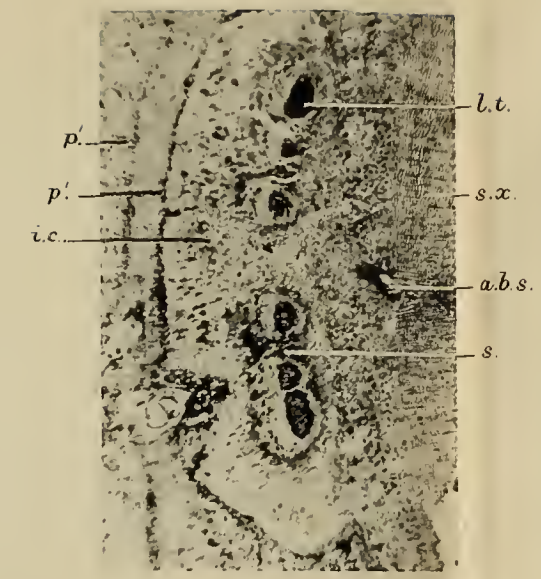

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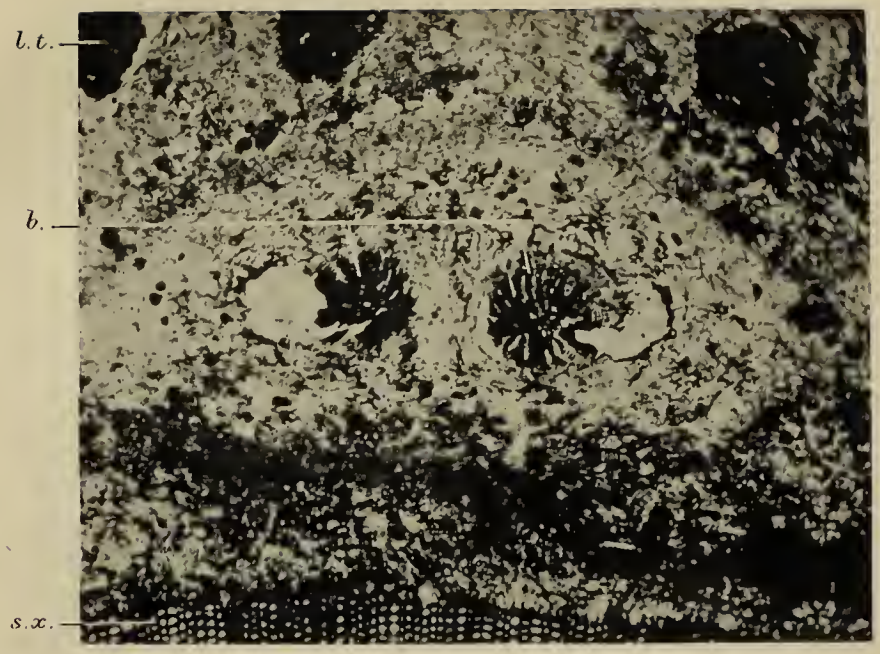

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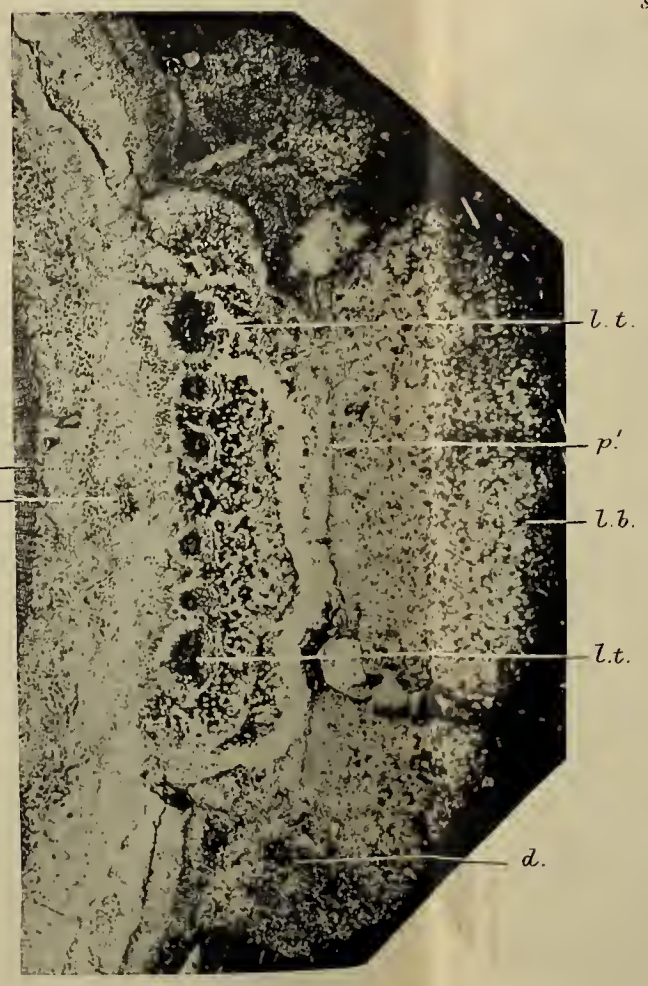

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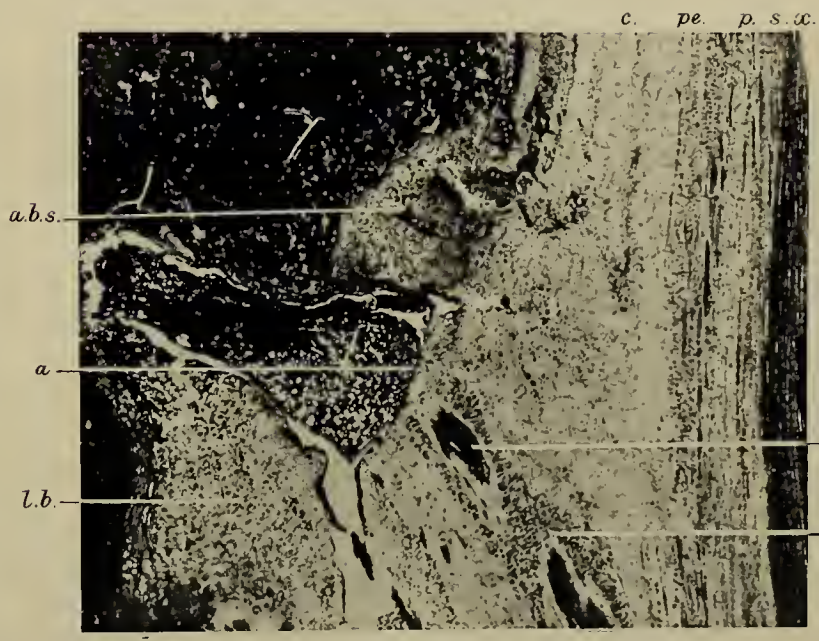

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L. A Boodle & W. Tams, Photo.

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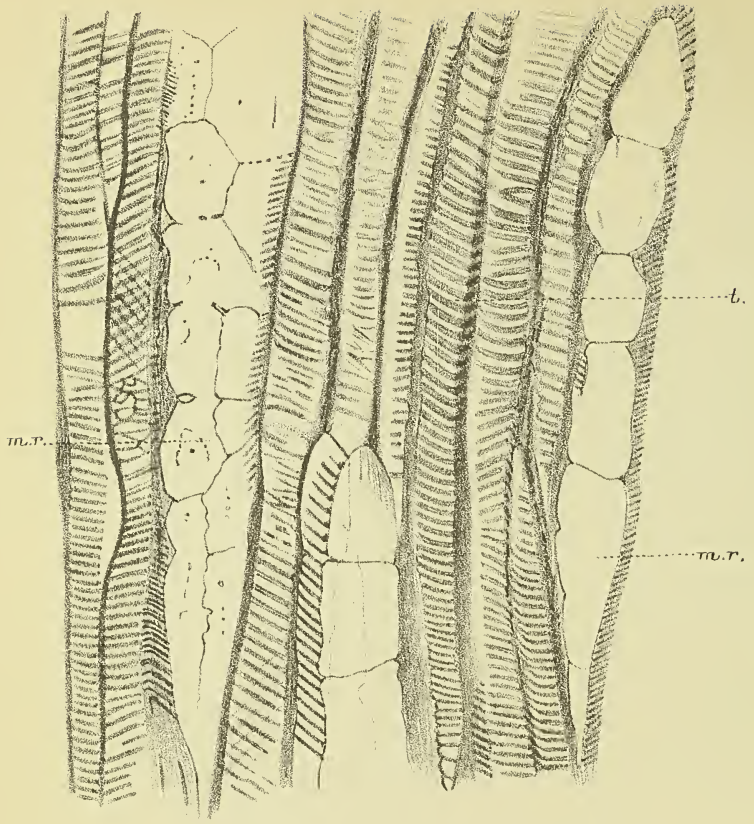

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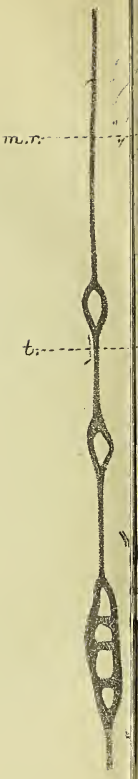

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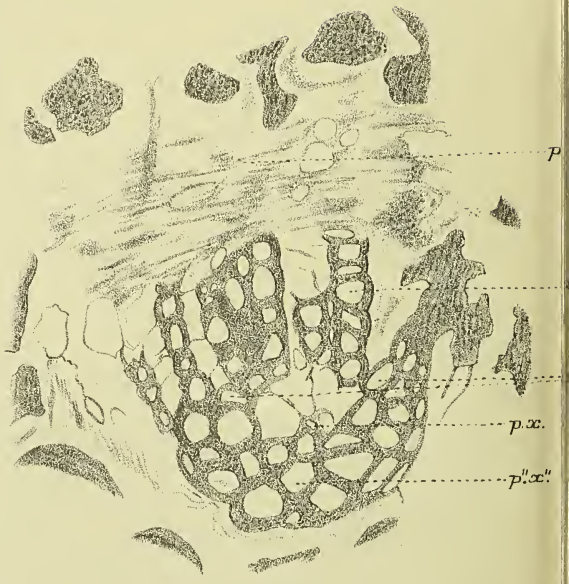

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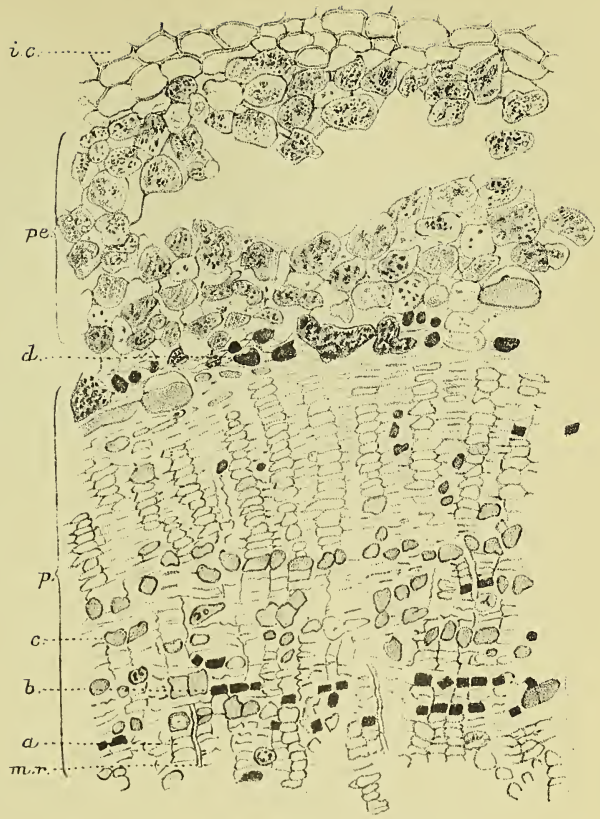

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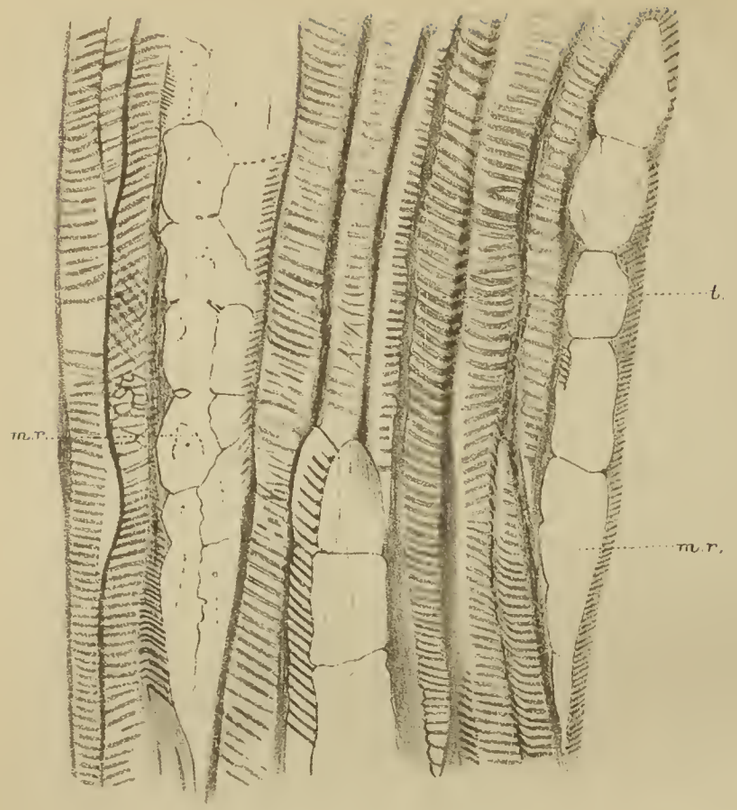

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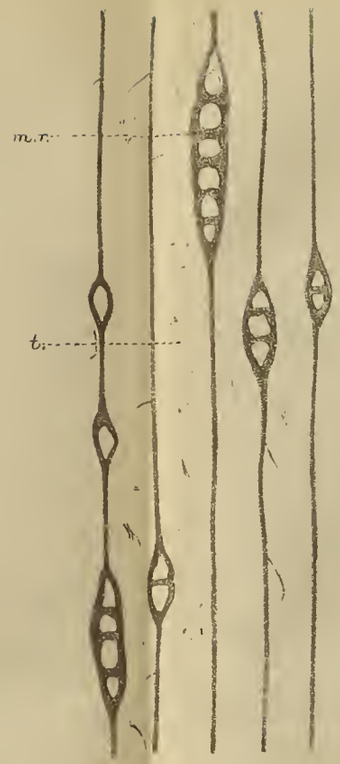

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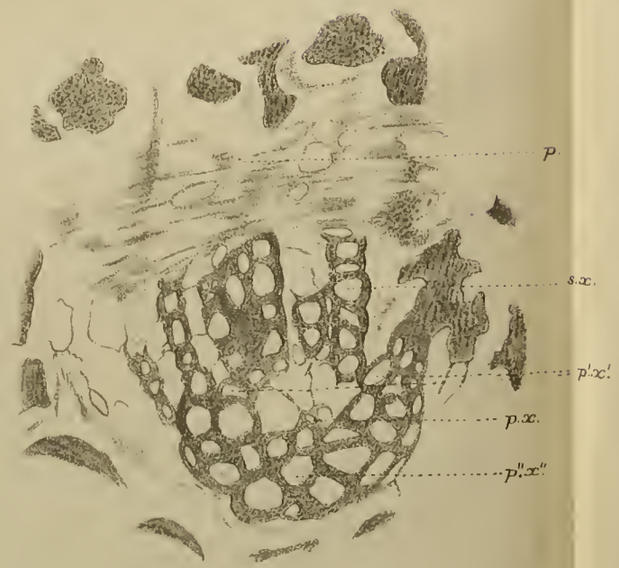

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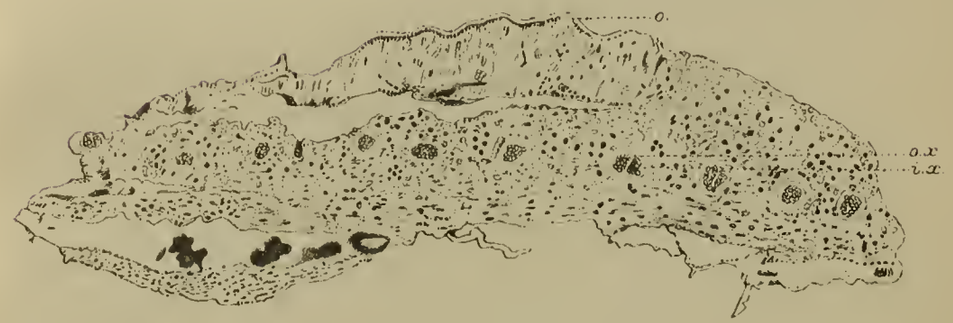

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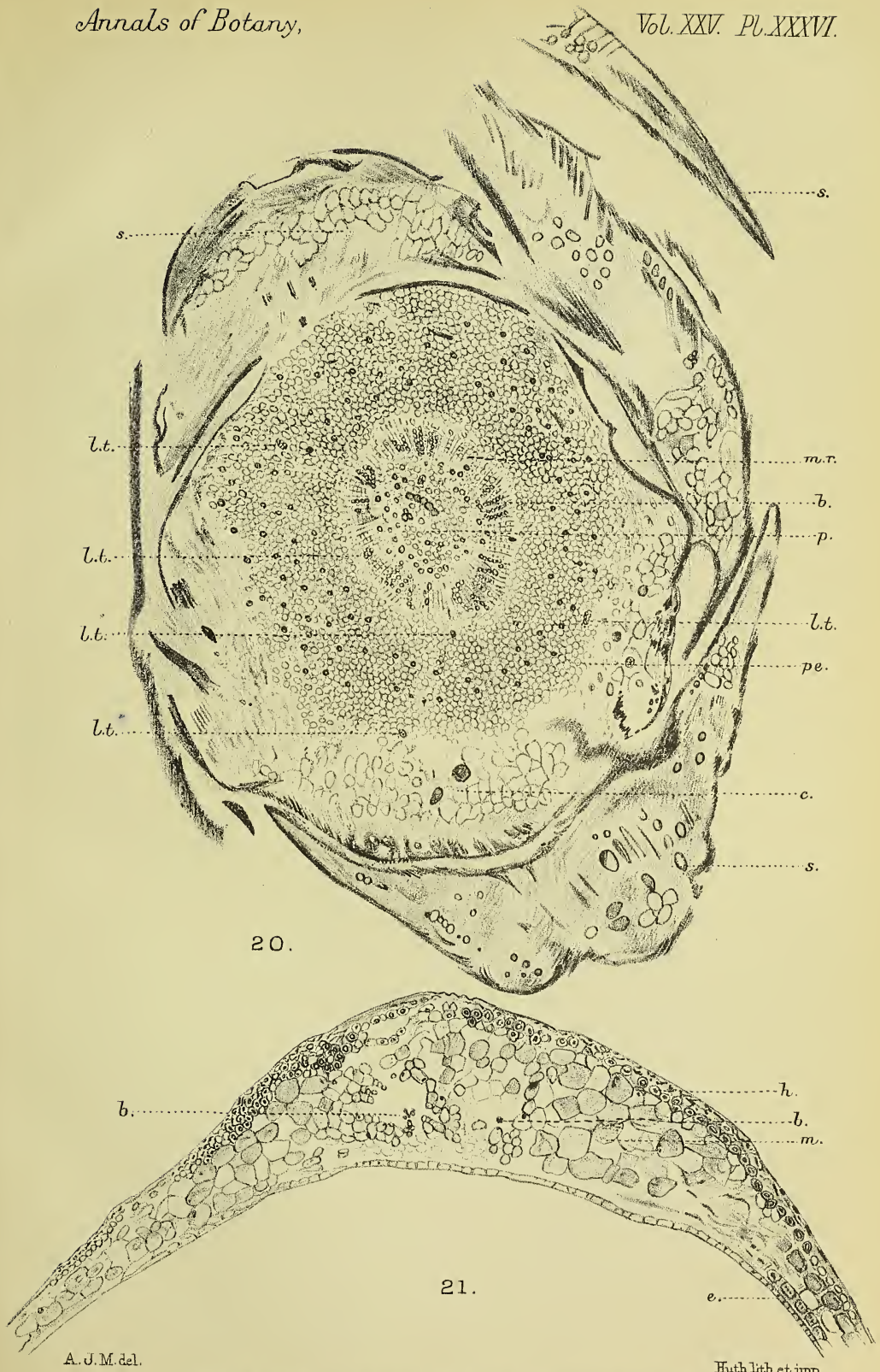


MASLEN — MESOXYLON SUTCLIFFII.

Spermatogenesis in the Bryophyta

BY

MALCOLM WILSON, B.Sc., A.R.C.S.

Senior Demonstrator in Botany, Royal College of Science, London.

With Plates XXXVII and XXXVIII and three Figures in the Text .

ALTHOUGH the structure and development of the spermatozoids in the Bryophyta have been the subject of numerous investigations, comparatively little information is available upon the earlier history of the spermatogenic cells. The majority of investigators have confined themselves to descriptions of the formation of the spermatozoid from the spermatid. Several papers have recently appeared on the spermatogenesis of the Hepaticae, but those dealing with the Musci are very few in number. J. and W. Docters van Leeuwen-Reijnvaan (40, 41, 42) have recently published several on this latter group, and, as pointed out by Strasburger (66), the conclusions arrived at by these observers have emphasized the necessity for further investigation.

The first reference to spermatozoids in plants was made by Schmiedel (55) in 1747. In his 'Icones plantarum et analyses partium', he described the movement of the spermatozoids in *Fossombronia pusilla*.

Other scattered observations followed, but the earliest comparative investigations in this subject were made by Hofmeister (28) and described in his 'Vergleichende Untersuchungen' in 1850. In several of the Hepaticae he found that the small rectangular cells of the nearly ripe antheridium each gave rise to one spermatozoid. The latter in *Pellia epiphylla*[1] consists of a spirally coiled body with two cilia attached to the thicker end, and, on its escape from the antheridium, it is still enclosed in the wall of the mother-cell. This subsequently ruptures, and the spermatid is set free, provided at the thin end with a vesicle. In the Musci the development is similar. In *Sphagnum acutifolium* the developing antheridia are figured, and the mature spermatozoid is shown, provided with two cilia at the anterior end. In *Phascum cuspidatum* spermatozoids are figured still enclosed within the walls of the polygonal mother-cells. The account given by Schimper (54) in 1858 of the spermatozoid of *Sphagnum* is very similar.

With the exception of *Pellia*, Hofmeister was unable to find cilia in the spermatozoids of the Hepaticae, but in the following year they were described and figured by Thuret (68) in *Pellia*, *Marchantia*, *Fossombronia*,

[1] p. 30, Eng. Trans.

[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.]

and *Targionia*. Thuret also noted that the mother-cells of the spermatozoids of *Fossombronia* and *Targionia* were discoid in shape with one convex and one plane surface, and that they were often united together in pairs by their plane surfaces. In *Funaria hygrometrica* he described the polygonal mother-cells, each of which gives rise to one spermatozoid. He also described and figured the monoecious habit of this plant, an account of which has been recently given by Boodle (14).

Schacht (51) in 1852 found in *Pellia epiphylla* that the nucleus of the spermatogenetic cells becomes smaller and elongated, and finally forms the body of the spermatozoid. Later (52), after the examination of a number of different plants, he concluded that the nucleus takes some part in the formation of the spermatozoid, but that it usually disappears in consequence. There is a complete protoplasmic covering around the body of the spermatozoid, and at the posterior end this is enlarged to form the vesicle; the cilia are also protoplasmic outgrowths.

Kny (38) in 1867 described the 'tesseralen Zellen', i. e. the rectangular cells, in the developing antheridium of the Ricciaceae, and concluded that each of these gave rise to one spermatid.

The disappearance of the nucleus was also described by Strasburger (61) in *Marchantia polymorpha*. Each spermatozoid mother-cell at first possesses a distinct nucleus, but during development this gradually breaks up, leaving the mother-cell filled with homogeneous contents. The spermatozoid is formed from a band-like thickening which appears later on the upper side of the cell. Sachs (50) gives a similar account of the development in *Nitella*. On the other hand, Schmitz (56) found that the nucleus did not disappear in the Characeae and Musci. The body of the spermatozoid is formed by the thickening and elongation of the outer parts of the nucleus, while the inner portion gives rise to the vesicle; only the cilia have a protoplasmic origin.

The opinion that the body of the spermatozoid was of nuclear origin was confirmed by the chemical investigations of Zacharias (77) in 1881. He showed that in the Characeae and Musci the nucleus of the mother-cell and the body of the spermatozoid agree in their reactions when treated with various substances. From these investigations he concluded that the body of the spermatozoid is formed from the nucleus, the protoplasm giving rise to the thin skin surrounding the body, the cilia, and the vesicle.

The production of two spermatozoids by the division of each of the rectangular cells of the antheridium was first noted in *Pellia* by Goebel (25) in his article on the Muscineae in Schenk's Handbuch, but in the remaining Hepaticae he states that one spermatozoid is formed in each mother-cell.[1] The nucleus does not disappear, but produces the spermatozoid directly; its peripheral layer thickens, and this by splitting gives rise to the body, whilst

[1] p. 342.

the middle part loosens and forms the vesicle. In the Musci [1] a spermatozoid develops in each cell of the inner tissue of the antheridium.

Buchtien (15) in 1887 gave a short account of the development of the spermatozoid in *Pellia epiphylla*. The body is formed directly from the nucleus of the mother-cell, while the cilia are outgrowths of the cell plasma. The discoid spermatids are figured in pairs, and a wall is shown separating the two, while at a later stage each developing spermatozoid is represented completely surrounded by a wall.

The formation of two spermatozoids from each mother-cell of *Pellia epiphylla* was also described by Campbell (16) a few years later, but no wall is shown between the two spermatids resulting from the division. In the Musci Campbell emphasizes the presence of a highly refractive mass found at the posterior end of the spermatozoid; this, in *Sphagnum acutifolium*, still contains starch at the end of development.

Leclerc du Sablon (39) in 1888 gave a description of the formation of spermatozoids in several of the Hepaticeae. In *Metzgeria furcata* the nucleus passes to the surface of the mother-cell, still retaining its usual form. Meanwhile, a protoplasmic band differentiates around the cell, touching the nucleus at one point. This band gradually thickens and becomes intimately fused with the nucleus, which decreases in size, owing to the transference of its material to the band. Finally, the nucleus completely disappears, while the band opens out and by growth in length forms the body of the spermatozoid. The cilia appear when the body is completely formed. The development in *Radula*, *Frullania*, and *Alicularia* is similar.

Guignard (26 A) in 1889 gave a careful account of development of the spermatozoid in the Characeae, Bryophyta, and Pteridophyta, and in all these groups he emphasizes the fact that the principal part of the spermatozoid is derived from the nucleus. In *Pellia epiphylla* he found that the mother-cells of the spermatozoids are placed in pairs and are discoid, and he figures them separated by a distinct wall. The spermatozoid is produced by the growth in length of the nucleus, the cilia arising from a mass of hyaline protoplasm at the anterior end. The development in *Anthoceros laevis* is similar, but here the mother-cells are described as being originally rectangular, becoming biconvex at a later stage. Other genera of the Hepaticae were examined, and these, together with the Mosses, agree in general with the above account. In *Sphagnum fimbriatum* each spermatid is shown surrounded by a wall; during the development an amylaceous mass of protoplasm is found at the posterior end of the nucleus, and this persists undiminished in amount in the vesicle. At the anterior end of the body a small highly refringent 'bouton' is present which carries the cilia.

The account given by Schottländer (58) in 1892 differs from those of other investigators in the discovery of two distinct portions in the body

[1] p. 376.

of the spermatozoid. Both in the Hepaticae and Filicineae he described a red-staining contractile ground substance surrounded by a non-contractile spirally arranged skin which takes up the blue stain. This structure persists in the mature spermatozoid. The development in *Aneura* and *Marchantia polymorpha* is similar in most respects. In the latter plant a centrosome is present at each pole of the spindle during the division of the spermatogenic cells; in the mother-cell two bodies, probably centrosomes, are found, and these persist in the spermatozoid near the place of attachment of the cilia.

Strasburger (62) in 1892 was unable to identify the structures described by Schottländer. In *Pellia calycina* he found that the cilia are attached some small distance behind the anterior end of the body of the spermatozoid. On staining with a mixture of iodine green and fuchsin the middle part of the body becomes blue, while the anterior portion, cilia, and posterior plasma mass take up the red stain. *Marchantia polymorpha* and *Polytrichum commune* also agree in this staining reaction. The anterior cytoplasmic portions of the spermatozoids of *Chara* and *Pellia* are homologous, but in the latter this portion remains short; on the other hand, the posterior cytoplasmic process is not present in the Muscineae. In other respects there is a great correspondence between the spermatozoids of the two groups, and the resemblance also extends to their development. In *Pellia calycina* the mother-cells remain together in pairs even when forced out of the antheridium. The nucleus elongates considerably, so that it finally reaches quite to the posterior end of the body. A strongly refractive Cytoplasmahöcker ' appears at the side of the nucleus, and this forms the short anterior portion which bears the backwardly directed cilia. A vesicle such as occurs in the Filicoideae is not present, but the cytoplasm is spread along the length of the spermatozoid. Strasburger agrees with Guignard that the body is not completely enclosed in a thin cytoplasmic layer.

After Strasburger's discoveries in 1892 a considerable period elapsed before the appearance of further investigations on the Bryophyta, although several important papers were published on the spermatogenesis in other groups. During this period Campbell (17) noted the rectangular ' sperm cells ' of *Fimbriaria Californica* and described and figured the walls separating the spermatozoids of *Funaria hygrometrica*, which still persist after ejection from the antheridium. Miyake (45) also described the spermatozoids of *Makinoa crispata*, which are the largest yet discovered in the Hepaticae. He figures an antheridium showing the rectangular spermatogenic cells, and the spermatozoids are shown still enclosed with the walls of the mother-cells.

Ikeno's (32) work in 1903 was carried out with modern cytological methods and was the first detailed description of spermatogenesis in the Bryophyta. He confined himself to the investigation of *Marchantia poly-*

morpha. In this plant the rectangular cells of the developing antheridium possess vacuolated protoplasm and a nucleus bounded by a distinct membrane. During the early stages of division a small body appears in the nucleus and soon moves towards the membrane, producing a beak-like swelling in it. This body passes into the cytoplasm, becomes constricted, and then divides, giving rise to two structures which pass to the opposite sides of the nucleus; these are the centrosomes. Although no cytoplasmic radiations are present these can be clearly distinguished, as no other granules are present in the cytoplasm. The nucleus now elongates towards the centrosomes, and spindle fibres stream out from the latter; eight chromosomes are found on the equatorial plate. During the later stages the centrosomes are only occasionally seen, and they finally disappear, being probably taken up in the daughter nuclei. All the divisions in the developing antheridium are similar, the centrosomes arising afresh during each prophase, but the last division is characterized by the diagonal position of the spindle and by the persistence of the centrosomes in the resulting daughter-cells. The latter are at first triangular in section, and no wall is present between them. Up to this time the majority of investigators had regarded the rectangular cells in the antheridia of the Hepaticae as the spermatozoid mother-cells. This was the view taken by Strasburger (65) in the 'Botanisches Praktikum' in 1902, although Thuret (68), as early as 1851, had described the discoid mother-cells in several species, and Goebel (25) in *Pellia epiphylla* had pointed out that each rectangular cell produced two spermatozoids. The triangular cells soon become rounded, and the centrosome which now functions as a blepharoplast moves towards the end of the cell, becomes elongated, and develops the two cilia. Meanwhile, a fairly large spherical body which takes up the chromatin stain has appeared in the cell; the origin of this 'chromatoider Nebenkörper' was not determined, and at a later stage it disappears, taking no part in the formation of the spermatozoid. A cytoplasmic band now becomes differentiated which connects the blepharoplast and the nucleus, and the latter, which has become homogeneous in structure, elongates, and together with the cytoplasmic process forms the body of the spermatozoid.

Ikeno considered that the production of two spermatids from a mother-cell is a general rule for all Bryophytes, both Mosses and Liverworts. He also concluded that typical centrosomes are present in all cell generations in the antheridia of the Bryophyta, and that after the last division they change their function and act as blepharoplasts.

In a later communication Ikeno (33), as a result of further investigation, modified the above conclusions. He found that in *Atrichum angustatum* and *Pogonatum rhopalophorum* no centrosomes are present during the cell-divisions in the young antheridium; in both these Mosses eight chromosomes appear during mitosis. In *Makinoa crispata* also no chromosomes

are present at a similar stage. In consequence of these discoveries and of Chamberlain's (18) statement as to the absence of centrosomes during the earlier stages of the spermatogenesis of *Pellia epiphylla*, Ikeno concluded that, although centrosomes are constantly present in the antheridium of *Marchantia*, in the other Bryophytes they are gradually disappearing and occur only in a few cell generations.

Johnson (37) in 1904 shortly described the spermatogenesis of *Monoclea Forsteri*, and in this, as in *Marchantia*, the final division is diagonal.

The spermatogenesis of several Liverworts was investigated by Miyake in 1905 (44). In a preliminary note he stated that he was unable to discover centrosomes in the earlier divisions in the antheridium of *Marchantia polymorpha*. Just before division the nucleus elongates and becomes elliptical. An aster appears at each pole, but no centrosome is present; the aster entirely disappears when the spindle is formed. At the final division a deeply staining body is found at each pole of the spindle, and this, no doubt, is the blepharoplast. *Fegatella conica* is similar. Asters are present just before spindle formation in the antheridia of *Pellia*, *Aneura*, and *Makinoa*, but in these, too, no centrosome is present. Just before the final division in *Makinoa* the cells become rounded; neither centrosomes nor blepharoplasts are present at the spindle poles, but a group of granules is found some distance from each pole, and it is not improbable that these function later as a blepharoplast. In the same year Ikeno (34) reaffirmed his statements concerning the centrosomes in *Marchantia*.

The account given by Bolleter (13) of the spermatogenesis of *Fegatella conica* agrees closely with that of *Marchantia* as described by Ikeno. No centrosomes or asters are found in the earlier divisions of the antheridium in material preserved in alcohol, but their presence is considered probable. The final division is diagonal, and no wall is produced between the resulting daughter-cells; centrosomes are present during this mitosis and persist in the corners of the spermatids. Later on they pass to the acute angles, become elongated and pressed against the surface of the cell plasma, and finally give rise to the cilia. Meanwhile, the cells have become rounded and a 'chromatoider Nebenkörper', such as is described by Ikeno, has appeared. A 'Verbindungsstück', probably partly derived from the 'Nebenkörper', is developed between the centrosome and the nucleus. The latter decreases in size and becomes elongated and curved, and apparently, together with the 'Verbindungsstück', forms the body of the spermatozoid. On the dehiscence of the antheridium the spermatozoids are ejected in pairs, each pair being still surrounded by the wall of the mother cell.

Humphrey (30) in 1906 described the spermatogenesis of *Fossombronia longiseta*. In the half-grown antheridium the cell-division is normal, and no centrosomes are present. The final division only differs in the diagonal position of the spindle; no wall is produced between the resulting daughter-

cells. The spermatid becomes rounded, and later on a blepharoplast is found in the cytoplasm which soon migrates to the acute angle of the cell, becoming closely applied to the membrane. Previous to this a 'chromatoider Nebenkörper' appears in the cell and passes to a position just beneath the blepharoplast, where it becomes elongated, connecting up with the cytoplasm. The chromatin of the nucleus condenses, hiding the nucleolus, and the whole mass then becomes joined with the 'Nebenkörper', sharing in its elongation, and with it giving rise to the body of the spermatozoid.

Lewis (43) in *Riccia natans* gives a somewhat similar account of the spermatogenesis. Centrosome-like bodies are present throughout the development, and these at the final diagonal division are very obvious; no walls are formed between the daughter-cells resulting from this division. The bodies persist one in each spermatid in the neighbourhood of the nucleus, but later move to the end of the cell, coming into contact with the membrane and producing the cilia. No 'Nebenkörper' was found.

Ikeno (35), in a discussion of the homology of the blepharoplast, after a consideration of these investigations, has concluded that in the Marchantiales the centrosomes perform their normal function as well as acting as blepharoplasts. In the course of the phylogeny of the Hepaticae, the original function of the centrosome has been lost to allow for its specialization as the cilia-producing organ.

Escoyez, in 1907 (22), in an examination of the divisions in the antheridium of *Marchantia polymorpha*, confirms the statements of Miyake. In the earlier divisions no centrosomes are present; at the prophase the chromatic network is massed in the middle of the nucleus, no nucleolus being found. Then threads of chromatic material often pass outwards towards the membrane, and one or more granules are often found at the edge of the central chromatic mass, but these never pass out of the nucleus. No bodies are present at the spindle poles. Just before the final diagonal division bodies are found in each corner of the cell touching the cell-wall. Their origin could not be determined, and they probably arise *de novo*. Later on these bodies are found at the spindle poles, and they are still present at the telophase, persisting as blepharoplasts in the spermatids. The early divisions in the antheridium of *Fegatella conica* are similar, no centrosomes being present. Escoyez concludes that centrosomes are not present, and that the bodies which appear at the last division function as blepharoplasts only.

Up to this time no detailed description had been given of the early development of the spermatogenic cells in the Musci, although Ikeno (33) had already noted the absence of centrosomes during the spermatogenesis of certain species. Arens (2) was the first to investigate this subject, basing his description on *Polytrichum juniperinum*. Shortly afterwards a series of papers dealing with the spermatogenesis of several species of *Polytrichum*

and *Mnium* sp. were published by J. and W. Docters van Leeuwen-Reijnvaan (40, 41, 42). Detailed references to these papers will be given in the course of the following description.

A preliminary note on *Mnium hornum* has already been published (75), and in the present communication a full account of the spermatogenesis of this and of other Bryophyta is given.

Methods.

The methods employed for preservation and staining were similar in each of the Mosses investigated. A small amount of material was fixed in the field, but in the majority of cases clumps of the Mosses were brought into the laboratory or cool greenhouse, and the male plants were fixed at various times in the next few days. In some cases the plants were kept at a temperature of 28° C. for twelve hours before preservation.

The following fixing reagents were employed :—

Flemming's strong and weak mixtures, Hermann, Merkel, chrom-acetic, acetic alcohol, the sublimate-acetic-formalin mixture recommended by J. and W. Docters van Leeuwen-Reijnvaan (42), 70 % alcohol.

With the exception of acetic alcohol, which was allowed to act from 10 to 15 minutes, the material was allowed to remain in the fixing fluid for a period of 12–24 hours. On the whole, the Mosses are difficult plants to fix, and in several cases the preservation was unsatisfactory. Well-preserved material was, however, obtained by the use of Flemming's mixtures, the strong formula proving the most satisfactory. The sublimate-acetic-formalin mixture gave moderate results as far as the nucleus is concerned, but the fixation of the cytoplasm with the reagent leaves much to be desired. Acetic alcohol proved unsatisfactory. In all cases air was removed from the tissues by means of the air-pump. Concentration was carried out by the glycerine method (74) or by the use of successively increasing strengths of alcohol.

Longitudinal sections varying from 3μ–7μ in thickness were cut through the male receptacle, but in addition to these transverse sections of the antheridia were also prepared.

The following stains were employed :—

Heidenhain's haematoxylin in combination with orange G, Congo red or safranin, Flemming's triple stain (safranin, gentian violet, and orange G), Breinl's (70) triple stain (safranin, methylene blue, and orange tannin).

Mnium hornum.

Mnium hornum is dioecious, the male plants usually occurring in distinct groups, although they are often intermingled with the female individuals. The antheridia begin their development about the middle of

February, and ripe spermatozoids are produced from about the middle of March to the end of May. Material preserved during the middle and later parts of this period generally shows all stages of development. In the young antheridium the spermatogenic cells can easily be distinguished from the wall-cells by their dense cytoplasm and large conspicuous nuclei. In the wall-cells the nucleus is small and is almost hidden by the large chloroplasts with which the cell is closely packed. The spermatogenic cells are generally cubical in form and are regularly arranged, but the shape and arrangement are not so constant as in the antheridia of many of the Hepaticae, the cells being occasionally oblong or polygonal.

In their structure the cells closely resemble those previously described (74) in the archesporium (Pl. XXXVII, Fig. 1). The protoplasm is very finely alveolar, without vacuoles, and no deeply staining granules are present. The nucleus is large in proportion to the cell, and possesses a large deeply staining nucleolus. The nuclear network is fine and closely resembles the cytoplasm in structure, no chromatin being present in it during the resting condition.

