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# ANNALS OF BOTANY

EDITED BY

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*ASSISTED BY OTHER BOTANISTS*

VOLUME XXIV

With Sixty-seven Plates, and One Hundred and Seventy-one Figures in the Text

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## The Nature of the Fertile Spike in the Ophioglossaceae.

BY

MINTIN A. CHRYSLER,

*Associate Professor of Botany, Univ. of Maine.*

With Plates I and II, and sixteen Figures in the Text.

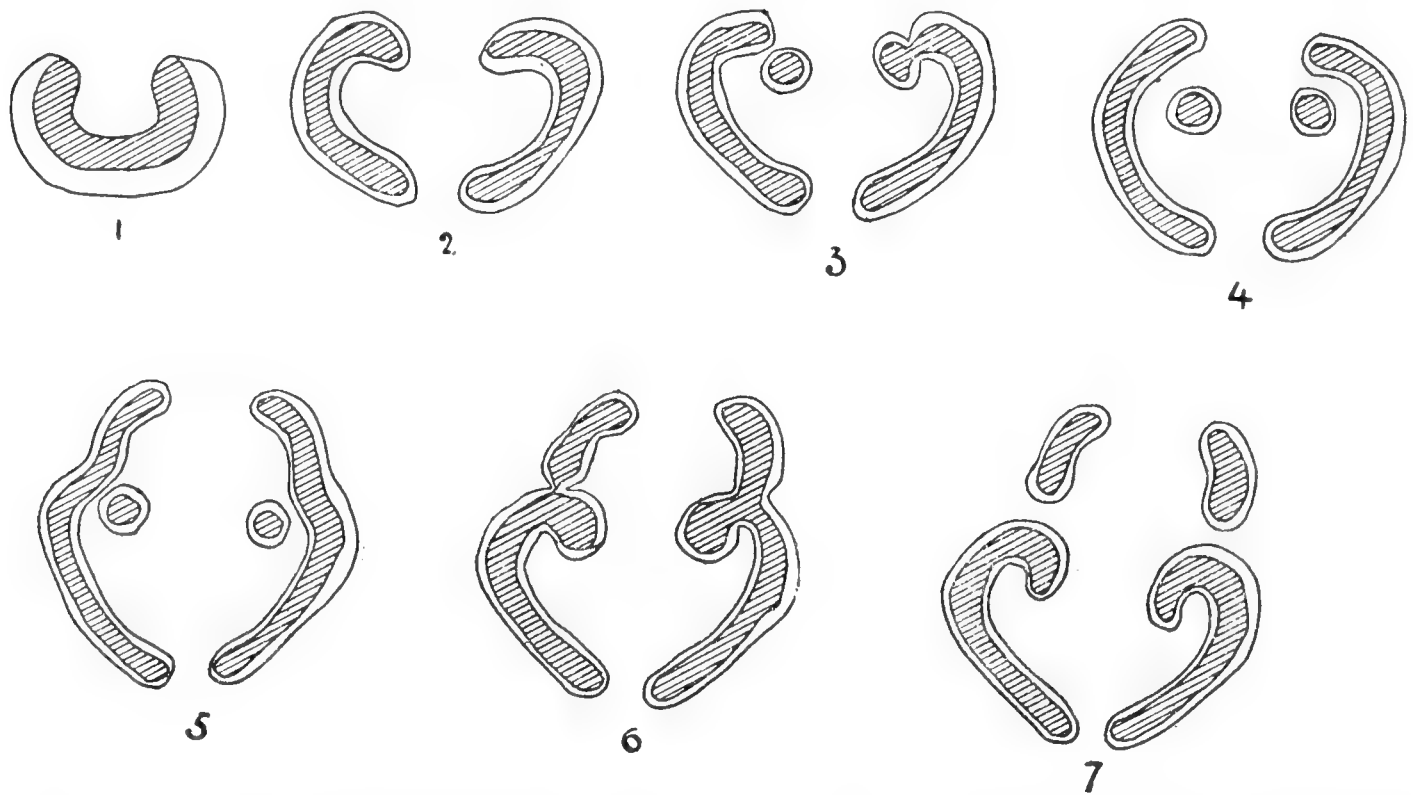
SPECULATION on the morphological nature of that unusual organ of the Ophioglossaceae known as the fertile segment or fertile spike dates at least from the time of Roeper, who in 1826 (20) suggested that in *Botrychium Lunaria* the leaves arise in pairs, one sterile and one fertile, with their petioles fused. In 1843 Roeper extended his views to the whole family (21). But Presl (19) pointed out that the base of the petiole ('stipe') contains only a single bundle, and hence he considered the fertile spike to be a pinna of the leaf. Apparently influenced by this criticism and that of Mettenius (17), Roeper in 1859 (22) replaced his earlier suggestion by the view that the fertile spike represents two fused pinnae, namely the basal ones of a leaf, the rest of the pinnae of which are sterile. He also adduced teratological evidence in support of his view, and noted that the vascular supply of the fertile spike is double in species of *Botrychium*. It was shown by Holle (14) that Roeper's theory might be applied to *Ophioglossum*, since the origin of the vascular supply of the fertile spike is similar to that in *Botrychium*.

The suggestion of Braun (7) that the fertile spike of *Ophioglossum* is the first leaf of a bud in the axil of the ordinary sterile leaf, with which its stalk is confluent, has not received wide acceptance.

Goebel (12, 13) has adduced the view that the fertile spike of *Botrychium* is the lowest fertile pinna of a leaf, but that it arises in a ventral instead of a lateral position.

All of the theories so far outlined assume that the aerial organ of the Ophioglossaceae consists of one or more leaves which have been derived from the leaf of some fern-like plant by a process of specialization; in other words, that the ancestry of the Ophioglossaceae is to be looked for among the ferns. Since *Botrychium* is the most fern-like genus of the family, it is regarded as the primitive genus.

Of quite different opinion is Bower, who since 1891 (3) has offered evidence for considering the fertile spike of *Ophioglossum* to be a septate sporangium arising on the ventral face of a sporophyll. Thus the aerial organ of *Ophioglossum* represents one of the sporophylls of the cone of *Lycopodium*. From *Ophioglossum* are derived the different species of *Botrychium*, which are arranged in an ascending series, and *Helminthostachys* takes its place as an elaborated pattern from the same origin. According to this view the ancestors of the Ophioglossaceae are microphyllous forms such as the Lycopods. Other writers, especially Celakovsky (10), have suggested a common origin for Ophioglossaceae and Lycopod-



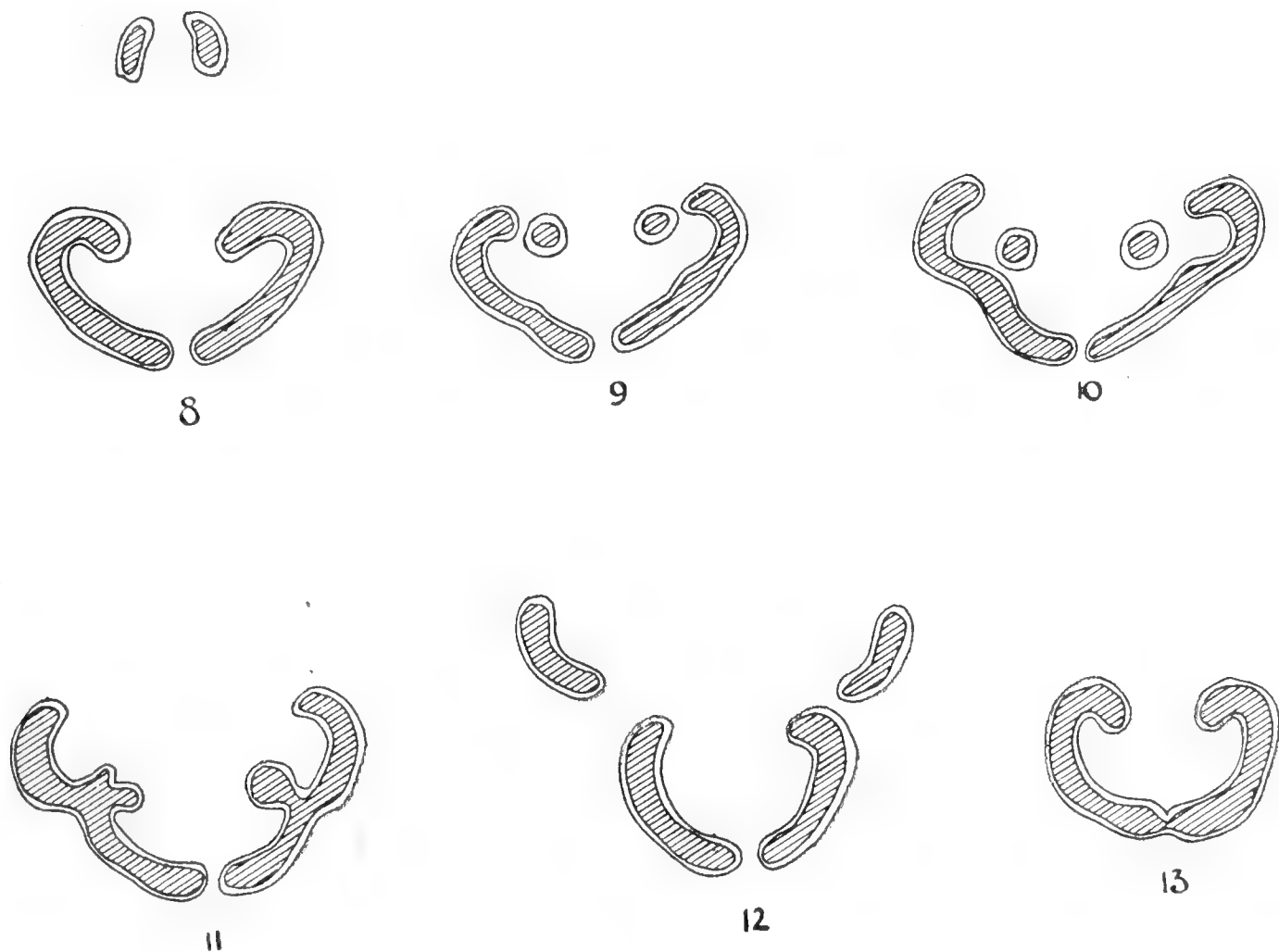
FIGS. 1-7. Diagrams illustrating the origin of the strands which supply the fertile spike in *Botrychium virginianum*. Fig. 1 is from the lowest section. Xylem is shaded, ploem left clear. The adaxial side of the petiole is placed upward.

iales, but none have supported the theory by any such array of evidence as Bower presents.

Campbell (8, 9) would give *Ophioglossum* a place much nearer the base of the genealogical tree, deriving the fertile spike directly from a sporogonium such as that of *Anthoceros*. An extended account of the different theories will be found in Bower's monograph on the Ophioglossaceae (4).

When the affinities of a group are subject to such wide variations of opinion, evidence should be sought in all quarters, and the attempt is here made to bring forward anatomical data which bear in no uncertain way upon the question. The work here described has to do chiefly with the origin of the vascular strands which supply the fertile spike. Although this has been examined in several European forms, the modern method of using serial sections does not seem to have been employed; further, *Botrychium virginianum*, which affords the key to the situation, has strangely enough been overlooked from this point of view.

The vascular supply of the aerial part of *Botrychium virginianum* arises as a single curved collateral strand (Text-fig. 1) from one side of the hollow cylinder of the underground stem, leaving a distinct gap. At a height of a centimetre or so the strand divides into two, and assumes a somewhat horseshoe shape, with its adaxial side concave. At about this level it gains an internal phloem and thus becomes concentric (Fig. 2); it retains this character as it rises through the stipe. At a distance of 1-2 cm. below the point where the fertile and sterile segments separate, each arm of the horseshoe-shaped strand becomes hooked, and soon the tip of each hook breaks off as a small circular concentric strand (Fig. 3),



FIGS. 8-13. Diagrams illustrating the origin of the strands which supply the first pair of pinnae of the sterile segment in *Botrychium virginianum*. Fig. 8 follows Fig. 7 in the sequence.

which a little higher up joins the inner face of the large strand (Fig. 5). Just at this level the adaxial portion of each arm of the main strand breaks off (Figs. 6, 7), and the two bundles so formed approximate to a certain extent and constitute the vascular supply of the fertile spike. Thus the fertile spike has a pair of bundles, as was pointed out long ago by Roeper. Before inquiring into the meaning of the peculiar course of the strands just traced, it will be well to follow up the main vascular supply into the sterile segment. The two main strands may unite at about this level or may remain slightly separated, and in any case they again begin precisely the same series of changes as has been described above, namely, the double strand assumes the form of a horseshoe with each point incurved

(Fig. 8), these points break off (Figs. 9, 10) and the two small bundles so formed join the main strands on their inner face at a slightly higher level (Fig. 11), then the adaxial portions of the main strands break off (Fig. 12) and supply leaflets or pinnae on each side. Here of course the branching strands do not approximate, but diverge as they pass out to pinnae. The same process is gone through in connexion with the exit of the traces of the next pair of pinnae. The identity of the mode of origin of the vascular supply of the fertile spike and that of a pair of pinnae of the leaf indicates, unless there is evidence to the contrary, that the fertile spike represents two fused basal pinnae of a leaf, thus supporting the theory proposed by Roeper (22). But it is necessary to show that the mode of origin so described corresponds to the mode exhibited in other organs admitted to be leaves.

If the figures just referred to are attentively examined it will be seen that each of the strands which supply the fertile spike and the pinnae arises from a *gap* in the curved leaf-trace. This appearance is obscured by the fact that the branch strand arises from the side instead of the base of the gap, which closes just as the strand makes its exit; or in other words the branch strand adheres to one side of the gap for practically the whole length of the gap. That this interpretation of the appearance is the correct one is borne out by the observation of Bertrand and Cornaille (2) that in the basal part of the petiole there are fourteen 'divergents' which run more or less united edge to edge; four of these, that is, two on each side, supply the fertile spike. In view of the upright position of the spike it is not to be expected that the divergents which supply it will at once turn outward, as occurs for instance in an ordinary fern leaf, but they maintain their original direction for some distance, thus altering and obscuring the gap. It is not necessary to seek far among the large-leaved pteridophytes for petiolar traces with gaps. Fig. 17 shows a transverse section through the midrib of *Todea barbara* at the level where two leaflets arise. It will be seen that the vascular supply of a leaflet leaves a wide gap at its exit from the curved leaf-trace. In the related genus *Osmunda* the same may be seen, but in this genus the gap is sometimes so narrow that a section taken just above the point of origin of the traces of pinnae shows no gap. This is represented in Fig. 18, near which (Fig. 19) has been placed a photograph of the corresponding region in *Botrychium virginianum*; the similarity is too marked to be overlooked. Sinnott (23) has recently shown that this feature is of general occurrence in the Osmundaceae. In Polypodiaceae the leaf-trace has the same general shape, and the gaps left by the departure of the vascular strands of the pinnae are generally so narrow that no fundamental tissue is to be seen in them, but it can easily be made out that these strands do not break off from the free edges of the petiolar trace, but are pinched off from the projecting corners of the trace. Only in com-

paratively small-leaved genera or species (e. g. *Pteris cretica*) does evidence of the gap disappear, and the lateral bundles spring from the edge of the petiolar trace. Bertrand and Cornaille (l. c.) have argued that the condition found in Osmundaceae is to be regarded as primitive, and the conditions seen in Polypodiaceae seem to be derived by reduction. It may be added that the structure of the midrib of the pinnae in *Marattia* is of the same general structure as the petiole of *Todea*, namely, the vascular supply of the pinnules arises by distinct gaps. The split or divided U seen in the petiolar strand of *Botrychium virginianum* recurs in numerous Polypodiaceae, e. g. the stipe of *Onoclea*, *Pteris*, spp., *Asplenium*, spp., so this feature forms no drawback to the derivation of *Botrychium* from fern ancestors. In *Onoclea* the leaf-trace is double near the base of the leaf, but higher up the two halves join to form a U-shaped strand; the same sometimes happens in *B. virginianum*. In both these cases the leaf-trace arises as a single strand, while the supply of the fertile spike of *Botrychium* is double from its origin upward. The diagrams shown in Fig. 1-13 were made from a fairly small specimen; in well-grown specimens the plan is less apparent on account of the parting asunder of some of the 'divergents' which compose the leaf-trace, though the plan is in reality precisely the same as in smaller plants.

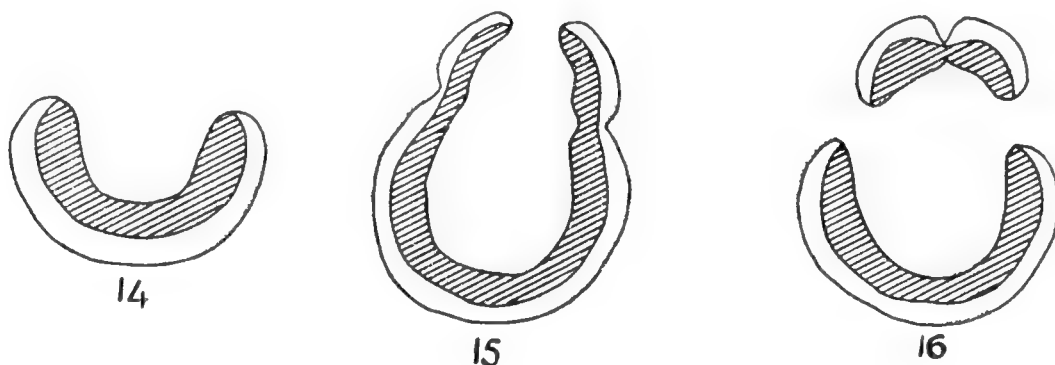
Of the species examined, *B. virginianum* seems to be the most fern-like. This appears not only in the mode of origin of the vascular supply of the pinnae, but in the amphiphloic feature of the leaf-trace and the distinctness of the divergents. Several varieties of *B. ternatum* have also been available for study, namely, *intermedium*, *dissectum*, and *obliquum*, which has lately been recognized as a distinct species.<sup>1</sup> These varieties merge into one another in a way that makes distinctions difficult. The anatomy of these varieties offers no basis for distinguishing them, and they may here be considered together.

The leaf-trace of *B. ternatum*, like that of all other species, leaves a wide gap where it parts from the central cylinder. At first it is only slightly curved (Fig. 14), but it rapidly assumes the form of the letter C, and may almost form an O. From the free edges arise the two strands (Fig. 15) which supply the fertile spike. These strands may fuse more or less just above their point of origin (Fig. 16), but separate higher up. All of the strands so far mentioned are collateral, in distinction to those of *B. virginianum* which are concentric. The branches of the fertile spike show interesting transitions from the condition seen in the sterile part to that described for *B. virginianum*. In some specimens these branches manifestly arise from gaps, as is illustrated in Fig. 20. A small strand (hereafter called the marginal strand) arises from each edge of the main

<sup>1</sup> Lyon (Botan. Gaz., xl. 455-8, 1905) even proposes to separate it into a new genus, *Sceptridium*, on the basis of gametophyte characters.



vascular strand, and higher up joins again as shown in the figure; but a new feature appears, in that half of the marginal strand remains with the main strand, while the other half passes out with the branch, which is accordingly C-shaped. The branch becomes concentric by acquiring an internal phloem as it passes out. In other specimens only the upper part of the marginal strand is present, that is, the part shown at the right-hand side of Fig. 20; following the series downward the marginal strand parts from the main strand (Fig. 20, right-hand side), swerves towards the edge of the latter but does not reach it, for it dwindles away and disappears. In other cases the marginal strand passes down only a short distance before it disappears, or its upper end may even be represented merely by a slight projection from the main strand. In such cases the branches which supply a group of sporangia appear to arise from the edge of the main strand. Since only the upper part of the marginal strand is present in these transitional cases, the strand can have no function; its xylem cannot conduct water when it has



FIGS. 14-16. Diagrams illustrating the origin of the strands which supply the fertile spike in *Botrychium ternatum*.

no attachment below; hence it must be regarded as a vestigial structure, derived from the condition seen in all regions of the leaf of *B. virginianum* and in the fertile spike of some specimens of *B. ternatum*. This evidence plainly goes to support the view that *B. virginianum* has been derived from some less specialized fern, and that *B. ternatum* has been derived from some such plant as *B. virginianum* by a process of reduction in respect to the vascular system. The location of the internal phloem also supports this view. In most ferns this is present in both stem and leaf; in *B. virginianum* it is absent from the stem but occurs in the leaf through most of its length; in *B. ternatum* it is to be seen only in the remote branches of the leaf, especially in its fertile part; in other species its presence has not been recorded. It is becoming pretty well recognized that the leaf is one of the places where primitive features are apt to persist, and in the genus *Botrychium* we have a case where a structure is seen to gradually disappear from this organ.

The two species *B. ramosum* (Roth), Aschers (*B. matricariaefolium*, A. Br.), and *B. lanceolatum* (S. G. Gmel.), Angs., are usually associated in schemes of classification, and the anatomical features bear this out.



Material of these species was secured from Maine and Massachusetts. These species show their relation to *B. ternatum* by the similar form of the petiolar strand, the two parts of which almost meet adaxially, so that the form is nearly that of the letter O. From the adaxial edges break off the two strands which supply the fertile spike; these may approximate, but do not fuse. In both fertile and sterile segments the branches of the vascular system arise in the manner just stated. Thus the gap left by the exit of branch traces in *B. virginianum* has disappeared in the species under consideration.

*Botrychium Lunaria* was examined by Roeper and others, who found the double vascular supply of the fertile spike. By soaking out and imbedding herbarium material, I have been able to verify the earlier accounts. The whole vascular system of the leaf is markedly two-sided; the leaf-trace splits before the leaf has become free from the stem, and the two halves diverge rather widely. The strands which supply the fertile spike and also those which supply the lobes of the leaves arise from the free adaxial edges of the main strands. A slight prominence on the inner side of the latter at the point of departure of a branch may represent a vestige of the 'lateral strand' described under *B. ternatum*. If such is the case *B. lunaria* is to be regarded as a reduced species derived from more fern-like ancestors.

*Botrychium simplex*, material of which I owe to Mr. J. C. Parlin, looks externally like a reduced *Lunaria*, and its internal structure bears this out. Its vascular system is quite similar, but the strands are smaller, especially those of the sterile segment, so that those of the sterile and fertile parts are of about equal size. The two petiolar strands divide into adaxial and abaxial parts, so that the gap seen in *B. virginianum* has quite disappeared. The two strands of the fertile spike remain separate throughout their length.

Certain abnormal forms of *B. obliquum*, Muhl., may next be considered. Through the great kindness of Mr. F. W. Bachelder a supply of this interesting material, collected in the vicinity of Manchester, New Hampshire, has been available for study. The external features of the specimens have been briefly described by Mr. Bachelder (1), and the peculiarity consists in the presence of two or three fertile spikes in place of one, or in the presence of one or more fertile pinnae among the ordinary sterile ones. Certain of these specimens were photographed, then soaked up and swelled out by means of warm dilute ammonia, and afterwards imbedded and cut into serial sections in the usual manner.

In the simplest of these cases, two similar fertile spikes arise in the normal position (Fig. 24), diverging slightly right and left. Externally they appear to form a pair, and the structure corresponds to this view, for the petiolar strand has in transverse section the form of a U, from each

extremity of which arises a strand which supplies one of the two fertile spikes. Here we appear to have an actual realization of the theory of Roeper, and the facts are readily explained on the assumption that these two fertile spikes represent the two basal (fertile) pinnae of the leaf. *Botrychium* has often been compared with *Aneimia*; the resemblance of the present form to the tropical fern is so striking that there has been introduced for comparison a photograph of a specimen of *Aneimia tomentosa* kindly furnished by the Curator of the Gray Herbarium. The resemblance extends to the internal structure, for *Aneimia* has a trough-shaped leaf-trace from the edges of which are given off the strands that supply the fertile pinnae. Specimens of *B. obliquum* showing two fertile spikes have been collected also at Sandwich, N.H., Conway, N.H., Topsham, Me., Utica, N.Y., Friendsville, Pa. Mrs. Britton, speaking before the Torrey Botanical Club, refers to the 'frequency of the fronds forking' in specimens of *B. ternatum* (vars.) found in the Berkshire Hills, Mass.<sup>1</sup> These facts scarcely tally with Bower's statement (4, p. 42) that in the large-leaved forms abnormalities 'are, if existent at all, so rare that they are seldom or never represented in herbaria, or recorded in the books'. In some of the specimens (Fig. 28) the two spikes are fused for a short distance from the base, or for half-way up the stalk. A series of sections through one of these showed the usual double vascular supply with partial fusion of the two bundles in the lower part of the stalk.

Other specimens show three fertile spikes (Fig. 26), one in the normal position and two smaller ones forming a pair springing from a point 12–15 mm. higher on the petiole. The leaf-trace is as usual U-shaped in transverse section; from its adaxial edges arise two strands which soon meet to produce a shallow U and run up into the lowest fertile spike. After running onward for about a centimetre the leaf-trace gives off a strand on each side from a point near but not at the edge; the appearance is much like the figure (18) of *Osmunda*, indicating that the gap is too narrow to allow the fundamental tissue to be continuous through it. Each of these strands is concentric, and supplies one of the paired spikes. Certain specimens of this type show internal phloem in the region where the branches arise. Apparently these specimens show reversion in several particulars. The appearances receive ready interpretation by the theory here advocated; there are here two separate but upright pinnae and two fused basal pinnae.

Still other specimens show two fertile spikes, one arising in the normal position and another 8–10 mm. above it (Fig. 27). In a specimen of this sort which was sectioned, the lower spike had a double vascular supply, and therefore represents two fused basal pinnae, while the higher (smaller) spike was supplied by a single strand which arose from near one edge of

<sup>1</sup> See Bull. Torrey Bot. Club, xxiv. 1897, p. 585.

the U-shaped petiolar trace, and hence must be considered a single pinna.<sup>1</sup>

Cases where one or more pinnae of the sterile segment bear sporangia have been earlier figured for several species, especially *B. Lunaria* (see Bower, 6, p. 443); Fig. 25 represents a case of this kind occurring in *B. obliquum*. The branch of the sterile segment which appears to be median in this figure is really a lateral branch which has been turned upward in preparing the herbarium specimen. An examination of the specimens of *Botrychium* in the Gray Herbarium shows that (1) almost all species occasionally show the feature illustrated in Fig. 25, and (2) in the smaller-leaved species the sterile leaflets frequently bear a few sporangia along the margin. The latter case resembles the half-sterile pinnae which may be seen on almost any plant of *Osmunda regalis*. These cases indicate that the whole leaf was at one time fertile, and that in the course of evolution a division of labour has occurred (cf. *Osmunda Claytoniana*), resulting in the sterilization of all of the pinnae except the basal pair, which have become specialized by turning upright (as is the case in *Aneimia*) and finally fusing. But in certain cases reversions occur, giving a clue to the probable evolutionary history of the genus.

Bower rejects the evidence afforded by these 'sports', considering the cases of double spikes merely instances of splitting or chorisism. I do not think that the cases here adduced can be disposed of in this simple way. If three or four spikes arose from the same point, the objection might be more significant, but in the plants of *B. obliquum* with three spikes two are paired and the third larger one arises from a lower point on the petiole. It seems clear that in this case there are two pairs of fertile pinnae, the basal pair of which are fused. Even in case two spikes are present they may arise from different points and may have a single or double vascular supply, as has been shown. The structural features are completely and easily explained on the view that a spike represents one or more pinnae of a leaf. Bower admits the cogency of the argument derived from the occasional fertility of pinnae which are usually sterile, but refers to the view of Goebel that sporangia are parts *sui generis*, and hence may occur on members of different rank. Apparently Bower does not endorse this view, and surely such an argument should be reserved as a last resort, for to admit it would rob comparative morphology of much of its meaning.

Among the species of *Ophioglossum* which have had their petiolar structure examined, *O. lusitanicum* has a comparatively simple structure. Prantl's account (18) shows that the leaf-trace early divides into three strands, one central and two lateral; these three arrange themselves as the angles of a triangle, and from each lateral strand there breaks off

<sup>1</sup> See Note at end of paper.

another strand; the last two move towards each other, fuse, and supply the fertile spike. The plan shown in *O. vulgatum*, as described by Holle (14) and verified in my own specimens, is closely related to that in *O. lusitanicum*, differing chiefly in that the petiolar strands are more numerous, that is, the three strands break up into about ten (there may be as many as fifteen) and are disposed in an ellipse, or rather a semicircle, for the strands on the adaxial side generally form nearly a straight line. The latter strands represent the right- and left-hand edges of the original petiolar strand, and they higher up anastomose to form three to five strands which pass up into the fertile spike, while the remaining strands branch extensively as they pass up into the sterile segment. The plan here exhibited differs from that seen in *B. ternatum* in that the vascular supply of the petiole early breaks up into a number of strands instead of remaining a single U- or C-shaped strand. In both genera the vascular supply of the fertile spike is derived from the two free edges of the main petiolar system. This is well illustrated by the small species *O. Bergianum*, of which Bower (6, p. 463) says 'the single leaf-trace strand may long remain undivided, giving off two lateral strands which fuse on the adaxial side to form the supply for the spike: further up the strands of both sterile lamina and of fertile spike may branch again'. Campbell (9) has figured the disposition of the vascular bundles in the petioles of certain other species belonging to the section *Euophioglossum*. Assuming that the origin of the curved series of bundles in these petioles is the same as in those in which the origin has been traced, namely, from repeated division or branching of a curved leaf-trace, Campbell's figures show that several other species, including *O. moluccanum* and *O. californicum*, agree with *O. lusitanicum* in having the vascular supply of the fertile spike derived from the edges of the curved leaf-trace.

In the section *Ophioderma* the leaf-trace at its origin consists of from four to five strands arranged in a U, as is clearly shown by Bower's figures (5) of *O. pendulum* and *O. simplex*. In leaves which bear a fertile spike the edges of the U soon close in to form a circle, and several strands which represent the two free edges of the trace constitute the supply of the fertile spike, exactly as in *Euophioglossum*. This may be seen from Campbell's figures of *O. intermedium* and *O. pendulum*, though it is only fair to say that this writer draws a different inference from his studies of these species.

The section *Cheiroglossa*, represented only by *O. palmatum*, is the most specialized in the genus. As in *Ophioderma*, the vascular supply of the leaf arises as several strands (5) which form a leaf-trace, becoming more and more curved until a circle of over a dozen strands is formed. From the adaxial side of this circle break off about four strands to supply the fertile spike. So far the behaviour is exactly as in *Ophioderma*, but, as Bower's figures show, from what now constitute the free edges of the

leaf-trace there break off other sets of strands; those which break off from one edge supply the second spike, and those from the other edge supply the third spike. In other words, the vascular supply of the first spike is derived from both edges of the leaf-trace, while that of subsequent spikes may be derived from only one edge. 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. Whether any particular spike arises from one or from both edges of the leaf-trace can be determined only by the study of serial sections, and not by an inspection of the external surface, as has too often been done. 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.

The monotypic Asiatic genus *Helminthostachys* presents points of considerable difference from the other two genera. Farmer and Freeman (11) have given the best account of the mature sporophyte, while the young sporophyte is considered by Lang (16) in his paper on the gametophyte. By means of material kindly supplied by Dr. J. C. Willis, Director of the Royal Botanic Garden at Peradeniya, Ceylon, I have had the opportunity of verifying earlier accounts, while I have been able to study the structure of a young specimen kindly placed at my disposal by Professor Jeffrey of Harvard University. In the mature plant the single leaf-trace forks before it emerges from the cortex of the creeping rhizome, and soon repeats the process several times, forming a nearly circular row of eight to ten strands which soon become concentric. From *one* of the adaxial edges of this broken circle a strand breaks off,<sup>1</sup> turns towards the middle region of the petiole, and orients itself in the inverse position. Thus a section through the petiole shows a ring of bundles with an odd one in the middle, frequently somewhat nearer the adaxial side. Campbell (9) figures a specimen with two strands in the position of the odd bundle, and says that these strands 'remain separate and are those which later extend into the spike'. Inasmuch as this condition is not represented in any of the figures of Goebel (13), Bertrand (2), or Tansley (24), nor shown in my material, it would be very interesting to know whether it is the result of splitting of the odd bundle, or whether each of the bundles arises from one edge of the curved leaf-trace. Campbell's account gives no

<sup>1</sup> This fact, I believe, was first pointed out by Gwynne-Vaughan in his paper on *Archangiopteris* (Ann. Bot., xix, 1905, p. 259).



information on this point. The vascular supply of the fertile spike is derived partly from the odd bundle and partly from two strands which branch off from the right and left edges of the curved series of bundles which constitute the leaf-trace, but the odd bundle anastomoses with the main petiolar supply at the level of branching of the sterile segment, and emerges from this anastomosis as a pair of strands. Thus the vascular supply of the fertile spike consists of four strands. Upon this rather complicated system the young plant throws some light. Lang finds that the first leaves borne by the sporophyte contain only two bundles, derived by the splitting of a single leaf-trace. In this respect, therefore, the young plant resembles *Botrychium*. The small plant which came into my hands bore a fertile spike but possessed a simpler structure than the adult plant. The lower part of the petiole shows only four strands, two adaxial and two abaxial, i. e. the leaf-trace forks twice instead of three times as is the case in older plants. About half-way up the petiole, one of the adaxial strands gives off a rather small strand from the side which corresponds to one of the free edges of the curved leaf-trace (Fig. 22); the new strand takes a place midway between the two adaxial strands and runs up to the place where the fertile and sterile segments separate; here it anastomoses with the main strands (which in the meantime have united edge to edge) and finally emerges to supply the fertile spike. In this specimen therefore the vascular supply of the spike is derived from one edge of the curved leaf-trace, and it will be recalled that in the mature plant part of the supply is so derived. We have already seen that in *B. obliquum* a single pinna may be fertile or may even turn upward and constitute a fertile spike, and it has been argued that some of the spikes of *Ophioglossum palmatum* may represent single pinnae or lobes, so that it appears probable that in the highly specialized *Helminthostachys* a single pinna functions as a fertile spike.