The earlier divisions in the antheridium closely resemble those occurring in the archesporium. At the commencement of the prophase the nucleus becomes more granular and chromatin appears in the network. No body is cut off from the nucleolus, and no sign of centrosomes was found at this or at later stages in the division. The spireme stage is evidently of short duration, for few cells were discovered in this condition. At the metaphase six chromosomes appear on the equatorial plate (Fig. 2). Each is slender, elongated, and hooked, the short arm usually lying parallel with the equator of the spindle. In consequence of the statements made by J. and W. Docters van Leeuwen-Reijnvaan concerning the difference in size of the chromosomes of *Polytrichum* (41) and *Mnium* (42), a careful examination was made both in side and in polar views, but no definite variation in size was discovered—the chromosomes are of approximately equal length. In polar view (Fig. 3) each chromosome is seen as a sharply bent rod with the convex side towards the centre. Both Arens (2) and J. and W. Docters van Leeuwen-Reijnvaan (42) have given the chromosome number in *Mnium* as eight, but the former subsequently corrected this to six (3). Although the cell is small, there is no doubt as to the number present. This number has also been previously described in the meiosis. The spindle at this stage is ill-defined, but the fibres can be more clearly seen at the anaphase. No centrosomes are found at the poles. The absence of centrosomes can be stated with considerable certainty, since the cytoplasm is of very regular structure and there is a complete lack of deeply staining granules of any kind.

The subsequent divisions in the antheridium agree in their general characters with the description just given, and on this account considerable

difficulty was experienced in determining the relative ages of the various antheridia in which dividing cells were found; this was especially the case in antheridia which were almost mature. As several investigators have pointed out, the whole of the cells in an antheridium do not divide at the same time, but division takes place simultaneously in all the cells of a definite group. It is probable that a division is completed in the cells of all the groups before any group enters upon the succeeding mitosis. As, in the great majority of cases, the same number of spermatozoids is produced in each antheridium, it follows that at any given stage of development the number of cells present in different antheridia is approximately constant. The number of cells in the later stages is obviously very large, and no attempts were made to estimate the total. As will be described later, after the final division, the spermatids become rounded, and there is therefore no difficulty in distinguishing this stage. The number of spermatids occurring across the median longitudinal plane was ascertained, and this was compared with countings made in a similar manner in younger antheridia. The following results were thus obtained:— [1]

Average number of spermatids across antheridium . . . 13–14
Average number of cells across antheridium immediately before final division 10–11
Average number of cells across antheridium immediately before penultimate division 8
Average number of cells across antheridium immediately before antipenultimate division 4–5

In a few cases, apparently, an additional division had taken place, for antheridia, already possessing on the average fourteen cells across the median plane, showed groups in which divisions were still proceeding; but these cases were quite exceptional.

As the multiplication of spermatogenic cells goes on the antheridium grows considerably, but this increase in size does not keep pace with cell-division. The spermatogenic cells, therefore, become progressively smaller as maturity is approached. This fact affords confirmatory evidence as to age. Measurements of the cells found in antheridia at different stages of development were made, but these did not give constant results, probably on account of the variable shape of many of the cells. The distance

[1] By making certain assumptions the number of cells which should be found across the antheridium when cut in the median longitudinal plane can be calculated. After each successive division the number is increased in the proportion $1 : \sqrt[3]{2}$. If eight cells are found before the penultimate division $8(\sqrt[3]{2}) = 10{\cdot}08$ should be present after its completion and $8(\sqrt[3]{2})^2 = 12{\cdot}7$ after the close of the final division. How closely these numbers approximate to those obtained by actual observations may be seen by a comparison of those given above. I am greatly indebted to Mr. Odell, A.R.C.S., for information as to the factor $(\sqrt[3]{2})$ involved.

between the reconstituting nuclei in the early telophase was found to be much more constant, and here the results were as follows:—

Distance between daughter nuclei at telophase of last division, about 6·3 μ.
Distance between daughter nuclei at telophase of penultimate division, about 10·6 μ.
Distance between daughter nuclei at telophase of antepenultimate division, about 12 μ.

The importance of a method by which the ages of the various antheridia can be determined is made apparent by a consideration of the conclusions arrived at by J. and W. Docters van Leeuwen-Reijnvaan (41, 42). These investigators, who have described the spermatogenesis of several species of *Polytrichum* and of *Mnium* sp., state that, in all these plants, centrosomes are constantly present at the divisions in the antheridium. At the final division a reduction takes place whereby the haploid number of the chromosomes is reduced to half, in *Polytrichum* sp. to three, and in *Mnium* to four. Arens (2), who had previously examined *Polytrichum juniperinum* and *Mnium hornum*, found that the final division in these species was of the normal type. The later divisions in *Mnium hornum* were therefore examined with especial care.

During the early stages of the penultimate division a body is cut off by constriction from the nucleolus, although, as previously pointed out, nothing of this kind has been discovered in the earlier divisions. The course of events is the same as those previously described in the reduction division of this plant, where a similar process takes place. A small bud-like outgrowth is developed on the nucleolus (Pl. XXXVII, Fig. 4), and this by constriction is finally separated off, the body so produced lying close to the nucleolus within the nuclear membrane. This body has not been discovered outside the nucleus, and as it has not been seen during the later stages of division, it probably soon disappears.

During the prophase of the final division a somewhat similar process takes place, but here two bodies are produced. The nucleolus becomes slightly elongated and dumb-bell shaped, then by further constriction almost complete division into two spherical masses is effected (Figs. 6 and 8). Before complete separation, however, one of these masses buds off a comparatively small spherical body similar to that already described in the penultimate division (Fig. 7). The three bodies ultimately become free, but for some considerable time lie close together in a row within the nucleus. As before, these have not been seen to pass beyond the membrane, and no trace of such bodies has been discovered in the cytoplasm.

In view of these occurrences the statements regarding the origin of the centrosome made by J. and W. Docters van Leeuwen-Reijnvaan (41, 42) must be considered. According to these observers the centrosomes in *Polytrichum*

and *Mnium* are produced from the nucleus before each division. A small body is cut off from the nucleolus, which at first lies free within the membrane, but soon passes into the cytoplasm, becoming rod-like, and by constriction and division producing two centrosomes. These pass to opposite sides of the nucleus, and finally occupy the poles of the spindle. Before the final division in *Mnium* after the centrosome has been separated off, the nucleolus divides into two bodies of equal size, one of which passes into the cytoplasm and there disappears, the other persisting as the nucleolus.

Although the production of bodies from the nucleolus in the last two divisions in the antheridium can be confirmed in the case of *Mnium hornum*, the examination of a large number of preparations leads to the conclusion that these bodies do not pass outside the nuclear membrane as long as the latter is present. As already described, the cytoplasm of the spermatogenic cells is very regular in structure and is free from deeply staining granules, and it is exceedingly unlikely that any body equal in size even to the smaller of those cut off from the nucleolus would be constantly overlooked after its passage through the nuclear membrane. At the same time it may be pointed out that the size of the smaller body produced before the final division is considerably greater than that usually associated with a centrosome.

The importance of the separation of a body from the nucleolus at these divisions will be discussed later, but it may be stated here that a similar process has been observed in cells found in the neighbourhood of the stem apex. In these cases the cells in question are of considerable age, and it is probable that no further divisions would in any case have taken place.

In the spermatogenic cells the further history of these bodies has not been ascertained with certainty. During the following prophase a number of chromatin masses appear scattered throughout the nucleus and the nuclear membrane disappears (Pl. XXXVII, Fig. 9); at this stage neither the nucleolus nor the bodies in question can be distinguished. It could not be ascertained whether the latter take part in the formation of the spireme, but judging from the course of events in the reduction division it is probable that they do not function in this way. The spireme soon appears as a close network, and a little later the individual chromosomes can be distinguished. From the frequency of its occurrence the latter stage is of considerable duration, and in this respect the later antheridial divisions differ from those already described in the archesporium, where this condition is not well marked and is rarely found. The chromosomes are of the usual form, but are frequently curved and intertwined (Fig. 10), and, in consequence, it is often rather difficult to ascertain their number. As before, no difference in the size of the six chromosomes was observed. The metaphase is quite normal, and is quickly passed over. As a rule the axis of the spindle coincides with the long axis of the cell, but owing to the irregularity in the shape of the cells

it is sometimes found in an almost diagonal position. Even in these cases the resulting daughter-cells are rarely triangular in section, for the division wall does not usually strike the mother-cell wall at the intersection of two of its sides. The spindle is not well marked, and no centrosomes are found at the poles. The number of chromosomes present can be clearly seen both in polar (Fig. 12) and in side views (Fig. 11); six are constantly found.

Separation goes on in the usual way, and during the anaphase the daughter chromosomes can be distinguished on account of the small number present (Fig. 13). In polar view six can be again clearly seen (Fig. 14). On their arrival at the poles the chromosomes lose their sharp outlines, and on the appearance of the nuclear membrane can no longer be distinguished (Fig. 15). In the figure of this stage given by J. and W. Docters van Leeuwen-Reijnvaan distinct chromosomes are shown inside the daughter nuclei, but in the present investigation no such appearance has been seen. The reduction in number of chromosomes described by these investigators does not take place in *Mnium hornum*.

During the later stages of the final division vacuoles frequently appear in the cytoplasm, and these often persist in the daughter nuclei. The division wall arises in the usual way, but at a comparatively late period. The daughter nuclei at first contain several deeply staining granules, but later these are replaced by a single centrally placed nucleolus.

The cells after the final division is completed are somewhat irregular in shape, generally four or five sided, and at first occupy the whole cavity. But very shortly a shrinkage takes place, the protoplasm contracts from the wall, and the cell becomes rounded. The resulting spermatid is oval in form (Fig. 16). The nucleus is large and possesses a single centrally placed nucleolus, while the remaining part is made up of a fine network which contains little or no chromatin. The cytoplasm is finely alveolar, and one or more large vacuoles are usually found in it; no deeply staining granules can be distinguished. The original walls of the cells remain thin and sharply defined, and stain readily with orange G or Flemming's triple stain.

The nucleolus soon becomes slightly elongated and constricts in the middle, dividing into two bodies of almost equal size which at first remain in close contact (Fig. 17). The process is then repeated in one of the two resultant bodies, so that three are now found placed in a row within the nucleus. The nuclear membrane, which up to this time has been sharply defined, now loses its distinctness, and in consequence the limits of the nucleus can only be distinguished with difficulty. A separation of the bodies within the nucleus now takes place, and two of them pass into the cytoplasm.

Although the sequence of events just described is by far the most common, several variations of it may occur. In some cases it appears that

at this stage only one division of the nucleolus takes place, one of the bodies produced then passing into the cytoplasm. Or, of the three bodies formed from the nucleolus, only one becomes separated and two remain in contact with the nucleus for a considerable time. The same result is, however, arrived at in all cases ; a transference of the chromatic material from the nucleus to the cytoplasm takes place either in the form of one or of two bodies. These bodies, which are found scattered throughout the cytoplasm frequently in the neighbourhood of a vacuole, probably increase in number by division, as more than two can often be discovered (Pl. XXXVII, Fig. 18). Some or all of these now give rise to short irregular rod-like structures which are usually grouped together within or close to a vacuole (Fig. 19). At this stage the nucleus is only distinguishable as a denser mass which takes up the stain rather more strongly than the surrounding cytoplasm. The nucleolus is frequently found in a dividing condition, generally partially separated into two portions, but occasionally into three (Fig. 19). During these divisions of the nucleolus the rod-like bodies already described are always present in the cytoplasm, and on this account this stage can be distinguished from the earlier period of division, although the processes in the two cases are apparently identical. Later on the bodies produced by the division of the nucleolus become completely separated (Fig. 20) and probably pass into the cytoplasm, becoming associated with the rod-like bodies and perhaps giving rise to some of them.

The rod-like bodies now increase in length, becoming irregularly curved, and in this condition exhibit a remarkable resemblance to chromosomes. Their number is not constant, but three or four can often be distinguished, although counting is rendered difficult by their irregular form (Figs. 21–4).

A further consideration of the results obtained by J. and W. Docters van Leeuwen-Reijnvaan is of interest at this stage. In both *Polytrichum* (41) and *Mnium* (42) these investigators state that the spermatogenic cells shrink and become rounded before the final division. In *Polytrichum* a process of constriction goes on, and in the daughter-cells so produced chromosomes can still be distinguished. Although no constriction is described in the case of *Mnium*, chromosomes are similarly represented in the daughter-cells. It would seem probable that these investigators have seen spermatids containing the rod-like bodies and have considered that this is a stage in the final division. The number of the rod-like bodies as already pointed out is three or four, and this would correspond roughly to the number of chromosomes demanded by a double reduction. The partial superposition of two spermatids will account for the constriction shown in Fig. 56 of their communication (41).

The sequence of the following stages in *Mnium hornum* is very difficult to determine. During the early stages of development of the rod-like bodies the nucleolus is undivided (Figs. 21 and 22), but when these have

attained their greatest length two bodies are found within the nucleus produced, as in the previous cases, by the division of the nucleolus (Figs. 23–5). These two bodies remain close together for some time, and this circumstance aids in the determination of the order of events. The rod-like bodies, which are at first distinct from each other, become massed together in the vicinity of a vacuole which, at this stage, is always found in the cytoplasm. They decrease in length and at the same time become thicker, and finally a deeply staining mass is produced apparently by the coalescence of the greater part of the bodies in question (Figs. 26 and 27). The mass so produced is of variable shape and size, but often approaches an ovoid form. Fig. 25 probably shows an early stage in its production. All the rod-like bodies do not take part in its formation, but one or two remain free and are usually found stretching away from the mass towards the periphery of the spermatid (Figs. 26 and 27). At this time the nucleus is faintly distinguishable and still contains the two bodies formed by the division of the nucleolus, but soon almost all trace of it is lost. Changes take place in the deeply staining ovoid mass which result in the formation of a body which, although variable in form, is always recognizable in the spermatid at this stage. In view of the remarkable form of this structure, as well as its constancy of occurrence, it has been considered advisable to distinguish it in some way. The name limosphere is therefore proposed.[1]

The limosphere is usually situated towards one end of the longer axis of the spermatid. When fully formed it consists of a hollow sphere enclosing a vacuole. Its structure can be most easily explained by a full consideration of its development. The deeply staining mass formed by the coalescence of the rod-like bodies (Fig. 26) becomes more definite in form, and soon an almost spherical solid structure is produced (Fig. 27). This then becomes divided into two parts. As seen in optical section a curved interspace appears near its periphery, separating off an inner spherical portion from an outer shell which is usually incomplete on the side towards the periphery of the spermatid (Fig. 28). The interior portion of the inner spherical body now begins to lose its staining capacity (Fig. 29), and soon the greater part of it takes up only the cytoplasmic stain (Figs. 32 and 33); the remainder in the form of a segment of a hollow sphere still stains similarly to chromatin (Fig. 30). A little later the whole of this internal body takes up only the cytoplasmic stain, and finally it completely disappears (Fig. 31). At the stage the outer shell alone remains, enclosing a vacuole which in some cases contains a few ill-defined granules.

Although the course of events just described is the usual one, variations doubtless occur. In some cases it is probable that the outer shell referred to above is separate from the beginning, and is derived from one or more of

[1] λιμός = hunger, σφαῖρα = sphere. The name limosphere was suggested by Professor Farmer, F.R.S.

the rod-like bodies, while the remainder produce the inner spherical portion. In other spermatids it is possible that the limosphere is produced more directly by fusion and expansion of the rod-like bodies without the intervention of a solid mass. The structure of the body finally produced is, however, similar in all cases.

The conclusions arrived at concerning the formation of the limosphere have resulted from the examination of a large number of spermatids which had been 'fixed' in various ways and stained by different methods. The difficulties encountered in the determination of the stages are obvious, and these are added to by the small size of the bodies in question. The length of the longer axis of the spermatid varies from 5 to 7 μ, while the limosphere is about 1·5 μ in diameter. These structures also stain with difficulty. The best results were obtained by the use of Heidenhain's haematoxylin when this was allowed to act for long periods. In preparations stained for twenty-four hours and carefully washed out the rod-like bodies and limosphere are stained an intense black, but when sections are stained for only a short time these structures can only be faintly seen in the cytoplasm. Similar results were obtained by the prolonged use of Flemming's and Breinl's triple stains.

The stage at which the rod-like bodies are present is apparently quickly passed over. Although only a few preparations were obtained showing these structures, a large number of spermatids were found in them in this condition. The fixation in these preparations, judging from the younger antheridia, was particularly good. Stages showing the divisions of the nucleolus and the formation of the limosphere were frequently found, and it may be concluded that both of these are comparatively long processes.

The limosphere in a large number of spermatids is not a complete sphere, and is seen in optical section as a ring interrupted at one point. In some cases, however, a complete ring is found (Pl. XXXVII, Fig. 35). A somewhat similar structure has been observed in the developing spermatids of *Polytrichum* by J. and W. Docters van Leeuwen-Reijnvaan (41), who consider that it is homologous with the 'chromatoider Nebenkörper' described by Ikeno (32) in *Marchantia*. According to these investigators the structure is not spherical but ring-like in form. Arens (2) in *Mnium hornum* has also described the occurrence of a sickle- or ring-shaped 'Nebenkörper'. As already pointed out, the limosphere is seen in optical section as a complete or interrupted ring, and since this form is retained from whatever direction the structure is viewed it necessarily follows that the shape is spherical. A ring, when seen from the side, would appear as a band, but the limosphere has never been seen to assume this form.

It has already been mentioned that one of the rod-like bodies is generally found stretching away from the mass produced by the fusion of the remainder. During the changes just described this body is found

attached to the outer shell-like portion, and still persists as a deeply staining appendage of the limosphere. At or near the distal end of this appendage the blepharoplast is found as a small spherical deeply staining body (Figs. 28 and 29).

At this point, for the sake of clearness, a recapitulation of the various bodies produced from the nucleolus may be given. The first bodies cut off pass into the cytoplasm and form the rod-like bodies. During the elongation of the latter the nucleolus enters upon a second period of division, and the fate of the bodies produced is somewhat doubtful. A third period of division results in the formation of two bodies within the nucleus, one of which, in all probability, is identical with the blepharoplast. The origin of the latter structure was not definitely determined, but there is little doubt that it is the last body produced by constriction from the nucleolus. At a later stage, as seen in Fig. 27, one of the bodies produced by this final division is found in the cytoplasm, and when the formation of the limosphere is almost completed no deeply staining bodies can be seen within the nucleus. The blepharoplast is now found at the end of the appendage, while a second body is present in the cytoplasm in the vicinity of the limosphere (Figs. 28, 29, 31, and 32). It appears that the substance of the nucleolus is completely used up in the production of these bodies, and that both pass out of the nucleus, one functioning as the blepharoplast, while the other persists without change in the cytoplasm, and is finally found in the vesicle of the spermatozoid. It is possible that this second body corresponds to the 'Nebenkörper' described by Ikeno (32) in *Marchantia*, but further investigation is necessary before any statement can be made with regard to this matter. This body will in future be referred to as the accessory body. In view of the considerable number of similar bodies present in the spermatoid an earlier origin of the blepharoplast is not absolutely excluded. One of the bodies first produced from the nucleolus may persist throughout the changes described above, and finally function as the blepharoplast, but this is considered improbable.

In the following description the portion of the spermatid in the vicinity of the blepharoplast will be spoken of as anterior; the limosphere occupies the opposite or posterior part, while the appendage connecting the blepharoplast and the limosphere run approximately in an antero-posterior direction. Arens (2) has pointed out that in *Polytrichum juniperinum* the blepharoplast is situated on that part of the periphery of the spermatid which is directed towards the apex of the antheridium, but in *Mnium* no such regularity in its position has been discovered. The anterior (previously spoken of as the distal) end of the appendage extends to within a short distance of the membrane of the cell. The blepharoplast is at first found at the same spot, but a little later it changes its position and comes to lie on the actual periphery. In some cases the appendage elongates and

remains attached to the blepharoplast, but a short interval is often found between them.

Considerable changes have meanwhile gone on in the structure of the nucleus. At the time when the rod-like bodies are present in the spermatid it is almost spherical in form. During the formation of the limosphere a considerable increase in size goes on, and the nucleus passes to the periphery, occupying a lateral position in the cell (Pl. XXXVII, Fig. 32). Its structure is now homogeneous, for, as already described, the nucleolus is no longer present. The bodies shown in Figs. 32 and 33 apparently in the nucleus probably lie above or below it. The blepharoplast now elongates, producing a short thick bar-like structure which lies closely pressed against the periphery of the spermatid (Figs. 30 and 33). A similar elongation of the blepharoplast has been described by Ikeno (32) in *Marchantia*, but in this case, judging from the figures given, the extension is away from the spot which will finally be occupied by the apex of the spermatozoid. In *Mnium*, on the other hand, the elongation is towards the anterior part of the spermatid. Shortly afterwards a thread-like structure develops in connexion with the blepharoplast and passes over the inner contour of the cell membrane in a direction opposite to the previous elongation (Figs. 34 and 35). It was not determined whether this thread is produced by an outgrowth from the blepharoplast substance or by a differentiation of the cytoplasmic membrane of the cell. The thread soon reaches the portion of the cell periphery in contact with the nucleus and passes some distance beyond it (Figs. 35–8). At this stage the attachment of the appendage of the limosphere to the blepharoplast can almost always be distinguished, the connexion being found at the junction of the thread and the thick bar-like portion (Fig. 37). In some cases, however, the appendage, although directed towards this point, does not quite reach it (Fig. 34); occasionally a projection of the appendage beyond the blepharoplast has been discovered (Fig. 36).

During the elongation of the blepharoplast the limits of the nucleus are difficult to define, and can only be distinguished in preparations which have been subjected to prolonged staining in Flemming's or Breinl's triple stains. As a general rule, the nucleus does not extend quite up to the blepharoplast. Very soon after the production of the thread elongation takes place, and the nucleus soon reaches the anterior end of the bar-like structure produced by the elongation of the blepharoplast (Fig. 39). The nucleus continues to elongate (Fig. 40), and at the same time stains more deeply. It can be easily seen extending around almost the entire periphery of the spermatid in contact with the thread, and even at this stage the appendage is still faintly distinguishable (Fig. 41).

At the latest stage discovered in the preparations the spermatozoid was almost mature. The nucleus has now attained a length of about one and a half turns of a spiral, and at the same time has decreased considerably

in breadth. The limosphere and the body already referred to are still to be seen within the spermatid. The cytoplasm is scanty, and with the exception of a small mass at the posterior end of the developing spermatozoid stains feebly. The staining capacity of the mass in question increases as the spermatozoid approaches maturity. Guignard (26 A) in his investigation of *Sphagnum fimbriatum* states that an amylaceous mass is present in the vesicle of the mature spermatozoid, and it is probable that this mass corresponds to the deeply staining portion of the cytoplasm found in *Mnium*. When the spermatid of this latter plant is treated with iodine solution the mass referred to stains reddish brown, and does not give the blue-black coloration characteristic of true starch. The nucleus cannot be distinguished, but the thread and limosphere stain deeply (Fig. 44). No cilia could be distinguished even at this late stage, but it may be presumed that they arise from the blepharoplast, and in this case would be found attached to the anterior end of the spermatozoid.

The occurrence of 'double' spermatids has been described in *Marchantia* by Ikeno (32), and in *Mnium hornum* similar structures have been discovered in this investigation (Fig. 43). These spermatids are approximately double the normal size, and very probably contain two nuclei, although in consequence of the difficulties in staining the outlines of these bodies could not be clearly distinguished. Two limospheres, two blepharoplasts, and two thread-like structures could, however, be seen in each (Fig. 43). These 'double' spermatids were discovered in an otherwise normal antheridium. In *Marchantia* each pair of normal spermatids produced by the final division is surrounded by the mother-cell wall, and no wall is formed between the two individuals. Since each 'double' spermatid is surrounded by a wall it appears that this is produced by the failure of the mother-cell to complete its division. In *Mnium hornum*, however, each normal spermatid is surrounded by a wall. The 'double' spermatids in this plant may also be produced by the failure of the spermatogenic cells to complete the final division, but obviously, similar evidence to that used in the case of *Marchantia* cannot be advanced.

Atrichum undulatum.

The structure of the antheridium of *Atrichum undulatum* is very similar to that of *Mnium hornum*. Here again the spermatogenic cells are easily distinguished from those forming the wall on account of the numerous large chloroplasts found in the latter. The spermatogenic cells are rather irregular in shape, being four or five sided. Each contains a large nucleus in which a deeply staining nucleolus is found. In this plant Beer (5) has pointed out that the nucleoli 'consist of a lightly coloured matrix in which are embedded a number of grains of chromatin'. Although this appearance has occasionally been seen, it is not of constant occurrence.

The cytoplasm is rather denser than that of *Mnium*, but, as in that plant, its structure is very regular; neither deeply staining granules nor vacuoles are found in the cells. The various stages of division have not been studied in great detail in this species, but as far as they have been observed they closely resemble those already described in *Mnium*. One point of difference has, however, been observed. In *Atrichum undulatum* division of the nucleolus has been discovered in spermatogenic cells forming part of young antheridia (Fig. 45), and it seems probable that in this case the separation of a body from the nucleolus takes place prior to all the divisions of the spermatogenic cells. As in *Mnium*, no trace of centrosomes has been discovered at any stage in the divisions.

On account of the statements made by J. and W. Docters van Leeuwen-Reijnvaan (41, 42) the final division of the spermatogenic cells was carefully investigated. As in *Mnium hornum*, this mitosis closely resembles the previous ones, and in consequence the same methods were here employed to determine the comparative ages of the antheridia. Although the results obtained were not so constant as those given by the former species, the final mitosis could still be recognized with considerable certainty. In the metaphase of this division the spindle is not well marked, and no centrosomes are present. Its orientation is variable, for while in some cases its long axis coincides with that of the cell (Pl. XXXVIII, Fig. 48), in others it is diagonally arranged (Pl. XXXVII, Fig. 46). The number of chromosomes is considerably greater than that found in *Mnium*. As the result of several countings made in polar views of the metaphase, it has been concluded that seventeen chromosomes are present, although in a few cases only sixteen could be distinguished (Fig. 47). Separation of the daughter chromosomes goes on in the usual manner, and during the anaphase the number could be again determined in polar views; as before, seventeen chromosomes are found (Pl. XXXVIII, Fig. 49). It is therefore obvious that no reduction has taken place. The telophase is normal, and a wall is formed between the resulting cells in the usual manner.

Shortly after the final division is completed shrinkage takes place, and each cell becomes free from its investing walls. The spermatids so produced are approximately spherical in form. Each possesses a large nucleus which contains a deeply staining nucleolus. The cytoplasm is regularly alveolar in structure and contains no deeply staining granules (Fig. 50). A protrusion is now produced on the nucleolus, and soon a small spherical body is cut off by constriction, which at first remains in contact with it. Shortly afterwards this body migrates into the cytoplasm and passes towards the cell membrane (Fig. 51), finally coming to rest on the periphery (Fig. 52). Later on this functions as the blepharoplast.

The nucleolus meanwhile continues to bud (Figs. 51–3) and produces two more small bodies which do not at once separate. In consequence,

at this stage a row of three bodies is found lying within the nuclear membrane (Fig. 54). After a time the two bodies formed by constriction become free and pass into the cytoplasm, but do not take up definite positions (Figs. 55 and 56). While these bodies are being cut off, changes take place in connexion with the blepharoplast. A thread-like structure is produced in contact with it, which elongates and passes over the inner contour of the cell membrane (Figs. 53–5). The remarks made with regard to the origin of the similar structure found in *Mnium hornum* apply equally in this case. It may, however, be concluded that the process of formation is identical in the two plants. The growth of the thread continues until it extends round about half the circumference of the cell (Fig. 54). Soon after this length has been attained the nucleus, which up to this stage has occupied a central position in the spermatid, passes towards the periphery and comes into contact with the thread at some distance from the blepharoplast (Fig. 56).

At the next stage observed two structures are found in the cytoplasm of the spermatid. One of these is clearly similar to the limosphere already described in *Mnium hornum*. It consists of a comparatively large hollow sphere which takes up the nuclear stain freely (Fig. 57). In *Atrichum* no obvious vacuole is found associated with the limosphere, the interior being filled with cytoplasm, or at least with a substance closely resembling it in structure and staining properties. Although this body often exists as a complete sphere, it frequently happens that only a segment of a sphere is present (Fig. 58). There can be no doubt as to its real form, as in all cases the limosphere in optical section is seen as a complete or interrupted ring from whatever direction it is examined. In Figs. 57 and 58 two spermatids are shown in planes perpendicular to each other, and it will be observed that in each the limosphere takes the ring-like form.

No direct evidence as to the origin of the limosphere could be discovered, but it seems highly probable that it arises from one of the two bodies derived from the nucleolus. It is usually found occupying a position not far from the nucleus and close to the periphery of the spermatid. On the opposite side of the cell a second structure is present, staining similarly to the limosphere but of a different form (Figs. 57 and 58). This is similar in shape and appearance to the bodies previously cut off from the nucleus, and there is little doubt that while one of these produces the limosphere the other persists in an unaltered form. It must be left to future investigation to determine whether this second body is equivalent to the 'Nebenkörper' described by Ikeno (32) in *Marchantia*. This in future will be referred to as the accessory body. The nucleus now begins to elongate along the course of the thread, and soon reaches the blepharoplast, which in future retains its position at its anterior end. The nucleolus, which up to this time has been distinguishable, now disappears, and the structure of

the nucleus becomes homogeneous. Elongation proceeds until the ends approach one another closely (Pl. XXXVIII, Fig. 59), but actual contact does not take place, the anterior usually passing to the outside of the posterior end (Fig. 60).

The limosphere and the accessory body are still distinguishable in the cytoplasmic mass enclosed by the elongated nucleus (Fig. 60), and apparently are finally found in the vesicle. Although the remaining cytoplasm is scanty in amount, the portion towards the posterior end of the spermatozoid stains strongly, and together with the limosphere and body, probably gives rise to a deeply-staining mass similar to that described by Guignard (26 A) in the vesicle of the spermatozoid of *Sphagnum fimbriatum*. At this stage the spermatozoid consists of about one and a half turns of a spiral, and although almost mature no cilia have been distinguished with certainty in the preparations. Presumably these are produced from the blepharoplast, and are therefore attached to the anterior end of the spermatozoid.

Pellia epiphylla.

The antheridia of *Pellia epiphylla* usually appear at about the beginning of April, and development proceeds until the latter part of June. Towards the end of the period plants which have already borne antheridia frequently develop archegonia. Plants were preserved at intervals during the whole of this period in order to obtain all the stages of development. Several fixing fluids were employed, and satisfactory results were obtained with both of Flemming's mixtures, Merkel's fluid, and acetic alcohol. This last medium gave much better results with *Pellia* than with *Mnium* or *Atrichum*.

Material was obtained in the field and from Chelsea Physic Garden. After collection in the field, clumps of the undisturbed plants were brought into the laboratory and preserved at various times in the next few days. In some cases the plants were kept at a temperature of 28° C. for twelve hours preceding fixation. At the Physic Garden material was fixed either in the cool greenhouse where the plants were grown or after they had been placed for several hours in a greenhouse kept at a higher temperature. In all cases the air was removed from the tissues by means of an air-pump. Sections varying from 3 μ–7 μ in thickness were cut, some being parallel to the longitudinal axis of the plant, some at right angles to this axis, and some parallel to the surface. The following stains were used: Flemming's triple, Heidenhain's haematoxylin, Breinl's triple stain, and a mixture of acid fuchsin and iodine green.

The spermatogenic cells in the young antheridia of *Pellia* generally appear four-sided in section, but their form is not nearly so regular as that of the corresponding cells in *Marchantia* and *Fegatella* (Pl. XXXVIII, Fig. 61). The large nucleus is bounded by a definite membrane and contains a large

deeply staining nucleolus; the remaining portion is made up of a network containing very fine particles of chromatin. The cytoplasm is finely alveolar and contains few or no deeply staining granules.

The first sign of approaching division is seen in the increase in size and number of the chromatin particles. At first a few of these attain a greater size than the remainder, and these are frequently found near the periphery of the nucleus, sometimes in actual contact with the membrane (Fig. 62). A little later they tend to accumulate in the vicinity of the nucleolus, and an irregular ring of deeply staining masses is seen around this body (Fig. 63). The nucleus now increases somewhat in size and becomes elongated and bluntly pointed at each end, while the chromatin begins to aggregate into short, irregular threads.

The above appearances have been seen in antheridia which do not show more than twelve spermatogenic cells in median transverse section, but unfortunately the immediately succeeding stages have not been observed in these young antheridia. In the succeeding metaphase the spindle is well marked, the fibres converging to a point at the poles, but no polar radiations have been distinguished. This agrees with the observations of Chamberlain (18), who was unable to find centrospheres in the development from the initial cell up to the stage where the antheridium shows thirty or more cells in transverse section.

After this stage has been reached centrospheres have, however, been observed in the spermatogenic cells, and it is a singular fact that although these are apparently absent in the earlier divisions, they are here found as well-developed structures. These centrospheres are similar to those described by Farmer and Reeves (23) in the germinating spores. In these older antheridia the early stages of division are similar to those already described. Soon after the chromatin granules begin to aggregate together, the membrane becomes drawn out into two points on opposite sides of the nucleus (Fig. 64). From them distinct cytoplasmic radiations extend (Fig. 65), but these are not so numerous as those figured by the latter observers in the germination of the spores. At the centre of these radiations a small granule can in some cases be seen, but as its presence could not always be determined, the occurrence of a centrosome is considered somewhat doubtful. At the following metaphase radiations were not discovered with certainty, but here again a small granule was occasionally seen at each pole.

It may be noted in this connexion, that although Farmer and Reeves (23) were unable to ascertain the existence of any definite particle which would indicate the presence of a centrosome within the centrosphere, Strasburger (63), after examining the same preparations, concluded that a centrosome was always present. The constant occurrence of this body in the spores increases the probability of its existence in the centrospheres

found in the developing antheridium. When first distinguished, the centrospheres are found lying in the cytoplasm at opposite ends of the nucleus, and no evidence as to their origin was obtained. According to J. and W. Docters van Leeuwen-Reijnvaan (42), a single centrosome is at first present in the cytoplasm of the spermatogenic cells of *Pellia*, and this by division produces the two found later at the spindle poles, but in spite of careful search, no structures resembling those figured by these investigators were found.

Before the final division the spermatogenic cells shrink slightly and free themselves from their surrounding walls, becoming ellipsoidal or almost spherical in form. The walls swell considerably, so that each cell comes to be in an almost spherical space, separated by a considerable thickness of wall from the surrounding cavities. Each possesses a large nucleus which extends over more than half the cell and contains a well-marked nucleolus. The early stages of division resemble those already described (Pl. XXXVIII, Fig. 66). Small deeply staining granules were sometimes seen near the pointed ends of the elongated nucleus, but owing to their close proximity to the periphery of the cell, their presence could not always be determined; radiations could not be distinguished for the same reason. A little later the nucleus increases in size, and the pointed ends come into contact with the cell membrane (Fig. 67). The chromatin is now found in a threadwork which is arranged in an equatorial band just within the nuclear membrane, while the remainder of the nucleus is free from staining substance. The chromosomes then become distinct, and are found scattered throughout the central part of the protoplasm. At the next stage discovered, the daughter chromosomes had almost reached the poles, and since here only an extremely thin layer of cytoplasm exists between the ends of the spindle and the cell membrane, no bodies resembling centrosomes could be distinguished.

After their arrival at the poles the chromosomes become crowded together and lose their sharp outlines, finally forming a number of irregular deeply staining masses. These are pressed closely against the periphery of the cell, and in consequence it is impossible to see whether centrospheres are present at this stage (Fig. 68). Meanwhile, thickenings of the spindle fibres form at the equator, and a little later by coalescence give rise to a wall separating the two daughter-cells or spermatids. At the same time retrogressive changes have gone on in the daughter nuclei, and a nuclear membrane appears; no definite nucleolus is present, but each contains a number of deeply staining, irregular masses of chromatin.

Owing to the circumstance that the mother-cell is almost spherical, each spermatid is approximately hemispherical in form, and in the preparations appears either circular or semicircular in outline, depending on the direction from which it is observed. In the following description the spermatid, when seen in section as a semicircle, is spoken of as viewed from

the side, and when circular in outline it is described as seen from above or below. After the division is completed each spermatid shrinks slightly, but retains its original shape; the wall separating the individuals of each pair does not swell, but remains thin and sharp in outline (Fig. 71). At this stage the appearance of the antheridium is very characteristic; the pairs of spermatids are easily distinguished since each is separated from the neighhouring groups by the greatly swollen mucilaginous walls of the mother-cells; between the individuals of each pair there is a small space in which the thin wall separating the two cells is found. The swollen walls of the mother-cells, as well as the walls separating the spermatids, are easily stained with orange G and colour deeply with Congo red. They consist at least partially of pectic substance, since they are also stained with ruthenium red.

The occurrence of a wall between the spermatids of each pair is of especial interest. The wall was figured by Buchtien (15) and Guignard (26 A) in *Pellia epiphylla*, but is omitted by Campbell (16) in a drawing of the paired spermatids of the same plant. Ikeno, after special search, failed to find a similar wall in *Marchantia polymorpha*, and similar results have been obtained by Bolleter (13) in *Fegatella conica*, Humphrey (30) in *Fossombronia longiseta* and *Aneura*, and Lewis (43) in *Riccia natans*.

In view of the diversity of the above statements, preparations[1] of the developing antheridia of *Aneura pingius* were examined in order to determine whether in this plant a wall is present at a similar stage. The material had been fixed in acetic alcohol and the preparations were stained with Flemming's triple stain or with Heidenhain's haematoxylin and orange G. With either of these stains a wall can be clearly distinguished separating the spermatids of each pair. The antheridia of this plant closely resemble those of *Pellia*; the spermatids are of a similar shape, and the pairs show the same space relationships. In consequence, both in *Pellia* and *Aneura*, this wall does not divide up a cubical mother-cell, but one which is approximately spherical in shape. This is also the case in *Makinoa crispata*, in which Miyake (44) states that the cells in the antheridium assume a more or less spherical form just before the last mitosis. Although there is no doubt that this is the equivalent to the final division described by Ikeno (32) in the antheridium of *Marchantia*, yet it is obvious from the account just given that it cannot, at any rate in *Pellia* and *Aneura*, be described as 'diagonal'. In these latter plants the form of the mother-cells is determined by their early separation, while in *Marchantia* no such process goes on and the cells remain cubical. In *Pellia* and *Aneura*, even if the division is phylogenetically 'diagonal', yet, ontogenetically, there is little evidence of it.

[1] The preparations in question were made by Professor Farmer, F.R.S., who has very kindly allowed me to have the use of them.

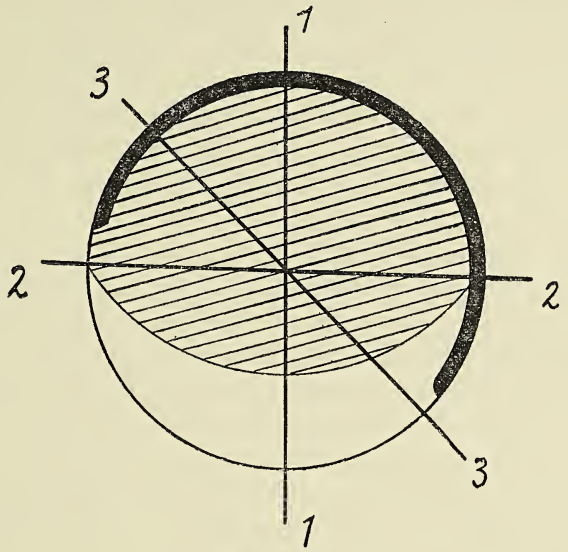
The nucleus of the spermatid is centrally placed, and is very large in comparison with the size of the cell, almost extending across its whole width. It is oval in shape and contains a number of irregularly shaped masses of chromatin usually massed towards the centre, while no definite nucleolus is present. The cytoplasm is very regular in structure and contains no deeply staining granules. The blepharoplast soon appears as a small intensely staining spherical body. It can be first distinguished at about the middle of the curved wall of the spermatid lying in the protoplasm midway between the nucleus and the periphery of the cell (Pl. XXXVIII, Fig. 69). The origin of the blepharoplast was not determined, but the position in which it was first observed is suggestive. This spot was occupied by the pole of the spindle in the division of the mother-cell, and it is the place at which a centrosome, if present, would probably be found. Although a complete investigation has not been made, it has been shown that there is considerable evidence for the presence of a centrosome at the final division. It is difficult to avoid the inference that this body persists as the blepharoplast, or, at any rate, is genetically connected with it. The latter is larger and stains more deeply than the bodies seen at the spindle poles, but this increase of size and of staining capacity in the developing spermatid is not surprising. It must, however, be borne in mind that the appearance of the blepharoplast in this position does not exclude the possibility of its nuclear origin. It is hoped to deal with this matter fully in a subsequent paper.