The various theories regarding the fertile spike may now be considered in the light of the foregoing observations. The early suggestion of Roeper (20) that two fused leaves are present in the aerial part of *Botrychium* is easily disproved by the well-known fact that a single bundle arises from the central cylinder of the stem, leaving a well-marked gap, hence the whole structure constitutes a single leaf. The same fact disposes of Braun's surmise (7) that the fertile spike represents an axillary bud, for the vascular supply of the spike arises from the single leaf-trace, not from the central cylinder.

The position of those who hold with Goebel that the fertile spike is a ventral lobe of a leaf is, as Bower remarks, somewhat obscure. If the organ is really ventral in origin, the vascular system ought to exhibit this feature, but it has been shown that in *Botrychium*, and probably in the other genera of the family, the origin of the vascular supply of the fertile



spike is marginal or slightly away from the margin on the abaxial side. No examples of true ventral lobes have been adduced; the sporocarp of *Marsilia* is stated by Van Tieghem (25, p. 1405) to be such, but sections through the petiole at the point of attachment of the sporocarp (see Fig. 21) show that the origin is similar to that of the leaflets, namely, from one edge of a curved leaf-trace. Goebel (13) has described a species, *M. polycarpa*, in which several sporocarps arise in acropetal succession on one side of the petiole. The fertile lobes of *Aneimia* and of *Schizaea* are likewise lateral in origin, as a study of their vascular structure shows. Apparently the 'ventral lobe' has a merely hypothetical existence outside of teratology.

To bridge over the gulf which exists between the bryophytes and pteridophytes, as Campbell proposes to do in deriving *Ophioglossum* from *Anthoceros*, were indeed a consummation devoutly to be wished, but the gulf seems still to exist, for even in *Ophioglossum simplex* the aerial organ is borne laterally on a stem, the central cylinder of which is perforated by a gap at the point of exit of the traces supplying this organ. Even if it could be proved that the fertile segment is the main structure in the aerial organ and the sterile segment only an appendage, the sporangiophore is still a lateral organ, which arises from the stem just as does a leaf. Campbell's latest contribution (9) on the subject seeks to show that the vascular supply of the fertile spike is not secondarily derived from the main bundles of the petiole, but that it can be traced to the base of the petiole. Hence the fertile spike is considered to be not a secondary but a terminal structure. According to this mode of reasoning, the basal pinnae in the leaf of *Osmunda* are terminal, for their 'divergents' (see 2) may be traced far down through the petiole. Similarly, in *Botrychium virginianum*, the divergents which pass off to the fertile spike may be seen for some distance below the point where the strands of the spike break off from the main supply, but the same is true of the divergents belonging to the divisions of the sterile segment. In this species the strands which supply the fertile spike do not break off until within a short distance below the point where the spike is visible externally, but appear very distinctly to be 'secondarily given off from the main bundles of the petiole'. The evidence for Campbell's view would be more conclusive if his figures left less in doubt as to the actual course of the vascular bundles which form the basis of his argument. All the cases where serial sections have been studied support the view that the strands which supply the fertile spike arise from near the edges of the leaf-trace, as do those supplying the pinnae of a fern leaf.

The theory of Bower and others who consider the aerial part of the *Ophioglossaceae* to be a single sporophyll of a strobilus receives small support from the present investigation. The examination of the vascular supply of the fertile spike and the sterile pinnae of the leaf has shown the close similarity between *Botrychium* and an ordinary fern. The similarity

between *B. virginianum* and *Osmunda* in this respect is so striking that, when considered in connexion with the other filicinean characters of *Botrychium*, we must conclude either that this genus has been derived from fern ancestors, or that ferns have been derived from Ophioglossaceae. The latter alternative is adopted by Campbell (8), but is not countenanced by Bower. Signs of high specialization are apparent in the family, which accordingly is not a favourable point of departure for other groups. Without attempting to discuss in detail the evidence adduced by Bower for linking Ophioglossaceae with the Lycopods, certain filicinean features of the former group may here be mentioned, in addition to the structure of the petiole:—

1. The Ophioglossaceae belong distinctly to the large-leaved group. Although it is not impossible that large leaves may have arisen more than once in evolution, it is hard to believe that so great similarity of structure should have been attained independently.

2. The endarch xylem (mesarch in *Helminthostachys*) is in sharp distinction to the exarch condition seen in most Lycopods. Since *Tmesipteris* has mesarch xylem this criterion is not so reliable as others.

3. The central cylinder of Ophioglossaceae is hollow and is perforated by leaf gaps. This fact places the family in the group *Pteropsida*. Bower's effort to show that a phyllosiphonic stele occurs also in *Tmesipteris* (6, p. 487) has been shown by Jeffrey (15) to be a misconception. *Ophioglossum* cannot be linked with *Lycopodium* by means of *Tmesipteris* or *Phylloglossum*, which are both outspoken members of *Lycopsidea*.

4. Those who have studied the gametophytes of the three genera of Ophioglossaceae are agreed in considering their characters unmistakably filicinean, though specialized in connexion with the subterranean habit. The multiciliate sperm merits special mention.

In view of the evidence in favour of the filicinean affinities of the family it is appropriate to inquire which of the ferns are most closely related. When one considers the isolation of the family as a whole, and the differences between the genera, especially shown in the monotypic *Helminthostachys*, it becomes apparent that the family diverged from the main filicinean stock at a very remote date. The large sporangia opening simultaneously and lacking an annulus, the eusporangiate habit, the usually vertical rhizome, the gap in the leaf-trace formed at the point of exit of the strands which supply pinnae, indicate that the affinities of the family are with the group Simplicis, especially with Osmundaceae and Marattiaceae. More than this cannot be safely inferred from the data at present available.

The evidence brought forward in the present study supports the earlier view that this family shows a series of reduced forms. Starting with a fern whose leaves bore sporangia over the whole of the lower surface, there has apparently been a sterilization of certain leaflets, similar to what may be seen in *Osmunda Claytoniana*, except that in the present case it is the basal

ones which remained fertile. We may surmise that the next step was the raising of the fertile pinnae into the vertical position, so that the ancestral plant at this stage resembled *Aneimia*, as is shown in the instances of reversion in *Botrychium obliquum*. The next step was the fusion of the two fertile pinnae to form the fertile spike, and this produced the genus *Botrychium*, of which the large-leaved forms are, according to this view, the most primitive. Bower (6) has shown that there is a remarkable sequence of forms, which we may take the liberty of arranging backwards thus: *Botrychium Lunaria*, *B. simplex* (various forms), *Ophioglossum vulgatum*, *O. Bergianum*. Within the genus *Ophioglossum* there are signs of increasing complexity leading to *O. palmatum*; also, as Bower suggests (p. 479), a probable line of reduction including *O. pendulum*, *intermedium*, *simplex*. On the other hand both the rhizome and the leaf of *Helminthostachys* indicate that this genus represents an ascending line which early branched off from the composite form which is believed to have resembled *Botrychium*.

My hearty thanks are due to a number of friends who have kindly supplied material used in the present study: to Dr. J. C. Willis, Director of the Royal Botanic Garden at Peradeniya, Ceylon, for *Helminthostachys*; to Professor E. C. Jeffrey, for a young specimen of *Helminthostachys*, and for the use of the photographic equipment of the Laboratory of Phanerogamic Botany at Harvard University; to Mr. J. C. Parlin, for several species of *Botrychium*; to Mr. F. W. Bachelder, for a generous supply of abnormal *Botrychium obliquum*; to Professor G. L. Goodale and Mr. R. Cameron for many ferns from the Harvard Botanic Garden; and to Professors A. F. Blakeslee and N. L. Britton, Mr. A. J. Eames, Professor J. W. Harshberger, Dr. O. W. Knight, Mr. C. H. Knowlton, and Mr. C. S. Ridgway for other material.

#### SUMMARY.

1. The pair of vascular bundles which supply the fertile spike in *Botrychium virginianum* arise from near the two edges of a trough-shaped leaf-trace which is generally split into halves. Each of these bundles leaves a gap in the leaf-trace, which is obscured by the slight divergence of the bundles from the trace. Similar gaps occur in Osmundaceae, Polypodiaceae, and other families of ferns.

2. The main vascular supply of the petiole runs into the sterile segment, where the bundles which supply the pairs of leaflets arise in exactly the same manner as those of the fertile spike. It is therefore inferred that the fertile spike represents two fused leaflets or pinnae, namely the basal pair, of a fern leaf.

3. In *B. ternatum* and *B. obliquum* the bundles which supply the fertile spike arise directly from the edges of the trough-shaped leaf-trace. The

bundles of the branches of the fertile spike, however, leave either a distinct gap, or a more or less degenerate one. The condition found in these species is therefore considered to be derived by reduction from that found in *B. virginianum*.

4. Certain other species of *Botrychium* show no trace of this gap, and are considered to be further reduced.

5. Abnormal specimens of *B. obliquum* show either a pair of fertile spikes, in which case they resemble *Aneimia*, or a pair of spikes with an additional larger one inserted somewhat below the pair and corresponding to two fused spikes. The internal structure of these and other similar specimens bears out the view here adopted (see 2 above), hence such cases are to be regarded as reversions.

6. In *Ophioglossum* the bundles leading to the fertile spike break off from the two edges of the curved row of strands which represents the leaf-trace. This condition is considered to be derived from that found in *Botrychium*.

7. The vascular supply of the fertile spike in *Helminthostachys* is derived principally from one edge of the curved row of bundles which forms the leaf-trace; this is more clearly seen in a young specimen. It is inferred that the fertile spike in this genus represents a single pinna.

8. The Ophioglossaceae are considered to be related to the ferns and to have sprung from near the level of Osmundaceae. They appear to have branched off from the primitive stock at a very remote period.

9. A study of the internal structure of the leaf in Ophioglossaceae strongly supports the view of Roeper that the fertile spike represents two fused basal pinnae, though in certain cases a spike represents a single pinna, which, however, does not arise ventrally. No support is afforded to the view of a strobilar origin or of a direct derivation from Hepaticae.

ORONO, MAINE,  
August, 1909.

NOTE.—During September, 1909, the writer visited at Sandwich, N.H., one of the stations where abnormal *Botrychium obliquum* had been found. A number of additional specimens were collected, and it was observed that nearly all of the unusual forms mentioned on pp. 7-9 are exemplified also in the variety *dissectum*. One specimen approaching *dissectum* had the fertile spike sterile with the exception of part of one of the basal branches. At this station and at Manchester, N.H., the number of abnormal specimens amounts to at least 10 per cent.

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## EXPLANATION OF PLATES I AND II.

Illustrating Dr. Chrysler's paper on the Ophioglossaceae.

### PLATE I.

Fig. 17. *Todea barbara*. Transverse section through midrib of leaf at level of origin of a pair of pinnae. The vascular supply of one pinna is visible on the right side. The adaxial side is placed upward in this and all the other figures. × 10.

Fig. 18. *Osmunda Claytoniana*. Transverse section through midrib of leaf at level of origin of a pair of pinnae. The gaps left by the exit of the branches supplying the pinnae have closed. × 20.

Fig. 19. *Botrychium virginianum*. Transverse section through petiole, showing the origin of the strands which supply the fertile spike. These strands appear at the upper side of the figure. × 20.

Fig. 20. *Botrychium ternatum*. Transverse section through axis of the fertile spike, showing the mode of origin of two branch strands. × 25.

Fig. 21. *Marsilia quadrifolia*. Transverse section through petiole, showing the mode of origin of the strand which supplies the sporocarp. This strand appears at the left, and is enclosed in dense sclerenchyma.  $\times 90$ .

Fig. 22. *Helminthostachys zeylanica*. Transverse section through petiole of a young specimen. The odd strand in the upper part of the figure arises from the strand at its right and supplies the fertile spike, the base of which is visible as a ridge on the surface of the petiole.  $\times 30$ .

PLATE II.

Fig. 23. *Aneimia tomentosa*, Swartz, var. *fulva* Hook and Bak. Introduced for comparison.

Fig. 24. *Botrychium obliquum*, Muhl. Specimen with a pair of fertile spikes.

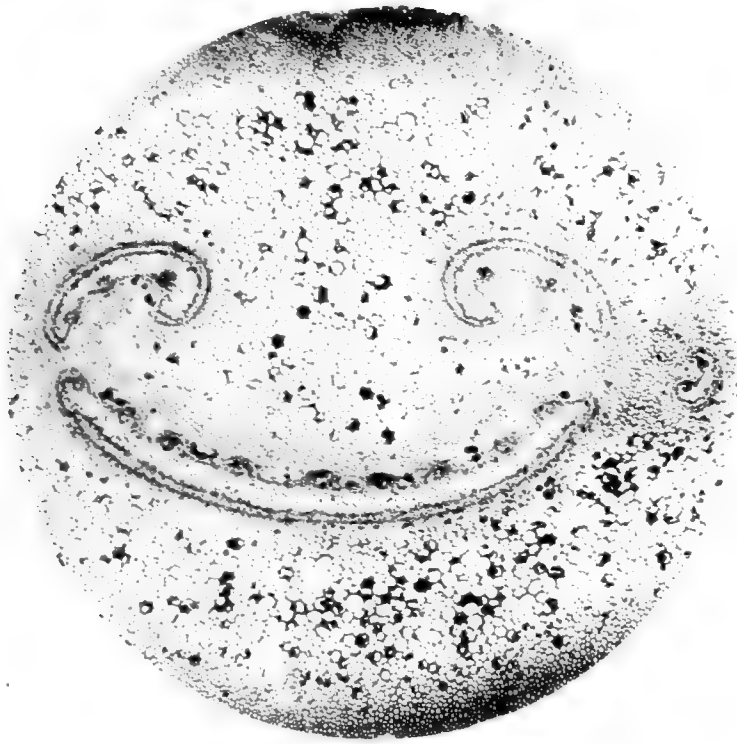
Fig. 25. Same. Specimen with one fertile pinna in the sterile segment. This pinna is really lateral, but has been bent upward in pressing the plant.

Fig. 26. Same. Specimen with a pair of fertile spikes in addition to the ordinary one, which is seen to be inserted further down than the pair.

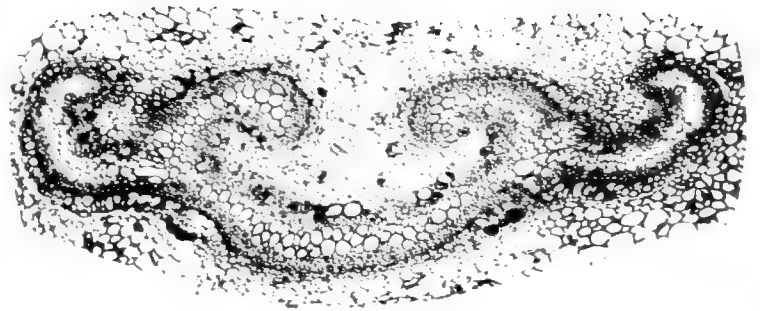
Fig. 27. Same. Specimen with an extra fertile spike, which in this case represents a single pinna.

Fig. 28. Same. Specimen with two fertile spikes partly fused.