The blepharoplast now approaches the plane surface, always remaining during its passage a short distance from the curved wall (Fig. 70). As seen in side view it frequently passes towards one end of the spermatid, but this is not constantly the case, as it may finally occupy any position along the junction of the curved and the plane surfaces, and hence appear at any spot on the flat wall. The blepharoplasts are often similarly orientated in each spermatid of the pair, and in this case may be found in side view occupying the correspondingly adjacent angles (Fig. 73). On the other hand, they may be seen in the widely separated angles (Fig. 72). The nucleus, up to this time, has retained its central position in the cell, but now passes towards the periphery and is finally discovered in contact with the membrane at a short distance from the blepharoplast (Fig. 74).

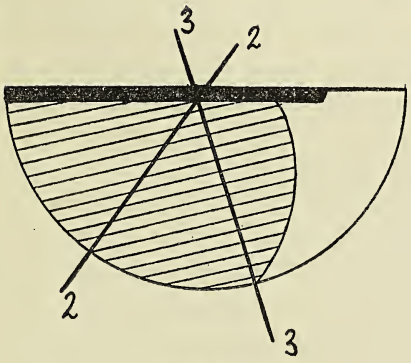
A thread of stainable substance is now formed in connexion with the blepharoplast, extending over the inner contour of the cell membrane and passing along the edge of the plane surface. It soon reaches the area in contact with the nucleus and passes beyond it, terminating at the opposite side of the cell (Fig. 77). At present it must be left an open question whether the thread is due to an extension of the blepharoplast substance or whether it is produced by a differentiation of the cytoplasmic membrane in connexion with the blepharoplast.

At this stage it is necessary to consider in detail the appearances pro-

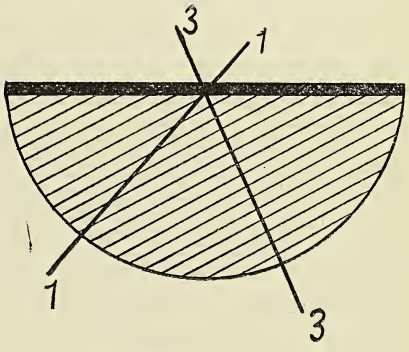
duced when the spermatid is cut in various directions. As already explained, when the spermatid is viewed from above or below, the outline is circular (Text-fig. 1), and semicircular whenever it is viewed from the side (Text-figs. 2 and 3). Since the nucleus is not centrally placed, it is obvious that all sections taken perpendicularly to the plane wall will not be similar.



TEXT-FIG. 1.



TEXT-FIG. 2.



TEXT-FIG. 3.

Text-figs. 1, 2, and 3 represent diagrammatically the appearances respectively seen when the spermatid is viewed in three planes perpendicular to one another.

Text-fig. 2 shows the appearance when the spermatid is seen in optical section through the line 1, 1 (Text-fig. 1) in a plane perpendicular to that of the flat surface, and Text-fig. 3 when similarly seen in section through the line 2, 2 (Text-fig. 1). Fig. 75, Pl. XXXVIII, is an accurate drawing

of a spermatid seen in a similar position to that shown diagrammatically in Text-fig. 2. Here the nucleus apparently occupies one end of the cell, and the thread passes about half-way along the flat side. In this case the two halves of the thread (seen to the left and right of 1, 1, Text-fig. 1) are exactly superposed, and in consequence no change in position is noticed on altering the focus. Seen in one plane only, the thread should appear in section as a dot, but on account of the depth of focus possessed by the objective, this appearance is never realized. When the spermatid is seen in optical section along the line 2, 2 (Text-fig. 1), the nucleus appears to occupy the whole of the cell, and the thread, on altering the focus slightly, apparently extends along the whole length of the flat side. Optical sections in the planes 3, 3 and 4, 4 (Text-fig. 1) produce appearances somewhat similar to that given along the plane 1, 1.

Optical sections in a plane making an angle of less than 90° with the plane of the diagram shown in Text-fig. 1 produce different appearances. Such will in future be referred to as oblique sections. The direction of a section passing obliquely through the line 1, 1 (Text-fig. 1) is shown by 1, 1 (Text-fig. 3). In such an optical section the outline of the spermatid, if examined at one focus, is bounded by one flat and one curved side as before. The nucleus apparently occupies one end of the cell, and the thread is seen as a slightly elongated structure at the end of the cell in contact with the nucleus. On focusing up and down the flat wall is apparently replaced by a slightly curved one, and the two halves of the thread (shown right and left of 1, 1 in Text-fig. 1) come into view, one occupying the slightly curved and the other the more strongly curved wall. Fig. 78, Pl. XXXVIII, is a composite drawing made at several foci, and shows the appearances just described. In an oblique optical section taken through the line 2, 2 (Text-fig. 1), the direction of which is given by line 2, 2 (Text-fig. 2), the nucleus apparently occupies the whole cell; as before, the spermatid is bounded by one curved and one flat wall. At one focus the thread is seen as two short rods, one at each end of the cell; on altering the focus the flat wall is apparently replaced by a slightly curved one, and the rods appear to approach one another along the more strongly curved wall. The total impression given is that the thread passes along the curved wall.

Fig. 76, Pl. XXXVIII, shows the appearance of an oblique optical section through the line 3, 3 (Text-fig. 1), the direction of which is indicated by the lines 3, 3 in Text-figs. 2 and 3. Here the nucleus apparently occupies one end of the spermatid, while the blepharoplast is found near the opposite end. The thread appears to pass along the more strongly curved wall.[1]

[1] The description just given has been written with the aid of a model. Without the assistance thus provided it was found to be extremely difficult to appreciate the space relationships in such a body as the spermatid of *Pellia*.

At this period two other structures appear in the cytoplasm of the spermatid (Figs. 77 and 78). One of these consists of a deeply staining spherical body somewhat larger than the blepharoplast and usually occupying a position midway between the nucleus and the periphery of the cell. This possibly corresponds to the 'Nebenkörper' described by Ikeno (32) in *Marchantia*, and subsequently recognized in other plants by several investigators; this in future will be referred to as the accessory body. In close proximity to this another structure is found staining similarly to the 'Nebenkörper', but not so deeply.

This often takes the form of a sphere in which a portion of the surface stains more deeply than the remainder (Fig. 77). It is of considerable size, being three or four times as large as the blepharoplast. In other cases it has the appearance of a short curved rod from whatever direction it is observed; its real form must, therefore, here correspond to the segment of a hollow sphere. Occasionally two such rods have been observed in optical section joined together and making an angle with each other (the lower spermatid shown in Fig. 78). On account of its similarity in form, this structure strongly recalls the limosphere already described in the Mosses. In *Pellia*, as in *Atrichum*, the limosphere is not found in connexion with a vacuole. It is probable that the sphere just described is the earlier stage, and that the segment is derived from it by the disappearance of the more lightly staining portion. No statement as to the origin of the limosphere or the body accompanying it can be made at present. They have not been recognized with certainty at any later stage and probably soon disappear, taking no direct part in the formation of the spermatozoid.

At this stage, when the spermatid is viewed from above or below, the nucleus is found to occupy the greater part of the cell, extending round about half the circumference. The blepharoplast is situated on the periphery a short distance from the nucleus and connected with it by the thread-like structure already described (Fig. 77).

It is probable that the blepharoplast and the portion of the thread which projects beyond the nucleus together correspond to the 'Cytoplasmahöcker' described by Strasburger (62) in *Pellia calycina*. This investigator describes the 'Cytoplasmahöcker' as a highly refractive, slightly elongated body occurring on the outer edge of the nucleus and bearing the two cilia. He gives no details as to its formation, but the resemblance of the structures in the two species suggests similarity of development in each.

Up to this time a number of small irregular masses of chromatin can usually be distinguished, but the substance of the nucleus now becomes homogeneous. It begins to increase in length and at the same time decreases in width. The elongation proceeds in both directions along the course of the thread-like structure, and at the anterior end the blepharoplast is soon reached; this body retains its position at the end of the

nucleus during the subsequent extension. The thread, which is constantly found on the outer contour of the nucleus, must consequently undergo an equal increase in length.

The elongation as shown by Guignard (26 A) and Campbell (16) takes place in a plane parallel to that of the wall which divided the mother-cell, and on this account can be most easily observed in those cells so placed as to appear circular in optical section. In this respect *Pellia* agrees with *Monoclea Forsteri*, in which Johnson (37) states that the axis of the spiral formed by the spermatozoid is perpendicular to the plane dividing the mother-cell. A comparison of the Cycads and *Ginkgo* is also of interest in this connexion. In these plants, as far as they have been investigated, the axis of the spermatozoid is always perpendicular to the wall dividing the mother-cells, and here again there is complete agreement with *Pellia* in this respect. On the other hand, in Ikeno's account of *Marchantia* (32), although no statement is made as to the orientation of the plane of elongation, judging from the figures given, this plane is at right angles to that of the divisional plane of the mother-cell. An examination of the drawings of *Fossombronia* given by Humphrey (30) leads apparently to the conclusion that this plant resembles *Marchantia* in this respect.

The elongation of the nucleus proceeds until the ends come almost in contact (Pl. XXXVIII, Fig. 80), and then further extension goes on in a spiral above or below the level of the first coil (Fig. 81). This increase in length is accompanied by contraction of the substance of the nucleus and consequent decrease in width. The cytoplasm meanwhile diminishes in amount, and is found in the portion of the spermatid enclosed by the first coil. When the spermatozoid has reached its full length it consists of a spiral of about three turns.

No observations have been made on the free swimming spermatozoid, and during the various stages of development no traces of the cilia were found, although special staining methods were employed to render them visible. Consequently no statement can be made as to their exact place of attachment, although they may be presumed to arise from the blepharoplast.

Discussion and Conclusions.

In the above description it has been shown that the mitoses which take place during the development of the antheridia of *Mnium hornum* and *Atrichum undulatum* are of the normal type. The final division of the spermatogenic cells does not differ essentially from those mitoses which immediately precede it, but in fact resembles them so closely that there is considerable difficulty in distinguishing it. It is clear that no reduction in the number of chromosomes takes place in the antheridia of these plants. It may also be concluded with considerable certainty that no reducing

division similar to that described by J. and W. Docters van Leeuwen-Reijnvaan in the antheridia of several species of *Polytrichum* and in *Mnium* sp. takes place in the Musci.

The final divisions in the antheridia of the two species show, however, several points of interest. In *Mnium hornum* the axis of the spindle usually coincides with the longitudinal axis of the cell, and is not diagonally arranged as is the case in many of the Hepaticae. In a few cases, however, an almost diagonal arrangement was observed. At the corresponding division in *Atrichum undulatum*, although the axis of the spindle is sometimes almost diagonal, it is usually parallel to the longitudinal axis of the cell. The cells resulting from the final division in both species are, however, never triangular in section, but rectangular or polygonal. Since no shrinkage takes place in the cells before the final division, these cannot be easily distinguished from the spermatogenic cells at earlier stages of development.

It is therefore doubtful whether the term 'mother-cell' should be applied to the cells immediately before the final division or to the cells resulting from this division.

In the Hepaticae the mother-cells each give rise to two spermatids. In *Marchantia* and *Fegatella* these mother-cells can be distinguished by the fact that they divide diagonally, and in *Pellia*, *Aneura*, and *Makinoa* by their contraction before the final division. It must therefore be concluded that either the mother-cells in the Musci do not divide, and only one spermatid is produced in each, or that at their division the axis of the spindle is generally not diagonal. Since divisions of approximately the diagonal type are sometimes found, it appears that the second of the above possibilities may exist. The occasional occurrence of such divisions might be adduced as evidence for the Hepatic ancestry of the Musci, but in view of the irregularity in shape of many of the spermatogenic cells, too much importance must not, perhaps, be attached to this fact. The discovery of 'double' spermatids in *Mnium hornum* can be cited as additional evidence for the formation of two spermatids from each mother-cell in this plant. It may also be pointed out in this connexion that the production of two male cells from each mother-cell is almost universal in the Pteridophyta, Gymnospermae, and Angiospermae.

Several different structures have been described in the developing spermatozoid in the various groups of plants. The most important of these is the blepharoplast, and this is the only one which is constantly found in the spermatid at some stage in its development. A considerable literature has appeared dealing with the formation of this body. Four methods of origin have been assigned to it in various plants.

I. In a large number of cases the blepharoplast is said to arise *de novo* in the cytoplasm, either in the spermatid itself or in the spermatogenic cell before its final division. It is said to originate in this manner in *Cycas*

(Ikeno 31), *Zamia* (Webber 73), *Ginkgo* (Hirasé 27), *Nephrodium* (Yamanouchi 76), *Marsilea* and *Onoclea* (Shaw 57), *Marchantia* and *Fegatella* (Escoyez 22), *Fossombronia* (Humphrey 30). In several cases the blepharoplast is described as arising in the cytoplasm near the nucleus, as in *Adiantum* and *Aspidium* (Thom 67), *Derbesia* (Davis 19), *Polytoma* (Dangeard 20).

II. In several Algae the blepharoplast arises by differentiation of the plasma membrane ('plasmodermale Blepharoplasten' of Ikeno). Strasburger (62), as the result of his investigations on the zoospores of *Oedogonium, Vaucheria*, and *Cladophora*, concluded that in these plants the blepharoplast was formed from the plasma membrane (*Hautschicht*); Mottier (47), in the case of *Chara*, came to a similar conclusion.

III. In several Pteridophyta and Bryophyta the blepharoplast is stated to be identical with the centrosome or to be derived directly from it ('zentrosomatische Blepharoplasten' of Ikeno). This view was advanced by Belajeff (7–12), who based his opinion on the examination of *Marsilea* and *Gymnogramme*. It has been strongly upheld by Ikeno (32–5), as the result of his investigation of *Marchantia polymorpha*. Lewis (43) in the case of *Riccia*, and Bolleter (13) in *Fegatella*, have come to similar conclusions. Jahn (36) states that in *Stemonitis flaccida* the centrosomes function as blepharoplasts, the cilia growing out from them while they are still at the poles of the spindle.

From the present investigation it appears probable that in *Pellia epiphylla* the blepharoplast is derived directly from the centrosome.

IV. In a few cases the blepharoplast has a nuclear origin ('Karyo- oder Kernblepharoplasten' of Ikeno). In *Mnium* sp. and various species of *Polytrichum*, J. and W. Docters van Leeuwen-Reijnvaan (40, 41, 42) state that the blepharoplast originates from the nucleolus by division. In the present investigation it has been found that a similar process takes place in *Mnium hornum* and *Atrichum undulatum*. Another instance of the nuclear origin of the blepharoplast has been given by Schaudinn (53) in *Trypanosoma* and *Spirochaete*.

Up to the present time, the nuclear origin of the blepharoplast has been discovered amongst plants only in the Musci. The fact that the process has now been discovered in three genera suggests that it is widespread in this group.

As already described, centrosomes are not present at any of the divisions of the spermatogenic cells in *Mnium hornum* and *Atrichum undulatum*. In the latter plant, however, a small body is separated from the nucleus prior to each division in the antheridium. A similar body is found in *Mnium hornum* at certain divisions only. In a former communication (74) it has been shown that during the division of the archesporial cells of this plant, no such body is separated, although at the reduction division its presence is easily discovered. During the earlier mitoses in the antheridium

it is again absent, but appears in the penultimate and final divisions in that organ. It is interesting to note that the stages at which this body is produced in *Mnium hornum* agree closely with those at which centrospheres and centrosomes have been described in several Liverworts. These latter structures, if present at all, are generally discovered at the meiosis and during the divisions of the spermatogenic cells. It may therefore be suggested that in the case of *Mnium hornum* the production of a body from the nucleolus during certain periods represents a late stage of reduction in centrosome formation.

It may be supposed that at an early period in its phylogenetic history *Mnium* possessed centrosomes of nuclear origin similar to those described in *Marchantia* by Ikeno. During the specialization that is generally admitted to have taken place in the Musci these have become functionless, and in consequence have almost disappeared, being represented at the present time only by the bodies cut off from the nucleolus prior to certain divisions.

If this supposition is admitted the direct formation of the blepharoplast from the nucleus in the Musci can be more easily correlated with its centrosomic origin in *Marchantia* and other Hepatics. In *Marchantia* the centrosome derived from the nucleus just before the final division persists in the daughter-cells, and functions as the blepharoplast in the spermatids. In *Mnium*, the body produced at the final division (phylogenetically the centrosome) having no function soon disappears. In the spermatid a similar body arises from the nucleus, and now, having taken on an additional function, persists as the blepharoplast. The formation of bodies from the nucleolus in the cells in the vicinity of the stem apex has already been mentioned. In these cases, as pointed out, this production of bodies is not associated with cell-division, and the mass separated is not regarded as possessing any phylogenetic relation to the centrosome. A similar production of bodies from the nucleoli of resting cells has been described in several animals and plants by Walker and Tozer (71).

It is possible that the nuclear origin of the blepharoplast is more widespread than is generally supposed. In several cases where this body is described as arising *de novo* in the cytoplasm and in the vicinity of the nucleus, it is conceivable that it really originates from the latter structure.

The plasmoderm origin of the blepharoplast described by Mottier in *Chara* is of interest in connexion with the similarity which, as long ago as 1892, was pointed out by Strasburger (61) to exist between the spermatogenesis of this plant and that of the Musci. The spermatid of *Atrichum* shown in Fig. 56, Pl. XXXVIII, bears a considerable resemblance to the drawing of the spermatid of *Chara* given by Mottier (47) in his Fig. 1, although in the former case the blepharoplast is of nuclear, while in the latter it is of plasmodermic origin. The results obtained by a re-examination of

the spermatogenesis of *Chara* would be of considerable interest in this respect.

The present writer is in agreement with Ikeno concerning the phylogenetic origin of the blepharoplast from the centrosome in the great majority of plants. This view is considerably strengthened by a comparison of its origin in animals, where in the great majority of cases it is identical with or directly derived from the centrosome.

The term blepharoplast was introduced by Webber (73) in 1897, who defines it (footnote, p. 30) as 'the cilia-forming organ of the spermatogenous cells of *Zamia* and *Ginkgo*, which so nearly resembles a centrosome or centrosphere'. This investigator states 'there would seem to be no possible doubt' that the subsequent formation of the cilia-bearing band in *Zamia* is organized at the expense of and by the granules of the blepharoplast. The production of a thread or band from the blepharoplast has also been described in *Onoclea* and *Marsilea* by Shaw (57) and by Yamanouchi (76) in *Nephrodium*. Until the present, however, no thread-like structure produced in connexion with the blepharoplast has been definitely described in the Bryophyta. Ikeno (32) in *Marchantia* describes the slight elongation of the blepharoplast, but states that the latter becomes connected with the nucleus by a 'cytoplasmatischer Vorsatz', which grows, not from the blepharoplast, but towards it. J. and W. Docters van Leeuwen-Reijnvaan (41) state that a similar band is found in *Polytrichum*, in this case growing from the blepharoplast towards the nucleus. Humphrey (30) in *Fossombronia* describes the slight elongation of the blepharoplast, but states that the connexion with the nucleus is completed partly by the cytoplasm and partly by the elongated 'Nebenkörper'. Bolleter (13) gives a somewhat similar account in the case of *Fegatella*.

A slight elongation of the blepharoplast has been described in *Mnium* in this investigation, but the thread is quite distinct from this and is present in addition to it. The elongation takes place towards the anterior part of the spermatid, while the thread is produced in an opposite direction. The thread has been described in *Mnium hornum*, *Atrichum undulatum*, and *Pellia epiphylla*, and there is no doubt that it is homologous in these three species. In each of these plants the nucleus elongates right up to the blepharoplast, and consequently no cytoplasmic material is intercalated between the two. In this respect these observations agree more closely with the accounts of spermatozoid formation in *Pellia* given by Buchtien (15) and Guignard (26 A) than with the more recent accounts given of this process in the Hepaticae. With the exception of the blepharoplast and the thread-like structure, the whole of the body of the spermatozoid is produced from the nucleus. A thread-like structure closely resembling that found in the two mosses and in *Pellia* has been described by Mottier (47) in the spermatid of *Chara*. In this case, however, the thread does not

grow out in connexion with a blepharoplast, but is produced by a differentiation of the cell membrane. At present it must be left an open question whether this thread in *Chara* is homologous with that described in the Bryophyta.

It has already been pointed out that the origin of the thread-like body in the plants examined has not been definitely determined. Two methods of formation are possible :—

1. The thread is formed by an extension of the blepharoplast substance, and forms a part of the latter body.

2. It arises by a differentiation of the plasma membrane, and is distinct from the blepharoplast.

If the first view is accepted, it appears that elongation of the blepharoplast goes on in two directions in *Mnium*, towards the anterior part of the spermatid forming a short bar-like structure, and in the opposite direction producing the thread. Such a process has, however, not been previously described in connexion with the blepharoplast. On the second assumption the thread is a structure distinct from the blepharoplast. In this case the thread is not homologous with the blepharoplasts described in the Bryophyta, Pteridophyta, and zoidogamous Gymnosperms. As additional evidence for this, it may be pointed out that the thread has apparently no direct connexion with the cilia. Further investigation is necessary before any decision can be arrived at regarding this matter.

The processes involved in the actual production of the spermatazoid in *Pellia* and the two masses examined are very similar, as is shown by a comparison of Fig. 57 of *Atrichum* and Fig. 79 of *Pellia* (Pl. XXXVIII). Subsequent to the production of the thread and limosphere, the changes which take place in *Pellia* and *Atrichum* are almost identical.

In many of the cases in which the spermatogenesis of plants has been carefully investigated, one or more chromatic bodies in addition to the blepharoplast and nucleus have been discovered in the spermatid. This has been emphasized by Ikeno in his description of *Marchantia*. In the spermatids of this plant he described the occurrence of a fairly large spherical body which takes up the nuclear stain freely. He refers to this as the chromatoid 'Nebenkörper', and suggests that it is homologous to the body described by Meves (46) under this name in the spermatids of several animals. The origin of this body was not determined, and it soon disappears, taking no direct part in the formation of the spermatozoid. He considers the 'Nebenkörper' is homologous with the blepharoplastoids described by Shaw (57) in *Marsilea*, and the 'corps sphérique' found by Hirasé (27) in *Ginkgo*. It is interesting to note these bodies also are stated to take no direct part in the formation of the spermatozoid. A similar body was subsequently described by Bolleter (13) in *Fegatella*, and by Humphrey (30) in *Fossombronia*, but in the latter plant it is stated that the

'Nebenkörper' forms a part of the body of the spermatozoid. In a recent communication Yamanouchi (76) has described a body in the spermatid of *Nephrodium* which arises near the nucleus, but the origin of which was not ascertained. This body, which he designates the 'Nebenkern', is found persisting in the vesicle of the mature spermatozoid.

In the present investigation two bodies have been constantly discovered in the spermatids, and these in the three plants examined take no direct part in the formation of the spermatozoid, but are found ultimately in the vesicle. The limosphere, as already shown, is easily distinguished in all cases by its peculiar form, and no doubt corresponds to the 'Nebenkörper' described in *Polytrichum* by J. and W. Docters van Leeuwen-Reijnvaan (41) and by Arens (2) in *Mnium hornum*. Up to the present no such structure has been discovered in the Hepaticae. The accessory body is probably similar to the body described by J. and W. Docters van Leeuwen-Reijnvaan in *Polytrichum* produced by the third constriction of the nucleus. With this exception it has not yet been recorded in the Bryophyta or elsewhere. At present it cannot be stated which of these two bodies is equivalent to the 'Nebenkörper' described by Ikeno in *Marchantia*.

There seems to be no doubt that in *Atrichum undulatum* and *Mnium hornum* both the limosphere and the accessory body are derived either directly or indirectly from the nucleus, and although in the case of *Pellia* their origin has not been determined, it seems probable that here, too, they are derived from the nucleus.

The occurrence of structures corresponding to the rod-like bodies discovered in *Mnium hornum* has not, up to the present, been recorded in the spermatids of plants. Although these structures have not been found in *Atrichum*, it is still possible that they exist in this plant and that their occurrence has been overlooked on account of the short period during which they are present. Somewhat similar structures have been discovered in the spermatocytes and spermatids of a considerable number of animals, and these are variously referred to as chromidia, chondromites, mitochondria, pseudochromosomes, &c.

It would be out of place here to attempt even a partial summary of the results obtained in animals in this connexion, but a few will be mentioned. Gross (26) in *Pyrrhocoris* describes pseudochromosomes in spermatocytes I, II, and the spermatids. In the latter these give rise to the 'Nebenkern', which is somewhat ring-like in form. Schreiner (59) states that chondromites and chromatic bodies are present during the spermatogenesis of *Myxine*. Oettinger (48) describes the occurrence of mitochondria in the spermatids of some Myriapods. Some of these are 'fadenförmige Mitochondrien', but others exist in the form of a sphere, which at first possesses a dark-staining periphery. Popoff (49) in *Helix* and *Paludina* states that chromidia are formed from granules extruded from the nucleus

in spermatocyte I. These chromidia persist in spermatocyte II, and in the spermatid give rise to a triangular or polyhedral body, the 'Nebenkern'. This, together with a plasma mass, is thrown off finally from the sperm.[1] Von Baehr (4) in *Aphis Saliceti* states that after the final division of the spermatocytes a 'Nebenkern' in the form of a 'Bläschen' with stainable walls appears. This probably originates from the mitochondria. Later on it becomes shaped like a split ring, and finally disappears during the formation of the sperm. Wasilieff (72) states that the mitochondria found in the spermatocytes of *Blatta germanica* originate from the nucleus.

A consideration of the above results leads to the conclusion that the limosphere bears a close resemblance to the 'Nebenkern' described in several animal spermatids, and that at least in some cases the two have a similar origin. It is not desired, however, to state that the two structures are homologous. At present little evidence is available as to the occurrence and formation of the limosphere in plants, and such a generalization would not be justified. The elimination of chromatic material from the nucleus during spermatogenesis seems to be almost universal in animals and plants. The widespread nature of this phenomenon suggests that it is of far-reaching importance. It is possible that some such separation of material must take place before the cell can undergo the changes which result in the formation of the sexual cell. The material eliminated probably differs considerably in composition from the cytoplasm which surrounds it, and during the chemical changes which take place after its separation from the nucleus it is not surprising that structures of more or less constant form are produced. The appearances already described, which are seen during the formation of the limosphere, strongly suggest that the material of which it is formed is gradually changing in composition, so that finally the greater part of it loses its capacity for taking up the nuclear stain. A similar elimination of chromatic material takes place in many plants during the formation of the egg. An instance of this in the Musci has been given by Holferty (29), who states that masses of chromatic material are present in the cytoplasm of the egg of *Mnium cuspidatum*.

The extrusion of chromatic substance from the nucleus during meiosis is a widespread phenomenon in plants.[2] Miss Digby (21) has recently given an account of the extrusion of bodies from the nucleus at this stage in *Galtonia candicans*, and a considerable number of examples of this process are here quoted. It is a highly suggestive fact that at two important stages in the life of the organism chromatin is eliminated from the nucleus, while during the intervening periods little or none is thrown out.

[1] Popoff's results have been adversely criticized by Murray, Ancel, and Bolles Lee. These investigators admit the existence of the chromidia but question their nuclear origin.

[2] Since in animals meiosis always coincides with the maturation of the sexual cells, the two periods during which extrusion of chromatic material from the nucleus takes place in plants cannot be here distinguished.

Summary.

1. In *Mnium hornum* and *Atrichum undulatum* the divisions of the spermatogenic cells are normal, and no centrosomes are present. The final division is not of the diagonal type which is found in several of the Hepaticeae. No reduction in the number of the chromosomes takes place at the final mitosis.

2. In *Pellia epiphylla* centrospheres and probably centrosomes are present during the later divisions in the antheridium. The blepharoplast is probably derived directly from the centrosome.

3. In the spermatid of *Mnium hornum* a number of bodies become separated from the nucleolus. These pass into the cytoplasm and there give rise to a number of rod-like structures. The rod-like structures, by coalescence, finally form a hollow spherical body, for which the name limosphere is suggested. The nucleolus then divides into two masses, which both pass into the cytoplasm; one of these functions as the blepharoplast, while the other gives rise to the accessory body.

4. In the spermatid of *Atrichum undulatum* three bodies are separated from the nucleolus and pass into the cytoplasm. The body first produced functions as the blepharoplast. The limosphere arises from one of the remaining bodies, while the other gives rise to the accessory body.

5. In *Pellia epiphylla* a limosphere and accessory body are present in the cytoplasm of the spermatid. Their origin was not determined.

6. In all of the three plants under consideration the blepharoplast passes to the periphery of the spermatid. A thread-like structure is produced in connexion with the blepharoplast, which passes along the inner contour of the cell membrane. The nucleus passes to the periphery and lies in contact with the thread. Elongation takes place in the nucleus, and the latter, together with the thread, produces the entire body of the spermatozoid. The limosphere and accessory body persist in the almost mature spermatozoid, and in all probability are found ultimately in the vesicle.

In conclusion, I wish to express my thanks to Professor Farmer, F.R.S., for his constant help and valuable advice throughout the course of the investigation. I also wish to thank Mr. F. J. F. Shaw, B.Sc., A.R.C.S., for the collection and preservation of material of *Pellia epiphylla*, and for the preparation of a considerable number of sections of this plant.

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EXPLANATION OF PLATES XXXVII AND XXXVIII.

Illustrating Mr. Wilson's paper on Spermatogenesis in the Bryophyta.

All the figures were drawn with the camera lucida under a 2 mm. apochr. hom. imm. Zeiss, N.A. 1·40 with comp. oc. 18, × 2250, except Figs. 65, 70, and 71, which were drawn under a 3 mm. apochr. hom. imm. Zeiss, N.A. 1·40 with comp. oc. 18, × 1500.

Figs. 1–44 refer to *Mnium hornum*; 1–15 to divisions in the antheridium, and 16–44 to spermatids. Figs. 45–60 to *Atrichum undulatum*; 45–9 to divisions in the antheridium, and 50–60 to spermatids. Figs. 61–81 to *Pellia epiphylla*.

PLATE XXXVII.

Mnium hornum.

Fig. 1. Resting cell from antheridium just before antepenultimate division.
Fig. 2. Metaphase of antepenultimate division in side view.
Fig. 3. Metaphase of antepenultimate division in polar view.
Fig. 4. Constriction of body from nucleolus before penultimate division.

Fig. 5. Late prophase of penultimate division.
Fig. 6. Constriction of large body from nucleolus before final division.
Fig. 7. Constriction of two bodies from nucleolus before final division.
Fig. 8. Body separated from nucleolus and lying in the nucleus before the final division.
Fig. 9. Early prophase of final division.
Fig. 10. Late prophase of final division.
Fig. 11. Metaphase of final division in side view.
Fig. 12. Metaphase of final division in polar view.
Fig. 13. Anaphase of final division in side view.
Fig. 14. Anaphase of final division in polar view.
Fig. 15. Telophase of final division.
Fig. 16. Shortly after contraction from the enclosing cell-wall.
Fig. 17. Nucleolus constricting.
Fig. 18. Bodies produced from nucleolus in cytoplasm. Outline of nucleus not shown.
Fig. 19. Formation of rod-like bodies. Outline of nucleus not shown.
Fig. 20. Views of the same spermatid at two foci: (*a*) showing rod-like bodies, (*b*) nucleus with nucleolus divided into three bodies.
Figs. 21 and 22. Nucleus with undivided nucleolus and rod-like bodies in cytoplasm.
Figs. 23 and 24. Nucleus with nucleolus divided into two and rod-like bodies in cytoplasm.
Fig. 25. Commencement of fusion of rod-like bodies.
Figs. 26 and 27. Fusion of rod-like bodies.
Figs. 28–31. Stages in formation of limosphere, appendage, and blepharoplast. Outline of nucleus not shown.
Fig. 32. Elongating nucleus and developing limosphere.
Fig. 33. Elongation of blepharoplast.
Figs. 34–8. Formation of thread from blepharoplast. Outline of nucleus not shown.
Figs. 39–41. Stages in elongation of nucleus.
Fig. 42. Almost mature spermatozoid enclosed by original cell-wall.
Fig. 43. Double spermatid.
Fig. 44. Spermatid stained with iodine solution.

Atrichum undulatum.

Fig. 45. Cell from young antheridium, showing constriction of nucleolus.
Fig. 46. Metaphase of final division in side view.
Fig. 47. Metaphase of final division in polar view.

PLATE XXXVIII.

Fig. 48. Anaphase of final division in side view.
Fig. 49. Anaphase of final division in polar view.
Fig. 50. Shortly after contraction from the enclosing cell-wall.
Fig. 51. Nucleolus dividing; blepharoplast in cytoplasm.
Fig. 52. Nucleolus dividing; blepharoplast on periphery.
Figs. 53–5. Nucleolus dividing; formation of thread from blepharoplast.
Fig. 56. Nucleus in contact with thread; bodies passing into cytoplasm.
Fig. 57. Limosphere and accessory body in cytoplasm.
Fig. 58. Limosphere and accessory body in cytoplasm. Spermatid viewed in plane perpendicular to that seen in Fig. 57.
Fig. 59. Elongation of nucleus; limosphere and accessory body.
Fig. 60. Almost mature spermatozoid, showing limosphere and accessory body.

Pellia epiphylla.

Fig. 61. Resting cell from young antheridium.
Fig. 62. Early stage of prophase, showing chromatin masses near periphery of nucleus.
Fig. 63. Slightly later prophase.
Fig. 64. Prophase, showing elongated nucleus and centrospheres (?). From antheridium showing fifty-two cells in cross-section.

Fig. 65. Slightly later stage, showing centrosphere and radiations.

Fig. 66. Cell in early prophase of the final division.

Fig. 67. Prophase of final division, showing nucleus pointed at each end.

Fig. 68. Telophase of final division.

Fig. 69. Spermatid seen from side, showing blepharoplast in position where first distinguished.

Fig. 70. Spermatid seen from side, showing blepharoplast half-way to angle.

Fig. 71. Pair of spermatids seen from side, showing swollen mother-cell walls and dividing wall; in the lower cell blepharoplast is at the angle, while in the upper one it is a short distance from the angle.

Fig. 72. Pair of spermatids seen from side, showing blepharoplasts in widely separated angles.

Fig. 73. Pair of spermatids seen from side, showing blepharoplasts in adjacent angles.

Fig. 74. Spermatid from below, showing blepharoplast.

Fig. 75. Spermatid from side, showing blepharoplast and thread.

Fig. 76. Spermatid seen obliquely from side, showing blepharoplast and thread.

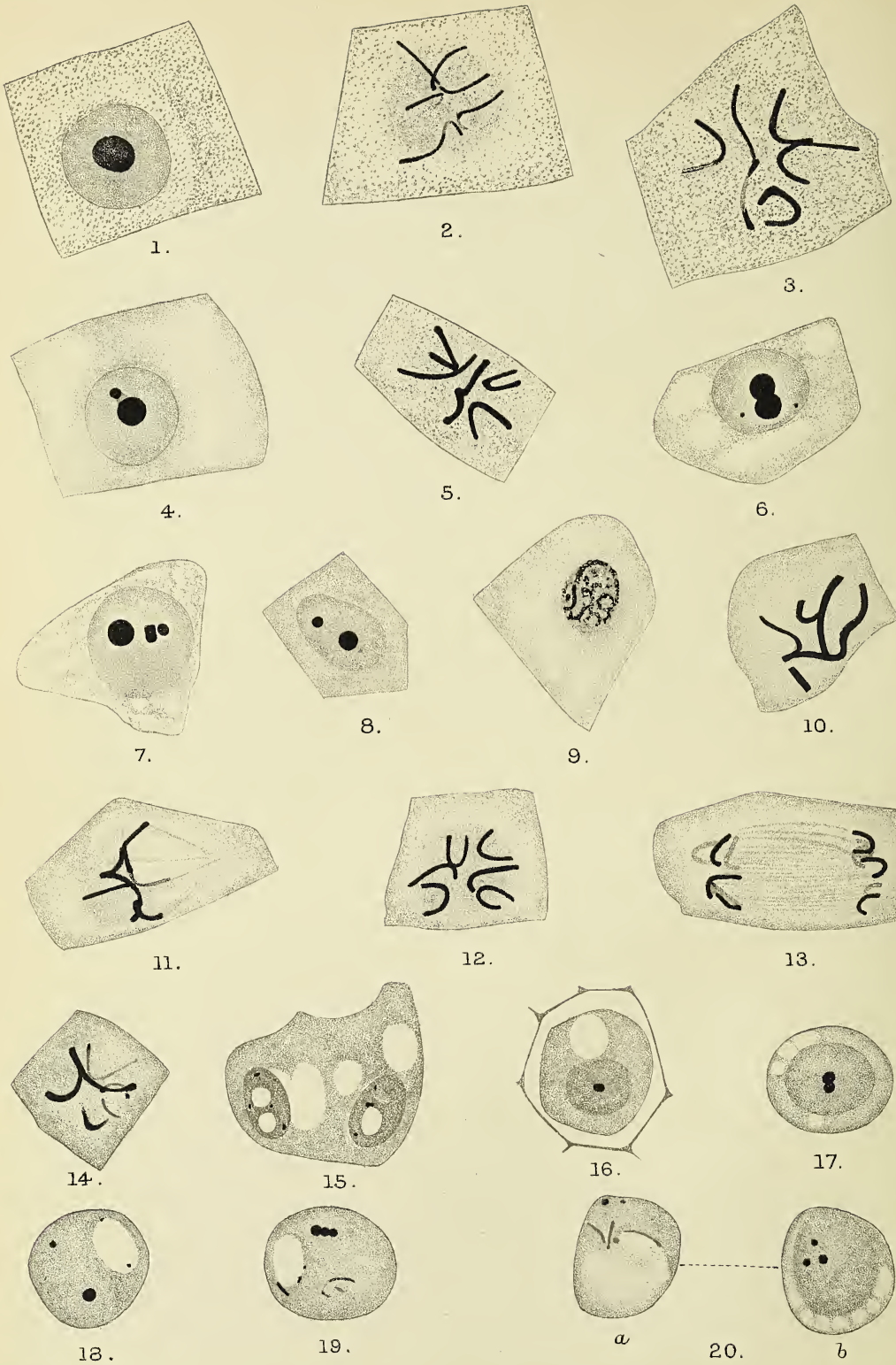
Fig. 77. Spermatid from below, showing limosphere, accessory body, blepharoplast, and thread.

Fig. 78. Pair of spermatids seen slightly obliquely from side; in each the limosphere, accessory body, and thread can be seen.

Fig. 79. Spermatid from below with limosphere, accessory body, blepharoplast, and thread.

Fig. 80. Spermatid from below, showing elongating nucleus.

Fig. 81. Spermatid from below, showing the nucleus elongated into a spiral.