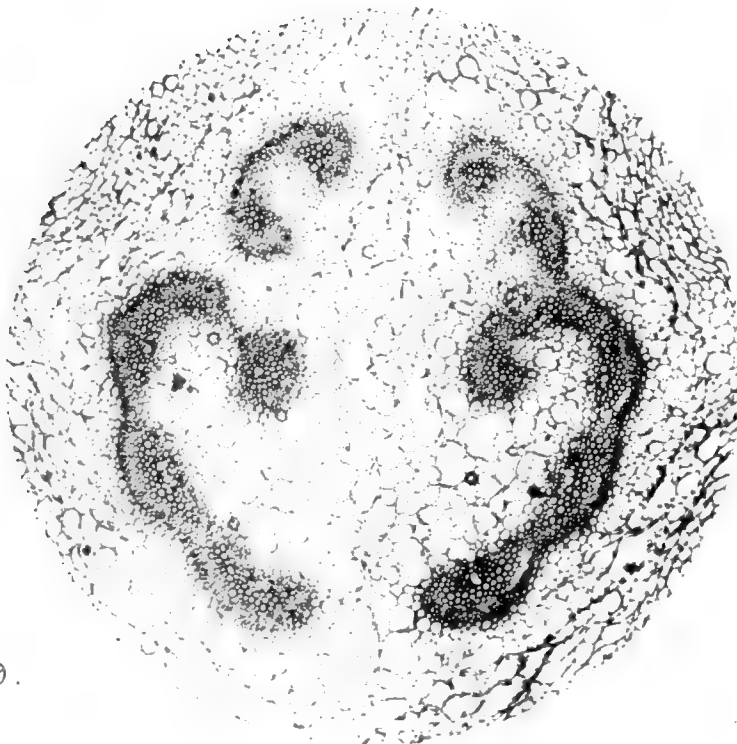




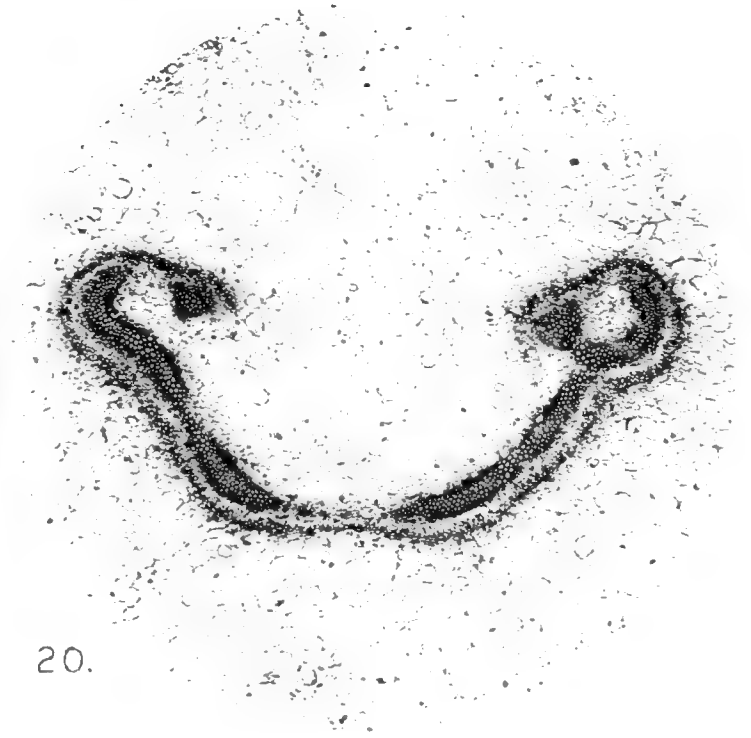
17.



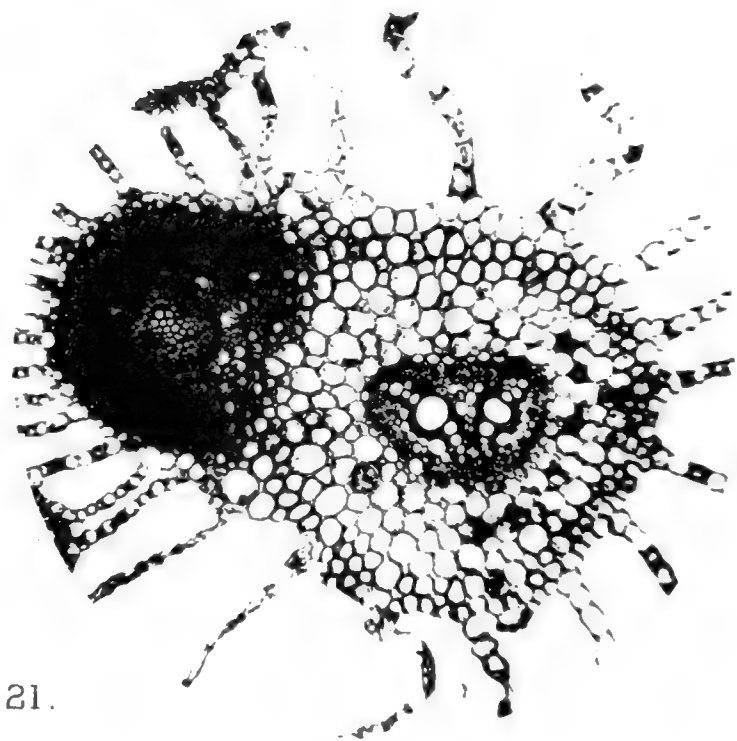
18.



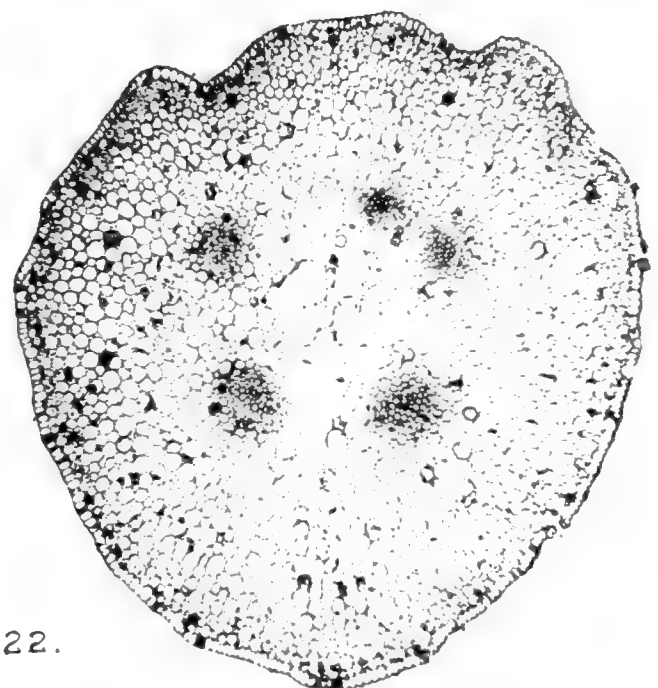
19.



20.



21.



22.

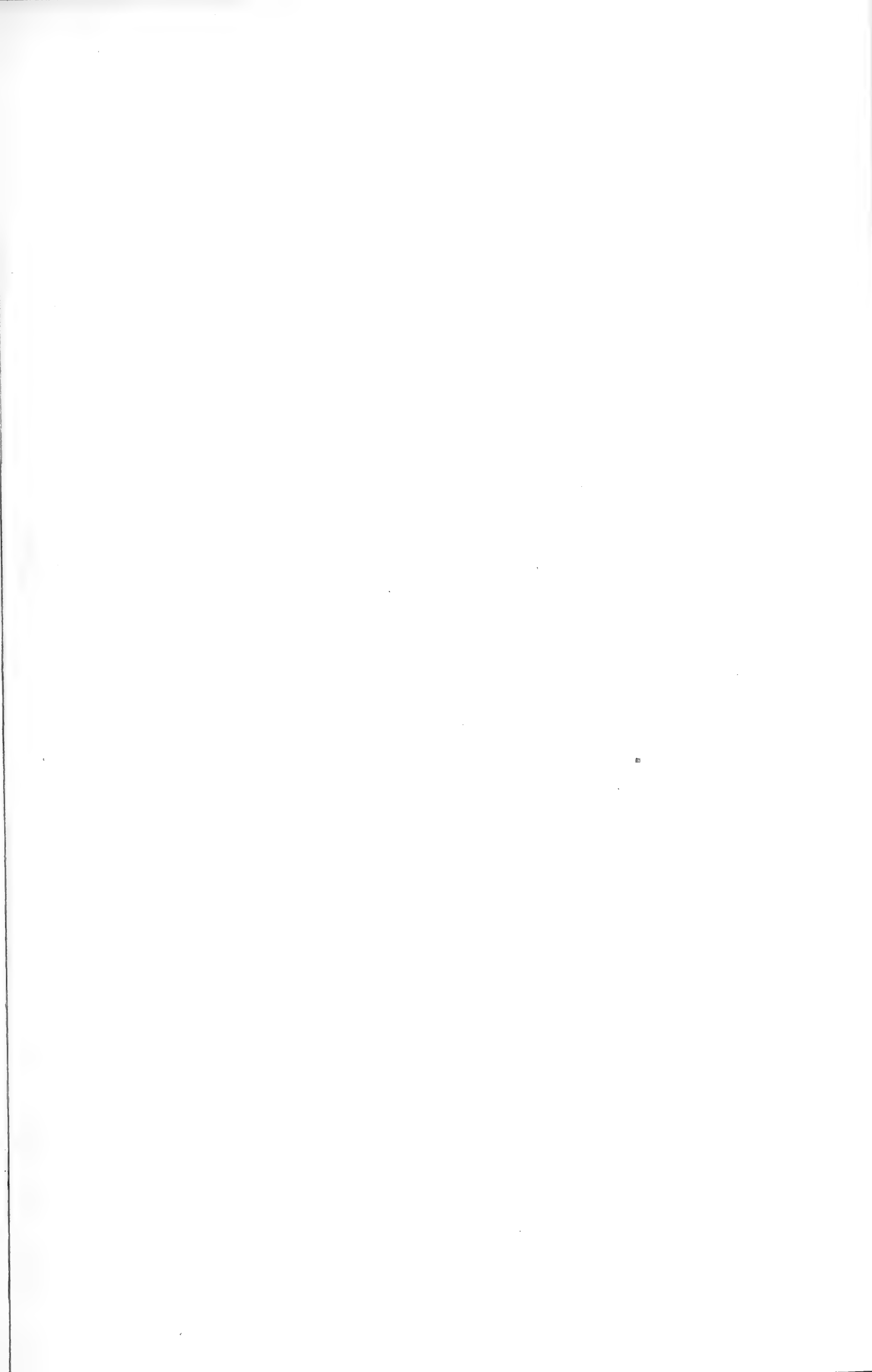
M.A.C. photo.

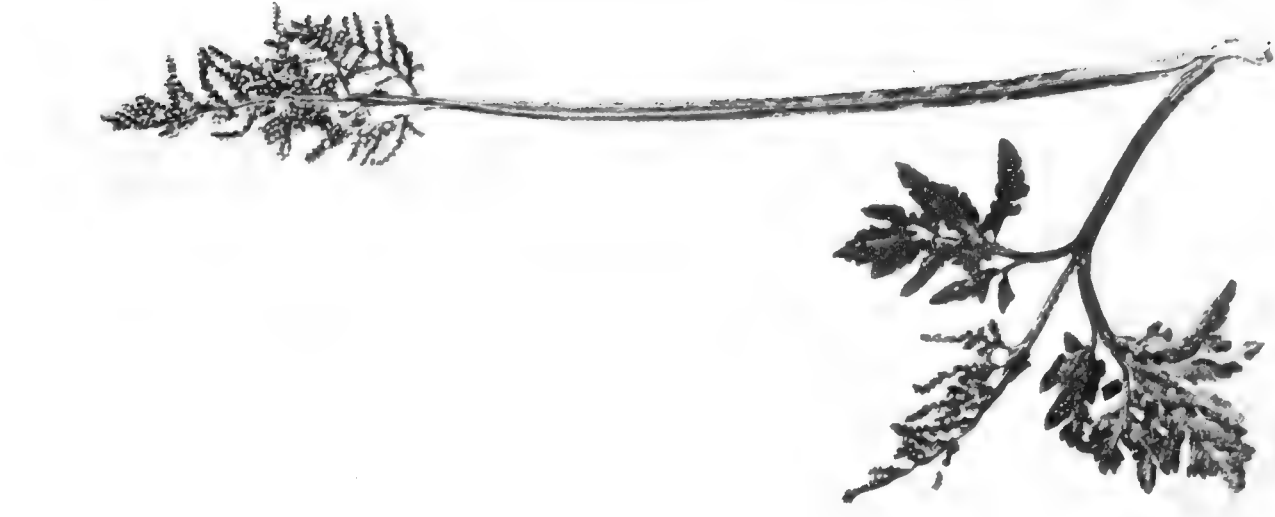
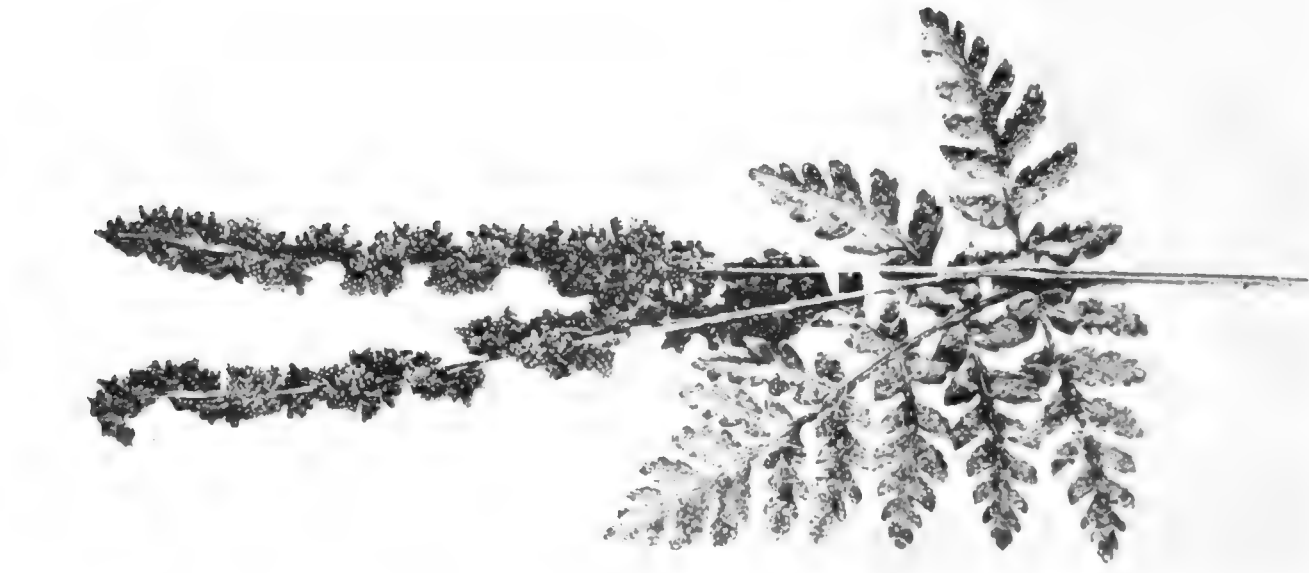
Huth coll.

CHRYSLER — OPHIOGLOSSACEAE.











26.

M.A.C. photo.



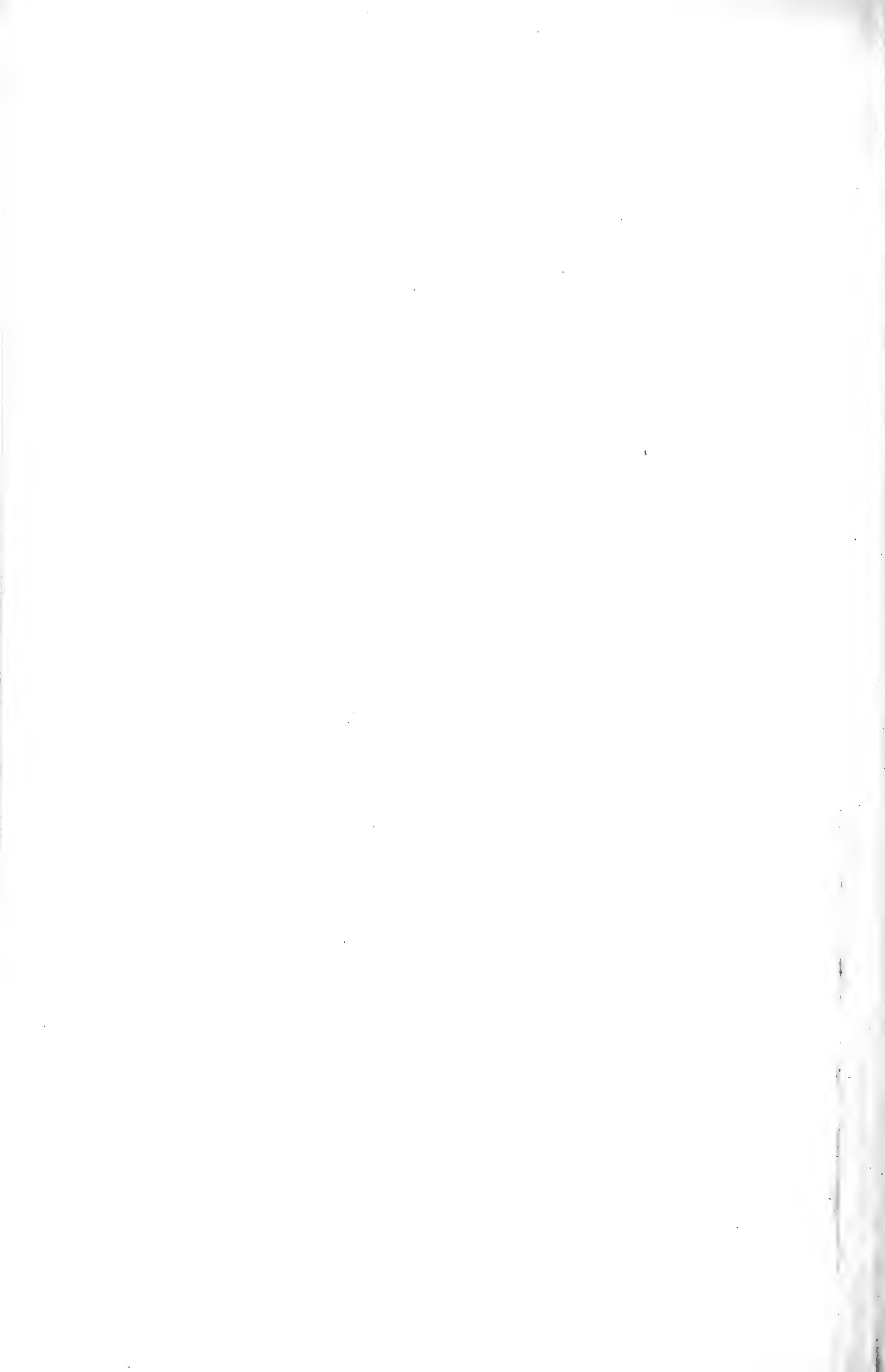
27.

CHRYSLER — OPHIOGLOSSACEAE.



28.

Hirth coll.



# Contributions towards a Knowledge of the Anatomy of the Genus *Selaginella*, Spr.

## Part V. The Strobilus.

BY

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With Plates III and IV.

[PRELIMINARY NOTE.—In previous volumes of the *Annals of Botany* I recorded certain observations made by myself on the Comparative Anatomy of the stem, root, leaf, and ligule of *Selaginella*. Pressure of other duties has hitherto prevented me from recording observations on the anatomy of the strobilus, as I had originally intended to do.

Since a very considerable amount of work has already been carried out on the structure of the sporangia and on the gametophyta by various investigators, more especially by Pfeffer, Millardet, Belajeff, Lyon, Bruchmann, and Bower, the present paper cannot expect to do more than fill up, at least in part, some gaps left by these authors or confirm or controvert statements made by them. Being myself unable to devote the necessary time to the work, I asked one of my pupils, Miss Gertrude Mitchell, B.Sc., to undertake the investigation of the material I had collected. This she has done under my supervision, and the results of her work are now submitted. Miss Mitchell has confined herself to a study of the structure of the strobilus and its parts from a morphological point of view, and has made no attempt to deal with the spore-contents, since the material available had, unfortunately, not been preserved with a view to cytological research.

R. J. HARVEY-GIBSON.]

IN the present paper I purpose recording some observations on the structure of the adult strobilus of the genus *Selaginella*, omitting, however, the gametophyta. The work, which was carried out in the Hartley Botanical Laboratories, University of Liverpool, was undertaken at the suggestion of Professor Harvey-Gibson, with the view of completing his series of papers dealing with the anatomy of the genus, and I am indebted to him for his help and advice during the prosecution of the work.

The material examined comprised thirty-eight species, for which I am indebted to Professor Harvey-Gibson, who placed his collection at my disposal; I am also indebted to the Director of the Royal Gardens, Kew, who kindly forwarded me fresh material of *S. Lyallii* and *S. Wildenowii*.

The literature referring to the cone of *Selaginella*, apart from the development of the gametophyte, is very scanty. Goebel, Schwendener, Steinbrinck, and others have investigated the structure of the sporangium wall, with the view of ascertaining the mechanism of spore discharge. The recent researches of Miss Lyon on *S. apus* and *S. rupestris* have demonstrated very clearly the progress *Selaginella* has made in the direction of the seed habit. The study of the cone is, however, specially interesting as supporting in some measure the theory advanced by Professor Bower as to the origin of the strobilus in Archegoniate plants. It is, of course, not to be expected that an investigation of *Selaginella*, a type essentially specialized in having acquired a definite strobilus and heterosporous habit, can be as enlightening as a study of the simpler Lycopodiaceae, but detailed examination has revealed numerous examples of reversions to more primitive conditions, such as branching of the fertile region, alternation of sterile and fertile portions in the strobilus, continued apical growth beyond its apex, re-establishment of dorsiventrality, &c., and the occasional occurrence of sporangia in the axils of purely vegetative leaves, all of which anomalies are not only easily explained on Bower's hypothesis of the evolution of the sporophyte, but would naturally be expected to occur, and are to be read as emphasizing the phylogenetic affinity between the Selaginellaceae and the Lycopodiaceae, and confirming the assumption that the former are but specialized derivatives of the latter.

The following is a list of all the papers I have had occasion to consult, and these will be referred to in the text by the prefixed numeral:—

1. BOWER: Origin of a Land Flora, 1908.
2. ———: Abnormal plurality of sporangia in *Lycopodium rigidum*. *Annals of Botany*, xvii, 1903.
3. ———: Imperfect sporangia in certain Pteridophyta—are they vestigial? *Annals of Botany*, xv, 1901.
4. ———: Theory of the Strobilus in Archegoniate plants. *Annals of Botany*, viii, 1894.
5. ———: The Morphology of spore-producing members. (Equisetales and Lycopodiales.) *Trans. of Royal Society, Ser. B.* clxxxix, 1897.
6. LYON, F. H.: Sporangia and gametophytes of *S. apus* and *S. rupestris*. *Botanical Gazette*, xxxvi, No. 4, 1903.
7. ———: Another seed-like characteristic of *Selaginella*. *Botanical Gazette*, xl, No. 1, 1905.
8. ———: Two megasporangia in *Selaginella*. *Botanical Gazette*, xxxvi, No. 4, 1903.
9. GOEBEL: Sporangien, Sporenverbreitung und Blütenbildung bei *Selaginella*. *Flora*, lxxxviii, 1901.
10. STEINBRINCK: Ueber den Schleudermechanismus der *Selaginella*-Sporangien. *Berichte der deutsch. bot. Ges.*, xx, 1902.
11. SCHWENDENER: Ueber den Öffnungsmechanismus der *Selaginella*-Sporangien. *Berichte der deutsch. bot. Ges.*, xxi, 1903.

12. STEINBRINCK: Kohäsions- oder 'hygroskopischer' Mechanismus. Ber. der deutsch. bot. Ges., xxi, 1903.
13. HARVEY-GIBSON: Contributions towards a knowledge of the anatomy of the genus *Selaginella*, Spr. I. The Stem. Ann. of Bot., viii, No. xxx, 1894.
14. —————: Contributions towards a knowledge of the anatomy of the genus *Selaginella*, Spr. II. The Ligule. Ann. of Bot., x, No. xxxvii, 1896.

### THE ANATOMY OF THE CONE.

*General description.* In the great majority of the species the fertile region is well marked off from the purely vegetative part as a definite strobilus, sometimes distinctly pedunculate (as in *S. helvetica*), frequently tapering towards the apex, and varying in dimensions according to the species. The length of the cones, which occur singly or in clusters at the tips of the branches (e. g. *S. Kraussiana*), ranges from one-quarter inch in the more delicate types (*S. viridangula*, *S. haematodes*, &c.) to the comparatively massive structures of *S. helvetica* and *S. spinosa*, which often attain a length of two or even three inches. The leaves are generally smaller and less expanded than they are on the vegetative region of the stem, being more in the nature of protective bracts (though the reverse is true of *S. helvetica*, *S. spinosa*, &c., where the axes have small isophyllous leaves, and the sporangia are extremely large), and are hollowed to accommodate the sporangia, which arise one in the axil of each leaf. In some cases, notably *S. Lyallii* and *S. rupestris*, the leaves have a strongly recurved portion at the junction of the leaf-base and the upturned lamina, so that the sporangia come to be enclosed in a definite chamber—an obvious adaptation to the environment. Homophylly is not a constant feature of the cone; *S. molliceps*, for example, has a resupinate arrangement of leaves. The degree of compactness and regularity of outline are determined by the set of the leaves. The sporophylls are, in all cases, spirally arranged round the axis (as can easily be seen in the straggling cones of *S. helvetica*), but the spiral may become so condensed that the leaves arise almost opposite to each other, in four distinct rows, the lower ones considerably overlapping those immediately above. Beyond the somewhat narrowed leaf-base the lamina curves sharply upwards, parallel with the axis of the cone, thus giving the regular quadrangular outline so characteristic of *S. Wildenowii*, *S. Wallichii*, &c. As a general rule the cone is terminal and unbranched. The following interesting variations from the normal type may be referred to:—

*a. S. patula* and *S. cuspidata* (cf. Pl. IV, Fig. 14):—

Beyond the fertile homophyllous cone, the axis continues to grow vegetatively, reverting to the dorsiventral structure characteristic of the ordinary stem.

*b.* An unnamed species from India, probably *S. pennata*, exhibited the same phenomenon, save that abortive sporangia were produced in the axils



of the vegetative leaves immediately following the tip of the cone, illustrating the gradual transition between the purely sterile and entirely fertile regions.

*c.* In *S. erythropus* a second cone was produced on a fertile branch after an intervening sterile region, entirely devoid of any vestiges of sporangia; in other words, two definitely fertile regions occurred on the same branch.

This alternation of sterile and fertile zones in the four species quoted suggests the condition normally occurring in the more primitive Lycopodiaceae.

*d.* Fig. 11, Pl. IV, represents an abnormal cone of *S. oregana*. That this is one branched strobilus, and not two cones, is demonstrated by the character of the leaves, and the distribution of sterile and fertile regions. The intervening leaves in the sterile regions retain, however, the external form of sporophylls.

#### *Distribution of the Sporangia on the Cone.*

Both mega- and microsporangia occur as a general rule on the same cone, and arise from the axis, one in the axil of each leaf. The disposition of these on the cone is not constant for the genus, there being four chief types of arrangement.

A. One large basal megasporangium subtended by a specially large leaf, e. g. *S. Kraussiana*, *S. Lyallii*, *S. convoluta*, *S. Bakeriana*, *S. sulcata*, *S. Galeotti*, *S. rubella*, *S. Wildenowii*, &c.

B. Several basal megasporangia followed apically by microsporangia, e. g. *S. spinosa*, *S. Vogelii*, *S. molliceps*, *S. helvetica*, *S. Braunii*, *S. oregana*, *S. rupestris*, &c.

C. Cones wholly megasporangiate or microsporangiate, e. g. *S. atroviridis*, *S. inaequalifolia*, *S. gracilis*, *S. Lobbii*, *S. viridangula*, &c.

D. Indiscriminate arrangement of mega- and microsporangia, e. g. *S. Martensii*, *S. haematodes*, *S. caulescens*, *S. patula*, *S. serpens*, *S. involvens*, *S. cuspidata*, *S. erythropus*, *S. flabellata*, *S. Wallichii*, *S. viticulosa*, &c.

Occasionally, exceptions from this rule are found; for example, (*a*) *S. molliceps* and *S. viticulosa* sometimes produce cones which are megasporangiate only, and (*b*) *S. erythropus* and *S. flabellata* entirely microsporangiate ones. This is not very surprising since, normally, the number of megasporangia in (*a*) and microsporangia in (*b*) greatly predominates.

A detailed examination of the cones with special reference to the distribution of the sporangia leads to the following generalizations:—

1. The megasporangia usually occupy the basal region.

2. The species which have acquired a definite arrangement (notably groups A and B) are those which show the greatest difference in shape and size between the micro- and megasporangia and the greatest elaboration of structure of the latter.

In group D the two kinds of sporangia are approximately of the same size, neither showing any great degree of complexity. It is of interest to note that the species in this group belong almost exclusively to the *S. Martensii* group (13), and that the indefinite character of the cone is associated with a corresponding simplicity of stem structure; otherwise the classification of the species as based on the anatomy is not followed definitely by peculiarities of sporangial arrangement.

*The occurrence of imperfect sporangia.*

Bower (3) states that 'no cases of alternating sterile and fertile zones are recorded, but in some species the fertile spikes revert at the apex to a vegetative character. Abortive sporangia have been seen at the base of the strobilus of *S. spinosa* and *S. Martensii*, and would doubtless be found in many species, but no isolated sporangia have been seen in the sterile region.'

The occurrence of abortive sporangia in *Selaginella* is a far more widespread phenomenon than one would be led to suspect by Professor Bower's statement. In most cones, scattered but imperfect sporangia are to be found. In *S. Kraussiana*, the majority of the sporangia never reach maturity, but are arrested at the stage figured in Pl. IV, Fig. 12, the whole being of a distorted shape, consisting of a two-layered wall, stalk, disorganized tapetum, and sporogeneous cell-mass in the interior. Those which do develop are large, massive structures, but they are few in number.

In *S. helvetica*, *S. Wallichii*, *S. oregana*, and *S. flabellata*, all of which produce elongated cones, the sporophylls of the three latter being particularly crowded and numerous, the whole of the middle region is frequently sterile (cf. *Lycopodium Selago*).

At the base of every cone in *S. involvens*, the megasporangia are persistently abortive. The mature megasporangia are found above this region, and are large, and contain four dark, thick-walled spores. The same is true also of *S. gracilis*, where the basal sporangia are invariably shrivelled. *S. viridangula* progresses a step further. Here, the basal sporangia are entirely abortive, and the basal sporophylls are purely vegetative in function.

That the definite sterilizations quoted above (whether occurring in the middle, or at the base, of the strobilus) are utilitarian, and make for the increased efficiency of the spore production as a whole, is well illustrated by the case of *S. Kraussiana*. This species is fairly delicate, and the strobili are developed in profusion, in clusters at the tips of the branches. The supply of nourishment is insufficient to bring all the sporangia to maturity. Most of them, therefore, abort at an early stage in development, and the subtending leaves become purely vegetative. These sterilizations preserve the balance between the spore-producing and the vegetative parts, and are best explained as a continuation of the process by which the

strobilus itself was originally produced. The occurrence of sporangia in the sterile region is very rare, only one case (*S. molliceps*) having come under my notice. The sporangium was, of course, abortive, but was situated in the axil of one of the large stem leaves, at a distance of quite three inches from the cone. Remains of one abortive megaspore could be seen.

*Plurality of Sporangia.*

Miss Lyon (8) has recorded a case of two megasporangia in the axil of one leaf in *S. rupestris*, one situated in the normal position, and the other in the line joining this one to the base of the ligule. Bower (2), on examining *Lycopodium rigidum*, discovered a similar case, the two sporangia, however, lying side by side, and arising probably 'by the separation of the sporogenous group of a normal sporangium'.

In *S. Lyallii* I found a further anomalous condition. The two sporangia (which were found on a leaf quite at the tip of the cone) were widely removed from each other; one was perfectly normal in character, but the stalk of the other occupied the position of the glossopodium of the ligule (Pl. IV, Figs. 1, 2). The interpolated sporangium had taken the place of the lamina of the ligule, or possibly the lamina of the ligule had changed into a sporangium. I have not met with any other case of a double sporangium either in *S. Lyallii* or in any other species. If the cases cited above be regarded as merely sports, Bower's statement (2) that 'the interpolation of accessory sporangia is entirely absent in the Lycopods' is quite supported by the Selaginellaceae.

*The variation in the number of spores in the sporangium.*

The number of spores in the microsporangium is indefinite, but always exceedingly large; the megasporangium contains typically four, and frequently remains of the degenerate spore mother-cells may be seen.