M. Wilson, del.

WILSON — SPERMATOGENESIS IN MOSSES AND LIVERWORTS.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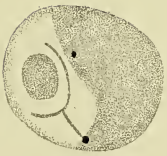
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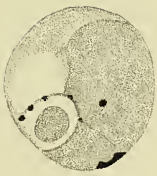
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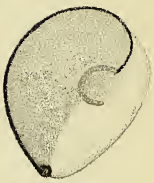
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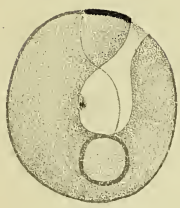
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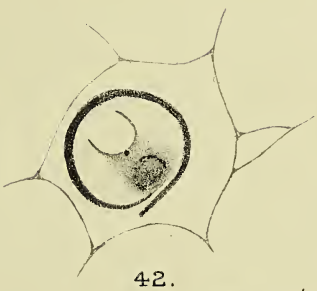
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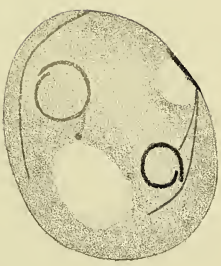
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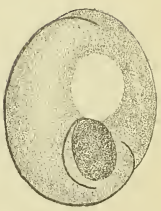
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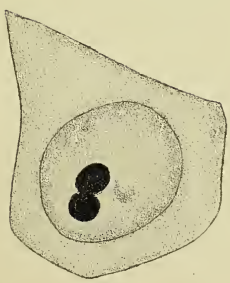
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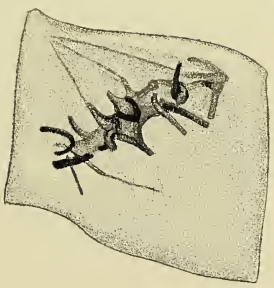
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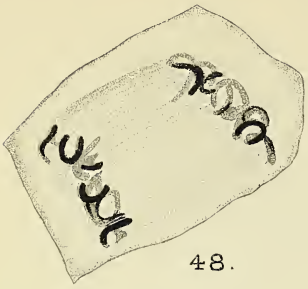

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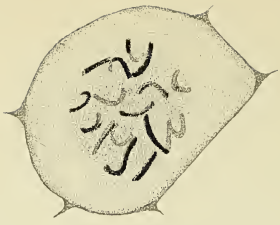

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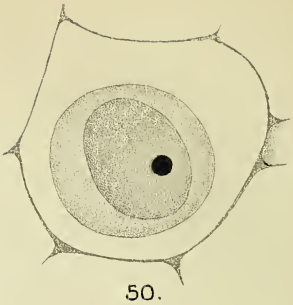

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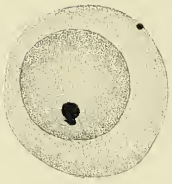

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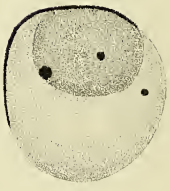

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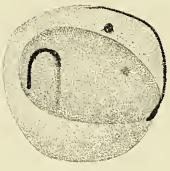

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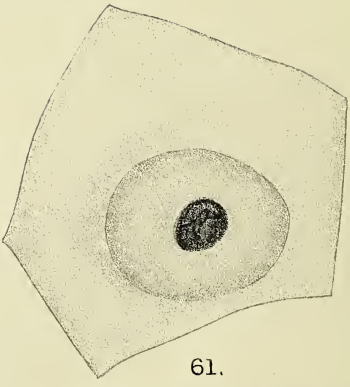

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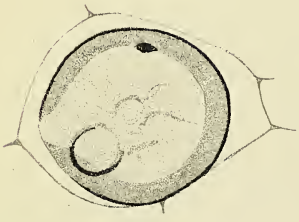

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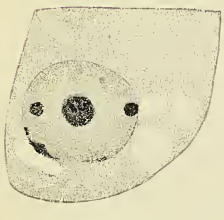

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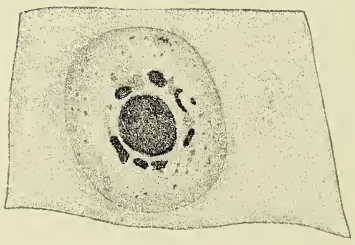

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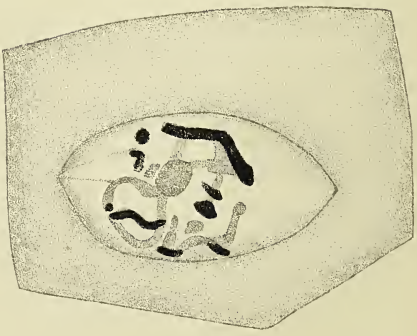

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WILSON — SPERMATOGENESIS IN MOSSES AND LIVERWORTS.

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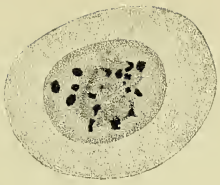

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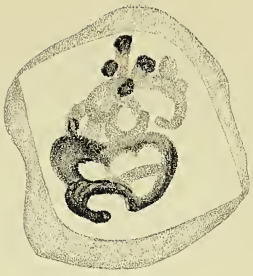

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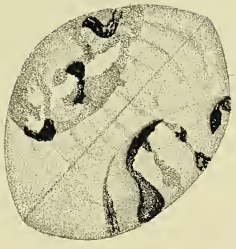

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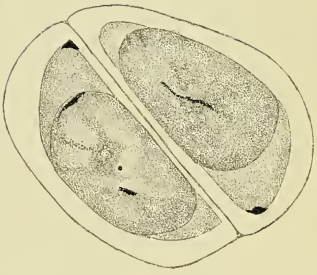

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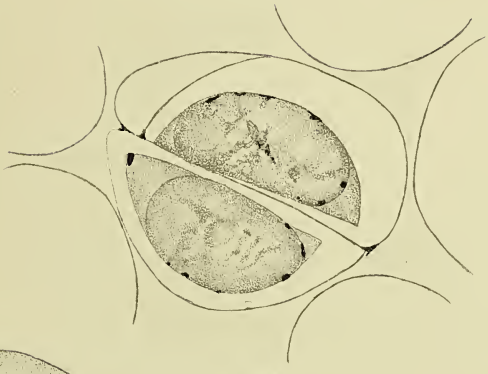

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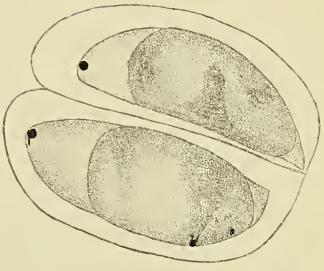

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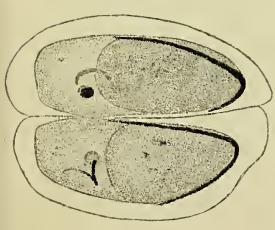

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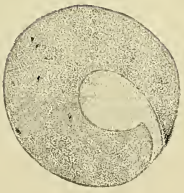

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On Traquairia.

BY

RINA SCOTT (Mrs. D. H. SCOTT), F.L.S.

With Plates XXXIX and XL and four Figures in the Text.

THE genus *Traquairia* was named by Mr. W. Carruthers, F.R.S., after his friend, Dr. Traquair, then of Dublin, in 1872. The specimens were originally described by him before Section D, at the meeting of the British Association at Brighton, in 1872, in a paper entitled: 'On *Traquairia*, a Radiolarian Rhizopod, from the Coal Measures.'

I append a part of his description: 'A spherical spiniferous body, the hollow globular cavity is included in a clearly defined structure, probably a fenestrated shell. Beyond this, there is a considerable thickness of a spongy substance, which rises externally into numerous cones, the bases of which are in close proximity. From the apex of each cone, there proceeds a hollow echinate spine. The echinations are also hollow, and at the apparent base of the spine these echinations are produced into hollow tubes which, repeatedly branching and anastomosing and increasing in number downwards, enclose the radial hollow spines in the mass.'

I have also had a reproduction made of Mr. Carruthers's original diagram, which he kindly gave me two years ago (Text-fig. 1).

Since this paper was written, there has been a great diversity of opinion as to the real nature of *Traquairia*. Count Solms-Laubach [1] says: '*Traquairia* and *Sporocarpon* appear to me and also to Strasburger, if comparable to anything, to be like either the massulae of *Azolla* or the sporocarps which contain them.' After a description of these genera he adds that they are well worth further investigation. Dr. Schenk [2] and Professor Zeiller [3] agree with Count Solms's view.

The subject has been dealt with in greatest detail by Professor Williamson,[4] who produced a paper in the Transactions of the Royal Society

[1] Einleitung in die Paläophytologie, 1887, p. 188.

[2] Handbuch der Botanik, Bd. iv, 1890, pp. 52, 53.

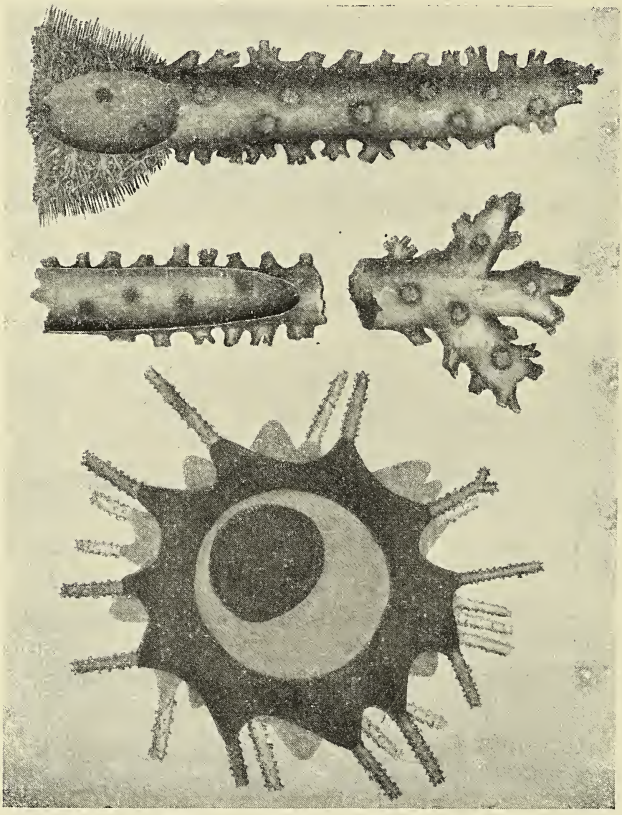
[3] Éléments de Paléobotanique, p. 130.

[4] On the Organization of the Fossil Plants of the Coal Measures, Pt. x. Phil. Trans. Roy. Soc., Pt. 2, 1880, Pl. XVIII, Figs. 40, 41, 42, 45, 46, 49; Pl. XIX, Figs. 40, 44, 49, 50; Pl. XX, Figs. 85, 86, 78.

[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.

in 1880, in which some fine vigorous drawings of these objects are to be found.

Professor Williamson suggested that *Traquairia* bore more resemblance to the spore of a Cryptogam, and concluded that it was a reproductive organ of some unidentified Cryptogamic plant. Professors Haeckel and Strasburger examined his specimens and agreed with him that they were vegetable and not animal structures.



TEXT-FIG. 1. *Traquairia Carruthersii.* From Mr. Carruthers's original diagram.

This view has met with some acceptance. Dr. Hinde[1] says in one of his papers: 'The minute spherical bodies in the Coal Measures, known as *Traquairia*, have been shown by Professor Williamson to be vegetable structures.'

No new work has been done on this subject for a long time; many botanists have come to the conclusion that the *Traquairiae* are not plant structures, and do not seem to think it part of their duty to investigate them. The zoologists, however, seem to take no interest in them either. During

[1] Dr. Hinde, Annals Nat. Hist., Ser. 6, vol. vi, p. 46.

the cataloguing of Dr. Scott's Collection of fossil slides, each specimen of *Traquairia* occurring was noted, and as a result of this work it has become quite clear that there is more than one species of *Traquairia* amongst the Coal Measure plants.

Two years ago I found many good specimens in the material from Burntisland, and also in that from Ostrau and the Karwin district in Moravia, in sections lent by Dr. Kubart. So that now specimens are known from the following horizons :—

Lower Carboniferous : Burntisland.

Lower Coal Measures said to be older than the English : Ostrau and Karwin, Moravia.

Lower Coal Measures : Dulesgate, Oldham, Halifax hard bed.

It seemed to be worth while to describe these new species in the hope that some biologist would be found who would take up the study of these objects, and try to decide what they really are. I hope that Dr. Kubart will also shortly add a description of his species.

Before beginning to describe the various species, I will give a rather more detailed account of Professor Williamson's work. In his notes on the catalogue of his slides now at the Natural History Museum, he says that many of the sections are serial and all show *Lepidostrobus* sporangia. In slide W. 1064 [1] there are three very good *Traquairiae* in a *Lepidostrobus* sporangium, and he thought that they were three spores of a tetrad belonging to the plant.[2]

W. 1063 also shows a specimen in a *Lepidostrobus* sporangium. On looking at these slides it will be seen that the sporangial wall is very rotten ; it is quite a usual thing to find all sorts of objects apparently in these *Lepidostrobus* sporangia. In one of Professor Williamson's own slides there is a good specimen of *Zygosporites* apparently in a sporangium, and in another a *Sporocarpon*. One constantly comes across very deceptive examples of this sort. So that the fact of finding *Traquairiae* inside a *Lepidostrobus* sporangium is not in itself satisfactory evidence that they belong to it.

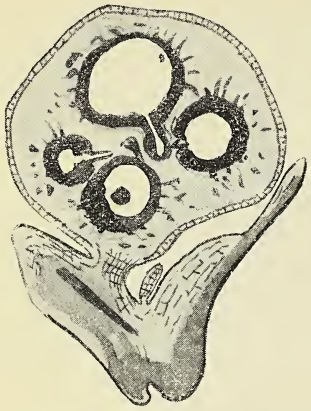
It is true that these *Traquairiae* are usually found in association with *Lepidodendron*, but the *Lepidodendron* is always in a very rotten condition. They are generally found associated with Fungi and coprolites of insects. If one wishes to be sure that the contents of a sporangium really belong to that sporangium, it is a good rule only to judge by examples in which the sporangial wall is intact. In Text-fig. 2 will be seen an example of a sporangium with perfect walls, evidently containing its own megaspores (*Bothrodendron mundum*).

The megaspore belonging to the *Lepidostrobus* in Professor Williamson's slides is now well known. As a matter of fact *Traquairia*

[1] W. = Williamson Collection, S. = Scott Collection.

[2] loc. cit., Pl. XXI, Fig. 85.

occurs in all sorts of cavities in the tissues of *Lepidodendron* and other plants, and as they usually occur in groups it is very easy to find three together (see Figs. 14 and 16, Pl. XL).



TEXT-FIG. 2. *Bothrodendron* megasporangium on spirophyll. × 30. S.2561.

For some reason Mr. Carruthers's view, that *Traquairiae* were Radiolarians, was never generally accepted, yet there seems to be a good deal of evidence to prove that he was not far wrong. Perhaps one reason was that Radiolarians were always supposed to be deep-sea organisms and people did not see how this was compatible with their being associated with land plants, but Professor Sollas[1] is of opinion that the deposits in which Radiolarians occur may be of a shallow-water character, and Dr. Hinde and other observers have noticed that impressions of *Lepidodendron* occur in the same rock with Radiolarian casts. An example of this is found in the Devonian rocks of New South Wales, where the Radiolarians are associated with *Lepidodendron australe*. This latter fact is of especial interest, as fragments of *Lepidodendron* and *Traquairia* are also so often found together in the British petrifactions.

Many of the Radiolarians figured in Dr. Hinde's paper bear a striking resemblance to *Traquairia*, e.g. his Pl. IX, Fig. 1; Pl. VII, Fig. 23, is very much like another object found in the Coal Measures, *Sporocarpon elegans*.

Dr. Hinde, who has kindly written to me on the subject, says, however: 'My present opinion is that this genus is not related in any way to Radiolaria.'

Mr. Carruthers, in his paper on the subject, gave as a reason against his own arguments, that *Traquairia* had no 'central capsule', constantly found in Radiolarians. In the specimens which we now have at our disposal, every species shows a 'central capsule' (see Pls. XXXIX, XL, Figs. 9, 15, 16).

It now remains for me to describe the specimens and to record the new species.

Traquairia is a spherical organism, consisting of two parts each surrounded by a sharply defined membrane: an inner capsule, often containing spores, and an outer part, which is surrounded by a thick gelatinous envelope. In this are embedded numerous hollow spines. The apparent bases of these spines are produced into hollow anastomosing tubes, which spread over the surface of the sphere, forming a complicated network. The spines are hollow and are perforated in every direction by projecting tubular pores. Emanating from these pores are delicate threads (see Pl. XXXIX,

[1] Discussion on Dr. Hinde's paper on Devonian Radiolaria from New South Wales. Geol. Soc. Journ., vol. lv, 1899, p. 64.

Fig. 3) which appear to lose themselves in the gelatinous envelope. Sometimes the threads form a regular network in it.

The inner capsule, a definite brown membrane, can only be observed in the more perfectly preserved specimens. Spores are generally present, which appear to produce smaller spores. The *Traquairiae* occur in groups in the decayed wood of *Lepidodendron* and other plants.

The first species to be described is that figured by Williamson.[1] I propose to call it *Traquairia Carruthersii* after Mr. Carruthers, who was the first to describe these organisms.

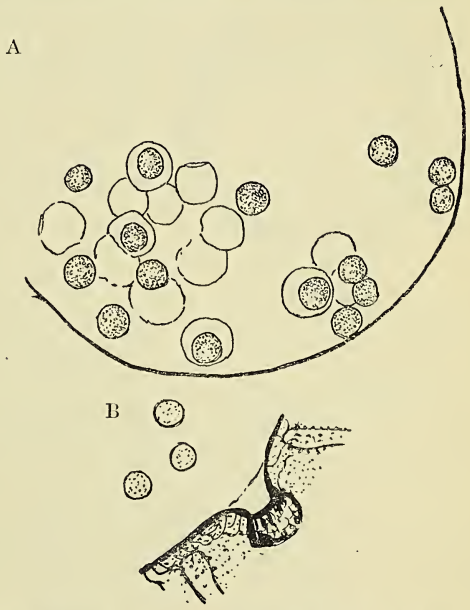
TRAQUAIRIA CARRUTHERSII (Pl. XXXIX, Figs. 5, 6, 7 ; Pl. XL, Fig. 14 ; Text-figs. 1 and 3).

The form is spherical.

The spines appear to have been brittle. They are uniformly distributed over the sphere. They are sometimes branched and do not taper. At their apparent base they give off anastomosing tubes, which connect one spine with the other and form an elaborate network covering the surface of the sphere.

Each spine has numerous projecting tubular pores, generally arranged in about six longitudinal rows ; from these pores emanate threads, which divide and branch, eventually losing themselves in the gelatinous substance enveloping the sphere. An inner capsule is present.

There are often large spores, which have a delicate membrane. Inside these are smaller thick-walled spores (see Text-fig. 3, A), which in some specimens are found coming out of the larger spores. There is an apparent operculum in the outer capsule, but it has only been observed in one case (see Text-fig. 3, B).



TEXT-FIG. 3. *Traquairia Carruthersii.* A. Inner capsule and spores. × 170. S. 1789. B. Operculum. × 200. W. 1077.

Measurements :—

Diameter of the sphere without spines :	from 0·32–0·48 mm.
Length of spines :	about 0·2 mm.
Width of spines :	from 12 μ–18 μ.
Diameter of large spores :	from 36 μ–72 μ (average 40 μ).
Diameter of small spores :	from 16 μ–18 μ.

[1] loc. cit.

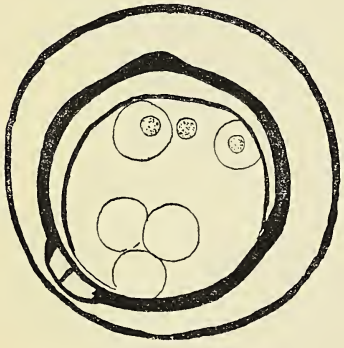
Locality: Coal-balls from the Lower Coal Measures of Lancashire and Yorkshire.

The second species I propose to call *Traquairia Spenceri*, as it was first observed by the late Mr. Spencer and occurs in slides from his collection.

TRAQUAIRIA SPENCERI (Pl. XXXIX, Figs. 8, 9, 10; Pl. XL, Figs. 15, 16; Text-fig. 4).

The form is spherical.

The spines are much more delicate and apparently less brittle than in *T. Carruthersii*, and rapidly taper to a point. At the ends they are often curved like a shepherd's crook. At their apparent base they give a network of tubes much finer than in *T. Carruthersii*.



TEXT-FIG. 4.
Traquairia Spenceri. × 150. S. 22.

Each spine has very numerous fine pores, from which emanate very fine threads. The spines are less numerous than in *T. Carruthersii*; about twenty can be counted round the circumference of the sphere seen in median section.

The inner capsule appears to have two membranes. Sometimes an operculum seems to be visible (see Text-fig. 4).

The inner capsule is filled with large spores containing smaller ones.

Measurements:—

Diameter of sphere without spines:	about 0·42 mm.
Length of spines:	from 0·18–0·24 mm.
Width of spines at base:	24 μ.
Width of gelatinous envelope:	0·1 mm.
Diameter of large spores:	from 36 μ–42 μ.
Diameter of small spores:	18 μ.

In association with *Traquairia Spenceri* I have found an object (Pl. XXXIX, Fig. 10) which might possibly be a stage in the development of *Traquairia*, perhaps the first product of germination of a spore. It appears to have one spine only, the whole object being barely one-third the size of the usual specimens. It measures 120 μ × 96 μ. The whip-like shape of the spine strongly resembles that of *T. Spenceri*.

In the Burntisland material there are two *Traquairiae* very different in form, always occurring together. In fact, in my first investigations I always called them Form A and Form B. However, I have been unable to trace any connexion between the two, so at present they must be

described as distinct species. They occur in association with *Lepidodendron Veltheimianum*. The first very much resembles *T. Carruthersii*. I propose to call this

TRAQUAIRIA BURNTISLANDICA (Pl. XXXIX, Figs. 1, 2, 3; Pl. XL, Figs. 11, 12, 13).

The form is spherical.

The spines appear to have been brittle, as in *T. Carruthersii*. They do not branch or taper. At their apparent base they give off anastomosing tubes, which connect one spine with another and form a coarse network over the sphere. The spines are arranged in groups of 4–6. The total number of groups was probably from 24–30.

Each spine has numerous projecting tubular pores in about six longitudinal rows. From these pores emanate threads much as in *T. Carruthersii*.

The spines are enveloped in a gelatinous mass. The specimens observed are not well preserved, and the internal structure cannot be made out.

Measurements :—

Diameter of the sphere without spines :	0·36–0·42 mm.
Length of spines :	about 0·24 mm.
Width of spines :	12 μ–18 μ.

In some tangential sections one sees five groups each composed of 4–6 spines cut across, and outside these seven or eight groups are seen.

Traquairia burntislandica is much the same size as *T. Carruthersii*, and differs principally in having its spines arranged in definite groups.

The second species I propose to call *Traquairia stellata*, as it has an appearance like that of a star.

TRAQUAIRIA STELLATA (Pl. XXXIX, Fig. 4).

The form is spherical.

The arms or spines are much fewer and broader at the base than in *T. Carruthersii* or in *T. burntislandica*. The general appearance of a radial section is something like a starfish in form. The number of arms is about twenty-four, uniformly distributed round the sphere. The arms are pointed, widening out at the base, so that the base of one almost joins the base of the next. They appear to be chambered, the chambers sometimes forming a single series, while in other cases they are further divided by longitudinal septa. They are deeply embedded in the gelatinous mass. One cannot feel at all sure that these chambers may not be due to disorganization. Tubes are given out from the base of the arms, but the structure is so imperfectly preserved that little can be said on this point.

The surface of the sphere is covered by a finer network than *T. Carruthersii* and *T. burntislandica*. The inner capsule is preserved in some specimens, but none of those observed contain spores.

Measurements :—

Width of sphere without arms :	0·216 mm.–0·252 mm.
Length of arm :	120 μ.
Width of arm at base :	108 μ.

The most characteristic feature in the organisms described is the very complicated structure of the outer envelope with its elaborate system of anastomosing tubes connected with prominent spines, which are themselves very complex organs.

Nothing parallel to this is known in the vegetable world.

The presence in well-preserved specimens of an inner capsule containing spores, in the interior of which smaller spores are produced, reminds one of Radiolarians, though some previous authors have seen an analogy on this point with the massulae of *Azolla*.

The spores, however, are not always confined to the inner capsule.

The inner capsule and spores are features common to another fossil genus, *Sporocarpon*. In *S. elegans* there are spines also. But on the whole the outer envelope of *Sporocarpon* is less complicated, and often has a more cellular appearance than that of *Traquairia*.

On the other hand, *Sporocarpon elegans* with its long spines is very much like a Radiolarian. The organisms of both genera urgently need further investigation.

Professor Dendy, F.R.S., in a discussion at the Linnean Society, suggested that *Traquairia* might belong to an extinct group of *Protozoa* allied to the Radiolarians.

My thanks are due to Dr. Smith-Woodward, F.R.S., Mr. Carruthers, F.R.S., Dr. Hinde, F.R.S., and Professor Judd, F.R.S., for help in connexion with this paper.

EXPLANATION OF FIGURES IN PLATES XXXIX AND XL.

Illustrating Mrs. D. H. Scott's paper on *Traquairia*.

PLATE XXXIX. Drawings.

Fig. 1. *Traquairia burntislandica*. Portion of the periphery showing hollow spines. × 280. Pettycur, Burntisland. S. 990.

Fig. 2. *T. burntislandica*. Portion of the periphery showing hollow spines and tubular pores. × 280. Pettycur, Burntisland. S. 986.

Fig. 3. *T. burntislandica.* Section near the surface, showing anastomosing tubes, which connect the spines. × 280. Pettycur, Burntisland. S. 990.

Fig. 4. *T. stellata.* Nearly radial section, showing five complete 'arms' and inner capsule; at *b* sections of 'arms'. × 100. Pettycur, Burntisland. S. 977.

Fig. 5. *T. Carruthersii.* Transverse section near the middle, showing spines and place of attachment of spines. × 100. Dulesgate. S. 923.

Fig. 6. *T. Carruthersii.* Section near top, showing spines in section. Dulesgate. S. 923.

Fig. 7. *T. Carruthersii.* Section near surface, showing spines in section. × 100. Dulesgate. S. 923.

Fig. 8. *T. Spenceri.* Spores from Fig. 9 more magnified. × 280. Halifax. S. 22.

Fig. 9. *T. Spenceri.* Section through middle, showing spines in section. Inner capsule containing spores. × 100. Halifax. S. 22.

Fig. 10. *T. Spenceri.* Possible young stage with one spine developed. × 100. Halifax. S. 22.

PLATE XL. Photographs.

Fig. 11. *T. burntislandica.* Section, nearly median, showing inner capsule. × 100. Pettycur, Burntisland. S. 986.

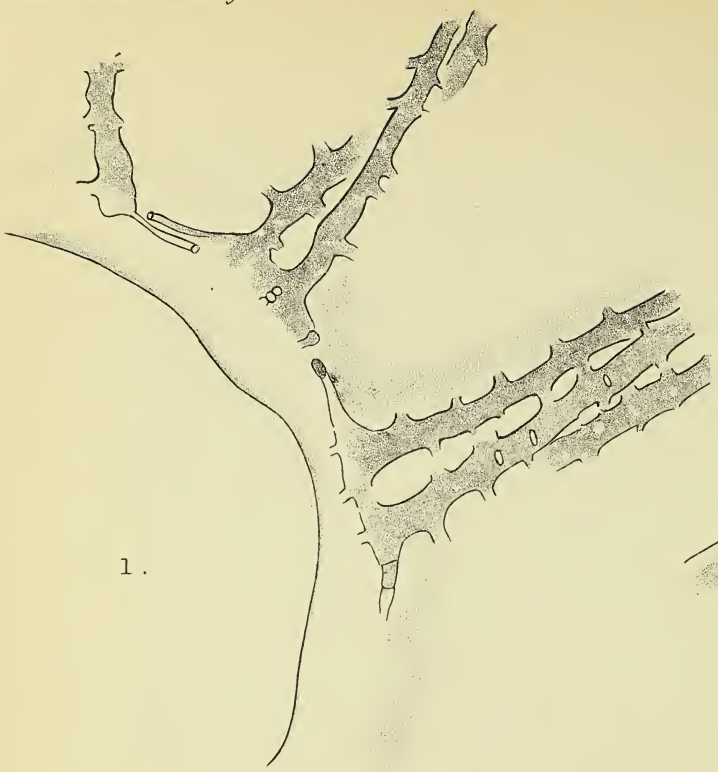
Fig. 12. *T. burntislandica* not quite median. × 100. S. 990.

Fig. 13. *T. burntislandica.* Section near the top, showing spines in section. × 100. S. 990.

Fig. 14. *T. Carruthersii.* Shows a large group. × 25. Dulesgate. S. 923.

Fig. 15. *T. Spenceri.* Section median, showing inner capsule with spores. × 100. Halifax. S. 22.

Fig. 16. *T. Spenceri.* Showing numerous sections *in situ* in wood of *Lepidodendron.* × 25. S. 22.



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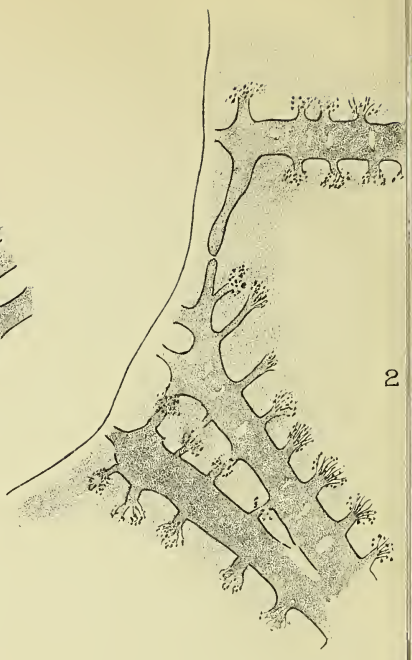

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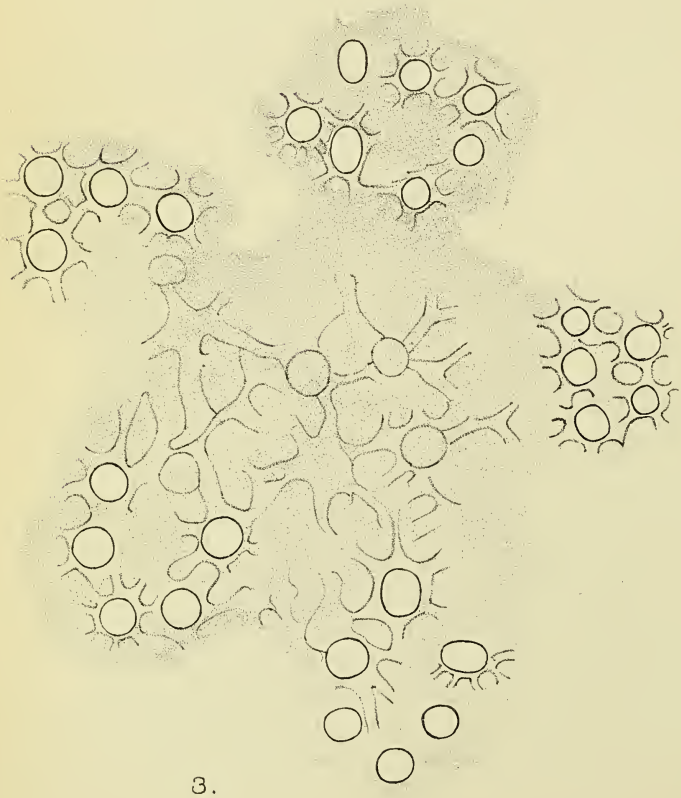

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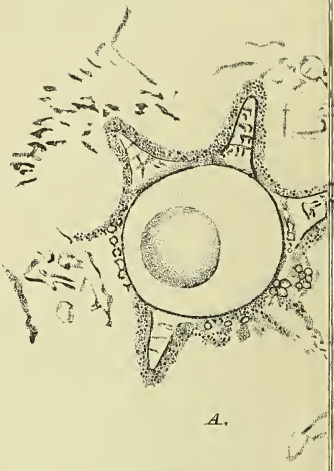

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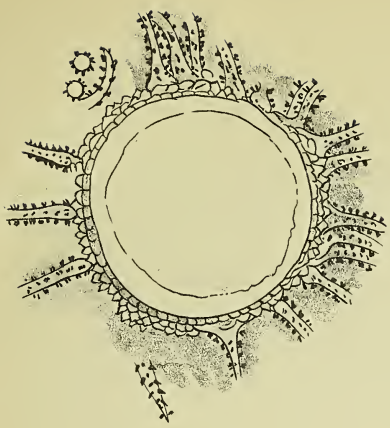
RINA SCOTT — TRAQUAIRIA.



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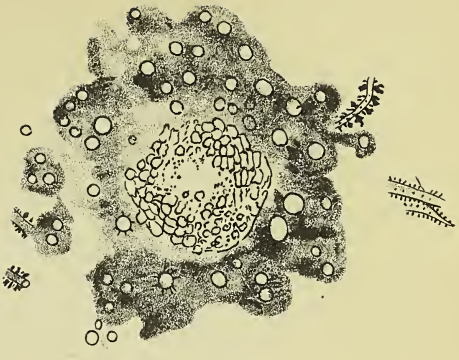

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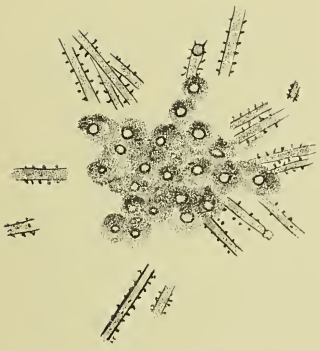

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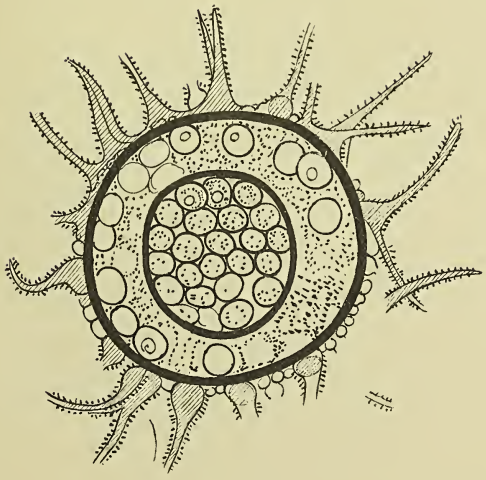

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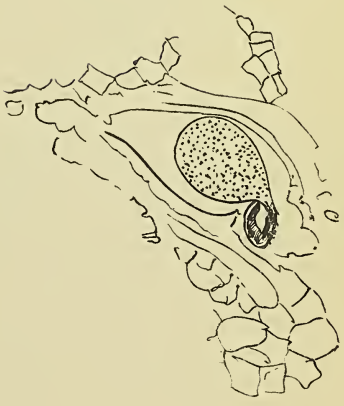

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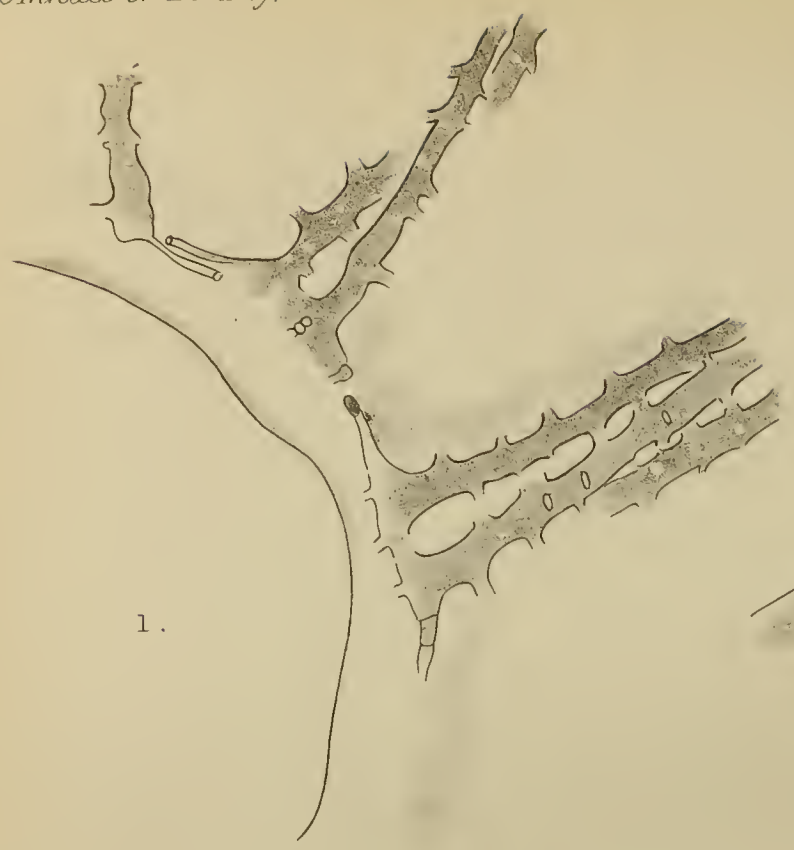

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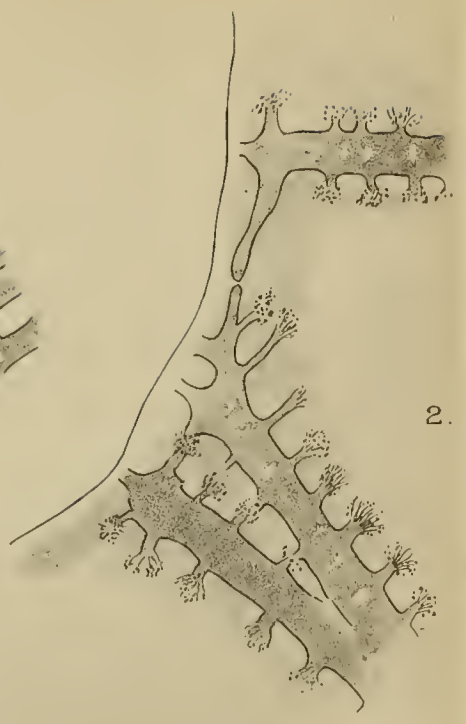

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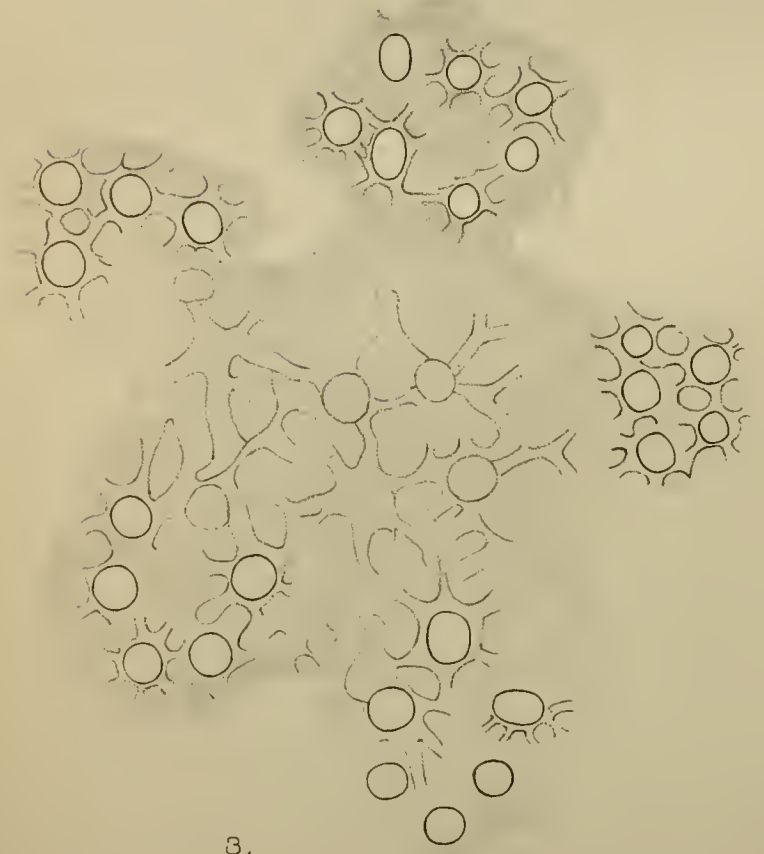

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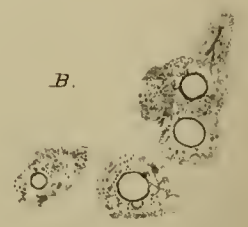

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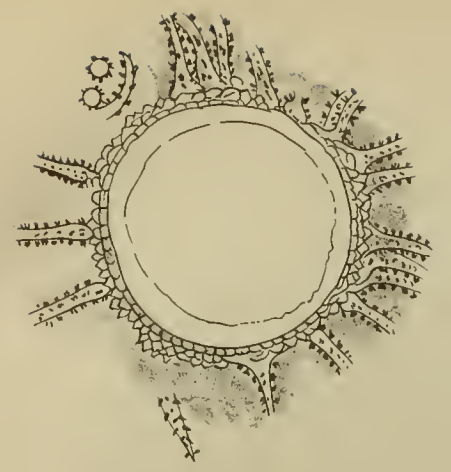
RINA SCOTT — TRAQUAIRIA.