Besides the cases described by Miss Lyon (6) in *S. apus* and *S. rupestris*, reductions take place in the following species also, and are classified according to the character of the reduction.

1. *Inequality of size of spores.*

<i>S. Vogelii</i> ,	2 cases	2 small, 2 large.
<i>S. rubella</i> ,	1 case	" "
<i>S. Kraussiana</i> ,	2 cases	3 large, 1 small.
<i>S. Lyallii</i> ,	1 case	3 " 1 "
<i>S. Braunii</i> ,	1 case	2 " 2 "
<i>S. rupestris</i> ,	several cases.	

2. *Reduction to three equal megaspores.*

*S. cuspidata* (2 cases), *S. molliceps* (2 cases), *S. Kraussiana* (1 case), *S. Braunii* (1 case), *S. oregana* (2 cases). In *S. Bakeriana* there are normally three megaspores. This is so constant a feature that the

sporangium has, as a rule, three lobes, instead of four. Occasionally there is evidence of the fourth abortive spore.

3. *Reduction to two equal megaspores.*

*S. molliceps* (2 cases), *S. patula* (1 case), *S. flabellata* (1 case).

In *S. rupestris* there are normally two spores.

4. *Reductions to one megaspore of correspondingly large size.*

*S. sulcata*, *S. molliceps*, *S. rupestris*.

Cases of increase in the number of spores are very rare. I have only discovered two—in *S. Vogelii* (12 spores) and *S. involvens* (8 spores). These are suggestive of the common ancestry of the micro- and megasporangia, whilst the reductions are indicative of a tendency towards the seed habit, specially exemplified in *S. Bakeriana* and *S. rupestris*. It is difficult to say how these reductions have arisen. In the case of two equal spores, one is tempted to explain them by premising one division only of the spore mother-cell. This has been proved conclusively for *S. rupestris* (6), none of the spores developing if two divisions occur. However, in face of the frequent occurrence of three equal spores, and occasional small abortive ones, it seems more probable to suggest, as the general rule, that division into tetrads takes place, but that some develop excessively at the expense of the others.

The reduction to one megaspore in certain species, and the confirmed tendency to reduction in others, become more significant when taken in conjunction with the fact that not only are archegonia developed, but that fertilization and development of the embryo with roots and cotyledons frequently take place whilst the spores are enclosed within the sporangium and still attached to the strobilus (6). With particular reference to *S. rupestris* Miss Lyon remarks: 'The strobili may fade and separate from the plant before fertilization, but the spores do not fall from the sporangium. In no case is there evidence of fertilization in the spores that are shed' (6). *S. rupestris* has, therefore, progressed a long way in the direction of the seed habit. Indeed if one could conceive a hypothetical case of a unispored megasporangium of *Selaginella*, whose spore had been fertilized and had developed an embryo whilst still surrounded by the protective sporangium wall, we should have what might be regarded as the morphological equivalent of a seed. With respect to this seed-bearing habit, the Selaginellaceae are far ahead of their lower allies, the Lycopodiaceae, and suggest a parallel, even though a remote one, with the Gymnosperms.

*Detailed structure of the sporangium wall, and mechanism for casting of spores.*

Both micro- and megasporangia are stalked, attached to the stem just above the origin of the leaf. The megasporangium is definitely four-lobed, owing to the relatively large size of the four enclosed spores, whilst the



microsporangium has a smooth, regular outline, and contains numerous small microspores, which are, however, tetrahedral in shape. The adult sporangium has two walls—an outer one, containing chlorophyll until after the spores are shed, and considerably modified in various parts as regards thickness, &c., and an inner one, uniform throughout, consisting of somewhat flattened, sometimes elongated cells, whose boundaries are difficult to distinguish. The tapetum, which is so characteristic and prominent a layer in developing sporangia, persists until the spores are nearly mature, but then degenerates into a pavement-like layer whose cellular structure can scarcely be determined (Pl. III, Figs. 1 and 2). Miss Lyon (6) records the occurrence of an incomplete septum in the sporangium of *S. rupestris*, and compares this with the similar, more complete, trabecular structure in *Lepidostrobis Brownii*. Bower suggests that the function of this may be either (*a*) supporting, or (*b*) nutritive; probably the latter, since the structure in question occurs in the microsporangium only. I have failed to discover any similar cases in the other species of *Selaginella* which I have examined.

The mechanism for the dispersal of the spores in the genus is the most elaborate contrivance in the whole of the Pteridophyta. It was at first suggested that when once the sporangium had opened, the spores were scattered by the wind. This hypothesis is feasible if reference be made to the microspores only, since they are very light, and are no doubt frequently blown about from place to place. The megaspores, however, are not only far too heavy, but are difficult of wind access, each being sunk in a depression of the sporangium wall. Moreover it is made perfectly evident, on watching the bursting of the sporangia, that the scattering of the spores is not left to chance; on the contrary, the spores are shot out with considerable violence by a definite mechanism, the force being so great that the sporangium itself may be jerked bodily to a distance of 3 to 4 cm., while the spores may travel much further, the megaspores from 6 to 10 cm., but the microspores from 1 to 1½ cm. only. It will be seen that the megaspores are shot out further than the microspores, a fact scarcely substantiating the 'wind agency' theory of dispersal. Goebel (9) suggests that this is an adaptation for cross-fertilization, and this explanation is supported by the proterogynous nature of the strobilus, and also the facts that (*a*) the microspores germinate sooner than the megaspores of the same cone, that (*b*) the archegonia develop six weeks after the antheridia taken from the same cone are empty, and that (*c*) no embryos are obtained if the micro- and megaspores of the same strobilus are sown together. Goebel's hypothesis, therefore, becomes not only feasible, but highly probable.

Schwendener, Goebel, Steinbrinck, and others have done a considerable amount of research on the structure of the sporangium wall, with special reference to the mode of distribution of the spores, and the observations which I have recently made on the subject are entirely in agreement with

those of the two later investigators, as may be seen on comparison of the following account with the account given by Goebel and Steinbrinck (9 and 10).

The type I selected for detailed examination was *S. spinosa*, since the sporangia in this form are large and well thickened, the movements of opening the sporangium and ejection of the spores taking place very vigorously even in dead sporangia which have been preserved in spirit.

Schwendener (11) has attributed the function of distribution of the spores to the hygroscopic action of the thin inner sporangium wall, and states that the outer wall not only takes no part in the shooting of the spores, but by its very nature militates against this. Goebel and Steinbrinck relegate the function to the outer wall.

The megasporangium is bivalved, and is attached in such a manner that one valve (the upper one) faces the axis of the cone, while the lower one faces the sporophyll, which is narrowed at the base so as to facilitate such movements. The sporangium is four-lobed, the spores being arranged so that they fit together in the centre, their inner faces becoming flattened, the outer faces remaining rounded, and creating a corresponding bulge in the sporangium wall. Two of the spores are situated below in the basal portion of the sporangium, two at right angles to these in the upper part. I have not as yet seen the alternative arrangement noted by Goebel (9), i. e. one spore lying superiorly and resting on the other three below, and therefore judge it to be extremely rare and abnormal.

In order to examine the various stages in the opening of the megasporangium, I dissected off several sporangia from the strobilus, fastened them down by their stalks with thick Canada balsam, so that the valves were quite free to move, although bodily movement of the whole was prevented, and watched them under a low power. On drying, two cracks first of all appear in the region marked *c, c'* (Pl. III, Fig. 4); the apex then opens fairly rapidly, though not with a jerk. The cracks divide the sporangium into two upper flaps and a basal boat-shaped portion. One of the flaps remains practically stationary, the other opens still further, until finally the sporangium gapes widely and all the four spores are exposed. After a short quiescent period, the four spores are jerked out with great violence, frequently to a distance of 10 cm.

A careful examination of the sequence of events shows quite clearly that the essential and functional part in the ejection of all four spores is the basal boat-shaped portion, and not the flaps (see Goebel and Steinbrinck), and the explanation is to be found in the extremely complicated nature of the outer wall of the sporangium. In Pl. III, Fig. 4, a sketch is given of the lower valve of the sporangium of *S. spinosa*. The crack regions can easily be distinguished before opening occurs, for the cells in the dehiscing region are small and shallow, standing out in sharp contrast with the much



thickened surrounding cells. They are easily ruptured on the drying of the sporangium. The basal portion is much more thickened than the flaps, the cells at the edge of the valve being specially large and thick (Pl. III, Fig. 1). Connecting the lower parts of the valves, and visible only as a deep furrow in the closed sporangium, is a definite layer of thin-walled tissue, easily seen on pulling the valves apart (Pl. IV, Fig. 4), and, as will be noted later, subserving a definite function. This tissue also extends between the upper flaps, but is not so evident in that situation. The cells in the basal region are not isodiametric, their long axes being orientated in different directions (Pl. III, Fig. 4), those near the edge of the valve being approximately parallel to that edge, whilst those between the cracks are exceptionally thick, and specially noticeable in travelling across in a directly transverse direction. Above this region, and in the upper flap, occurs the hollow prepared for the reception of one of the upper spores, and the cells round this become smaller and thinner walled, as they approach the edge of the flap. In this region the cells are arranged in rows approximately parallel to the margin of the flap. Transverse and longitudinal sections show (Pl. III, Figs. 1 and 2) that the structure of the active thick-walled cells is precisely the same as that of the cells of the fern annulus, i. e. the outer walls are thin, the inner ones strongly thickened, the thickening narrowing in the radial direction towards the outer margin; whilst the cells connecting the basal parts of the sporangium are thin, shallow, and entirely unthickened. The smaller cells figured at 1 c give additional flexibility to the sporangium wall.

The orientation of the cells in the basal portion is of importance. On examining the two valves of one sporangium it is evident, as Steinbrinck has already pointed out, that the transverse band of cells between the cracks is found only on one valve, viz. the lower one. This acts as a hinge on which the flap of the lower valve is turned back. The upper valve cannot move any considerable distance on account of the rigid axis of the cone; the lower valve, on the contrary, by its vigorous movement, presses back the sporophyll (which is narrowed at the base to allow of this), and thus gives greater space for the shooting out of the spores. The relative rigidity of the upper valve is necessary in order to give the lower valve full play.

When the sporangium opens apically, the flaps diverge, but at the same time continually change their shape. The edges of the flap curl over and turn back, thus widening the space between the valves and creating more space for the distribution of the spores. The changes take place gradually, so that when the flaps reach a stationary condition the upper spores are lightly poised on a flat or even sometimes convex surface (i. e. concave outwardly) and readily respond to any movement of the lower part of the sporangium.

(The fact that the two upper spores are sometimes shed first, and that the flaps are frequently convex instead of concave after ejection, led me at

first to seek an independent mechanism for these spores. I have not been able to discover one, and any such variation from the rule can easily be explained, as will be shown later.)

Whilst this change is taking place in the flaps, the boat-shaped portion has altered considerably. The sides, which were at first strongly convex, straighten out and approximate to each other, this process being facilitated by the thin tissue connecting the valves assuming a convex, instead of retaining its original concave, form. It is on this account described by Goebel as a 'hinge', whose function is to permit the elongation of the basal boat-shaped portion. The gradual approximation of the two lower walls (due to the thin outer walls of the active cells becoming concave) exerts a pressure on the two lower spores, and they are gradually squeezed outwards, the sporangium then appearing as represented in Pl. III, Fig. 3. Finally, they are shot out by the total collapse of the basal walls, just as a cherry-stone when pressed between the thumb and forefinger, to use Goebel's comparison. This rapid closing of the lower walls has a definite effect on the passive upper flaps, by which means they are jerked towards one another, and the spores lying on them, and only delicately balanced, are shed, simultaneously with the lower spores. After shedding of the spores, the basal portion of the sporangium is still open, and the flaps gape widely. The sporangium recovers its shape quickly on placing it in water, and will repeat the same vigorous movements again on drying. In cases where the two upper spores are shed first, this is due to a preliminary jerk on the part of the lower part. The pressure exerted by the approaching lower walls causes a slight movement in the upper flaps, and a consequent shooting of the upper spores.

A complete splitting of the sporangium down to the stalk sometimes occurs immediately after the spores have been shed. This is not, however, the normal method of procedure. The upper flaps have no independent power of shooting their spores; the thickening of the sporangium wall is therefore concentrated in the lower part.

Such an elaborate contrivance as that described for the megasporangium is unnecessary in the case of the microsporangium, for the spores, when once exposed, may easily be distributed by the wind. As a result there is here no definite 'hinge region', neither is the thickening confined to the basal portion, but occupies the central region of each valve (Pl. IV, Fig. 7), and is sometimes, as in *S. Kraussiana* (Pl. IV, Fig. 6), of a characteristic nature. A median longitudinal section of the microsporangium (Pl. IV, Fig. 10) shows that these cells again are identical in structure with those of the fern annulus, and of the basal portion of the megasporangium. They are arranged in approximately transverse rows, whilst those of the thinner marginal region are parallel to the edge of the valve. The two valves are prevented from splitting down to the stalk by the thickened basal portion

of the sporangium, which, although small and insignificant here, corresponds essentially to the elaborate boat-shaped structure in the megasporangium.

On drying, the sporangium opens at the top with a sudden jerk, the flaps separating quickly from each other. Each layer of thickened cells acts independently (strongly reminding one of the fern annulus), small spore masses being shot off by the snapping of different parts of the sporangium wall. The majority of the spores are, however, still unshed when the flaps have reached their maximum divergence, half lying poised on each flap. This condition is maintained for a time, the approximation of the small basal walls again causing the shedding of the spores, which are in this case, however, only just thrown clear of the sporangium wall, to a distance of 1 to  $1\frac{1}{2}$  cm. The microsporangia resume their normal shape with great rapidity on placing them in water, but I have not been able to induce them to perform vigorous movements on drying a second time.

The thickenings in the microsporangium are of a far more general character than those in the megasporangium, and it is easily seen how the specialized structure of the megasporangium may have been evolved from the simpler type of microsporangium by localization of the thickening in the basal region to form a powerful 'slinging' mechanism, and the development of the accessory 'hinge' tissue and crack regions to render more easy and effective the ejection of the spores.

#### *The Vascular System.*

The gross anatomy of the strobilus can best be seen on dissection, after boiling the cones in dilute caustic potash, on which it becomes evident that the vascular system here is essentially simpler than it is in the vegetative axes. All the species which I have examined (save *S. Lyallii*, which will be referred to later) have a single vascular cord, with, typically, two marginal protoxylems (although *S. Bakeriana* has four, and *S. spinosa* eight), from which are given off at regular intervals the spirally arranged leaf-traces. The stele is suspended in a definite lacuna, which broadens out considerably at the leaf base, ending abruptly just underneath the sporangium. It is traversed by the usual trabeculae, with the characteristic endodermal thickening peculiar to each species. The central core of xylem consists usually of well-thickened scalariform tracheides, but there may be a more or less well-marked procambial area extending from the tip downwards. The protoxylem elements are fairly large spiral and delicate annular tracheides, as are also those of the leaf-traces, which are always simple (save in the case of *S. Lyallii*, where occasional splitting takes place into two bundles), slender at first, but broadening out in the lamina of the leaf, and finally ending abruptly in a tuft of tracheides.

Frequently, as in *S. Wildenowii*, *S. helvetica*, &c., the leaf-trace shows a slight enlargement almost immediately on leaving the central strand.

This is due to the development of secondary tracheides round the base of the ligule (cf. 14). Transverse sections of the cone (whether these be homophyllous, or dorsiventral, as in *S. molliceps*) show that radial symmetry obtains here. The stele is circular and lies in a circular lacuna. The only exception to this rule is *S. Lyallii* (Pl. IV, Fig. 3). Here there are two distinct steles, in a somewhat elongated lacuna. Each is provided with one protoxylem which points to the outside, and the two may be sometimes united by their pericycles. Figs. 5 and 8, Pl. IV, illustrate the method of fusion of the several bundles of the stem into the simpler type of structure of the cone as seen in *S. inaequalifolia* and *S. Lyallii* respectively.

The vascular system of the cone is again differentiated into (1) a central cone of metaxylem with marginal protoxylems, (2) a region of phloem parenchyma, (3) one layer of sieve-tubes, frequently absent opposite the marginal protoxylems, (4) a large-celled pericycle, ranging from one to five cells in thickness, and (5) the endodermal trabeculae. Apart from the fact that radial symmetry is characteristic of the cone, there is naturally a great resemblance between the vascular system of the cone and that of the stem, which soon becomes evident on comparing the cases given below with the corresponding figures and descriptions given by Professor Harvey-Gibson in his paper on the stem of *Selaginella* (13).

*a. S. Lyallii* (Pl. IV, Fig. 3).

There are two circular steles, occasionally joined by their pericycles, each bearing an outwardly placed protoxylem, and surrounded by a common lacuna. The pericyclic cells, which are one or two layers deep, are large, and contain starch, &c., and surround a layer of sieve-tubes which are separated from the central cone of xylem by two or three layers of phloem parenchyma. A few isolated protophloem patches may occur.

*b. S. spinosa* (Pl. IV, Fig. 9).

The metaxylem consists of small elements, which are frequently not thickened towards the centre, and carries eight protoxylems (as in the tip of the vegetative shoot) which are partially sunk in the metaxylem. The phloem parenchyma is one to three layers thick, and is followed by one layer of sieve-tubes, which, however, may be about opposite the protoxylems. The pericycle is one to two cells in thickness.

*c. S. oregana* (cf. 13).

The pericycle is two to five layers deep, and there are no protophloem elements. The one layer of sieve-tubes is absent opposite the two marginal protoxylems, the phloem parenchyma (of which there are two or three layers) abutting directly on the pericycle.

*d. S. inaequalifolia* (cf. structure of central stele in stem).

The procambial area extends down to the base of the cone. The pericycle is generally one layer thick, and encloses a layer of sieve-tubes (whose continuity may be broken opposite the marginal protoxylems).



Round the outer edges of the phloem a few protophloem elements may still persist. There are one to two layers of phloem parenchyma.

*e. S. Martensii.*

The pericycle is one layer deep. Protophloem elements are rare. Generally one layer of sieve-tubes occurs (sometimes there are two), and these may be entirely absent opposite the protoxylems. The phloem parenchyma is somewhat elongated.<sup>1</sup>

*The Ligule.*

In a previous paper Professor Harvey-Gibson (14) has given a detailed account of the structure and development of the ligule in *Selaginella*, so that to describe in detail the ligule of the cone would be merely to repeat the facts recorded by him. In brief, it may be said that the ligule of the cone, down to the minutest details of glossopodium, glossopodial sheath, &c., has essentially the same structure as that in the vegetative axes; where a tracheal cup is present in the latter, as in *S. helvetica*, *S. inaequalifolia* (Pl. III, Fig. 5), &c., its counterpart is seen in the cone. As a general rule, the ligule of the cone is larger and more expanded than that in the stem. It always persists in a fresh condition quite down to the base of the strobilus, even when the ligules just below the cone are shrivelled and dead. (These facts are well exemplified in *S. Kraussiana*, *S. inaequalifolia*, &c.)

The cuticularization of the cells radiating out from the glossopodium to the vascular system does not take place in the cone until the spores have been shed (cf. 14). Fig. 13, Pl. IV, is a longitudinal section through the ligule of *S. Wildenowii*—in this case all the sporangia were empty.

The ligule commences its development early, and matures much more rapidly than the sporangium which it subtends. This, taken in conjunction with the above facts, suggests that the ligule is closely connected in function with the sporangium; the two commence their development together, and the ligule becomes functionless as soon as the sporangium has shed its spores. The proximity of the ligule to the vascular supply, and the mucilaginous nature of the cells of its lamina, which are, therefore, specially adapted for storing water, suggest that its function is to prevent the drying of the sporangium during the development of the spores (14).

The examination of the above species serves to emphasize the intermediate position in which *Selaginella* stands. On the one hand, it may revert to conditions characteristic of the distinctly primitive Lycopodia,

<sup>1</sup> It seems worth while drawing attention to the simplification of the vascular complexities of the stem in such types as *S. Lyallii* and *S. inaequalifolia* as the cone is approached, which suggests parallel arrangements seen in such forms as the Cycadaceae. The cone would appear to be more conservative of the primitive vascular state, and the simplification of anatomical structure might be regarded as evidence of the independence of evolution of the two regions, each on its own account when once fairly segregated. I am indebted to Professor Bower for this suggestion.

whilst on the other, its wonderful adaptations for cross-fertilization, bringing into play the most elaborate mechanism seen in the Pteridophyte group, and its near approach to the seed habit, render it one of the most interesting as well as instructive genera in the whole of the plant world.

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EXPLANATION OF PLATES III AND IV.

Illustrating Miss Mitchell's Paper on the Strobilus of *Selaginella*.

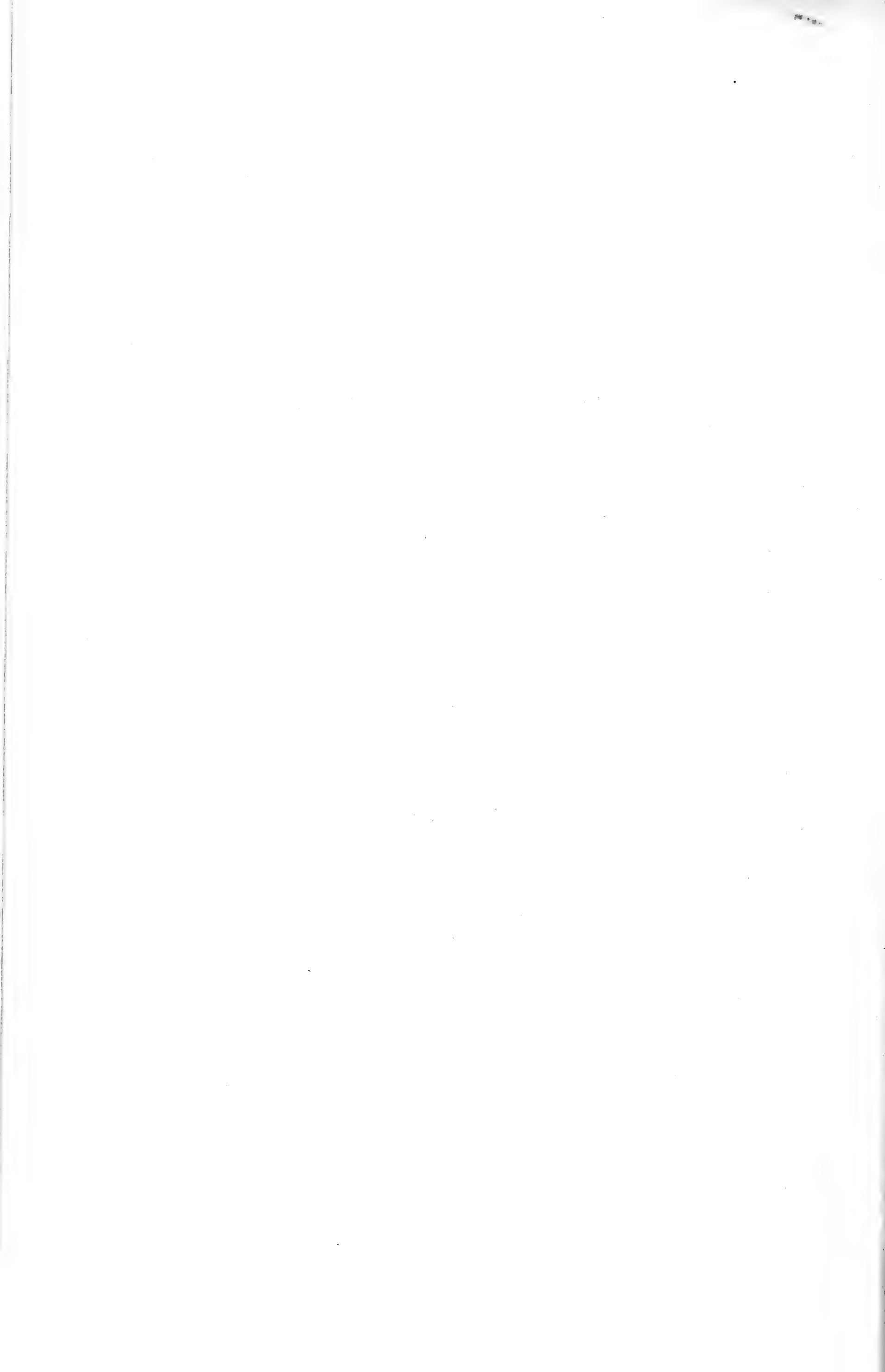
PLATE III.

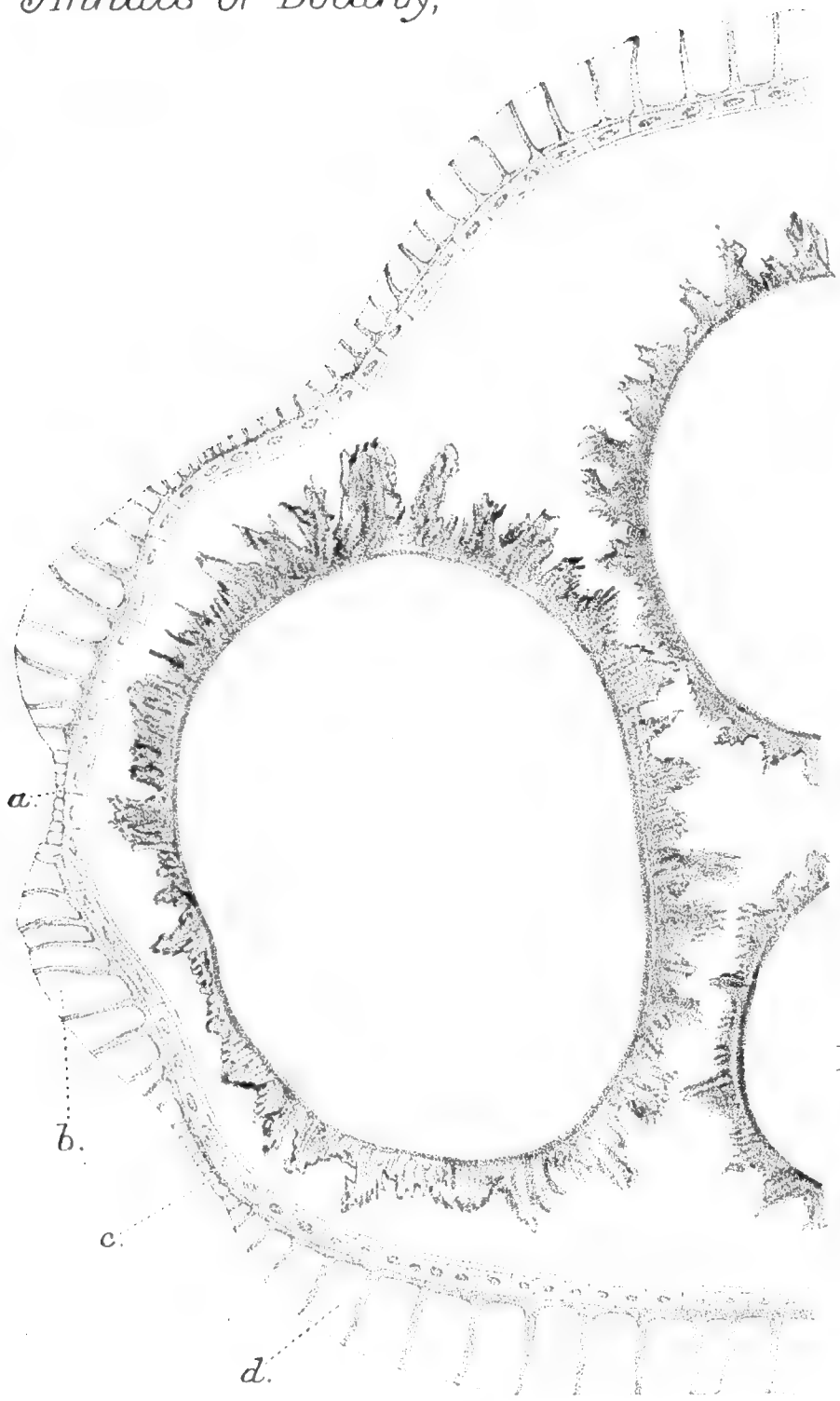
- Fig. 1. T.S. megasporangium of *S. spinosa*. *a.* hinge cells in section; *b.* thickened edge of valve; *c.* tract of shallow tissue giving flexibility; *d.* inner sporangium wall.  
 Fig. 2. L.S. megasporangium of *S. spinosa*; lettering as in Fig. 1.  
 Fig. 3. Opening sporangium of *S. spinosa*.  
 Fig. 4. Valve of megasporangium of *S. spinosa*. *sp.* position of spore; *a.* transversely orientated cells; *c, c'* cracks; *b.* thickened edge of valve.  
 Fig. 5. L.S. ligule of *S. inaequalifolia*. *gloss.* glossopodium; *g.sh.* glossopodial sheath; *tr.sh.* tracheal sheath.

PLATE IV.

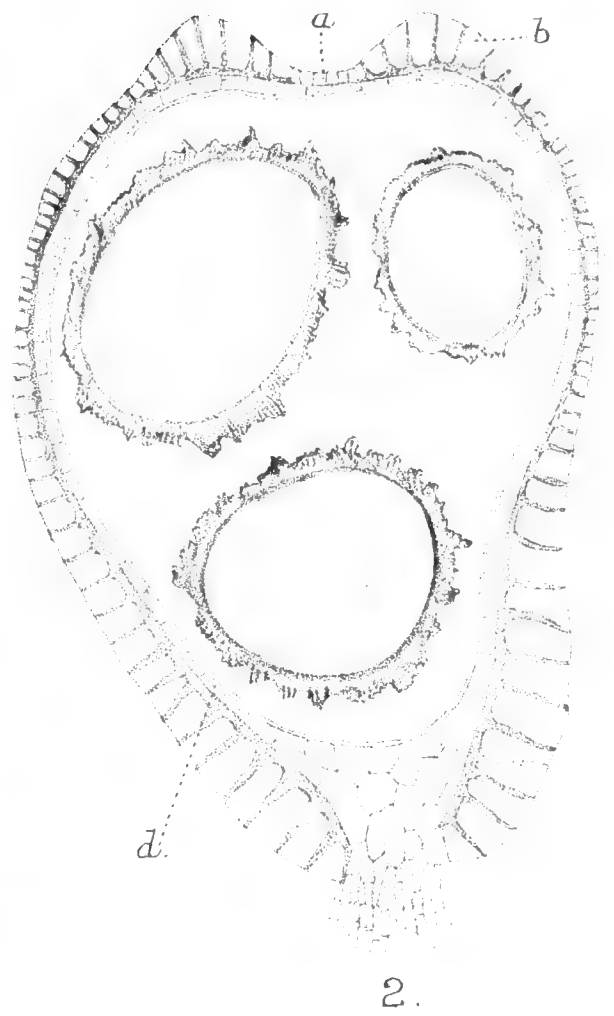
- Fig. 1. *S. Lyallii*, 'ligular' sporangium. *st.* sporangium stalk; *gloss.* glossopodium.  
 Fig. 2. *S. Lyallii*, two sporangium subtended by one leaf.  
 Fig. 3. T.S. stele of cone of *S. Lyallii*. *pcy.* pericycle; *end.* endodermis; *phl. par.* phloem parenchyma; *s.t.* sieve-tubes; *p.ph.* protophloem.  
 Fig. 4. 'Hinge' region of *S. helvetica*.  
 Fig. 5. Fusion of bundles in cone of *S. inaequalifolia*.  
 Fig. 6. Valve of microsporangium, *S. Kraussiana*. *a.* thickened band of tissue.  
 Fig. 7. Valve of microsporangium, *S. helvetica*.  
 Fig. 8. Diagram showing fusion of bundles in cone of *S. Lyallii*.  
 Fig. 9. T.S. stele of cone of *S. spinosa*.  
 Fig. 10. L.S. microsporangium of *S. helvetica*.  
 Fig. 11. *S. oregana*, branched cone. *a.* well developed sporangia; *b.* imperfect sporangia; *c.* entirely aborted sporangia; *d.* dehisced sporangia; *e.* young sporangia.  
 Fig. 12. *S. Kraussiana*, abortive microsporangium.  
 Fig. 13. L.S. ligule of *S. Wildenowii*.  
 Fig. 14. Cone showing reversion to vegetative characters, *S. sp.*