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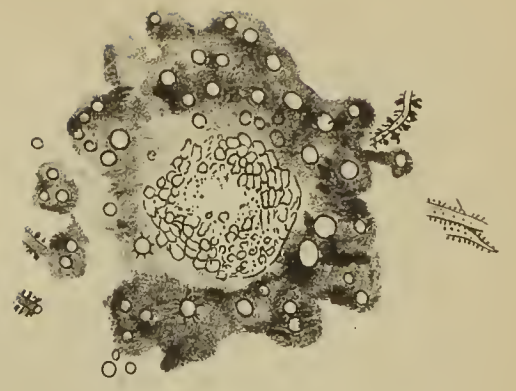

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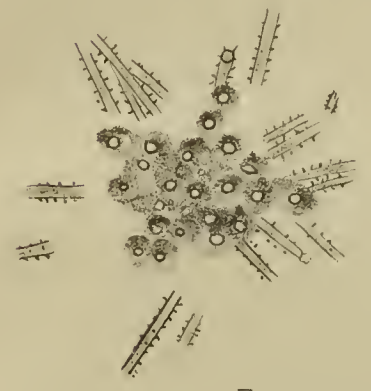

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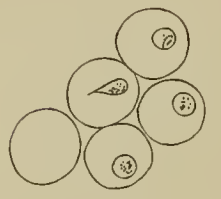

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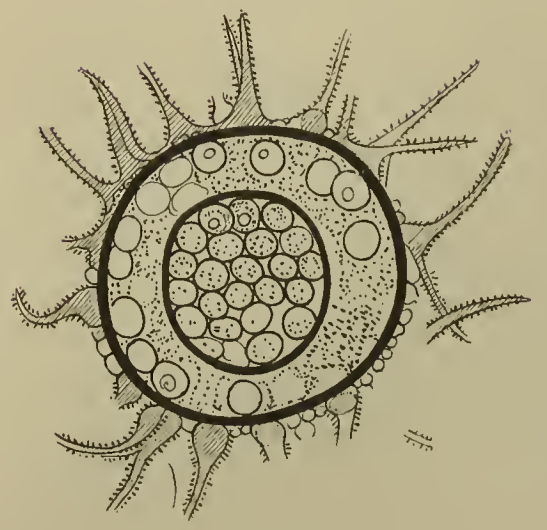

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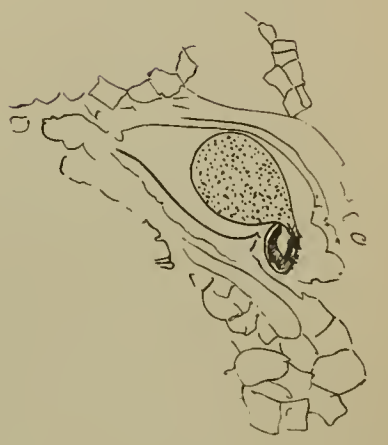

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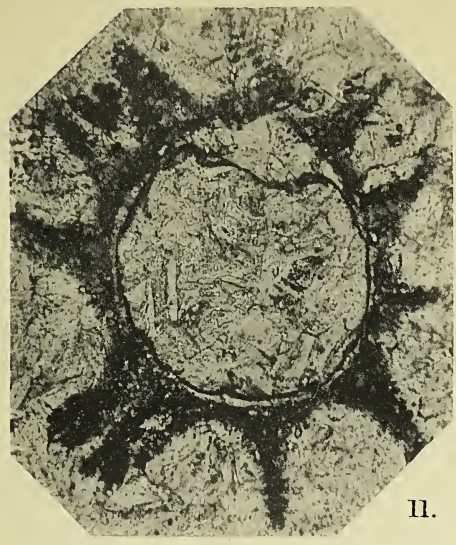

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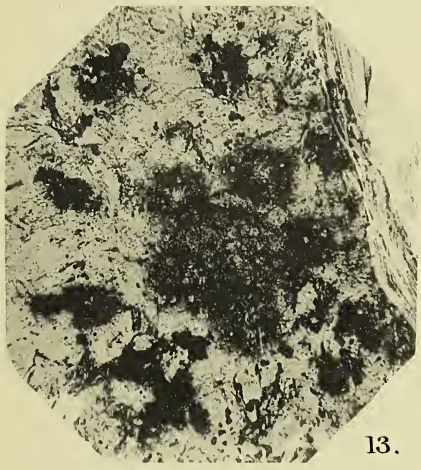

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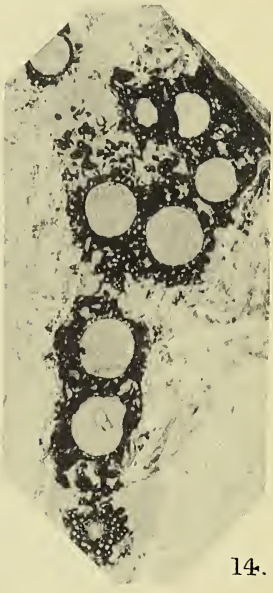

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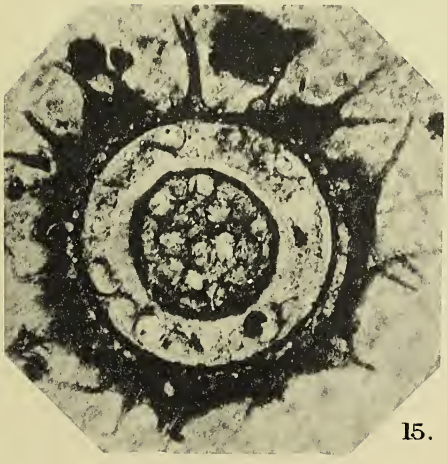

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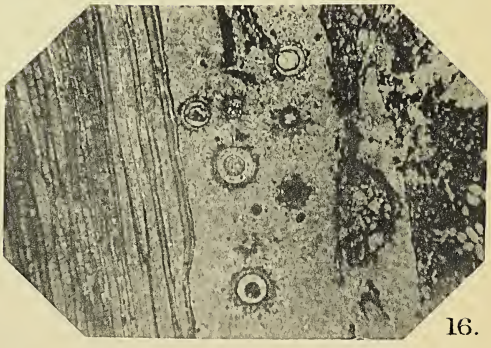

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RINA SCOTT.—— TRAQUAIRIA.

On the Life-history of Salvinia natans.[1]

BY

KONO YASUI,

Assistant Professor of Botany in the Higher Normal School for Women, Tokyo.

With Plates XLI–XLIII and one Figure in the Text.

ALTHOUGH the germination of the micro- and macrospore and the embryo-formation of *Salvinia natans* had already been observed by several of the older investigators, such as Mettenius ('46), Hofmeister ('51, 57), and others, the first careful and complete account of the subject was given by Pringsheim in 1863. In his classical paper he followed pretty closely the development of the male and female gametophytes, and the formation of the embryo, besides describing very carefully the further development of the sporophyte. Pringsheim's paper may, therefore, be looked upon as the starting-point upon which all subsequent morphological studies on *Salvinia natans* are based.

In 1873 Juranyi published the results of his studies on the development of the micro- and macrosporangia and the formation of spores. These parts of the life-history of *Salvinia natans* were entirely neglected by Pringsheim, and Juranyi's paper may be considered as the first careful description of this subject. Three years later Arcangeli ('76) described the development of both sporangia and prothallia of *Salvinia*. He noticed the three layers, i. e. the pseudo-epispore, the exospore, and the endospore, in the spore-coat of both the macro- and microspore. The pseudo-epispore of the latter is considered by him to be common to all microspores. He also noticed that there are two antheridia in one male prothallium.

In 1879 Prantl's short paper appeared, dealing chiefly with the development of the female prothallium of *Salvinia*. He clearly demonstrated the existence of three spore-coats in the macrospore. In the same year Bauke ('79) described the further behaviour of the unfertilized prothallia of *Salvinia*. He confirmed and extended the statements of Pringsheim. A few years later Heinricher ('82) studied the development of the macro- and microspores, and corrected and extended the results of Juranyi.

[1] The substance of this paper has been already published in Japanese in the Botanical Magazine, Tokyo, vol. xxiv, 1910.

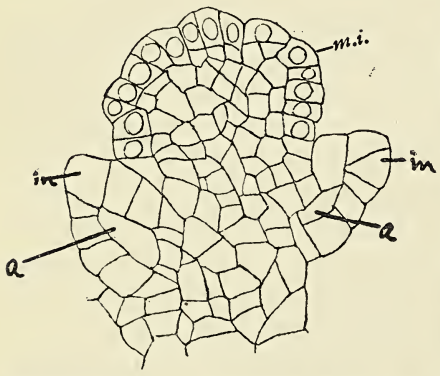
[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.]

In 1887 Campbell, in a paper dealing with the development of plant-spermatozoids, described the development of the male gametophyte of *Salvinia*. He agrees with Pringsheim ('63), who stated that there is one antheridium containing eight spermatozoids in each male prothallium. Belajeff ('98) made a careful study of the development of the male prothallium of *Salvinia*. He mentions that there are two antheridia in a single male prothallium, differing from the statements of Pringsheim and Campbell. Campbell still maintains his older view in the second edition of 'Mosses and Ferns' (1905).

The material for the study of spore-formation was collected in October, and the gametogenesis, fertilization, and embryogeny were studied from the material raised from spores in the laboratory from January to March during the last two years. The material was fixed either with Flemming's weaker solution or with chromo-acetic acid mixture. It was embedded in paraffin with the melting-point 52° C. The studies were made almost entirely with microtome sections.

Microspore Formation.

The microsporangium-initials are already formed on the surface of the very young sporocarp, as shown in the Text-figure. Each initial-cell undergoes a transverse division by which the stalk-cell is cut off from the apical cell (Pl. XLI, Figs. 1, 2). The stalk-cell then divides two or three times transversely. The apical cell segments again, cutting off the basal cell, the first primary wall-cell, below (Fig. 3, w_1). The apical cell then divides by a wall more or less perpendicular to that of the basal cell (Fig. 4, w_2). It is soon followed by a periclinal wall (Fig. 6, w_3), thus cutting off three primary wall-cells and the archesporial cell inside. According to Juranyi ('73), who made the first careful observations on the development of both the micro- and macrosporangium, one more wall-cell is cut off, thus forming four primary wall-cells enclosing the archesporial cell instead of three as I have observed (compare Juranyi's Pl. I, Figs. 6, 7).



Text-figure. Longitudinal section of a young sporocarp. *in*, indusium; *a*, air chambers; *m.i.*, microsporangium-initial.

The first-formed cell of two upper primary wall-cells then divides by a wall parallel to the long axis of the sporangium (Fig. 7). Then another upper primary wall-cell soon divides by a similar wall. These cells divide further several times, thus contributing to the greater part of the

wall of the mature sporangium. The basal cell divides but few times, and forms the part of the wall attached to the stalk.

The archesporial cell divides by the periclinal walls, forming three primary tapetal cells and a central cell (Figs. 8, 9). The central cell now divides by a wall more or less parallel to the long axis of the sporangium. It is soon followed by the next division, which divides two cells simultaneously by walls perpendicular to the first (Figs. 10–12). The quadrant-cells, thus formed, are again divided into equal octants (Figs. 12, 13). Each of these eight cells divides once more, and the sixteen microspore-mother-cells are formed (Fig. 14). During the divisions of the central cell the tapetal cells also divide by radial walls, but never by periclinal walls, so there is always one layer of tapetal cells, not two as described by Juranyi ('73). Juranyi adds, however, that the tapetal cells in the microsporangium are sometimes one-layered (Figs. 10–15).

The full-grown microspore-mother-cell has denser cytoplasmic contents and a much larger nucleus than the surrounding cells of the sporangium. Its nucleus contains a large nucleolus and a delicate linin-reticulum in which small chromatin-granules are embedded (Figs. 14, 18). The nucleus soon prepares for division and shows the characteristic synaptic contraction (Figs. 15, 17). The synapsis stage lasts comparatively long, and it is followed by the spireme which comes out from the contracted nuclear contents (Fig. 18). The spireme then becomes shorter and thicker, and at last it segments into chromosomes. After the chromosomes are formed, kinoplasmic fibres appear in the cytoplasm surrounding the nucleus. Then the nuclear membrane begins to disappear and the multipolar spindle enters the nuclear cavity and comes in contact with the chromosomes. The spindle, when it is completely formed, assumes a bipolar structure (Figs. 19–22).

The number of chromosomes is easily counted, as they are arranged at the equator of the spindle; the number is sixteen, and they are grouped in eight pairs (Fig. 21). Each half of the bivalent chromosomes now separates from the other and travels towards different poles of the spindle (Figs. 23, 24). When the daughter-chromosomes reach the pole they remain for a short time unchanged, and this stage is also favourable for counting their number (Figs. 24, 25).

As the spindle with a faint indication of a cell-plate at the equator disappears, two daughter-nuclei prepare for the second division. At the prophase of the division eight pairs of the chromosomes are distinctly visible (Fig. 27). The spindle is at first multipolar and later becomes bipolar. When the spindle is completely formed, eight bivalent chromosomes arrange themselves at the equator. The sister-chromosomes of the same pair then separate one from the other and travel towards the pole (Fig. 28). Each of the four granddaughter-nuclei thus formed receives

eight chromosomes. The walls are formed between the four nuclei, and thus four microspores are established (Pl. XLI, Figs. 29–32).

With the entering of the synapsis stage of the mother-cells, the tapetal cells commence to disintegrate, and soon the partition walls between individual cells disappear, the degenerating nuclei being embedded in the common cytoplasm (Fig. 15). As has already been described by Juranyi ('73) and Heinricher ('82), the fully-formed microspores separate from each other and lie free, side by side with the degenerating tapetal nuclei in the mass of the tapetal cytoplasm, which now completely fills up the inside of the sporangium (Figs. 33, 34).

The mature microspores later move towards the periphery of the sporangium, and the centre of the latter is occupied by a mass of vacuolated cytoplasm (Pringsheim ('63), Prantl ('79), Heinricher ('82)). No trace of disintegrating tapetal nuclei is found at this stage (Fig. 35). The mature microspore has a thin endospore and a thick exospore. It contains a rather large nucleus at or near the centre, and abundant starch-grains (Fig. 36).

Male Prothallium.

The microspore begins to germinate inside the sporangium in the early spring. The material kept in the laboratory was found to commence germination at the end of January, and to continue it until about the middle of May.

The development and structure of the male prothallium agrees on the whole with the description of Belajeff ('98). Belajeff studied the prothallium *in toto* and used the plasmolysis method in differentiating the outline of individual cells. He does not seem to have observed any dividing figures in the developing prothallium, and his observations lack histological details.

The microspore first divides into two cells by a wall tangential to the surface of the sporangium (Figs. 37, 38). The inner or lower of the two cells then gives rise to the two unequal cells—the lower small root-cell and the upper large prothallium-cell (Fig. 39). Early investigators such as Pringsheim ('63), Arcangeli (76), and Prantl ('79), who made some errors in describing the structure of the male prothallium, failed to observe any root-cell. Campbell ('87), who has studied the spermatogenesis of *Salvinia* more recently, was also unable to find the root-cell. He states: 'Die Spore theilt sich durch eine Querwand und die untere der gebildeten Zellen bleibt ungetheilt, die vegetative Zelle des Prothalliums darstellend.' Belajeff ('98) was the first to demonstrate the presence of the root-cell, but he was not certain when it is formed.

After the first division or sometimes just before, the exospore splits along three radiating lines on the surface of the microspore. The section of the germinating microspore at this stage is shown in Fig. 37.

The uppermost cell of the young prothallium now divides into two cells by a more or less oblique cross-wall (Fig. 40), and the outer one of these is divided again by an oblique wall, forming an apical cell and an antheridium-mother-cell (Figs. 41–43). The inner cell is also divided transversely, giving rise to a sterile cell above and an antheridium-mother-cell below (Fig. 44). From each antheridium-mother-cell a small sterile cell or wall-cell is cut off by an oblique wall which extends from the side-wall to the lower transverse wall, and a larger cell, the central cell or sperm-grandmother-cell, is formed (Figs. 45–6). The antheridium is made up of a wall-cell and a central cell which later divides into four sperm-cells. A single spermatozoid is formed in each sperm-cell. Now the development of the male gametophyte, consisting of a root-cell, a prothallium-cell, an apical cell, and two antheridia which are separated by a sterile cell, is completed (Figs. 47–53).

In the dividing cell of the male prothallium, the number of chromosomes has always been found to be eight.

In his classical paper on *Salvinia natans* Pringsheim ('63) states that in a male prothallium a single antheridium with eight spermatozoids is formed. He gives a figure of the apical cell and two sperm-grandmother-cells, but he mentions neither the presence of the wall-cells nor the sterile cell. Arcangeli ('76) takes the two antheridial cells for two antheridia. Prantl ('79) seems to be of the same opinion; and he states: 'Ich glaube, die beiden vorderen Zellen ebenso gut als zwei Antheridia deuten zu können, deren jedes eine Gliederzelle des rudimentären Prothalliums einnimmt.' Campbell's ('87) description of the mature male prothallium is as follows: 'Das ganze Antheridium (wenn wir den ganzen oberen Theil des Prothalliums als ein einziges Antheridium ansehen) ist jetzt aus fünf oder sechs Zellen gebildet: zwei innern (Fig. 26, *m*), welche die Urmutterzellen der Spermatozoiden darstellen, einer Deckelzelle (D) und zwei oder drei anderen peripherischen Zellen (P).' His 'peripherische Zellen' correspond to the wall-cells, and are, according to him, two or three in number. However, when he found three wall-cells it is very likely, in my opinion, that he mistook the sterile cell for one of them (compare Campbell's Fig. 26). According to Belajeff ('98) each male prothallium contains two antheridia which are separated from each other by a sterile cell. In regard to this sterile cell, Campbell ('05) states as follows: 'This cell, however, did not occur in the specimen studied by me, where the two groups of sperm-cells were usually in immediate contact (Fig. 233, E).' In my material it was found that the sterile cell always separates the two groups of sperm-cells, and thus forms two antheridia in each prothallium instead of one, agreeing with the statement of Belajeff.

When the sperm-cell is completely formed, a blepharoplast appears in its cytoplasm, and soon elongates towards the nucleus (Fig. 52). In the

meantime the nuclear reticulum becomes more prominent, and the nucleus begins to elongate. The elongated blepharoplast attaches itself to the dorsal side of the nucleus, which now begins to coil. The elongating nucleus which forms the greater part of the spermatozoid-body makes a coil of about two turns (Pl. XLI, Figs. 50, 51, 53). The numerous cilia evidently originate from the blepharoplast attached to the dorsal side of the nucleus.

The spermatozoid, when it comes out from the sperm-cell, swims about by means of cilia attached to the anterior dorsal side of its body, making two spiral coils. It carries a vesicle in the hinder end of its body, which contains several small starch-grains; the vesicle may, sooner or later, be detached from the spermatozoid.

Macrospore Formation.

The macrosporangium-initials appear at the apex of the young sporocarp (Pl. XLII, Fig. 54). They are characterized by denser cytoplasm and larger nuclei. Each initial is cut off at the base by two oblique walls, which are more or less perpendicular to each other, and thus the young stalk is formed. The third wall, parallel to the first oblique wall, is then formed; the last-formed cell corresponds to the basal cell, the first primary wall-cell of the young microsporangium. The fourth wall, parallel to the second oblique wall of the stalk, is then formed, being soon followed by a periclinal wall, thus forming an archesporial cell and three primary wall-cells (Fig. 55).

Three primary cells of the tapetum are cut off by periclinal walls from the archesporial cell (Fig. 56). In the meantime the wall-cells are divided by repeated radial divisions. The primary tapetal cells also undergo repeated radial divisions, and one layer of the tapetum is formed (Figs. 57–60). According to Juranyi ('73) the tapetum usually consists of two layers, as the following statement shows: 'Die Mantelzellen theilen sich nämlich in tangentaler Richtung; dieser Theilungsweise sind aber dieselben zu dieser Zeit gewöhnlich nur einmal unterworfen, in Folge dessen der Mantel in der Regel nur zweischichtig wird (Taf. II, Fig. 22).' This statement is adopted by Campbell in his revised edition of 'Mosses and Ferns'. Heinricher ('82), however, mentions that the tapetum is usually of a single layer. I was not able to confirm the statement of Juranyi, and no case was met with in which the tapetum consisted of two layers of cells.

Simultaneously the central cell continues to divide (Figs. 57–59), and after the third division it reaches the spore-mother-cell stage; so there are eight spore-mother-cells in one macrosporangium. The spore-mother-cells are easily distinguished from the surrounding cells by denser granular cytoplasm and larger nuclei (Fig. 59). There are two different statements concerning the number of the macrospore-mother-cells. According to

Juranyi there are sixteen, while Heinricher gives them as eight. My observation agrees with that of Heinricher, i. e. the number is eight.

The resting nucleus of the macrospore-mother-cell contains a delicate reticulum, which may consist of a network of linin in which are embedded some small chromatin-granules and a large distinct nucleolus (Fig. 61). The reticulum then assumes a more or less thread-like structure, and gradually becomes located on one side of the nuclear cavity, thus entering the synapsis stage (Fig. 62). The synapsis stage lasts comparatively long, and is followed by the spireme stage. Although I was not able to find the double nature of the thread in the early stages of the spireme, it was more or less apparent in the later stages (Figs. 63, 64). The spireme then segments itself into eight chromosomes, each of which is bivalent in nature (Figs. 65, 66).

The formation of the spindle now begins. The spindle is at first multipolar in origin, and assumes later a bipolar structure (Figs. 67, 69). Chromosomes then arrange themselves at the equator of the spindle. The number of chromosomes can easily be counted at this stage when viewed from a pole of the spindle (Fig. 68). Each half of the eight bivalent chromosomes now travels towards the poles (Figs. 69, 70). A sign of longitudinal splitting is observed in the chromosomes before they reach the pole.

As the daughter-chromosomes reach the poles their number can often be counted, and is always found to be eight (Fig. 71), but soon they come closely together until the individual outlines are lost. The nuclear membrane then appears (Fig. 72).

The two daughter-nuclei then divide. The division is homotypic and results in the formation of four granddaughter-nuclei (Fig. 73–77).

Simultaneously with the synapsis stage of the mother-cell, the tapetal cells begin to disintegrate, and macrospores that have not yet separated from each other float in the cytoplasmic mass of the tapetal cells, as has already been noticed by Heinricher (Fig. 78).

Afterwards the original wall of the macrospore-mother-cells disappears and the macrospores are separated from each other (Fig. 79). A single macrospore is surrounded by a dense mass of the cytoplasm and becomes enlarged very rapidly, while the rest of the macrospores move near the periphery of the sporangium and finally disintegrate (Figs. 80–82). So it can be said that the functional macrospore is nourished by the degenerating sister-spores and tapetal cells.

The development of only one macrospore within each macrosporangium at the expense of the degenerating sister-spores and tapetal cells was noticed by Juranyi ('73). But his description concerning the disintegration of tapetal cells and abortive macrospores seems to be not quite correct, as has been pointed out by Heinricher ('82). My observations agree on the whole with the statement of Heinricher.

Although there is, as a rule, only one functional macrospore in each

macrosporangium, I have observed in a few preparations that two macrospores were becoming enlarged instead of one. It seems that even in those cases one of the two finally develops, at the expense of the other, into the mature macrospore.

The mature macrospore is surrounded by three membranes. The outermost membrane, the epispore, shows a vacuolate structure, and is not of equal thickness, being thickest at the top, where it is divided into three folds. The folds are formed by three ridges at the sides (Pl. XLII, Figs. 84, 85).

The structure and development of the spore-membrane have already been described by Mettenius ('46) and Pringsheim ('63). Some more details were later added by Juranyi ('73), Prantl ('79), and Heinricher ('82).

The formation of the epispore by the vacuolization and the transformation of the protoplasmic mass surrounding the macrospore have already been described by Heinricher ('82). Fig. 83 shows an earlier stage of the epispore formation, in which the least differentiated cytoplasmic mass with one of the developing nuclei is found at the periphery, while the inner portion is transformed into a mass of vacuoles. A little later stage is shown in Fig. 84. The cytoplasmic mass is almost entirely transformed into the vacuoles. According to Heinricher ('82) the smaller vacuoles are found near the periphery, while the larger ones are arranged near the inner side. I have found, however, that the larger vacuoles are found near the periphery rather than towards the centre. In the young epispore a thin layer of protoplasm with a number of nuclei is found inserted between each episporic fold and the underlying portion of the epispore; Fig. 85 shows a tangential section of the epispore through the episporic folds at the apex of a macrospore.

The next inner membrane, the exospore, is homogeneous in structure, and almost equal in thickness throughout. Although the origin of the exospore is not quite clear, I am inclined to think that it is formed from the protoplasmic mass surrounding the macrospore. The innermost endospore is very thin and directly lines the inner surface of the exospore.

Female Gametophyte.

The macrospores germinate on the surface of the water after the wall of the sporocarp has decayed or even when it is only partly decayed. Macrospores used for the present study germinated during January and February in our laboratory. When germination begins, the cytoplasm at the upper part of the spore becomes very dense, and soon the nucleus is found to divide. One of the division-figures is shown in Fig. 86. A faint membrane is formed between the two resulting nuclei, thus dividing the whole macrospore into a smaller upper cell and a considerably larger lower cell.

The upper small cell forms, by further divisions, the prothallium tissue, while the nucleus of the lower large cell multiplies by free nuclear divisions, and the nuclei are finally distributed throughout the parietal layer of cytoplasm (Fig. 87). Before the prothallium pushes out from the spore cavity, a large cell appears on the upper surface of the prothallium, which is soon found to be the initial-cell of an archegonium (Fig. 88).

The archegonium-initial divides into two by a cross-wall (Pl. XLIII, Fig. 89), and the upper one is divided longitudinally twice (Figs. 90, 92, 94), by walls at right angles to each other, into four cells, every one of which is again divided twice transversely (Figs. 94–96). The uppermost four cells are cover-cells and the others are neck-cells.

Almost simultaneously with the first division of the upper cell, the lower one divides unequally by means of a curved wall into the smaller neck canal-cell and the larger central cell (Figs. 90–93). The central cell now divides by a curved cross-wall. The outer small cell is the ventral canal-cell and the inner large cell is the egg-cell (Figs. 94, 95).

Pringsheim gives no statement about the presence of the ventral canal-cell, but he seems to have observed it as his figures (Pringsheim '63, Pl. XXVI, Fig. 1) and the following statement shows: 'So sieht man, wie hier beiläufig bemerkt werden mag, den Inhalt der Canalzelle in zwei verschiedene Massen sich sondern (XXVI, 1), in einen grossen fädigschleimigen Klumpen, der die ganze Spitze ausfüllt, und einen kleinen tieferliegenden Klumpen, welcher der veränderte Zellkern der Centralzelle zu sein scheint.' The lower segment of the neck canal-cell in the above description may very likely represent the ventral canal-cell.

The nucleus of the neck canal-cell then divides into two, though there does not arise any membrane which would separate the nuclei. According to Campbell ('05), in *Azolla* 'the neck canal-cell may have its nucleus divide,' but mentions that 'this has not yet been observed in *Salvinia*'.

On both sides of this first-formed archegonium, there arise later two to four archegonia (Fig. 100). Pringsheim stated that on each female prothallium usually three archegonia arise, and he also mentions that he has sometimes seen the prothallium with a great many archegonia, which he attributes to the absence of fertilization. I have not been able to observe any prothallium with such numerous archegonia, as I have made no observation by preventing fertilization.

As already noticed by Pringsheim and later investigators of *Salvinia*, the vegetative cells of the prothallium contain many chlorophyll grains and continue to grow, until, after the appearance of the archegonia, they spread like wings on both sides of the prothallial tissue where the archegonia are located. The prothallium assumes, sooner or later, a dorsi-ventral structure, and the archegonia are found on its ventral side (Figs. 99, 100).

Fertilization.

When the egg-cell becomes mature, the neck of the archegonium opens widely, and the slimy substance, resulting from the disintegration of the canal-cell and ventral canal-cell, comes out of the opening (Pl. XLIII, Fig. 98).

The cytoplasm of the egg-cell is often denser around the nucleus, showing a more or less fibrillar structure. The nucleus contains a large nucleolus and a faint, poorly developed reticulum (Fig. 96).

A great many spermatozoids enter the neck of the archegonium and reach the exposed surface of the egg-cell (Fig. 98). Normally only one of them penetrates the cytoplasm of the egg. Fig. 97 shows the egg-nucleus just after the entering of the sperm-nucleus. The latter appears as a short curved rod near the nucleolus of the former. The sperm-nucleus then seems to disorganize into a mass of granules of various sizes. Fig. 98 shows the sperm-nucleus about half-way disintegrated, closely attached to the nucleolus.

Embryo.

The first division of the fertilized egg was not found. The wall separating the two cells of the embryo is parallel to the axis of the archegonium (Figs. 101, 102). The next division in each of the two embryonal cells is transverse, and thus the quadrants are formed which represent respectively the stem, the cotyledon, the foot, and the root (Figs. 103, 105). The cells of the cotyledon and the stem quadrants have always denser cytoplasm than the other two quadrants.

The cells of the quadrant both of cotyledon and stem divide by walls perpendicular to both of the first two walls (Fig. 104). In the stem-quadrant one of the two cells now formed becomes an initial of the apical cell, and it divides by a more or less oblique transverse wall, thus cutting off an apical cell above. The other cell of the stem-quadrant also divides like its sister-cell, and later develops into the first leaf of the young stem.

The early divisions in the cells of the cotyledonary quadrant do not differ much from those of the stem-quadrant mentioned above. But later they develop into a quite different structure, and thus, growing rapidly towards both sides and below, a triangular cotyledon is formed (Figs. 106–111).

In the root- and foot-quadrants the cell-divisions are not so regular as those of the stem or cotyledon. The development of the root ceases at an early stage, and later its tissue cannot be distinguished from that of the foot. The foot, on the other hand, develops to a massive structure. The lowest cells of the foot are filled with rather dense cytoplasm.

Pringsheim ('63) mentions that the triangular shape of the cotyledon is due to the presence of the three apical cells at its three corners, but I cannot confirm his statement. The cell at each corner of the triangle is not

an apical cell, being older than the surrounding cells, and ceasing to divide in the early stage of development (Figs. 109, 110). I found that two apical cells lying rather close to each other are situated at the upper sinus of the cotyledon, i.e. at the middle of the base of the inverted triangle.

The further development of the embryo is described in detail by Pringsheim, and I have nothing to add to his statement, as I can only confirm it.

Summary.

1. The primary tapetum-cells of both sporangia are formed by division of the archesporial cell, and they again divide into one layer of many cells.

2. The spore-mother-cells are eight in the macrosporangium and sixteen in the microsporangium.

3. The number of chromosomes of the spore-mother-cell is sixteen and the reduced number in the spore is eight.

4. During the reduction-division the tapetum-cells begin to degenerate and nourish the spores.

5. Sixty-four mature spores are formed in a microsporangium, but in a macrosporangium only one spore becomes mature.

6. The male prothallium consists of a large prothallium-cell, a small root-cell, two sterile cells and two antheridia, which consist of a wall-cell and a central cell. The central cell divides twice, and in each of the four cells a spermatozoid is formed.

7. The statement of Campbell, who denies the presence of a sterile cell between the two antheridia and the root-cell, was not confirmed, in agreement with the investigation of Belajeff. Belajeff was not clear about the time of the appearance of the root-cell. It has been determined by the present study that the root-cell is formed as a result of the second division of the germinating microspore.

8. The spermatozoid is a spirally coiled body having numerous cilia at the anterior end, and a large vesicle at the posterior end.

9. A blepharoplast, which first appears in the cytoplasm and later elongates towards the nucleus, was observed in each sperm-cell.

10. Three to five archegonia are usually formed in each female prothallium, and each archegonium consists of one egg-cell, a ventral canal-cell, a neck canal-cell with two nuclei, and the neck-cells.

11. Only one spermatozoid enters into the egg-cell. After the penetration of the spermatozoid into the egg-nucleus, there appears in the cavity of the latter a nucleolus-like body, derived very likely from the spermatozoid and somewhat smaller than the nucleolus.

12. The four quadrants are clearly distinguishable in the young embryo. The development of the root-quadrant ceases at an early stage, and later its tissue cannot be distinguished from that of the foot.

I am indebted to Dr. K. Miyake for his kind suggestions and advice during the progress of this work.

Note.—After this had been written, Professor Arnoldi's paper, 'Beiträge zur Morphologie der Keimung von *Salvinia natans*,' appeared in Flora (Bd. c, pp. 122–139, 1909). His results agree with mine in essential points, except in one important matter, i.e. the number of chromosomes. He gives four as the chromosome number of the gametophytes and eight for the sporophyte. I have found, however, that the number is twice as many as that given by Arnoldi, being eight and sixteen respectively.

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EXPLANATION OF PLATES XLI–XLIII.

Illustrating Miss Yasui's paper on *Salvinia natans*.

All figures are drawn with the aid of a camera lucida, from microtome sections.

PLATE XLI.

Fig. 1. Longitudinal section of the initial cell of a macrosporangium. × 600.

Fig. 2. Longitudinal section of the initial cell of a microsporangium which is cutting off a stalk-cell. × 600.

Fig. 3. The first wall-cell (w_1) is cut off from the apical cell. × 600.

Fig. 4. The second wall-cell (w_2) is cut off from the apical cell. × 600.

Fig. 5. Cross-section of the same stage as above. × 600.

Fig. 6. The third wall-cell (w_3) is just formed; the archesporium is now surrounded by three wall-cells. × 600.

Fig. 7. *a*, cross-section of a young microsporangium in which the second wall-cell is divided into two; *b*, the upper surface view of the same. × 600.

Fig. 8. The first tapetum-initial (t_1) is segmented from the archesporium. × 600.

Fig. 9. The third tapetum-initial is cut off from the archesporium; t_3, the nucleus of the third tapetum-initial. × 600.

Fig. 10. The second division of the central cell. × 600.

Fig. 11. The same as above. × 600.

Fig. 12. A later stage; the four cells are formed. × 600.

Fig. 13. A still later stage; the eight cells are formed. × 600.

Fig. 14. Cross-section of a microsporangium in which sixteen spore-mother-cells are formed × 600.

Fig. 15. Cross-section of a microsporangium in which the microspore-mother-cells are at the synapsis stage and the tapetum-cells are degenerating. × 600.

Fig. 16. A microspore-mother-cell in the resting stage. × 1400.

Fig. 17. The microspore-mother-cell in synapsis. × 1400.

Fig. 18. A microspore-mother-cell in spireme stage. × 1400.

Fig. 19. Multipolar spindle stage. × 1400.

Fig. 20. The same as above. × 1400.

Fig. 21. Cross-section of the equatorial plate. × 1400.

Fig. 22. Longitudinal section of a heterotypic spindle. × 1400.

Fig. 23. A later stage of the same. × 1400.

Fig. 24. The same in telophase. × 1400.

Fig. 25. A later stage; the reconstruction of the daughter-nuclei has begun. × 1400.

Fig. 26. Two daughter-nuclei are just formed. × 1400.

Fig. 27. Prophase of the second division. × 1400.

Fig. 28. A later stage of the second division. × 1400.

Fig. 29. The telophase of the second division. × 1400.

Fig. 30. The wall formation between four granddaughter-nuclei. × 1400.

Fig. 31. Young microspore just formed. × 1400.

Fig. 32. Young free microspore. × 1400.

Fig. 33. A microsporangium containing microspore tetrads. × 600.

Fig. 34. A later stage; individual spores are separated from each other. × 600.

Fig. 35. Longitudinal section of mature microsporangium, showing microspores at the periphery. × 600.

Fig. 36. A mature microspore. × 1400.

Fig. 37. A germinating microspore; the nucleus is in division. × 1400.

Fig. 38. The first division is completed. × 1400.

Fig. 39. The lower of the two cells is dividing to form the root-cell below. × 1400.

Fig. 40. A little later stage; the upper of the two cells has already divided into two. × 1400.

Fig. 41. The division of the apical cell. × 1400.

Fig. 42. A later stage of the same. × 1400.

Fig. 43. A still later stage. *a.c.*, apical cell; *a.m.c.*, antheridium-mother-cell. × 1400.

Fig. 44. The lower antheridium-mother-cell, being separated from the upper antheridium-mother-cell by a sterile cell, is differentiated. *s.c.*, sterile cell. × 1400.

Fig. 45. The wall-cell (*w.c.*) of the upper antheridium is formed. × 1400.

Fig. 46. The wall-cell of the lower antheridium is formed; the root-cell (*r.c.*) is shown. × 1400.

Fig. 47. The nucleus of the central cell of the upper antheridium is just divided, while that of the lower antheridium is in the telophase of the division. × 1400.

Fig. 48. A slightly later stage of the same. × 1400.

Fig. 49. The last division in the central cell of the lower antheridium. × 1400.

Fig. 50. A nearly mature male prothallium; spermatozoids are developing in the sperm-cells of both antheridia. × 1400.

Fig. 51. About the same stage as the preceding; the root-cell is shown. × 1400.

Fig. 52. Cross-section of an antheridium, showing an elongating blepharoplast in each sperm-cell. × 1400.

Fig. 53. A mature male prothallium; the root-cell is not shown. × 1400.

PLATE XLII.

Fig. 54. The initial-cell of a macrosporangium. × 600.

Fig. 55. Three young macrosporangia in various stages of development. × 600.

Fig. 56. A young macrosporangium in which the tapetum-initials are cut off. × 600.

Fig. 57. A little later stage in which the central cell is divided once. × 600.

Fig. 58. The second division of the central cell. × 600.

Fig. 59. Eight macrospore-mother-cells are formed. × 600.

Fig. 60. Cross-section of a macrosporangium, in which the macrospore-mother-cells are in the synapsis stage. × 600.

Fig. 61. A macrospore-mother-cell whose nucleus is in the resting stage. × 1400.

Fig. 62. Macrospore-mother-cell whose nucleus is in the synapsis stage. × 1400.

Fig. 63. The same in early spireme stage. × 1400.

Fig. 64. A later stage of the same. × 1400.

Fig. 65. Three macrospore-mother-cells in which the chromosomes are just formed. × 1400.

Fig. 66. A slightly later stage; the diakinesis. × 1400.

Fig. 67. The formation of the multipolar spindle. × 1400.

Fig. 68. Cross-section of an equatorial plate, showing eight bivalent chromosomes. × 1400.

Fig. 69. Spindle of the first division. × 1400.

Fig. 70. The same as above. × 1400.

Fig. 71. Telophase of the first division. × 1400.

Fig. 72. The two daughter-nuclei of the first division. × 1400.

Fig. 73. The prophase of the second division. × 1400.

Fig. 74. The telophase of the second division. × 1400.

Fig. 75. The granddaughter-nuclei are formed. × 1400.

Fig. 76. Macrospore-tetrad. × 1400.

Fig. 77. Young macrospores. × 1400.

Fig. 78. A macrosporangium containing macrospore-tetrads. × 450.

Fig. 79. A later stage with free macrospores. × 450.

Fig. 80. The functional macrospore (*m.*) is located at the centre, and all the other potential macrospores are moving towards the periphery of the macrosporangium. × 450.

Fig. 81. A later stage; the functional macrospore is surrounded by the cytoplasm and nuclei of disorganized tapetum-cells. × 260.

Fig. 82. A still later stage. × 260.

Fig. 83. Radial section of the spore-coat of a young macrospore. × 600.

Fig. 84. Radial section of the spore-coat of an older macrospore; *n*, nucleus of the tapetal cell; *ep*, epispore; *ex*, exospore. × 260.

Fig. 85. The tangential section of the same. × 260.

Fig. 86. The first division of a germinating macrospore. × 800.

Fig. 87. Upper part of a young female prothallium. × 800.

Fig. 88. The initial-cell of an archegonium. × 800.

PLATE XLIII.

Fig. 89. A young archegonium; a primary neck-cell is just formed. × 800.

Figs. 90–92. The division of the central cell. × 800.

Fig. 93. The division is completed and the neck canal-cell is formed above. × 800.

Fig. 94. The ventral canal-cell is formed by the second division of the central cell. × 800.

Fig. 95. The division of the nucleus of the neck canal-cell. × 800.

Fig. 96. A mature archegonium. × 800.

Fig. 97. An egg-cell just after the entrance of a spermatozoid. × 800.

Fig. 98. An archegonium, showing numerous spermatozoids entering it; the egg is already fertilized. × 800.

Fig. 99. The upper part of a germinating macrospore, showing young prothallium with an archegonium. × 100.

Fig. 100. Longitudinal section of a prothallium, with five archegonia. × 100.

Fig. 101. A fertilized egg-cell, preparing for the first division. × 600.

Fig. 102. Two-cell stage of the embryo. × 600.

Fig. 103. One of the two cells is dividing. × 600.

Fig. 104. The stem and cotyledonary quadrant-cells are dividing. × 600.

Fig. 105. The four-cell stage. × 600.

Fig. 106. The upper two cells are divided by cross-walls. × 600.

Fig. 107. An apical cell is formed in each stem and cotyledon quadrant. × 600.

Fig. 108. A later stage. × 600.

Fig. 109. A still later stage. × 600.

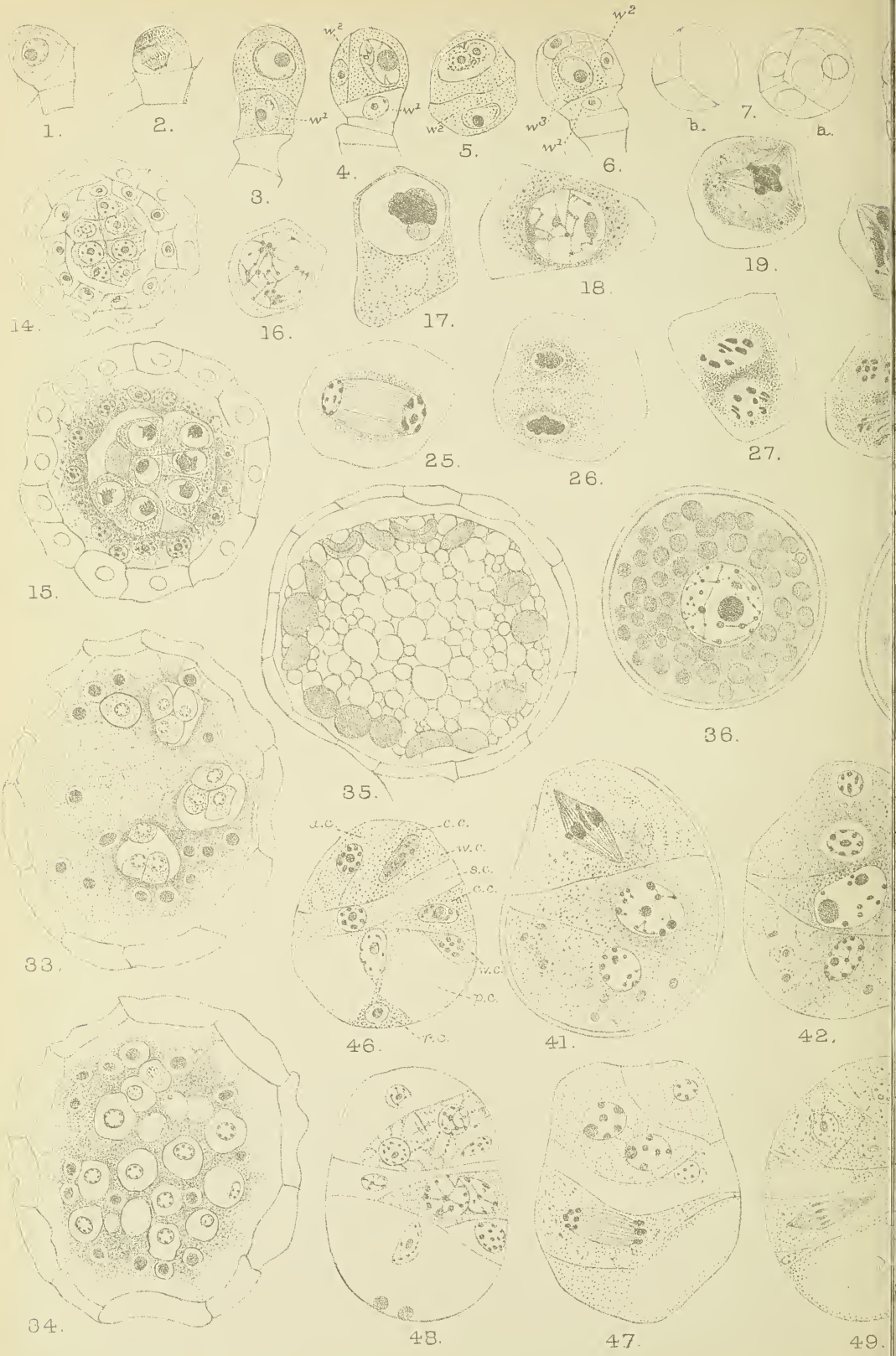
Fig. 110. Longitudinal section of a young plant. × 360.

Fig. 111. Longitudinal section of the cotyledon. × 360.

Fig. 112. Two embryos in the same prothalium. × 600.

Fig. 113. A cell of a young sporophyte. × 1400.

Figs. 114–119. Various stages of the division of the cells of a young sporophyte. × 1400.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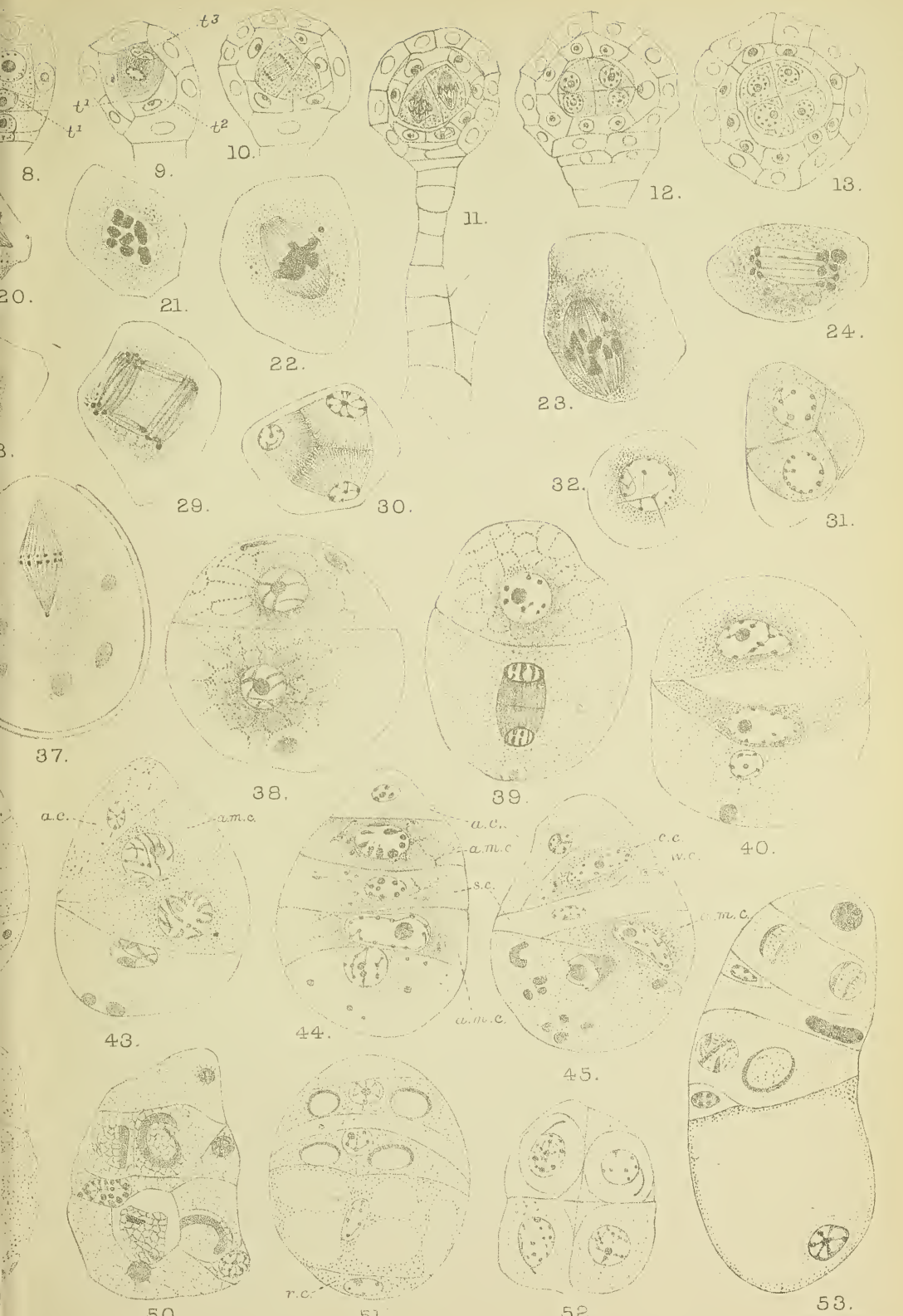

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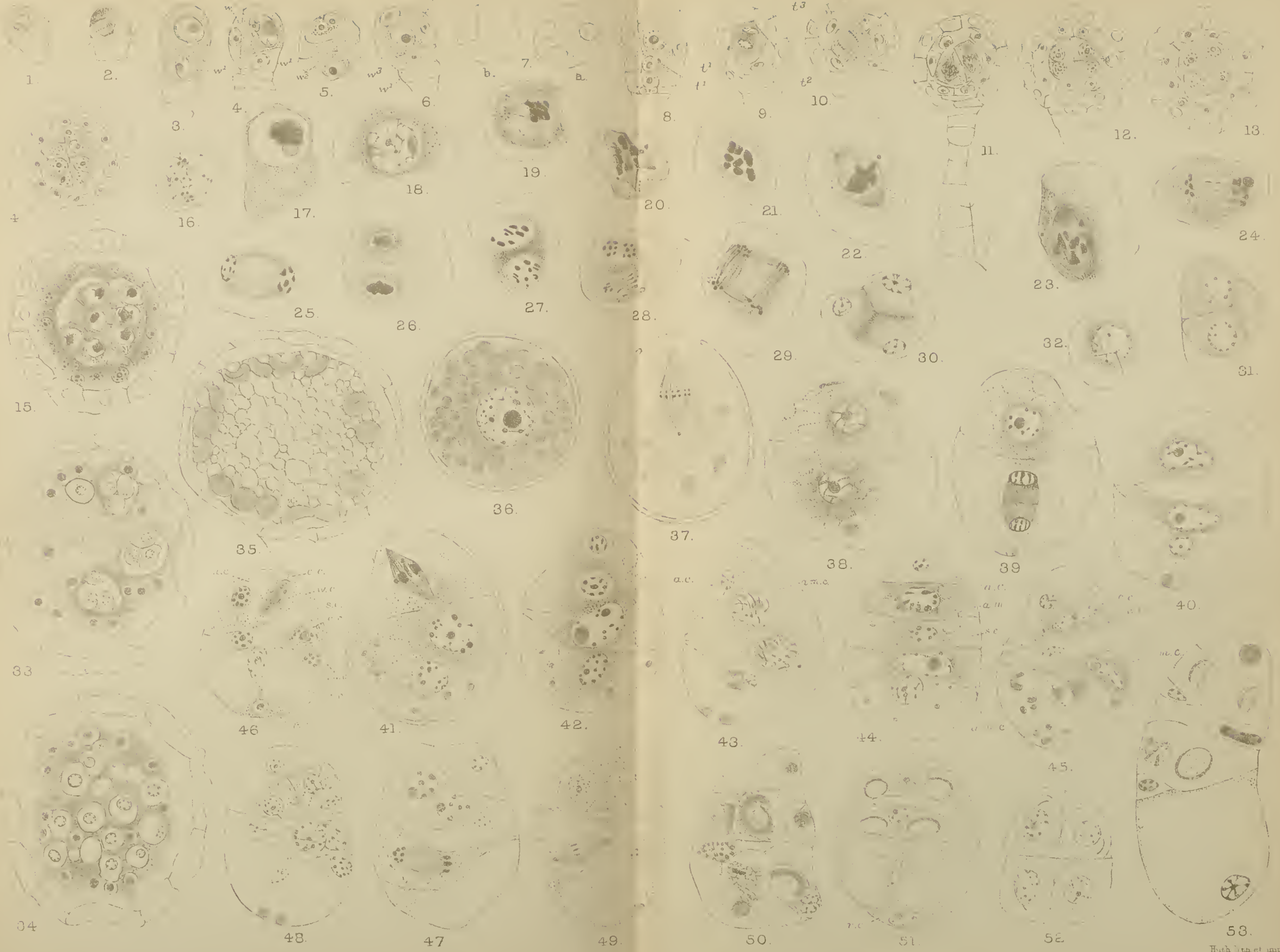

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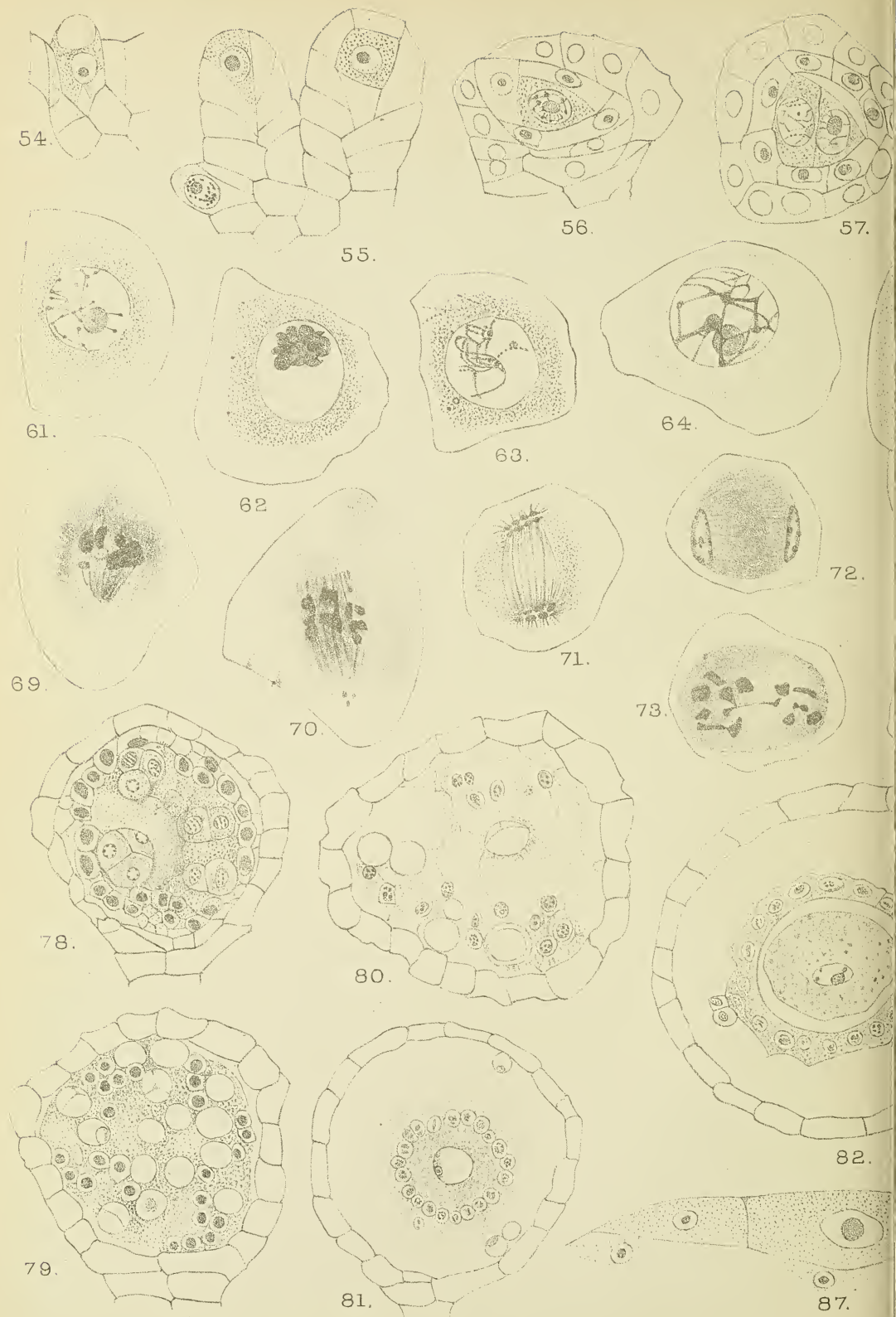

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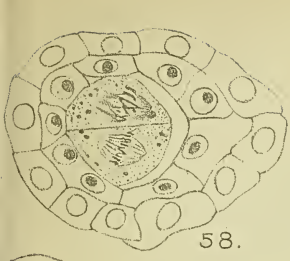
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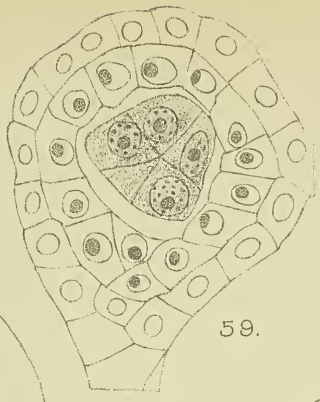
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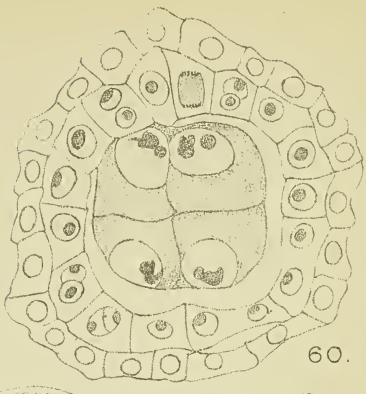
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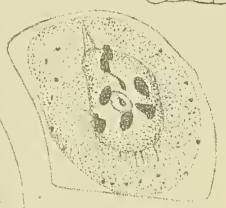
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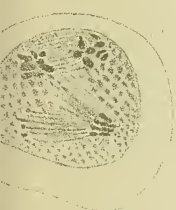
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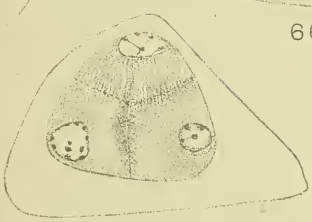
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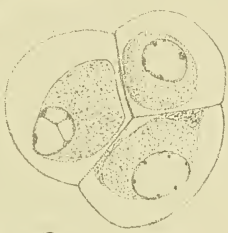
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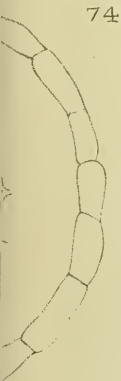

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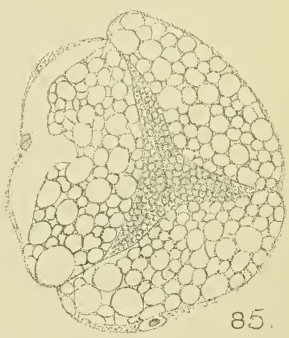
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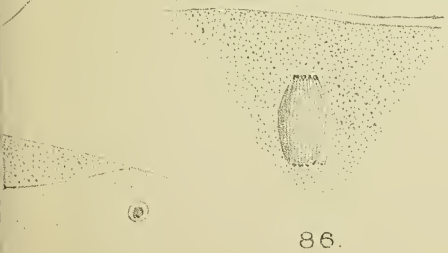
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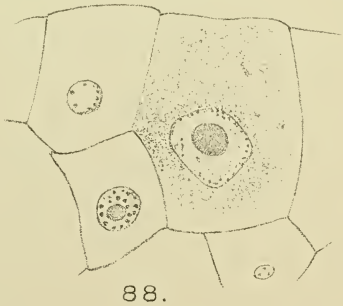
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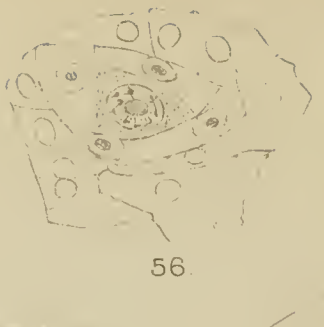
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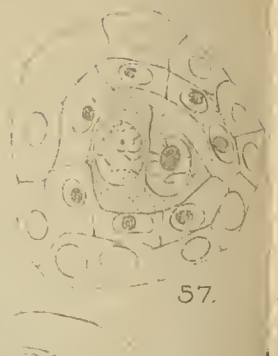
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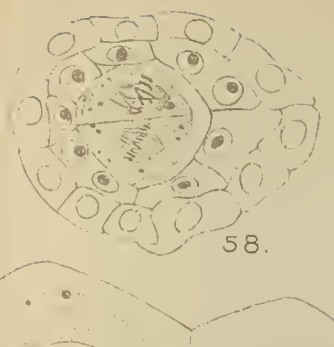
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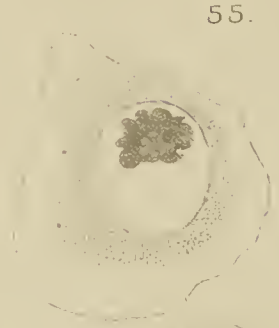
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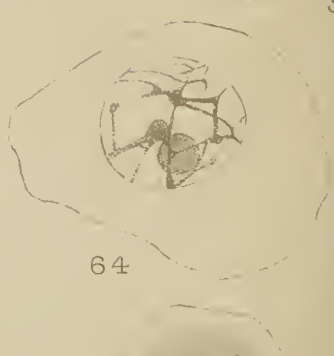
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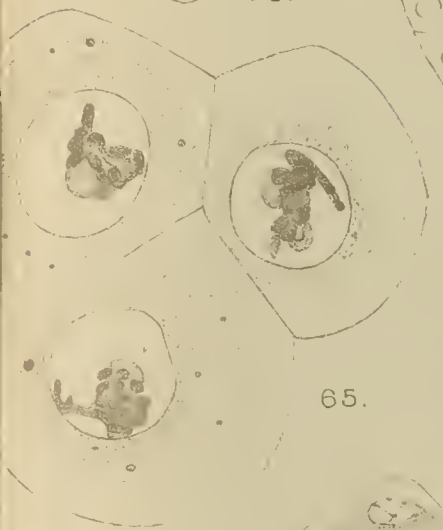
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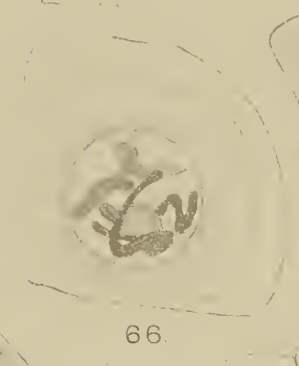
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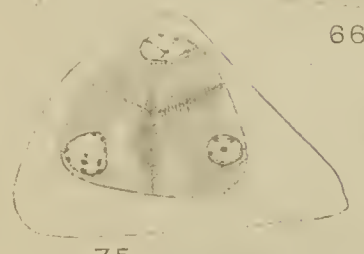
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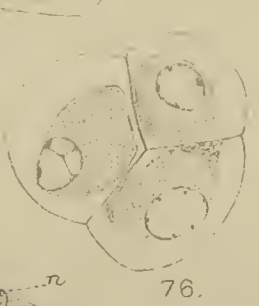
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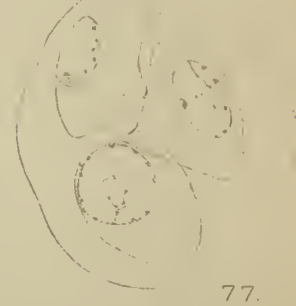
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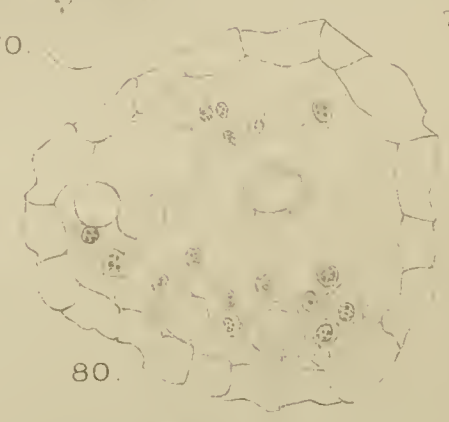
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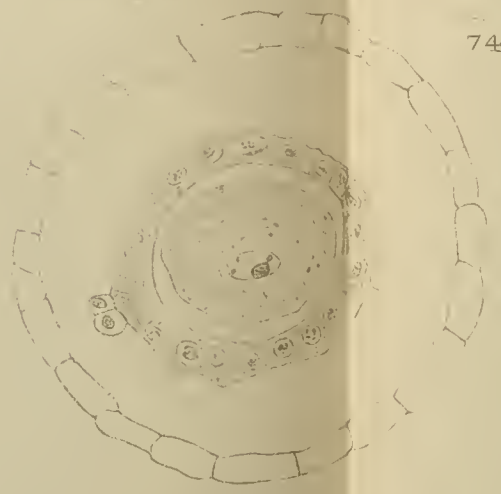
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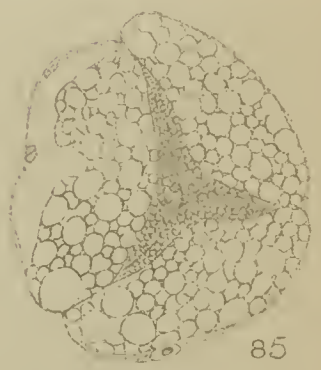
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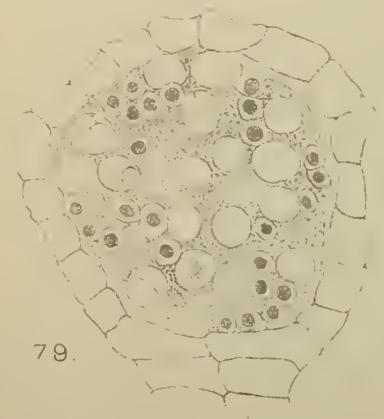
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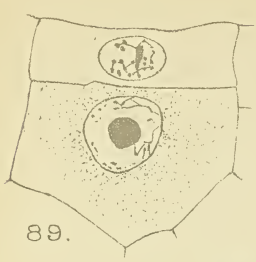

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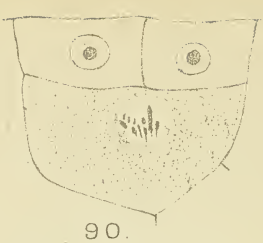

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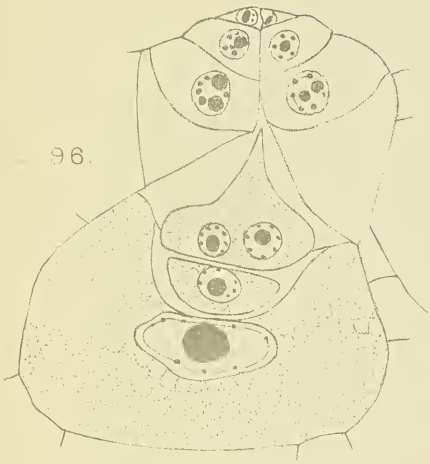

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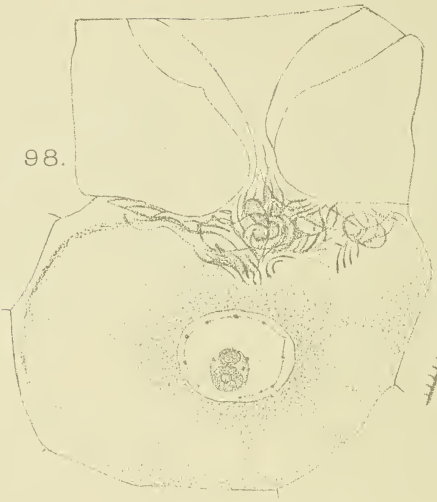

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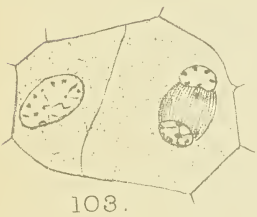

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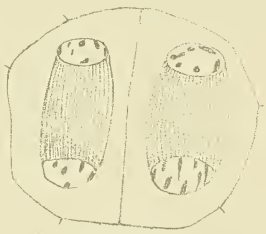

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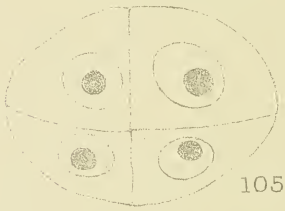

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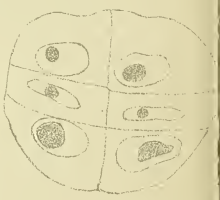

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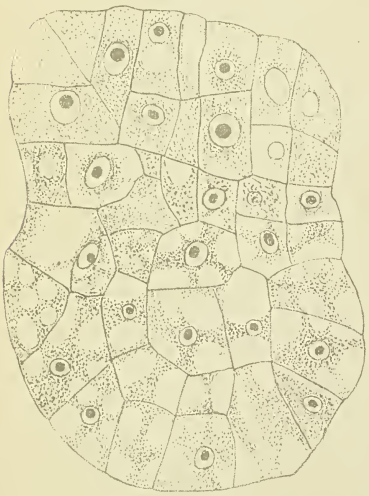

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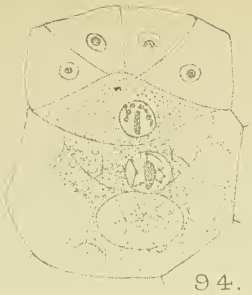

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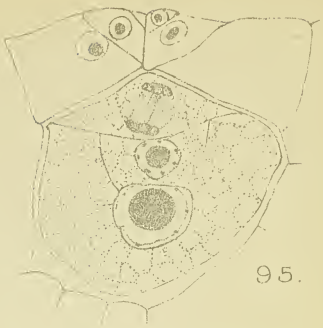

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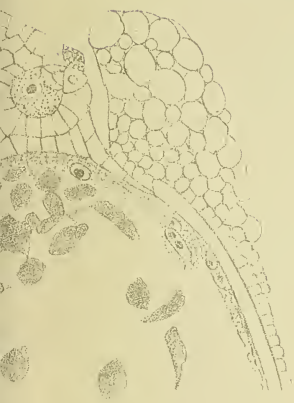

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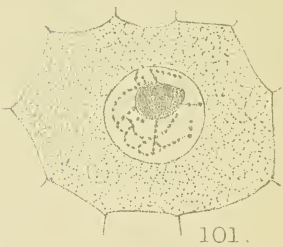

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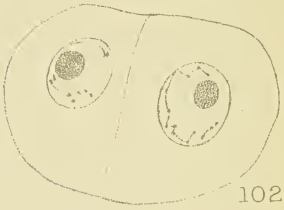

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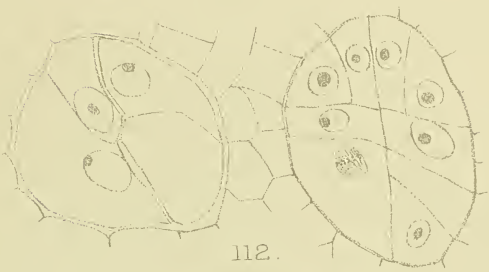

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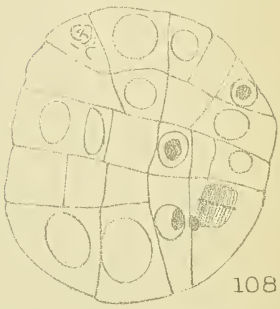

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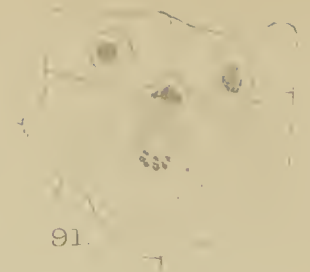

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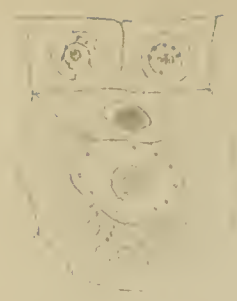

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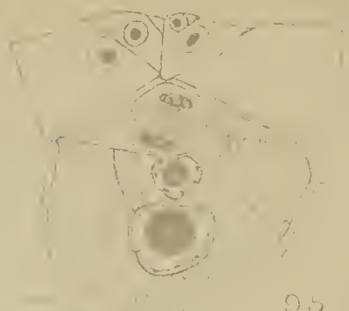

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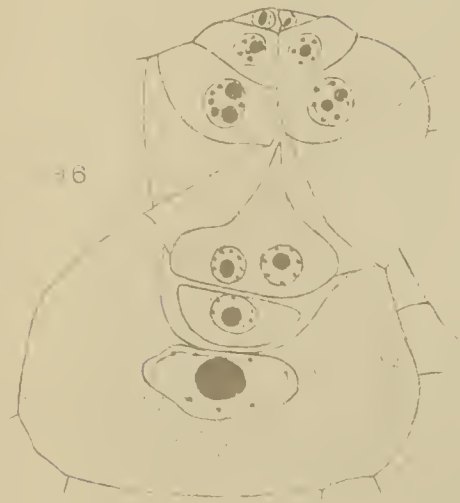

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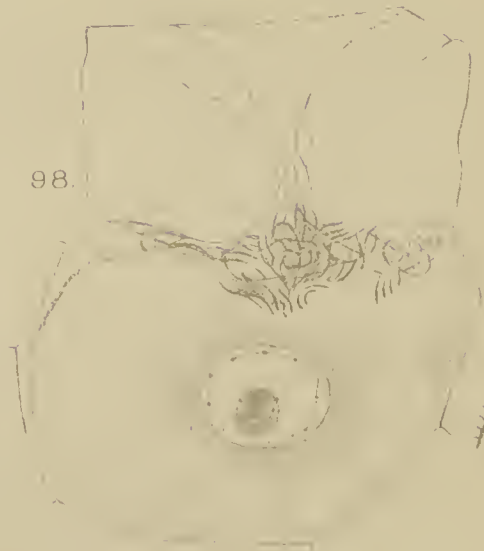

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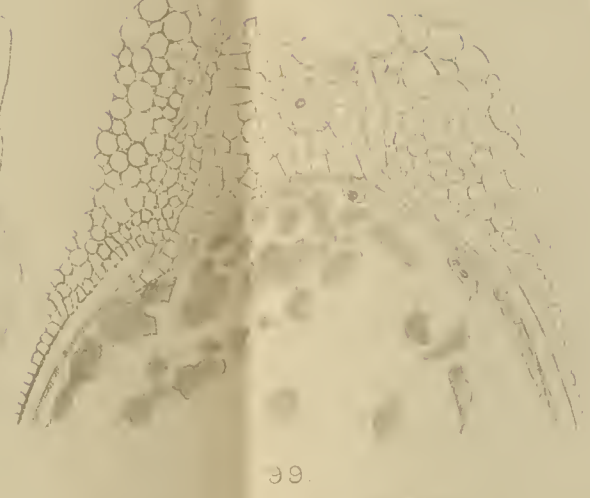

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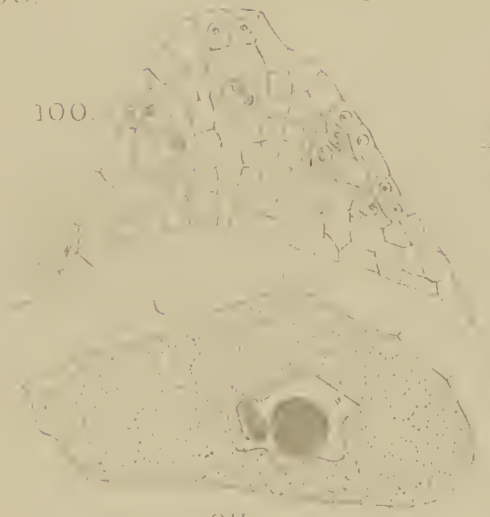

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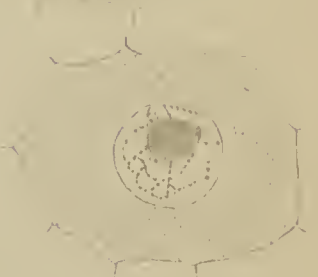

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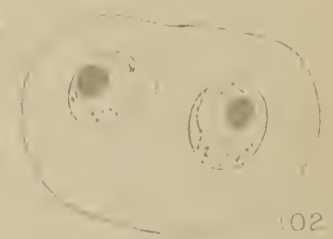

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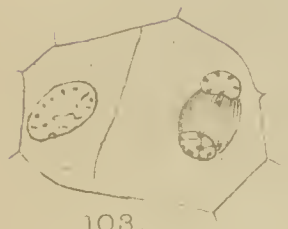

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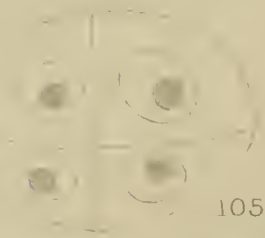

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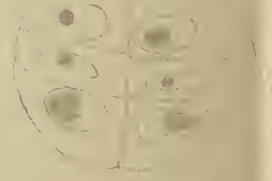

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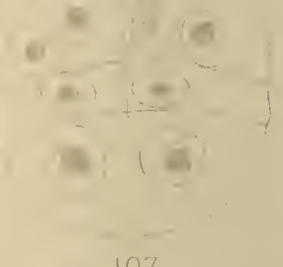

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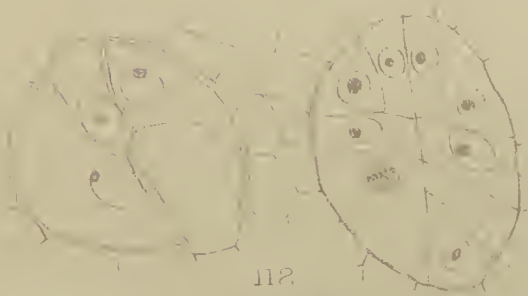

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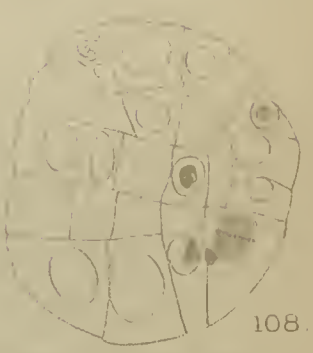

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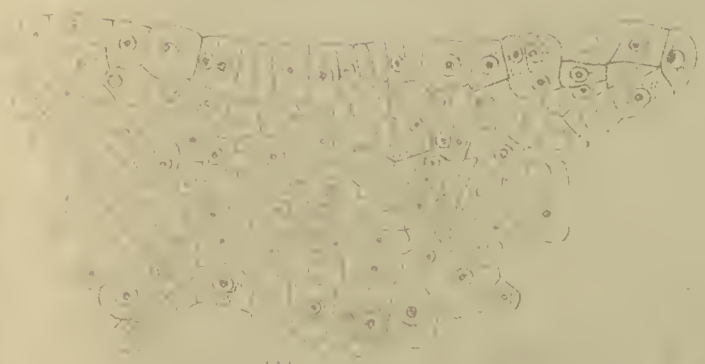

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YASUI—SALVINIA NATANS

Transpiration and Behaviour of Stomata in Halophytes.

BY

E. MARION DELF.

With thirteen Figures in the Text.

I. Introduction.

SALT-MARSH plants are of some interest because, whilst apparently aquatic in habit, they show features, such as succulence, reduction in leaf surface, and even hairiness (in tropical forms), which are commonly associated with a distinctly xerophilous habit.

As early as 1888 Lesage noticed that many plants common to littoral and inland regions were constantly rather more succulent when in the former position, and this led him to undertake an extensive series of culture experiments in order to see whether the thicker leaves could be produced artificially by watering with a solution of sodium chloride. The results were variable, but in *Lepidium sativum* and some other cases there was a distinct increase in thickness and a tendency towards diminution in the leaf surface. In 1891 Schimper found that watering inland plants with saline solutions caused a distinct reduction in the rate of transpiration, and in some cases appeared to have an injurious effect on assimilation; and that in certain cases plants watered with weak solutions showed a distinct accumulation of chlorides within the mesophyll cells of the leaf. The explanation advanced by Schimper and accepted by Pfeffer[1] and Dr. Ludwig Jost[1] was to the effect that these plants are unable to absorb water freely from the soil, owing to the danger of thereby bringing into the tissues injurious amounts of salts. Since the absorption is thus limited, the transpiration must needs also be diminished, and this is brought about by the various xerophilous adaptations to which allusion has already been made; or in the words of Schimper, the watery habitat must be regarded as being 'physiologically dry'.

[1] Pfeffer, Phys. of Plants, Eng. Edition, vol. i, p. 155. Cp. also Jost, Lectures on Plant Physiology, 1907, Eng. Edition, p. 97.

[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.]

In 1894 Stahl published an account of his cobalt paper method, and he found by means of this test that in many halophytes there was a considerable and sustained rate of transpiration. He asserted that in these plants the stomata appear to have lost the power to close, as is often the case in freshwater marsh plants. According to Stahl, the succulent habit has been developed as a compensation to reduce the transpiration, which can no longer be regulated by the stomata.

In 1897 Rosenberg repeated a number of Stahl's experiments, but worked in the field, testing each leaf immediately after detaching it from the plant. By this means he found that whereas immediately after being detached the leaf produced a pink coloration in from one to three minutes, thus showing a rapid rate of transpiration, yet five or ten minutes after being detached the same leaf gave little or no coloration when in contact with the cobalt paper for ten or even twenty minutes. Some of the plants were uprooted and brought to a laboratory, and on testing, gave the slower rate of transpiration; microscopic examination showed that in nearly every case the stomata were closed. Rosenberg suggests that this may have been due either to want of water, or to the darkness to which they were exposed while in the collecting-tin; but in either case his results clearly show that, contrary to the opinion of Stahl, the stomata in halophytes have some power of movement, whilst the results of both Stahl and Rosenberg are in opposition to the commonly accepted view of Schimper, that halophytes are essentially xerophilous in habit.

The present paper is the outcome of some experiments performed at Erquy in September, 1906, at the suggestion of Professor Oliver. Most of the work has been done in the laboratory at Westfield College, but in July, 1909, owing to the kindness of Professor Seward, some observations were made at the Cambridge Botanic Laboratory. My thanks are due to Professor Oliver for supplies of fresh material of *Salicornia annua*, and for advice and continued interest throughout.

In the following pages some account is given of measurements of the rate of water loss in certain typical halophytes, and of the power which these plants possess to absorb water by means of their green surface. Some observations on the stomata of *Salicornia annua* and *Aster tripolium* are also recorded, and these in the main support Rosenberg's conclusion that the guard cells possess the power of movement.

II. Measurements of Loss of Water due to Transpiration.

(a) *Method of determining transpiring areas.* The method most commonly employed in the determination of surface areas was that of tracing the outline on squared paper, and thus estimating the total surface exposed. When mesophytic leaves were used the tracing was done before detaching the leaf, or, where this was not possible, immediately after its removal from

the plant: this was done in order to avoid as far as possible the shrinkage which has been demonstrated to take place in many leaves even before withering becomes perceptible.[1]

For the very small boat-shaped leaves of *Suaeda*, and for the jointed swollen stems of *Salicornia*, I have used methods which were originally suggested to me by Mr. T. G. Hill. In the case of *Suaeda* ten or twelve leaves were cut off quickly from the stem and placed side by side on paper ruled in millimetre squares. The outline of the whole was then traced with a sharp pencil, and twice the area marked out was taken as a rough approximation to the transpiring surface of the leaves. This was repeated until all the leaves from the experimental shoot had been measured. The surface area of the stem, if this was herbaceous, was also included in the total transpiring surface thus estimated.

In the case of *Salicornia*, a number of measurements of the diameter were made by means of a micrometer screw reading to $\frac{1}{100}$ mm.; usually one measurement was made near the upper part of each internode. The

FIG. 1. Single leaf of *Suaeda maritima*.

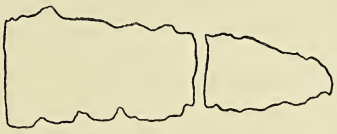

FIG. 2. Outline obtained by placing leaves of *Suaeda* side by side, and tracing area covered on squared paper.

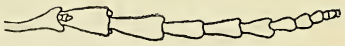

FIG. 3. A shoot of *Salicornia annua* with uneven internodes.

height of the shoot was measured, and the transpiring surface was then estimated as [average diameter $\times \pi \times$ height]. Now the diameter varied considerably amongst the specimens which were examined. Some possessed stems with a nearly even cylindrical surface, and tapering only slightly at the tip; others showed an uneven surface, each segment being wider above than below, and more or less laterally compressed in the upper part: the stem often tapered considerably at the tip. As far as possible, even, bluntly ending stems were chosen for experiment, but in the specimens with tapering internodes a very fair average diameter can be found by measuring just below the broadest region, taking if necessary the mean of the narrower and wider dimensions at that level. For example, in a small plant with markedly tapering internodes the following figures were obtained in measuring the internode next above the hypocotyl:—

[1] Thoday, D. ('09): Experimental Researches on Vegetable Assimilation and Respiration, V. Proc. Roy. Soc., B., vol. lxxxii, 1909.

TABLE I

Measurements of Diameter of a Single Internode at Different Levels.

Level.	(*i*)	(*ii*)
A	3·25 mm.	3·8 mm.
B	3·37 ,,	3·7 ,,
C	2·75 ,,	3·1 ,,
D	2·35 ,,	2·55 ,,
E	2·1 ,,	2·01 ,,

Average diameter 2·898 mm.

The levels at which the measurements were made are shown in Fig. 4 (i). At the level B the cross-section is roughly elliptical in outline; at the level E the outline of a cross-section would be almost perfectly circular. In this experiment measurements of the greatest and least diameter

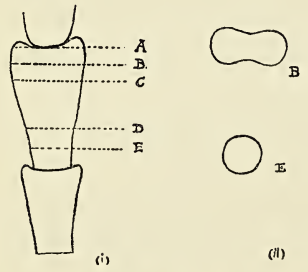

FIG. 4. (i) Internode of a shoot of *Salicornia annua*, from which measurements recorded in Table I were made, at the levels A, B, C, D, E. In (ii) are shown the outlines of cross-section at the levels B and E.

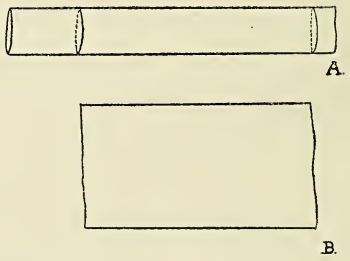

FIG. 5. Showing at A the short glass rod from which the celluloidin film B was obtained.

were made at each level (Table I, columns (i) and (ii)). The mean of all these readings is nearly 2·90 mm., and the mean of the two readings at the level C is 2·92 mm. Thus for all practical purposes the mean diameter just below the widest part of the internode represents the mean diameter for that internode. These readings can, with a little practice, be made easily and quickly on the growing shoot, and thus errors due to shrinkage during manipulation are avoided, but some care must be taken not to bruise the surface of the plant when adjusting the micrometer screw.

At first sight this method may seem too rough to be of any value for quantitative work, but the following experiments show that the error is less than might have been anticipated.

A length of 3 cm. was first marked off with Indian ink on a short glass rod. The distance between the marks was read with a lens magnifying ten times, and the mean of three readings gave the mean length

between the marks. The diameter was measured with a micrometer screw. The rod was now dipped into a solution made by dissolving Schering's celluloidin in equal parts of alcohol and ether. On withdrawing the rod and turning it gently for one to two minutes, a firm film was formed over the whole of the glass rod. This was now cut as nearly as possible above the Indian ink marks, and split along its length with a sharp knife, so that a small and nearly rectangular film was removed from the measured region of the glass rod. The film was *immediately* placed with the inner side downwards upon paper ruled in millimetre squares, and the area found. After a few trial experiments it was possible to make a film which gave the area correct to within 2 %.

Fig. 5 shows at A a short glass rod marked with Indian ink at two places, 3 cm. apart; and at B the outline of the film which was removed from this region and spread out on squared paper. Table II shows the preliminary measurements made on the glass rod, and the surface area found from these measurements and from the film, respectively.

TABLE II

Measurements on Rod.

	Length.	*Diameter.*
(i)	3·05 cm.	0·493 cm.
(ii)	3·07 ,,	0·497 ,,
(iii)	3·05 ,,	0·487 ,,
Mean	3·06 ,,	0·492 ,,

Area of surface of Rod = 4·71 sq. cm.
Area of Celluloidin Film = 4·80 ,,
Error = 1·9%

Using now the same solution of celluloidin and the same time of hardening the film (3 min.), this process was repeated, a shoot of *Salicornia* being substituted for the glass rod. The manipulation was more difficult in this case, owing to the delicate nature of the epidermis and the soft texture of the outer tissues; the crevices at the nodes between the leaf segments and the stem also made it difficult to remove the film intact. However, after a few trials good films were obtained, and these gave areas sufficiently close to those obtained by calculation from measurements with the micrometer screw.

Table III shows at A the measurements obtained from one such shoot of *Salicornia*, and at B the surface areas obtained by estimation from these figures and from the celluloidin film removed from the surface, and outlined in Fig. 6.

TABLE III

A. *Diameters of Successive Internodes.*

(*i*)	(*ii*)
0·354 cm.	0·470 cm.
0·334 ,,	0·342 ,,
0·338 ,,	0·357 ,,
0·326 ,,	0·357 ,,
0·281 ,,	0·331 ,,
0·268 ,,	0·270 ,,
0·184 ,,	0·233 ,,
0·160 ,,	0·203 ,,

B. *Results.*

Height of Shoot = 5·30 cm.
Mean Diameter of Shoot = 0·300 ,,
Surface Area of Shoot = 4·99 sq. cm.
Surface Area of Film = 5·00 ,,
Error = 0·2%

FIG. 6. Outline obtained by removing celluloidin film from shoot of *Salicornia*, and stretching it upon squared paper.

In a second experiment of the same kind the film was removed without injury to the shoot, which was therefore dipped again into the solution, so that a second film was obtained. The measurements are shown in Table IV, and the films in Fig. 7.

TABLE IV

A. *Diameters of Successive Internodes.*

(*i*)	(*ii*)
0.288 cm.	0·324 cm.
0·302 ,,	0·337 ,,
0·275 ,,	0·297 ,,
0·237 ,,	0·274 ,,
0·194 ,,	0·233 ,,
0·162 ,,	0·162 ,,

B. *Results.*

Height of Shoot = 0·257 cm.
Mean Diameter of Shoot = 3·35 ,,
Surface Area of Shoot = 2·70 ,,
Surface Area of Film (1) = 2·70 ,,
(2) = 2·72 ,,
Error = 0·7%

FIG. 7. Outline of two films obtained successively from the same shoot of *Salicornia*.

The most obvious source of error in working with a celluloidin film is the difficulty of removing the film when it is sufficiently firm not to suffer distortion, and is yet not wrinkled or shrunken. The preliminary experiments with a glass rod showed that if the film is not firm, it may stretch in its removal by as much as 20 % of the true area; on the other hand, in removing a film from such a stem as that of *Salicornia*, the edges of the part first lifted sometimes shrivel, and then a very low value for the surface area would be obtained. It will be seen, however, that good films successfully removed gave a very fair approximation to the calculated areas in the case of the glass rod, and they may also show a near approximation to the actual surface area in the case of the shoot. On the whole, then, it appears

that the calculated surface area of *Salicornia* shoots is somewhat high, but may probably be accurate to within 2–3 %.

(β) *Observations on the transpiration of detached shoots during first few hours of withering.* Through the kindness of Professor Oliver, a number of experiments were performed in the field at a salt marsh near Erquy, in the early part of September, 1906. The plants used were a green and a red form of *Salicornia annua*, *Suaeda maritima*, *Atriplex portulacoides*, and as a control, small plants of *Mercurialis annua*, which were taken from one end of a neighbouring cornfield, to serve as a typical mesophyte.

Early in the afternoon of September 8, clumps of *Mercurialis annua*, *Salicornia annua* (both the ' apple green ' and ' crimson plain ' varieties), and of *Suaeda maritima* were collected from their respective stations, and brought to the laboratory. Five shoots of *Mercurialis* were detached, set upright with the stems embedded in a shallow tin of wax mixture, and weighed. Ten shoots of each kind of *Salicornia* were set up similarly, the crimson plain shoots being in one tin together, and the apple green in a separate tin also together; and six small plants of *Suaeda* were placed in the same way at a little distance from each other in a fourth tin of wax. All the tins were placed in the open, after weighing, and the plants were left to wither. The dimensions of the *Salicornia* shoots were found immediately after the first weighing, but the determinations of the surface area of *Suaeda* and *Mercurialis* shoots had to be deferred until the end of the experiment. Since there is undoubtedly shrinkage in the leaves owing to water loss, the area estimations will be too low, and the transpiration values per 100 sq. cm. correspondingly too high; but this only emphasizes the fact that the halophytes examined had transpiration values comparable with or even greater than that of the typical mesophyte, *Mercurialis annua*.

TABLE V

Loss of Weight in Detached Shoots of Mercurialis, Salicornia, and Suaeda.

	Mercurialis.			*Apple Green Sal.*			*Crimson Sal.*			*Suaeda.*		
	Hour.	Time.	Actual Loss.	Hour.	Time.	Actual Loss.	Hour.	Time.	Actual Loss.	Hour.	Time.	Actual Loss.
	p.m. 12.5	hrs.	grm.	p.m. 12.10	hrs.	grm.	p.m. 2	hrs.	grm.	p.m. 2.15	hrs.	grm.
(1)	2.35	$2\frac{1}{2}$	0.075	2.45	$2\frac{1}{2}$	0.106	3.30	$1\frac{1}{2}$	0.058	3.45	$1\frac{1}{2}$	0.106
(2)	4.10	$1\frac{1}{2}$	0.025	4.20	$1\frac{1}{2}$	0.105	5	$1\frac{1}{2}$	0.019	5.15	$1\frac{1}{2}$	0.019
(3)	5.50	$1\frac{2}{3}$	0.017	6.30	2	0.007	6.50	$1\frac{5}{6}$	0.007	6.45	$1\frac{1}{2}$	0.024
	Surface Area 45·14 sq. cm.			24·43 sq. cm.			17·44 sq. cm.			40·38 sq. cm.		
	Loss per hour per 100 sq. cm.			Loss per hour per 100 sq. cm.			Loss per hour per 100 sq. cm.			Loss per hour per 100 sq. cm.		
(1)	0.066 grm.			0.173 grm.			0.226 grm.			0.350 grm.		
(2)	0.037 ,,			0.286 ,,			0.072 ,,			0.062 ,,		
(3)	0.022 ,,			0.038 ,,			0.027 ,,			0.079 ,,		

During the whole time of the experiment the plants were exposed outside the laboratory in a position sheltered from wind and from the morning sun; direct sunlight fell upon the plants only after 12 p.m., and until about 4.30 p.m. It will be seen that the weighings of the crimson *Salicornia* and *Suaeda* were begun later in the day than those of the other two plants; and accordingly both these plants show a higher initial transpiration value than the 'apple green' *Salicornia*. The maximum rate of transpiration per hour per 100 sq. cm. was attained by *Suaeda*. If the water content per 100 sq. cm. be taken as a rough measure of the degree of succulence of the plant, it will be seen (see Table VI) that *Suaeda* was also the most succulent of the plants examined, and it thus appears at once that the succulent habit is not necessarily due to or even coincident with a reduced rate of transpiration.

TABLE VI

Degree of Succulence of Mercurialis, Salicornia, and Suaeda.

	Mercurialis.	*Apple Green Sal.*	*Crimson Sal.*	*Suaeda.*
Fresh Weight	0·560 grm.	1·811 grm.	1·345 grm.	2·240 grm.
Dry Weight at 100° C. . . .	0·129 grm.	0·190 grm.	0·141 grm.	0·219 grm.
Water Content per cent. . .	76·97%	89·5%	89·5%	90·3%
Surface Area	45·14 sq. cm.	24·43 sq. cm.	17·44 sq. cm.	40·38 sq.cm.
Water Content per 100 sq. cm.	0·95 grm.	6·5 grm.	6·1 grm.	10 grm.

During the course of the experiment there is, except in the case of 'apple green' *Salicornia*, an initial transpiration value which quickly shows a con-

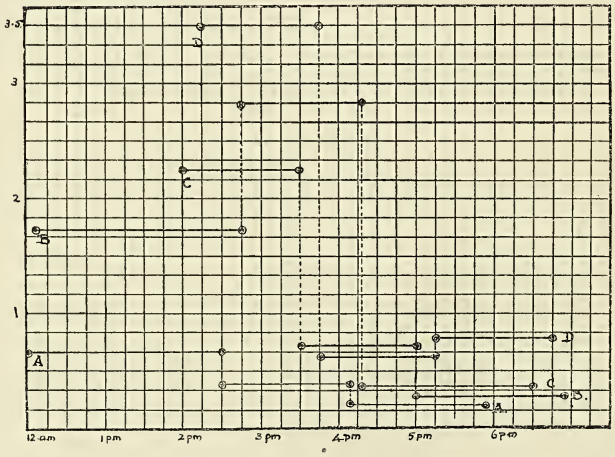

FIG. 8. Curves showing average transpiration per hour per 100 sq. cm. in cut shoots not supplied with water. A = *Mercurialis annua*; B = *Salicornia annua* (crimson red); C = *Salicornia annua* (apple green); D = *Suaeda maritima*. Ordinates represent loss of weight in grammes; abscissae represent time intervals of one hour. Figures taken from Table V.

siderable diminution. In Fig. 8 this is shown graphically; the horizontal line representing the average transpiration during any given period of time.

The *Mercurialis* and 'apple green' *Salicornia* were started practically simultaneously; both have the power of closing their stomata on wilting,[1] but in *Salicornia*, notwithstanding this, there is seen a considerable rise in the cuticular transpiration during the early afternoon; and this probably only fell off when increased sap concentration acted as an inhibiting factor, diminishing the possible water loss. *Suaeda* and 'crimson red' *Salicornia* were started under the atmospheric conditions which caused the increase in transpiration in *Salicornia*, and they show only a steady decrease during the afternoon hours.

Further experiments at Erquy, both on growing and on detached shoots, led to the conclusion that under ordinary conditions transpiration in the halophytes mentioned is of considerable magnitude; that in dry air it would be greater than the supply of water from the roots would allow; and that in nature this is to a large extent obviated by the fact that the layers of air next to the damp earth of a salt marsh contain much more water vapour than the higher layers, whilst periodic flooding renders a large amount of absorption of water possible over the whole surface of the plant; but this point will be considered more fully subsequently.

In July, 1909, with the permission of Professor Seward, further experiments were made at the Cambridge Botanical Laboratory. Some healthy and smooth-stemmed plants of *Salicornia annua* were very kindly sent by Professor Oliver from Cley; these were green, and no forms approaching the 'crimson red' type were available. The experiments were made in the dry air of the laboratory (humidity 60%), but more precautions were used than had been possible in the field laboratory at Erquy; an accurate chemical balance was used throughout.

On July 23 the transpiration of a detached shoot of *Salicornia annua* was compared with that of leaves of *Sedum spurium* and *Vicia cracca*. The former was the most suitable succulent xerophyte and the latter the most convenient mesophyte at hand.

A healthy shoot of *Salicornia* was measured, detached, and the cut end sealed with wax mixture of low melting-point, and a small loop of cotton was fixed to this end. It was then suspended in nearly saturated air under a bell-glass, while two leaves of both *Sedum* and *Vicia* were treated similarly. When all were hung up in the saturated air, they were weighed in order and the time noted; they were subsequently weighed at intervals of 20 minutes, during which time they had been suspended in bright diffuse light in the ordinary still air of the laboratory.

The following table shows the loss of water in these leaves at intervals of 20 minutes during the first three hours of exposure. Throughout this period the temperature varied from 19·8° C. to 20·8° C., and the humidity from 55% to 57%, chiefly owing to intermittent clouds: between 12.50 p.m.

[1] Cp. Rosenberg ('97). See also later experiments.

and 6 p.m. there was also fitful sunshine, but the observations were then made at longer intervals of time. At the end of the experiment the *Sedum* and *Salicornia* appeared fresh, but very slightly perceptibly flaccid, whilst the *Vicia* was very much withered and indeed almost brittle. These figures are shown graphically in Fig. 9.

TABLE VII

Loss in Weight of Salicornia, Vicia, and Sedum for given Time Intervals, per 100 *sq. cm.*

Time.	*Temp.*	*Humidity.*	*Sedum spurium.*	*Sal. annua.*	*Vicia cracca.*	*Water.*
9.40 a.m.	19·8° C.	57 %				
10 ,,	20 ,,	55 ,,	0·084 grm.	0·075 grm.	0·057 grm.	0·234 grm.
10.20 ,,	20 ,,	55 ,,	0·063 ,,	0·058 ,,	0·028 ,,	0·206 ,,
10.40 ,,	20·1 ,,	56 ,,	0·049 ,,	0·067 ,,	0·017 ,,	0·184 ,,
11 ,,	20·2 ,,	55 ,,	0·046 ,,	0·067 ,,	0·017 ,,	0·259 ,,
11.20 ,,	20·3 ,,	55 ,,	0·046 ,,	0·058 ,,	0·013 ,,	0·253 ,,
11.40 ,,	20·8 ,,	57 ,,	0·049 ,,	0·042 ,,	0·009 ,,	0·235 ,,
12.40 p.m.	20·3 ,,	62 ,,	0·115 ,,	0·091 ,,	0·018 ,,	0·684 ,,
6.5 ,,	21 ,,	51 ,,	0·699 ,,	1·099 ,,	0·109 ,,	0·746 ,,
6.30 ,,	21 ,,	65 ,,	0·038 ,,	0·042 ,,	0·005 ,,	0·655 ,,
	Surface Areas		14·32 sq. cm.	5·96 sq. cm.	50·54 sq. cm.	25·49 sq. cm.
	Fresh Weight		0·932 grm.	0·432 grm.	0·487 grm.	

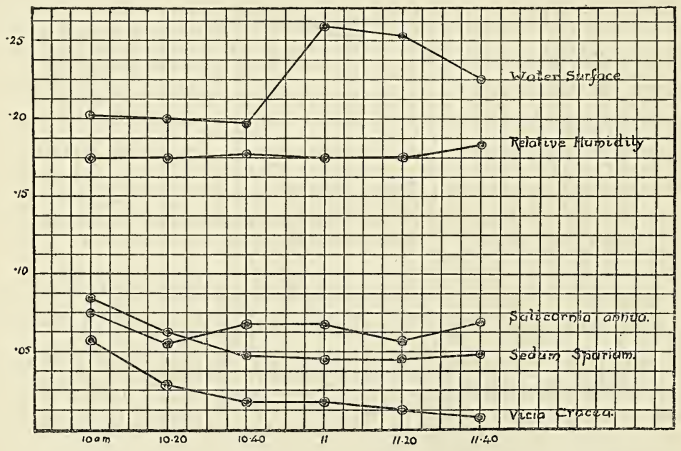

FIG. 9. Curves showing loss of water by weight per 100 sq. cm. in *Salicornia*, *Vicia*, and *Sedum* for first two hours of withering. Abscissae represent time intervals of 20 minutes; ordinates represent loss in weight in grammes. Figures taken from Table VII.

It will be seen that there is a striking resemblance between the behaviour of the two succulent types, and that the transpiration of both of these considerably exceeds that of the mesophytic type. It should, however, be observed that in the case of both *Sedum* and *Vicia*, the leaves used

were detached from the parent plant and were probably transpiring at a rate appreciably greater than would have been the case had they been attached to a shoot bearing other leaves. This phenomenon has been observed by Pringsheim [1] in certain species of *Sedum* and other succulent plants which store up water in the adult leaves, and it seems probable that the same would apply to mesophytes, although this point was not determined. In the case of a species of *Mesembryanthemum* [2] observed by me, a detached adult leaf transpired per unit area from two to four times as freely when withering, as a shoot of the same plant under the same conditions, but bearing two young as well as two adult leaves. If this were true in the case of the *Sedum* and *Vicia* mentioned in Table VI, it would make the contrast between the rates of transpiration in these cases only more strikingly remote from that of *Salicornia annua*.

In Table VIII the proportional or 'relative' transpiration values are given for the three plants, taking the loss per hour per 100 sq. cm. in a freely evaporating water surface as 100. The variations now shown in transpiration must be independent of the purely physical effect of the environment.[3]

TABLE VIII

Relative Transpiration per Hour per 100 *sq. cm. of Sedum, Salicornia, and Vicia.*

Sedum.	*Salicornia annua.*	*Vicia cracca.*	*Water Surface.*
36	32	26	100
30	28	14	100
27	36	9	100
17	26	6	100
18	23	4	100

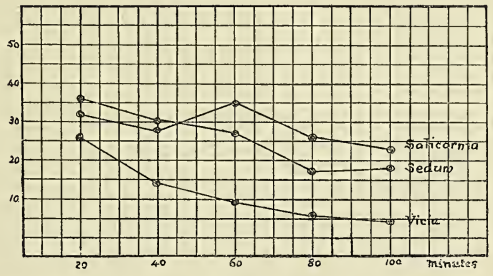

FIG. 10. Curves showing relative transpiration during withering of *Salicornia*, *Sedum*, and *Vicia*. Ordinates represent figures shown in Table VIII; abscissae represent time intervals of 20 minutes each.

[1] Pringsheim, E. ('06): Wasserbewegung und Turgorregulation in welkenden Pflanzen. Jahrbücher für wiss. Bot., 1906.

[2] Probably a variety of *M. speciosum*.

[3] Cp. Livingston ('06): The Relation of Desert Plants to Soil, Moisture, and to Evaporation.

III. Absorption of Water by Green Parts in Halophytes.

In Fig. 9 the relative[1] rates of transpiration during withering are shown graphically. It will be seen that, apart from effects of variations in the physical environment, the two succulent types, *Salicornia* and *Sedum*, show a water loss not only greater but more constant than that of the *Vicia* leaf. Probably the stomata in each case were closed, at any rate after the first half-hour, but in the first two plants, on account of the lack of cuticle and perhaps also the presence of aqueous tissue, this is an insufficient check to the transpiration in the dry air of the laboratory, although the wilting which ensues is less obvious than in the case of a mesophyte.

Notwithstanding the comparatively rapid transpiration any species of *Sedum* can be grown successfully in a dry atmosphere if a supply of water is given to the roots; but in my experience this is not true of *Salicornia* and some other halophytes; on the contrary, this plant appears unable to absorb enough water by its roots to make good the loss due to transpiration in the dry air of a laboratory

On July 19, 1909, a test experiment was performed to determine the transpiration and absorption of a young, healthy, and uninjured *Salicornia* plant in the comparatively dry air of the Cambridge Botanical Laboratory. The plant, which appeared to be perfectly turgid, was washed carefully free from soil, dried, weighed on an accurate balance, and placed with the root dipping into a known amount of water in a weighed vessel. A control vessel of water stood near, and was used to give the evaporation from the exposed water surface in the experimental vessel. After an interval of two hours, the plant was removed, dried, and weighed, and the vessels of water also weighed. On estimating the transpiration and absorption it was found that the plant had transpired 16 %, but had absorbed only 14 % of its own fresh weight, thus showing a loss of 2 % of its own fresh weight.

Such a deficit in the water supply obtained from the roots is, probably, common in nature, in spite of the moist habitat; and it is compensated, at least to some extent, by the power which this plant undoubtedly possesses of absorbing water freely over its whole surface,[2] which is capable of being easily wetted. It seemed worth while to make some observations on the absorptive power in this and other plants.

Three young plants of *Salicornia annua* were cut just above the level of the highest internode which was at all faded, and the cut ends were sealed with wax. The plants were then dipped into water, dried thoroughly with blotting-paper and a soft cloth, and weighed separately. They were then

[1] Livingston ('06): The Relation of Desert Plants to Soil, Moisture, and Evaporation.

[2] Cp. Pfeffer, W. ('00): The Physiology of Plants, English Edition, vol. i, p. 160.

allowed to wither for six, twelve, and forty-seven hours respectively, dipped in water and dried, and again weighed. Immediately after this last weighing the shoots were immersed in water, and the weight again determined after an interval of a varying number of hours. The results are shown in Table IX, at a, b, c. The remaining figures show the results of other similar experiments with the same plant. Shoots d and e showed a well-marked absorption of water after only two hours' immersion, and e withstood without any apparent injury two successive periods of withering; shoots f and g were immersed in a 2 % and 3 % solution of common salt respectively, and consequently showed much less absorption than the others, which were immersed in water only.

TABLE IX

Surface Absorption in Salicornia annua after withering.

	Withering.				*Absorbing (during immersion).*		
Plant.	Time.	Fresh Weight.	Loss.	Loss %.	Time.	Gain.	Percentage of Loss recovered.
a	6 hrs.	0·552 grm.	0·097 grm.	17·5	5 hrs.	0·035 grm.	35
b	12 ,,	0·628 ,,	0·157 ,,	25	12 ,,	0·094 ,,	59
c	47 ,,	0·172 ,,	0·090 ,,	52	26 ,,	0·049 ,,	53
d	2 ,,	0·232 ,,	0·010 ,,	4	2 ,,	0·006 ,,	60
e (1)	2 ,,	0·212 ,,	0·012 ,,	6	4 ,,	0·008 ,,	75
e (2)	11 ,,	0·229 ,,	0·044 ,,	21	15 ,,	0·054 ,,	108
f	4½ ,,	0·211 ,,	0·016 ,,	8	2 ,,	0·002 ,,	12
g	4½ ,,	0·084 ,,	0·006 ,,	7	2 ,,	0·001 ,,	16

It will be seen from the figures in the last column that, with equal intervals of time for withering and absorbing, about half the water loss is regained. The absorption is thus a slower process than that of transpiration; nevertheless, since these plants are frequently submerged in nature, it may often be of real advantage to the plant.

Finally, an experiment was made to determine whether a shoot of *Salicornia* could also absorb water vapour. For this purpose a detached, sealed shoot of *Salicornia annua* was weighed and suspended in darkness in an almost saturated atmosphere for fourteen and a half hours. No visible condensation had taken place at the end of that time, but on weighing a gain of 2 % had been made.

A few other halophytes which have been tested also show this power of absorbing water. In Table X are shown the results obtained with these, with *Sedum*, and with certain mesophytes.

TABLE X

Surface Absorption in certain Halophytes and Mesophytes.

	Withering.				*Absorbing (during immersion).*		
Plant.	Time.	Fresh Weight.	Loss.	Loss %.	Time.	Gain.	Percentage of Loss recovered.
Suaeda maritima							
(1)	3 hrs.	0.391 grm.	0·010 grm.	3	2 hrs.	0·029 grm.	290
(2)	24 ,,	0·552 ,,	0·095 ,,	17	15 ,,	0·018 ,,	20
(3)	63 ,,	0·297 ,,	0·105 ,,	35	9 ,,	0·027 ,,	26
Atriplex portulacoides							
(1)	6 ,,	0·283 ,,	0·039 ,,	14	12 ,,	0·024 ,,	61
					15 ,,	0·028 ,,	
					27 ,,	0·052 ,,	130
(2)	2 ,,	1·035 ,,	0·007 ,,	0·7	2 ,,	0·029 ,,	400
Arenaria peploides	17 ,,	0·392 ,,	0·070 ,,	2	7 ,,	0·070 ,,	100
					14 ,,	0·105 ,,	
					21 ,,	0·175 ,,	250
Sedum album							
(1)	8 ,,	0·246 ,,	0·016 ,,	7	10 ,,	0·019 ,,	120
(2)	12 ,,	0·265 ,,	0·030 ,,	14	15 ,,	0·031 ,,	100
Rumex	2 ,,	2·765 ,,	0·309 ,,	11	4½ ,,	0·282 ,,	90
Tropaeolum	2 ,,	1·118 ,,	0·113 ,,	10	4½ ,,	0·141 ,,	50
Plantago lanceolata							
(1)	2 ,,	0·488 ,,	0·131 ,,	27	4½ ,,	0·180 ,,	
					11 ,,	0·058 ,,	
					15½ ,,	0·238 ,,	182
(2)	12 ,,	0·289 ,,	0·219 ,,	75	15 ,,	0·129 ,,	59

All these plants show a considerable power of surface absorption,[1] and moreover, after immersion, the weight of the shoot is frequently greater than the initial fresh weight. Yet in every case freshly-gathered and apparently turgid shoots or leaves were used, and in the six succulent types, and in *Plantago lanceolata* among the mesophytes, it is almost impossible to tell at the moment of gathering whether or not the leaves are turgid at the beginning of the experiment. *Plantago lanceolata* is not itself a halophyte, but it comes very near to *P. coronopus* and *P. maritima*, which are typically marine ; and perhaps *P. lanceolata* is itself a facultative halophyte. At least it appears that halophytes possess a considerable range in their normal water content, and a power of absorption over the green surface which in nature is probably capable of supplementing the root absorption by the utilization of rain[2] and the standing water often found in a salt marsh.

[1] Cp. Pfeffer, W. ('00) : Physiology of Plants, English Edition, vol. i, p. 160.

[2] Cp. Lundström, A. N. ('84) : Die Anpassungen der Pflanzen an den Regen und den Thau. Henslow, Rev. Geo. ('08) : The Absorption of Rain and Dew by Green Parts of Plants.

Withering shoots of *Suaeda* and of *Sedum album* present a further point of similarity, since in both the old leaves act as water reservoirs for the young leaves at the growing apex. However, in *Sedum* each oldest leaf in turn yields up its water and shrivels;[1] just before shrivelling a single short white rootlet appears, standing just above the insertion of the leaf at right angles to the stem which bears it. During such withering the youngest leaves appear not to suffer in the least from want of water, and the rate of transpiration is maintained almost unimpaired for as long as five or six weeks. In *Suaeda*, on the other hand, no such rootlets were seen, and the old leaves withered several together, whilst the young leaves were perceptibly flaccid, although much less so than the oldest ones. *Suaeda* will withstand desiccation for one week, *Sedum* for as long as six weeks.

The foregoing experiments, although scattered over different seasons and performed under different physical conditions, present, when taken together, sufficient evidence to show that the transpiration of at least some typical halophytes is by no means as reduced in character as has been frequently asserted.

There is indeed little or no cuticle in *Salicornia*, in *Suaeda maritima*, and probably also in some other halophytes. This renders a considerable water loss inevitable, in spite of the frequently closed stomata[2] and the acidity[3] of the cell-sap in the green parts. This is compensated partly by the storage of water in the aqueous tissue and partly by the power which these plants possess of absorbing water over their green surface, a power which must be of much value in the damp and yet often exposed situations in which they are frequently found.

IV. Behaviour of the Stomata in Halophytes.

The behaviour of the stomata in halophytes was subjected to investigation by Stahl[4] in 1894, and by Rosenberg[5] in 1897, in both cases chiefly by means of the cobalt paper test.

Stahl found in this way that the stomata of certain halophytes which he had cultivated in an artificial salt marsh were constantly more or less widely open. He concluded that halophytes resembled freshwater marsh plants in possessing stomata which had lost the power to close.

Rosenberg applied the same method to the leaves of halophytes immediately after they had been detached from the plant growing *in situ*. In all the cases examined by him the stomata, open at first, closed shortly

[1] Cp. Pringsheim, E. ('06): Wasserbewegung und Turgorregulation in welkenden Pflanzen.

[2] Cp. Rosenberg, O. ('97): Ueber die Transpiration der Halophyten.

[3] Aubert, E. ('92): Turgescence et transpiration des plantes grasses. Ann. des Sci. Nat., 1892.

[4] Stahl ('94): Einige Versuche über Transpiration und Assimilation. Botanische Zeitung, 1894.

[5] Rosenberg ('97): Über die Transpiration der Halophyten. Öfvers. af K. Vetensk. Akad. Förhandl.

after the leaf was detached, and this was accompanied by a well-marked diminution in the rate of transpiration. Some of Rosenberg's plants (e.g. *Aster tripolium*) were the same as those previously used by Stahl, and he therefore concluded that the permanently open stomata of Stahl's experiments must have been the result of cultivation, rather than a natural phenomenon. The results of both these workers are opposed to Schimper's view of the reduced nature of the transpiration in these plants.

It would seem worth while to investigate the whole question of the stomata of halophytes in more detail, both on account of the discrepancy in these results, and also from the point of view of the influence of these organs on the process of transpiration; but this has had to be deferred. The following observations are therefore of a preliminary nature; but they indicate clearly a power of movement in the stomata of some halophytic genera, as already asserted by Rosenberg.

In the first place the distribution of the stomata was determined for such types as were available. The numbers are given in Table XI, and each figure represents the mean of ten, or sometimes twelve, countings from strips of epidermis taken from adult leaves and examined fresh. It will be seen that whilst there are comparatively few stomata in *Suaeda* and in *Arenaria peploides*, there are many in *Salicornia annua*, which compares in this respect more nearly with a typical mesophyte, such as *Vicia cracca*. The distribution of stomata in halophytes is therefore a variable feature, and throws but little light on the problem of transpiration in these plants.

TABLE XI

Distribution of Stomata in Halophytes.

Type.	*Stomata per sq. cm.*	
Suaeda maritima	Lower epidermis	6,300
Arenaria peploides	,, ,,	5,500
Aster tripolium	Upper ,,	9,375
	Lower ,,	8,750
Salicornia annua	Flanks	10,370
Sedum album	Upper epidermis	9,370
	Lower ,,	8,750
Vicia cracca	Lower ,,	13,250

In estimating the distribution of the stomata in *Salicornia* it was found that the number and size of the stomata varied much with the age of the internode from which the epidermis was taken. In one plant which had nine internodes in all, and which was six inches in height, epidermis from each internode was examined fresh, and the results are given in Table XII. The internodes are numbered from below upwards, and each number in the first column is the mean of ten readings, whilst the numbers in the remaining columns represent the mean of five readings. In each case the strip of epidermis was examined fresh, and mounted dry.

TABLE XII

Distribution and Size of Stomata taken from Different Regions of Salicornia annua.

No. of Internode.	*No. of Stomata per sq. cm.*	*Condition of Stomata.*	*Mean Diameter of both Guard Cells.*	*Diameter of Rift.*	*Diameter of Pore.*
I	10,500	Widely open	40 μ	20 μ	20 μ
II	9,750	Half shut	22 ,,	10 ,,	4 ,,
IV	11,875	Half shut	26 ,,	—	5 ,,
VI	23,125	Widely open	22 ,,	—	2 ,,
VIII	23,625	Widely open	16 ,,	—	2 ,,
IX	18,750	Unopened	—	—	—

In internodes VI and VIII the stomata were fully formed and open, but the epidermal cells were much smaller than on lower internodes, and probably neither these nor the guard cells had reached their full size. In internode IX the epidermal cells were in a state of active cell-division—the stoma mother-cells were in some cases just cut off; in others they had

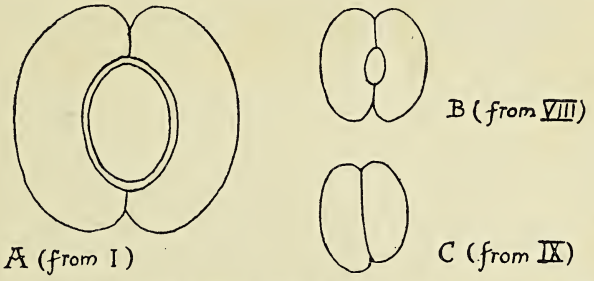

FIG. 11. Outline of stomata taken from the lowest and two uppermost internodes respectively of a shoot of *Salicornia*; drawn to scale and × 500.

just divided to form the guard cells, but in no case had any aperture been formed. It is of some interest to note that the guard cells in the upper and younger internodes open long before the stomata reach their full size. Typical stomata are shown drawn to scale in outline only in Fig. 11.

Some observations were made as to the effects of light and darkness on the stomata of *Salicornia annua* and *S. ramosissima*. From experiments made on July 14, 1909, it was found that stomata, previously half open, closed within three hours when placed in a dark room at nearly the same temperature and relative humidity. In this condition the pore appeared completely closed, but the rift was in every case still visible; within ten minutes all the stomata began to open, presumably owing to the illumination of the microscope stage. Measurements were made of the stomata in the open and closed condition, and from these and from the outlines shown in Figs. 12 and 13, it appeared that guard cells expand

both in the outward and downward direction when the pore opens, and conversely, the total diameter and the depth of the guard cells diminishes on closing: most commonly the rift is still obvious when the pore appears to be completely closed.

In September, October, and November, 1910, fresh material of *Salicornia annua* from various sources was examined for stomata. In some plants obtained from Burnham-on-Crouch in late September, the stomata were all closed and did not open when placed in either a damp or dry

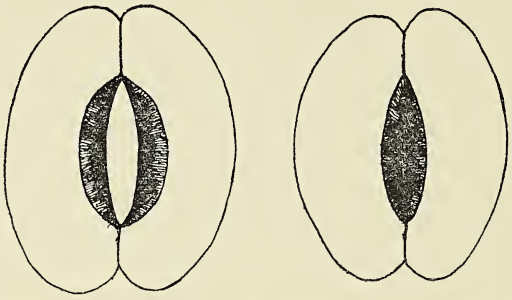

FIG. 12. Outline drawing of stomata of *Salicornia annua* in the half-open and closed condition respectively, seen in surface view from above. × 500.

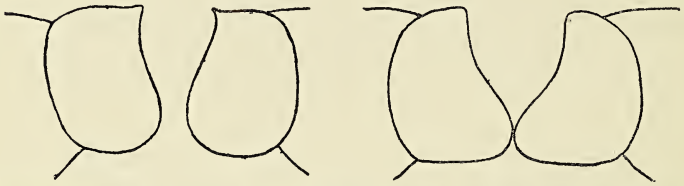

FIG. 13. Outline drawing of stomata of *Salicornia annua* in the half-open and closed condition respectively, but seen in cross-section. × 500.

atmosphere, with sunlight or bright diffuse light. The guard cells appeared to be turgid, but no opening was visible, and even when a strip of epidermis was mounted in water and exposed to bright diffuse light, only a narrow slit-like opening appeared. These plants were in their flowering period.

Other plants from Cley, received early in October, bore young seeds, and the stomata on these shoots behaved similarly; whilst material from Southampton later in the same month, and from Rye early in November, bore stomata which were all closed, and which seemed to have entirely lost the power of movement. It thus appears that in *Salicornia annua*, and presumably also in other annual species of the same genus, the stomata begin to lose their capacity for movement during the flowering season, and finally remain permanently closed.

The stomata of *Aster tripolium* are found on both sides of the leaf; they are from four to five times larger than those of *Salicornia annua*,

and are therefore much easier of observation. Plants examined from the Cambridge Botanic Gardens in July, 1909, and others growing in an artificial salt marsh in Dulwich, in September, 1910, possessed stomata, the guard cells of which moved with ease and rapidity. This is contrary to the result of Stahl,[1] who found that in his cultivated halophytes the stomata were open, but immovable.

In the artificial salt marsh at Dulwich there was but one plant of *Aster tripolium*. This had been grown for some months successfully, and had been watered at intervals with a 2 % solution of common salt. At 8 a.m. on September 20, just before the sun was on this part of the marsh, a strip of epidermis was removed from both sides of one of the leaves, mounted dry and examined on the spot under a microscope. The stomata were widely open in each case, but they half closed whilst still under observation in less than three minutes. At this time the relative humidity in the neighbourhood of the plant was found to be 93·5 %. The whole plant was now darkened for an hour by being covered with dense black cloth. A strip of epidermis from one of these darkened leaves showed about half the stomata with the pore narrowly open, whilst the remainder had the pore completely closed as far as could be seen in surface view. The relative humidity of the air around the plant during the time of darkening was 93·1 %, and this change is so slight as to have a negligible effect on the stomata.

The plant was then uncovered and was exposed to the sunlight, which now fell directly on the leaves. After an hour of such illumination half the stomata on a strip of epidermis were still closed, and the remainder had the pore about half-way open; the relative humidity had, however, fallen to 78 %, and very shortly after all the leaves stood less erect and began to appear limp and flaccid.

Some fine plants of *Aster tripolium* were received in November, 1910. These had many large and healthy leaves, all of which showed only closed stomata. Exposure to sunlight, bright diffuse light, and to electric light, either in dry air or nearly saturated air, did not cause these stomata to open, and this may have been due to the temperature or, more probably, to the time of year.

From the foregoing observations it may be concluded that the stomata of *Salicornia* and of *Aster tripolium* (two of the most typical British halophytes) do not show the features characteristic of either a xerophilous plant, as Schimper's theory would lead one to expect, or of a freshwater marsh plant as Stahl supposed. They rather resemble those of a typical mesophyte in being superficially placed, capable of opening and closing, and sensitive to light and to changes in the humidity of the atmosphere.

From preliminary observations it may be added that the stomata of

[1] Stahl, E. ('94): Einige Versuche über Transpiration und Assimilation. Bot. Zeit., 1894.

Sedum acre and of *Sedum album* are similarly unprotected and mesophytic in character, and these plants also show a high rate of transpiration per unit area. In the course of these experiments the epidermis of plants of *Suaeda maritima* has often been examined, but the stomata have never been found open; the same is true of the minute stomata of *Atriplex portulacoides*, which are further protected by a scaly covering of epidermal hairs.

Summary and Conclusions.

In the course of this paper it has been shown that :—

1. *Salicornia annua* and *Suaeda maritima*, both typical halophytes, have a high rate of transpiration per unit of surface area which is comparable with, or even greater than, that of a typical mesophyte, such as *Vicia faba.*

2. The transpiration rates obtained for these plants are not necessarily maximal, since no precaution was taken to ensure that the plants were absolutely turgid initially, and that the stomata were open; probably with wholly favourable conditions a still more rapid loss of water would ensue. The extent to which the rate of transpiration varies with the age of the plant has not yet been determined.

3. When not already turgid these plants are able to absorb water freely over their whole surface; in this respect they resemble certain mesophytes, such as species of *Plantago* and *Rumex*. They have, however, unlike mesophytes, a certain capacity for storing water.

4. The stomata in *Salicornia annua* and in *Aster tripolium* are not sunken and are not protected by cuticle to any extent; they have a distinct power of movement, and close in darkness. The stomata of *Salicornia* appear to lose their power of movement after the flowering period, and then remain permanently closed; those of *Aster tripolium* were also sensitive to the relative humidity of the atmosphere, being found open in the month of September in air nearly saturated with water vapour, and closed shortly afterwards on the same plant in air of 75 % relative humidity: in winter this power of movement diminishes, or perhaps completely disappears.

5. Observations on the transpiration and stomatal behaviour of other halophytic plants are needed before any conclusions applicable to the whole group of plants can be made.

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The Function of Hormones in regulating Metabolism.[1]

BY

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AND

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IT has long been known that a variety of substances which are generally regarded as chemically neutral are powerful stimulants of vital activity if used in very minute proportions but potent lethal agents if allowed to operate in larger amounts. Few botanists are unaware of the destructive effect that is exercised on plant tissues by the common anaesthetic chloroform and of the use that is made nowadays of hydrocarbons, such as toluene and naphthalene, as 'sterilizing' agents; the acceleration of the flowering processes in plants and of the ripening of fruits by means of ether vapour may also be cited as familiar cases of stimulative action: we have been led to study the effects produced by such neutral substances in the hope of arriving at an explanation of their phenomenal activity. In a recent communication to the Royal Society we have described and discussed experiments made with *Prunus Laurocerasus* in which leaves of this shrub were exposed to a variety of vapours and solutions in presence of Guignard's alkaline picrate paper; this test affords a means of detecting minute quantities of hydrogen cyanide, the yellow paper becoming orange and ultimately brick-red under the reducing influence exerted by this compound.[2] When a substance enters the leaf and conditions hydrolysis of the cyanophoric glucoside *Prulaurasin* ($PhCH(CN).O.C_6H_{11}O_5$), which the leaf contains, the intrusion is indicated by an escape of hydrogen cyanide. As the change that is initiated by the entering substance is cumulative, for reasons that will be apparent later on, a minute proportion of substance produces a relatively large effect; the test, therefore, becomes one of extraordinary delicacy.

The title of our communication[3] may be referred to as an indication, in our opinion, that the inquiry is one in which many issues are to be considered.

[1] Studies in Enzyme Action XIV; for XIII, see Roy. Soc. Proc., B., lxxxii, 1910, pp. 349-67.

[2] Cp. A. C. Chapman, The Analyst, 1910, with reference to limitations, pp. 35, 269.

[3] The Origin of Osmotic Effects. III. The Function of Hormones in stimulating Enzymic Change in relation to Narcosis and the Phenomena of Degenerative and Regenerative Change in Living Structures. Roy. Soc. Proc., B., lxxxii, 1910, pp. 588-602.

[Annals of Botany, Vol. XXV. No. XCVIII. April, 1911.]

It is obvious that the leaf, like the seed, must be provided with a protective mechanism preventing both escape and entry of all but a very limited number of substances. The invaluable series of observations made by Adrian J. Brown[1] with blue barley, *Hordeum vulgare*, var. *caerulescens*, have shown that the grain is surrounded with a membrane of extreme tenuity, which is penetrable by but few mineral substances and also not by sugars, &c.; our observations show that the leaf is protected in a precisely similar manner. The protection is obviously afforded by what is perhaps best termed a *differential septum*, the term semipermeable membrane being far too narrow and misleading as an indication of the functional significance of the membrane. As it appeared to be desirable to give a special name to the class of substances that pass through differential septa such as are met with in the barley grain and laurel leaf, exciting activity within the cells when they have thus gained an entry, we have proposed that the term *Hormone*—given by Starling to certain excitants of functional activity in the animal organism (including carbon dioxide)—should be applied to all such substances. The hormones as a class are substances which have but slight attraction for water and may therefore be spoken of as '*anhydrophilic*'; hydrophilic substances generally fail to penetrate septa which are selective to the degree manifest in cereal grains and the laurel leaf. We believe this criterion to be one of special importance.

In all our experiments freshly-picked leaves were either exposed to the vapour of the substance to be tried in corked tubes or nearly immersed in solutions. The tests were made both at room temperatures and at 37° with leaves picked at different periods throughout last year (1910). Young leaves picked in the spring responded far more rapidly to stimulation than older leaves collected in the late summer and autumn.

As in the case of the laurel leaf, the substances found to be inactive were: *weak* solutions of mineral acids, caustic soda and most metallic salts; the active salts were mercuric chloride (not nitrate nor sulphate), cadmium iodide (not chloride), sodium and potassium fluoride. Iodine, carbon dioxide and hydrogen sulphide act slowly, ammonia very rapidly.

Acids of the acetic series were found to be effective in the order observed by Loeb and others, activity being most marked in the case of the least soluble acids of highest molecular weight.

The same may be said of the alcohols of the methylic series. Lactic, benzoic, picric and salicylic acids act slowly, oxalic, tartaric and citric acids are inert.

Volatile hydrocarbons, especially benzene and toluene, carbon bisulphide, chloroform, ethers and ethereal salts, aldehydes, acetone, camphor, piperidine and phenols are all very active excitants; paraldehyde in aqueous solution is also very active.

[1] Annals of Botany, vol. xxi, 1907, p. 790; Roy. Soc. Proc., B., lxxxi, 1909, p. 82.

Not only does the colour change when the leaf is exposed to these various substances but the leaf becomes flaccid and the amount of reducing sugar in it is largely increased.

Mirande, in a recent communication to the French Academy,[1] has given a long list of organic substances of various classes which will penetrate into the leaf but has been unable to draw any definite conclusion as to the existence of a relationship between structure and activity. In our opinion, the difference is not one of structure, except in so far as structure determines the degree of affinity of the substance to water and its solubility relationships.

The picture that we have formed of the mode in which hormones gain an entry involves the conception that the surfaces of the intermolecular spaces in the differential septa are coated with a protective sheath of molecules of 'hydrone', OH_2—the fundamental molecule of water.

Anhydrophilic substances are able to escape the gauntlet and pass through almost without hindrance, whilst those to which the molecules cling are unable to run the blockade, because the hydrone molecules attached to them are so much attracted by and attractive of those attached to the passages in the septum, so much under the influence of the water in which they are dissolved, perhaps also too heavily laden with hydrone and therefore too big to get through, although not too big in themselves.

The hypothesis on which this conclusion is based has been developed in a communication made by one of us to the Royal Society [2] and also in two articles in 'Science Progress', Nos. 11 and 12, January and April, 1909. The chief contention, on which stress is laid, is that the unit molecule OH_2 is eminently unsaturated and active; consequently that water itself is probably a mixture of complex molecules formed by the union of hydrone molecules in various proportions—perhaps in twos, threes, fours and fives—this mixture being saturated with hydrone in proportions which must be supposed to vary with the temperature; hence, perhaps, the increase in the rate of change in aqueous solutions as the temperature rises. Foreign molecules, when dissolved in water, are supposed to have the effect of interfering with the formation of the various polyhydrones, the consequence being that solutions are richer than water in hydrone the larger the proportion of the solute: the attraction which the hydrone molecules have for each other is supposed to condition the so-called osmotic pressure effects.

The main effect produced by hormones when they gain entry into the living cell is the stimulation of enzymic activity. In our communication to the Royal Society we have pointed out that it is to be supposed that they exercise a determining influence in regulating metabolism in plants as well as in animals; as the section dealing very briefly with their influence on

[1] Comptes rend., t. cli, 1910, p. 481. [2] Roy. Soc. Proc., B., lxxxi, 1908, p. 80.

plants was omitted from the communication we may be allowed to reproduce it verbally as presented to the Royal Society:—

'It is the common practice among gardeners in the afternoon, after watering the plants in a glass house, to spray the leaves more or less heavily with water and then to close the house. During the daytime, while regenerative changes prevail, the proportion of diffusible hormones in circulation must be relatively small and water will have little tendency to pass in through the leaf surfaces; as the light diminishes, degenerative changes set in and gradually increase in intensity—these undoubtedly give rise to carbon dioxide and other hormones which serve to induce the entry of water at the leaf surface. The development of perfume by flowering plants, &c., in the evening, is clearly an external manifestation of the beginning of the degradation process; flowering, in fact, marks the onset of the period when degenerative processes begin to prevail and the accumulated stores of material are set in circulation to develop the reproductive organs and seed. The perfume itself must exercise a stimulative influence on the plant in which it is produced.

'Attention has been directed to the effects produced by sterilizing soils more or less completely through recent work done in the Rothamsted laboratory by Russell and others. Partially sterilized soils are more rapidly oxidizable than unsterilized and the fertility of a soil is greatly increased by partial sterilization. These results are attributed with reason to the destruction of protozoa, which normally fatten on the Bacteria and prevent these latter from determining the gradual break-down of organic matters in the soil into the materials which serve as plant food. The nitrifying Bacteria are killed off but other Bacteria survive and these tend to produce ammonia among other things—so that partially sterilized soils are rich both in carbon dioxide and ammonia in comparison with the unsterilized. We are inclined to believe that these two factors are of prime importance in facilitating plant growth by affording the stimuli required to determine the degenerative changes involved in the translocation of nutritive materials.

'Mr. Pickering has shown that, in soils which have been more or less effectually sterilized by heating, the germination of seeds takes place much less rapidly than in unsterilized soils, if at all. He attributes this result to the production of a substance or substances which have a directly toxic effect. We are inclined to think that in such soils the necessary stimulus to the inhibition of water and germination is lacking, chiefly because carbon dioxide and ammonia—the latter especially, perhaps—are produced only in abnormally small amounts, if at all. We doubt if seeds would germinate in pure sterile water.[1]

[1] It is well known that it is difficult to sterilize the exterior of seeds. Our observations show that ordinary sterilizing agents should not be used, as these will penetrate into the seed. Taking into account Adrian J. Brown's observations, it is clear that one of the best ways to sterilize cereal

'The experiments made at the Woburn Fruit Farm by the Duke of Bedford and Mr. Pickering have brought into prominence the highly deleterious effect of grass on the growth of trees and the earlier annual maturity of trees the roots of which are covered with grass.

'Mr. Pickering, after testing various possibilities, has been led to assume that toxic substances are formed by the growth of the grass and that these affect the underlying roots of trees. Here, again, taking into account the pronounced aminophilic habits of the Graminiae, we are inclined to think that the roots of trees under grass suffer because they are more or less completely deprived of the stimulus afforded by the greater proportion of ammonia and carbon dioxide in bare soils.

'It has long been known that under certain conditions sulphate of ammonia has advantages over nitrate of soda as a nitrogenous manure. It is customary to attribute the difference to the fact that ammonia salts are less easily washed out of the soil. We are inclined to think that the stimulative influence of ammonia should also be taken into account. It is possible that the value of ammonia in comparison with other forms of nitrogenous fertilizer has been somewhat overlooked and attention concentrated too much on nitrification in soils.

'Lastly, we may refer to the nodular growths on the roots of leguminous plants; these are known to be most essential to the proper growth of the plant but their function is by no means clear; it is well known that they are the seat of bacteroids and it may be that these function as assimilators of atmospheric nitrogen gas and convert it into ammonia; or it may be that they exercise digestive functions and serve to "deamidate" amino-compounds. At all events, they are distinctly alkaline, whereas the root sap is acid. Moreover, it has been shown by Hutchinson and Miller that, when distilled with magnesia under reduced pressure, the nodules furnish more ammonia than do the roots (0·043 per cent. against 0·016 per cent.). We suggest that some part at least of the influence exercised by the nodules may be due to their aminogenetic power. We propose to make this assumption the basis of experimental inquiry.'

Most leaves become more or less brown when exposed to the action of either chloroform or toluene; the most striking exemplification of this change that we have met with is afforded by *Aucuba Japonica*, the common spotted Japanese laurel; when subjected to the action of substances which

grains, at all events, would be to steep them in sulphuric acid and then to wash thoroughly with sterilized water under antiseptic conditions.

At a recent meeting of the Institute of Brewing (Journ. Inst. Brewing, No. 3, vol. xvi, 1910, pp. 253, 259), when German brewing practice was under discussion, surprise was excited by the statement that it had been found advantageous to steep barley for malting in weak sulphuric acid; it was suggested that sulphurous acid was meant but this was denied and reference was made to Adrian Brown's observations as showing that the acid would not enter the grain; but the obvious value of such treatment was in no way emphasized.

determine the liberation of hydrogen cyanide from the cherry laurel, the leaf of this shrub changes in colour from green, not merely to brown but to a rich chocolate brown-black and ultimately to black. On this account, as it carries its own indicator, it may be used even more effectively than the laurel leaf and with less trouble; otherwise its behaviour is precisely similar to that of the laurel leaf.

It has been shown by Bourquelot and Hérissey[1] that all the organs of *Aucuba*, the seed kernel especially, contain a crystalline glucoside, aucubin, $C_{13}H_{19}O_8$, which is resolved by 'emulsin' into glucose and aucubigenin, $C_7H_9O_3$(?). This latter, however, is so unstable a compound that it has not been isolated; as it is liberated it undergoes decomposition spontaneously into a black substance that is insoluble in water, alcohol, ether and even in a solution of caustic soda. According to Bourquelot and Hérissey, the change takes place in absence of oxygen; Maquenne and Demoussy,[2] who have used *Aucuba* together with other leaves in studying the effect of ultra-violet light in comparison with that of heat and chloroform, have arrived at a similar conclusion.

Maquenne and Demoussy have stated that if the leaf be kept in boiling water sufficiently long to destroy the enzyme, it nevertheless blackens subsequently; they have attributed the change to the slow hydrolysis of the glucoside under the influence of acid present in the leaf. We have not observed this effect when the leaf has been kept sufficiently long in boiling water; neither the leaf nor the aqueous extract in contact with it has changed in colour, although this latter has blackened rapidly after emulsin has been added to it.

Several varieties of *Aucuba Japonica* are in cultivation—most of these, especially the *longifolia* variety, behave precisely like the common form; but one (*A. Japonica*, var. *vera*) which we owe to the courtesy of Mr. L. R. Russell, of Richmond, does not blacken to any appreciable extent. Berries of this variety obtained by fertilizing with pollen from the male of the common form evidently contain much aucubin, as they blacken quite readily; it is not improbable, therefore, that *Aucuba* may furnish an interesting subject of study from a Mendelian standpoint and we hope to carry out experiments from this point of view.

Aucubin has been separated from a number of varieties of *Aucuba* by C. Lebas.[3]

The only other shrub we have met with that is at all comparable in its behaviour with *Aucuba Japonica* is *Azara microphylla*; the leaves of this shrub become a rich brown when exposed in chloroform vapour. We have not succeeded in obtaining from this an extract which changes colour when

[1] Ann. Chim. Phys., 8me sér., t. iv, pp. 289–318.
[2] Comptes rend., t. cxlix, 1909, p. 957.
[3] Journ. Pharm. Chim., t. xxx, 1909, p. 385.

mixed with 'emulsin'. Dogwood, the only English ally of *Aucuba Japonica*, does not blacken.

The leaves of the well-known shrub *Garrya elliptica*, the male of which is grown on account of its decorative green catkins, change to an ugly grey colour in chloroform. The colourless extract obtained on boiling the leaves in water assumes a beautiful blue colour when digested with 'emulsin'. We shall endeavour to extract the β-glucoside which is indicated by this behaviour.

Two other species of *Garrya*, *G. macrophylla* and *G. Thureti*, that we have tested behave similarly, although the latter gives but a weak effect. [Since this account was written, Hérissey and Lebas have recorded the isolation of aucubin from these two species and also from *G. elliptica*.[1]] Aucubin has also been separated from several species of *Plantago* (Bourdier[2]).

It is well known that the seed-pods of many leguminous plants ultimately become black. Apparently, in most cases, the effect cannot be much hastened by means of chloroform—as a rule, the effect can only be produced 48–24 hours before the seed is ripe; it would seem that either the enzyme or the substance which affords the black product comes into existence only at a late stage of growth. In the case of the ordinary Broad-bean, however, the pod can be caused to blacken at any time by exposure to chloroform. It is well known to botanists that the leaves of parasitic plants generally blacken with extreme readiness. We have found that the leaves of *Drosera* become intensely black when placed in toluene vapour.

The last case to which we will refer is that of Mangel-wurzel. It is well known that when the root is pulped it changes colour; if exposed in chloroform it soon blackens but the blackening is at first local. The blackening is shown also by sugar-beet but far less intensely. If a slice of the root, cut horizontally, be taken, the blackening takes the form of more or less circular rings of black dots; in slices cut vertically, the whole vascular system is seen outlined in black. Gradually the colour spreads to the soft tissue outside the vascular system—whether because of the diffusion of the colouring matter or because the action actually extends into this region, we cannot say at present. We are inclined to think that in this case the localization of the colour in the vascular system is due rather to the localization of an enzyme than to that of a colouring material. We intend, if possible, to study these and other cases of melanism induced by anaesthetics during the coming season, in the hope of deciding an issue that has already received attention at the hands of various observers several of whom have correlated the change with that occurring in cases of alkaptonuria in the human subject.

It would seem probable that in the case of many fruits the final

[1] loc. cit., 7me série, t. ii, 1910, p. 490. [2] Journ. Pharm. Chim., t. xxvi, 1907, p. 254.

appearance assumed by the fruit may be conditioned from within rather than by any environmental influence.

As the banana ripens, the green outer skin becomes yellow and finally a deep brown, if not black ; the fruit is then fully ripe. The brown-black colour is easily produced by exposing the fruit to an anaesthetic. Moreover, if the interior edible part of the fruit be corked up in a test-tube together with an *Aucuba* leaf, the latter blackens sooner or later, especially if the temperature be raised to 35–37°. There can be little doubt that under natural conditions the blackening of the outer skin is due to the escape of an ethereal salt produced within ; a signal is thus given that the fruit is ripe.

The autumn coloration of leaves and their fall may well, at least in part, be conditioned by processes of a similar order—by a sudden outburst of hormones which either determine the occurrence of special enzymic changes or hasten such changes. Similar considerations may apply to the ripening of seeds.

This last autumn, when green leaves of Mangel-wurzel taken from the various plots at Rothamstead were exposed to the action of toluene, those obtained from plots which had been highly manured with nitrogenous materials retained their green colour more or less completely ; those from roots grown on other plots became yellow, resembling in this respect those grown under ordinary farm conditions and judged to be about ripe.

Much discussion has taken place as to the physiological significance of glucosides.[1] Dunstan and Henry,[2] in summing up the position with reference to cyanophoric glucosides, speaking of the value of hydrogen cyanide to the plant, remark : 'At first it was regarded as merely a waste product of no metabolic importance ; later the view that it was possibly a means of protection was suggested ; and more recently a small number of botanists and chemists have put forward the idea that the acid is an intermediate product in the synthesis of proteids.' We are inclined to take the view that, in not a few instances at all events, the compound associated with glucose functions simply as a hormone. In some cases the cyanophoric glucoside disappears as the seed ripens—in *Linum*, for example ; maybe, hydrogen cyanide is of special service in hastening ripening ; or when present in the seed, as in mustard, the glucoside may undergo hydrolysis during germination and furnish a hormone which serves to stimulate the growth of the seedling. The extent to which hormones are producible may have something to do with the various degrees of readiness with which seeds germinate. It is well that we should point out that, from our point of view, it is improbable that hydrogen cyanide is ever present as such except in minimal amount, unless perhaps at certain special times ; it would necessarily escape at a rapid rate if it were thrown into circulation.

[1] Cp. E. F. Armstrong, The Simple Carbohydrates and Glucosides, p. 89.

[2] British Association Report, 1906, p. 145.

Again, the attack of plants by fungoid growths may be made possible by the excretion of hormones by the fungus. The behaviour of *Drosera* appears to us to be significant from this point of view. Before it will be possible to appreciate and interpret the whole of Darwin's wonderful observations on this plant, it will be necessary to repeat many of the experiments, taking into consideration our modern knowledge of enzymic changes. It is already clear, however, that most of the substances which Darwin found to be capable of inciting the digestive activity of the plant are substances that we class as hormones. Apparently the enzyme and the accompanying acid are not secreted until the glands are excited by the absorption of some soluble matter. The fact that acids generally do not act as excitants but only certain acids appears to us to be clear proof that the glands are provided with a differential septum which loses the differentiating power and then permits of the excretion of the enzyme *only after it has been sufficiently thinned by enzymic attack from within.* Darwin himself remarks: 'It is strange that allied acids act very differently; formic acid induces very slight inflexion and is not poisonous, whereas acetic acid of the same strength acts most powerfully and is poisonous. Lactic acid is also poisonous but causes inflexion only after a considerable lapse of time. Malic acid acts slightly, whereas citric and tartaric acids produce no effect.'[1]

The order given is precisely that which we find to be the order of the activity of acids towards laurel and *Aucuba* leaves and that which Adrian Brown has deduced as the order of permeability in the case of the barley grain. Our observations as to the activity of hormones generally, it may be remarked, are also in complete harmony with those made by Overton and by Fühner and Neubauer using blood corpuscles and with Loeb's remarkable studies of artificial parthenogenesis.

That one effect is to thin the leaf membrane and deprive it of differentiating power there can be little doubt—the enzyme could not well escape otherwise. We have had occular demonstration of such an effect in experiments made with young *Rhododendron* leaves with the object of ascertaining to what extent the leaves increased in weight—(*a*) when kept in water alone, (*b*) when kept in water to which toluene was added. In the course of several days a considerable increase took place only in the latter case. It so happened, moreover, that the bottles in which the leaves were immersed in water were left on the bench perhaps a couple of months. During the whole time, in the bottle in which water alone was used, the leaves remained green, only a few brown spots appearing here and there; no appearance of colour in the water was noticeable and no fungoid growth. In the other bottle, in which the leaves were brown, the solution soon became brown in colour and full of a mycelial growth; evidently sugar had passed out from the leaf in the one case but not in the other.

[1] Insectivorous Plants, 1875, p. 273.

If, in the case of *Drosera*, thinning and repair of the leaf are possible alternately, the leaf must vary in 'sensitiveness' according as it has been stimulated or not more or less recently; this indeed appears from Darwin's statements to have been the case.

Salts generally, with few exceptions, have but little effect on leaves of cherry-laurel and *Aucuba*. It will be remembered that many salts were found by Darwin to cause inflexion of the tentacles of *Drosera*—sodium salts, for example; yet the corresponding potassium salts were without effect. It is noteworthy that the effect of sodium salts was transient and that recovery usually took place when the leaves were placed in water. We are therefore inclined to think that such salts may have produced an exosmotic effect. In our experience, in cases in which a substance penetrates into the cell, if the action be sufficiently prolonged, necrosis inevitably follows; the quick recovery of *Drosera* leaves in water appears therefore to be evidence that the salt does not penetrate into the cells of the glands.

The greater activity of sodium salts may therefore be due to the greater osmotic tension of the solution. Darwin, as a rule, used a solution of one part of the salt to 437 parts of water; the solutions of potassium salts he used were weaker therefore than those of the corresponding sodium salts, both because the molecular weight of the former is greater and because sodium salts appropriate a larger proportion of water in solution.

Cadmium chloride, according to Darwin, caused inflexion but without discolouring the glands; mercuric chloride not only acted very rapidly but caused discoloration. This order of difference corresponds very closely with that observed by us.

Darwin has laid much stress on the extraordinary sensitiveness of *Drosera* to ammonia and many ammonium salts, especially the phosphates, which were the most active of all the salts he tried and much more powerful even than the carbonate, which was more powerful than the nitrate. This result was intelligible, he thought (p. 171), from the difference of the amount of nitrogen in the carbonate and nitrate and from the presence of phosphorus in the phosphate; the inflexion produced by other salts of ammonia, he suggested, was due to their nitrogen.

We are inclined to think that in all cases the stimulating effect is due to ammonia and that the activity of the salts in solution is conditioned by the extent to which ammonia is liberated by the hydrolysis of the salt. If an *Aucuba* leaf be partly immersed in a weak solution of ammonium carbonate, the part immersed is somewhat less rapidly and less intensely coloured than that which is not in contact with the liquid, the ammonia penetrating more rapidly from the atmosphere than from the solution; the difference is not improbably due to the fact that the film on the leaf surface is a more concentrated solution of ammonia than the solution itself.

At first our results with ammonium salts appeared to be in harmony

with Darwin's conclusion that they are very active; but the experiments were carried out in ordinary soft glass tubes and, suspecting that ammonia might be liberated, we were led to repeat them carefully in hard glass vessels. At room temperatures the chloride, nitrate, phosphate and sulphate were practically without effect, although as the temperature was raised they became slightly active; the ammonium salts of weak organic acids were more active.

Although we are not yet prepared to grade ammonium salts in their order of activity, we are convinced that the salts of the strongest acids are the least active and that the activity is consequent on the liberation of ammonia by hydrolysis.

Whatever explanation be given ultimately of Darwin's observations, a sense of wonderment at the perfection of the inquiry must be felt by all who study the record in the light of modern knowledge. The stimulus that 'Insectivorous Plants' gave to the study of the process of digestion is probably far greater than is realized; inspiration is still to be found in its pages; indeed, not a few of the problems to which attention was called by Darwin now deserve reconsideration in detail.

The information given by Loeb in the various accounts of his experiments on artificial parthenogensis in some respects supplements that to be derived from Darwin's account of the behaviour of *Drosera* and from the behaviour of leaves such as that of *Aucuba*. The characteristic feature in the development of the sea-urchin egg by artificial means appears to be the formation of a 'fertilization membrane' at an early stage in consequence of an inflow of water which determines a growth in size of the egg.

A similar effect may also be produced by placing the eggs in distilled water instead of in a solution of the hormone in sea-water or some similar liquid. If the action either of water or of hormone be allowed to continue, the cell breaks down entirely.

One effect of the hormone on the leaf, as we have shown, is that it conditions the entry of water; it is clear that this effect is also produced in the case of the sea-urchin egg. As the egg must be in equilibrium with the sea-water from which it is taken, it is to be expected that water would diffuse into it when distilled water is substituted; what is surprising is that so much water should be absorbed as a consequence of the entry of the spermatozoon or the introduction of a minute proportion of some hormone. Ovum and spermatozoon are reciprocally affected as soon as the contact is effective. It is obvious that the spermatozoon is affected as it soon merges in the ovum; and not only do changes become apparent within but also without the egg-cell—the superabundant spermatozoa in its neighbourhood being not merely warned off but even slain. Obviously, some hormone is produced which passes out rapidly into the medium surrounding the ovum.

The effect produced by the spermatozoon apparently is never exces-

sive; any hormone that is added incautiously and even distilled water will cause the ultimate break-down of the cell if the action be continued long enough.

We have contended in our communication to the Royal Society that one effect of the entry of a hormone is to condition the introduction of water and that the consequent dilution of the cell contents would determine the occurrence of down-grade changes. In the living cell, at any moment, the changes taking place forward (synthetic) and backward (analytic) must necessarily be in equilibrium; in the egg, presumably, the state is one of almost restful equilibrium. The effect of dilution should be to disturb the equilibrium previously existing in the analytic rather than in the synthetic direction. Loeb's observations show clearly that such an effect is produced. But the increase of katabolic activity cannot well be the only effect produced by the hormone; probably the protoplasmic structure is also affected by it. Darwin has called particular attention to the visible changes effected within the cell; Loeb's observations afford further proof that internal turmoil, eventually amounting to complete disaggregation of structure, is conditioned by the entry of foreign matter.

It appears to us probable that a primary effect of the hormone is to condition the separation from each other *of the successive layers* which may be supposed to constitute the protoplasmic complex; although deposited in close contact, these are perhaps sheathed with thin layers of hydrone molecules and disintegration sets in when the aqueous layers are penetrated by the hormone. A similar effect would be produced by freezing. Assuming such changes to happen, enzymes previously stored in the cells would be set at liberty and by their action the proportion of molecules in solution would be rapidly and largely increased—in other words, the osmotic tension would be raised and a flow of water determined to the region in which the hormone is active.

Waller has contended that the effect studied by us is a death phenomenon. This appears to us, however, to be merely a Wallerian *façon de parler* based on a definition of death peculiar to himself. Whether or no the action of a hormone be followed by death is apparently entirely a question of the amount administered—Darwin's observations and horticultural practice disprove Waller's statement. According to Loeb, benzene, toluene and a great number of other substances at once condition the formation of the fertilization membrane in sea-urchin eggs but this is rapidly followed by necrosis unless care be taken to initiate the change by a minimal dose. If the eggs are immersed in sea-water containing amylene or benzene, the membrane is formed at once while they are in the liquid; if fatty acids are used, the membrane is formed only when the eggs are taken out of the mixture and restored to fresh sea-water.

The process of alternate 'decay' and repair, obvious in *Drosera*, is

manifestly a phenomenon of supreme importance if it be of general occurrence. It is worth while to inquire whether, for example, the absorption of salts by plants may not be in part at least conditioned by such a process; whether the translocation of diffusible matter may not be dependent largely upon such a process? Obviously, the conditions are very different at different times of the day; it is to be supposed that up-grade changes are ascendant at one time (whilst light is active) and down-grade changes at another: during the latter period it may be well that the tissues are far more easily permeable than during the former. The degree of immunity under various conditions and at various ages, the occurrence of latent diseases at certain seasons, may be largely a question of the resistance afforded by septa to penetration—to their liability to become thinned under certain conditions. For such reasons, some hesitation may be felt in accepting all the results that have been obtained with blood corpuscles in ordinary glass tubes, for example; under such conditions they may well have properties somewhat different from those they possess in the body.

NOTE.

PRELIMINARY NOTE ON GASTRODIA ELATA AND ITS MYCORHIZA.—A peculiar habit of *Gastrodia elata* and its highly reduced vegetative organ led me to undertake the present work. After careful observations in the field, cultivation-experiments, and cytological study, I have arrived at results, the chief points of which may be stated as follows :—

The vegetative organ of *Gastrodia elata* is represented simply by a tuberous rhizome. It forms mycorhiza with the mycelium strand of *Armillaria mellea*, generally called *Rhizomorpha subterranea*. The cytological features tend to show the mycorhiza to be an endotrophic form. However, the direct connexion of the endophyte with the *Rhizomorpha* strands vigorously vegetating in the surrounding medium indicates the physiological relationship between the two symbionts to be similar to that in a typical ectotrophic mycorhiza.

The infection by the fungus is effected by a sucker-like branch of the *Rhizomorpha* strand, which penetrates into the cortical cell layers of the tuber, partly compressing the underlying cells and partly dissolving their walls. This process is essentially the same as that presented by the haustorium of *Cuscata*. The infecting strand sends out separate hyphae which spread intracellularly in a definite zone under a few layers of subcortical cells. The extension of the endophyte is limited within a certain area around the infected spot.

The mycorhizal cell-layers may be distinguished into three regions, according to the structure of the cells and the nature of the hyphae which compose them. The first region consists of the outer two or three layers, which contain a densely entangled mass of comparatively thick-walled hyphae. A similar convolution of hyphae is observed in the next one or two layers composing the second region. In this region the hyphae are generally thin-walled and of various breadths, and are often arranged in pseudo-parenchymatous form. The innermost layer constitutes the third region; these cells are the largest and contain each a few, slender, less curved hyphae.

The different hyphae of the endophyte have essentially the same structure as those composing the *Rhizomorpha* strand; the hyphae of the first, second, and third regions correspond respectively to the outer interwoven hyphal branches usually forming the gelatinous investment, the inner cortical hyphae, and the slender hyphae composing the secondary pith of the strand. The hyphae of each region show characteristic alterations. They are permanent in the first region, as may be seen in the so-called fungus host-cells, and undergo self-disorganization in the second, leaving their walls as irregular masses, while in the third region they are mostly consumed by the host-cells.

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The walls of the mycorhizal cells undergo certain chemical and physical changes. In the first region they become lignified, and in the second they are partly dissolved by the perforating hyphae. In the third region the walls become thickened, but do not undergo any chemical modification. Further, in both the first and second regions the wall develops a tubular sheath which always shows a distinct lignin reaction. Lignin reaction is also observed in the thick-walled hyphae of the first region.

In the mycorhizal cells the amount of the cytoplasm and the size of the nucleus are increased previous to infection by the fungus. After infection the protoplast is soon consumed by the fungus in the second region, but in the first region the cytoplasm invests the hyphal clump and the nucleus is stretched, often so much as to cause fragmentation into two portions. When the clump becomes larger the protoplast disappears entirely. In the third region the cytoplasm increases further in amount and acquires a granular and dense consistence, while the nucleus undergoes hypertrophy, hyperchromatophily, and various deformations by constriction. The constricted portions may be often pulled apart in a stellate form.

In the mycorhizal cells of the third region there appear prominent bodies which may be considered to comprise both secretion and excretion products of the endophyte. First, light yellowish, oil-drop-like globules (attaining to 0·08 mm. or more in diameter) and similar-sized vesicles with a hyaline membrane and containing yellowish granules become visible in the cytoplasm. They are both secretion-bodies, or, if not, their derivatives, to be consumed later by the host. While these are disappearing, there occur innumerable, small, hyaline, irregular masses, each in a vacuole of the cytoplasm. Afterwards they are thrown into a large common vacuole and by fusion form a mass, more or less resembling the so-called clump ('Klumpen') usually found in the digestive cells of many mycorhizal plants. Probably they are derived partly from the remnants or ground-substances of the secretion-bodies and partly from the undigested wall of the hyphae, and judging from their resistant property they are certainly useless excreta.

The cell of the third region is a metabolic centre of the higher symbiont, where the food materials are elaborated. The remarkable alterations in the cytoplasm and nucleus are indications of the great activities that are going on in the cell during this process; so that, when the latter is over, the nucleus resumes its original form and structure, while the cytoplasm again becomes fibrous and vacuolate.

The *Rhizomorpha*, besides forming mycorhiza, behaves towards *Gastrodia* as a true parasite, and under certain circumstances the strand penetrates deeply into the tissue of the tuber, then developing as *Rhizomorpha subcorticalis*. The infected tissue collapses and is apparently injured, as may be seen in potato tubers attacked by the same fungus.

Gastrodia multiplies usually by a tuber. It produces long rhizomes from its apex or node, upon which are developed stalked offsets. Sometimes the latter are produced directly on the node of the mother-body. At the end of autumn, the mother-body and the pedicel of the offset undergo degeneration, so that the daughter tubercles are all set free separately.

The association of the tuber with the *Rhizomorpha* takes place quite occasionally.

If the mother-tuber forms mycorhiza, it can produce a full-grown offset which remains dormant during the winter and develops the inflorescence axis in the spring of the next year. Otherwise, the offsets cannot grow larger than the mother-tuber, and under this condition the offsets become smaller and smaller in successive generations, until they are so much reduced in size and deficient in food materials as to be incapable of further multiplication.

The tubercles cultivated in the pot with sand, loam, or humus soil produce, as in the field, numerous offsets, but none of them can reach the flowering stage. This shows that they have no ability to provide themselves with nutriment from the surrounding medium.

The usually saprophytic development of *Armillaria mellea*, the extremely reduced vegetative organ of *Gastrodia*, and the cytological features involved in the symbiosis lead us to the view that the reciprocal exchange of nutritive substances between the two organisms is not equal, i. e. the fungus becomes the victim of the Orchid, perhaps receiving from the latter but little benefit for its whole organization. Therefore, it appears probable that physiologically the relation of *Gastrodia* to the fungus is similar to that of subterranean holoparasites to their host roots—*Gastrodia* is parasitic on the fungus.

The chief reserve material stored in an adult tuber is starch. The amyloplast contains a heavily staining body of nuclear nature, whose structure changes at successive stages.

A full account of this subject, with illustrations and references to the literature, will appear in the 'Journal of the College of Agriculture, Imperial University of Tokyo'.

S. KUSANO.

Agricultural College, Tokyo,
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