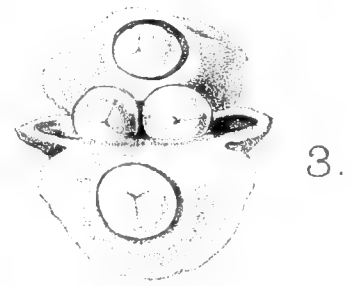




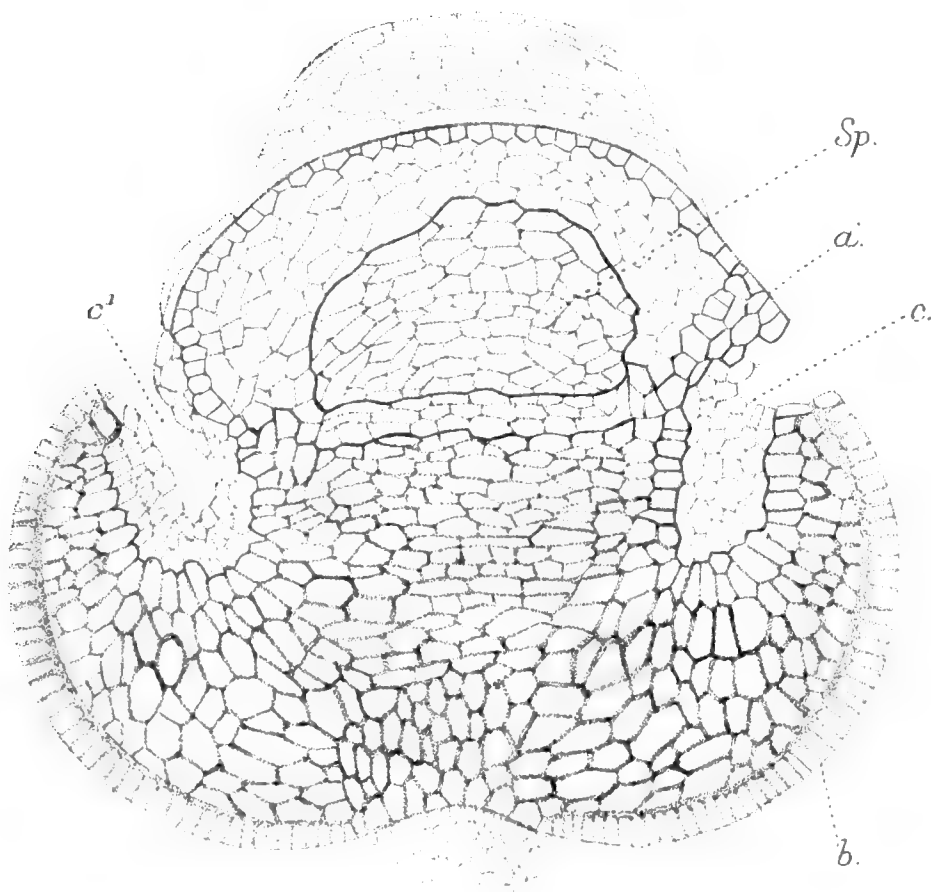
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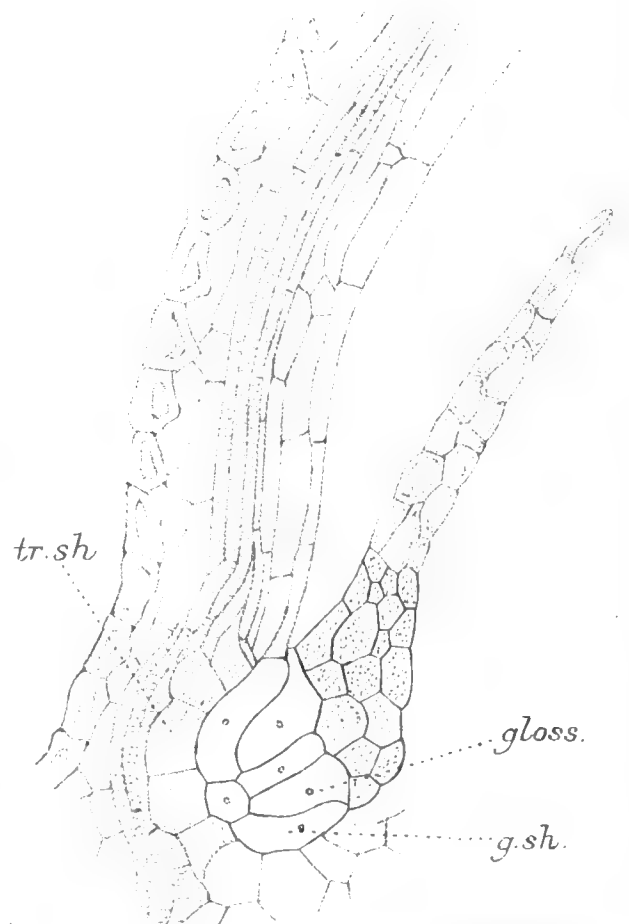
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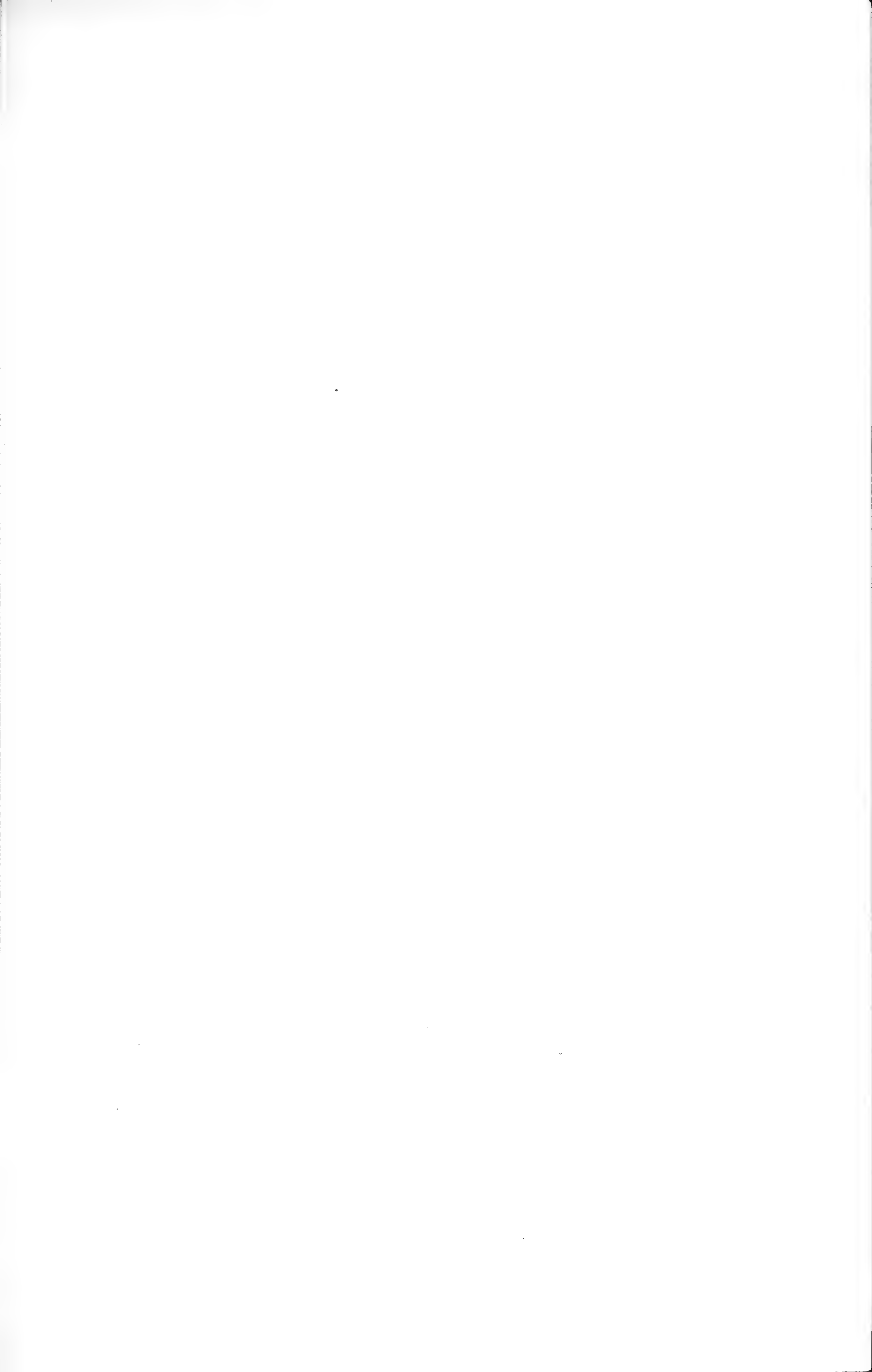
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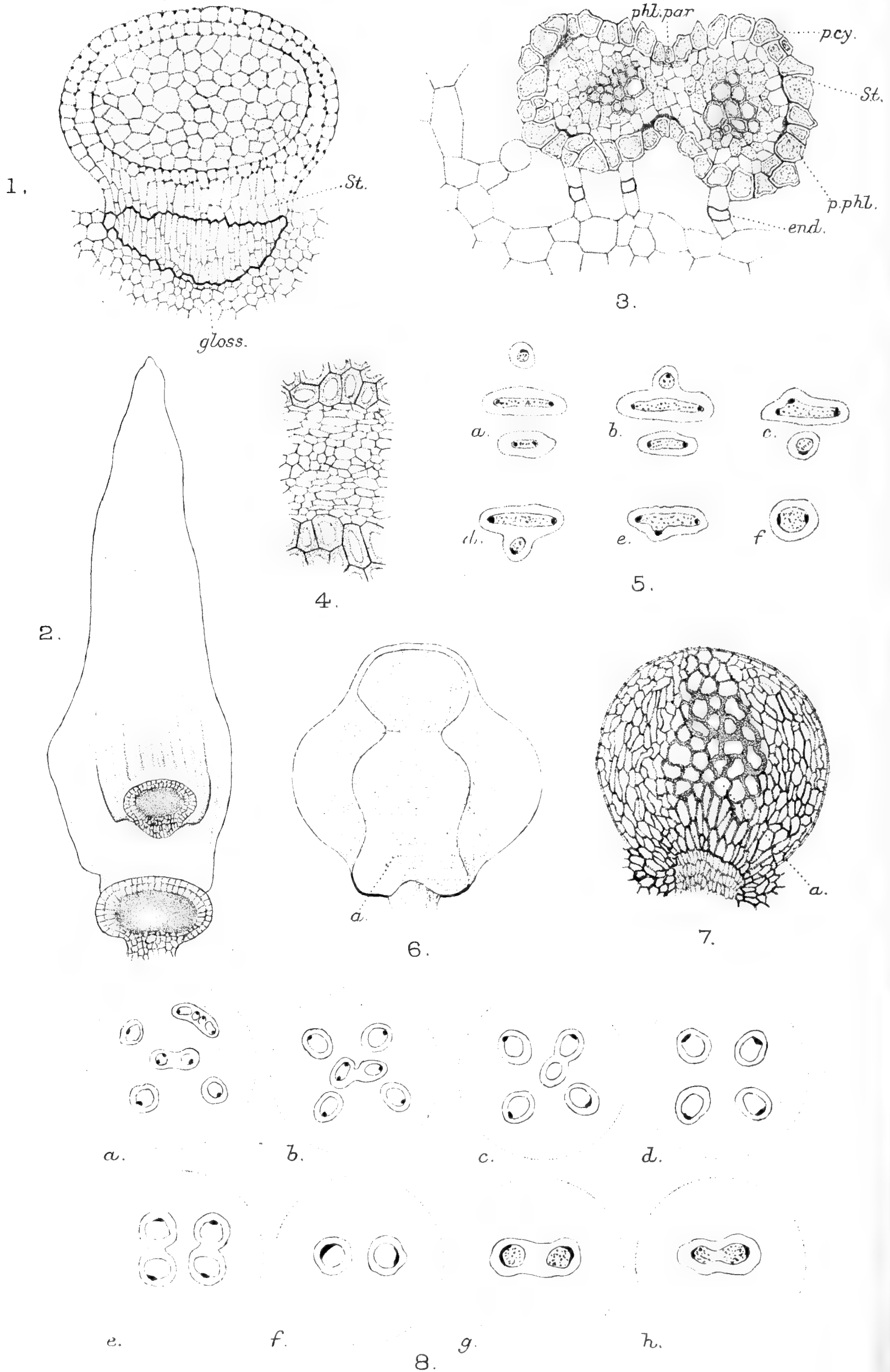


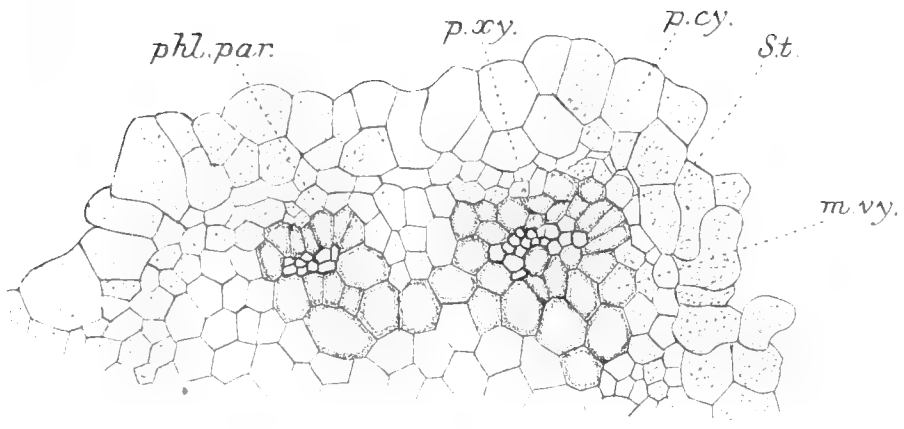
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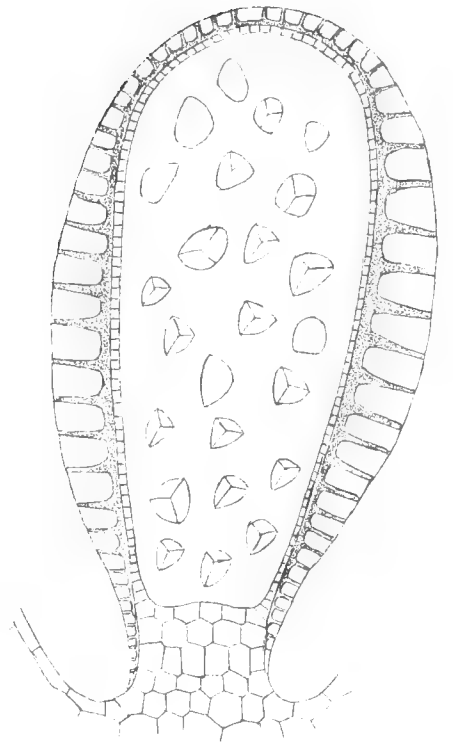




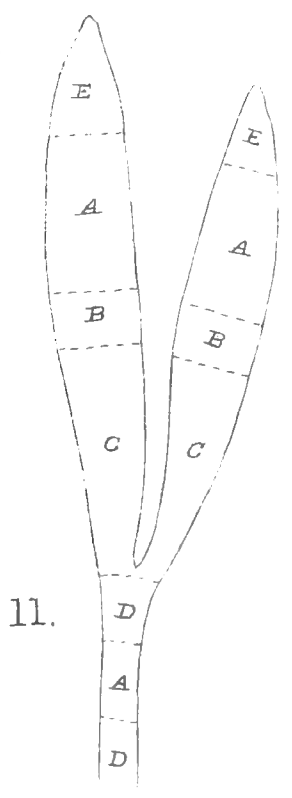




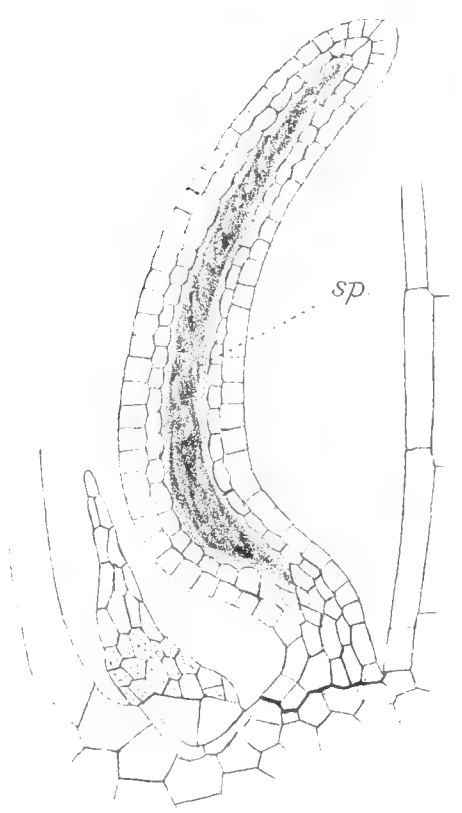
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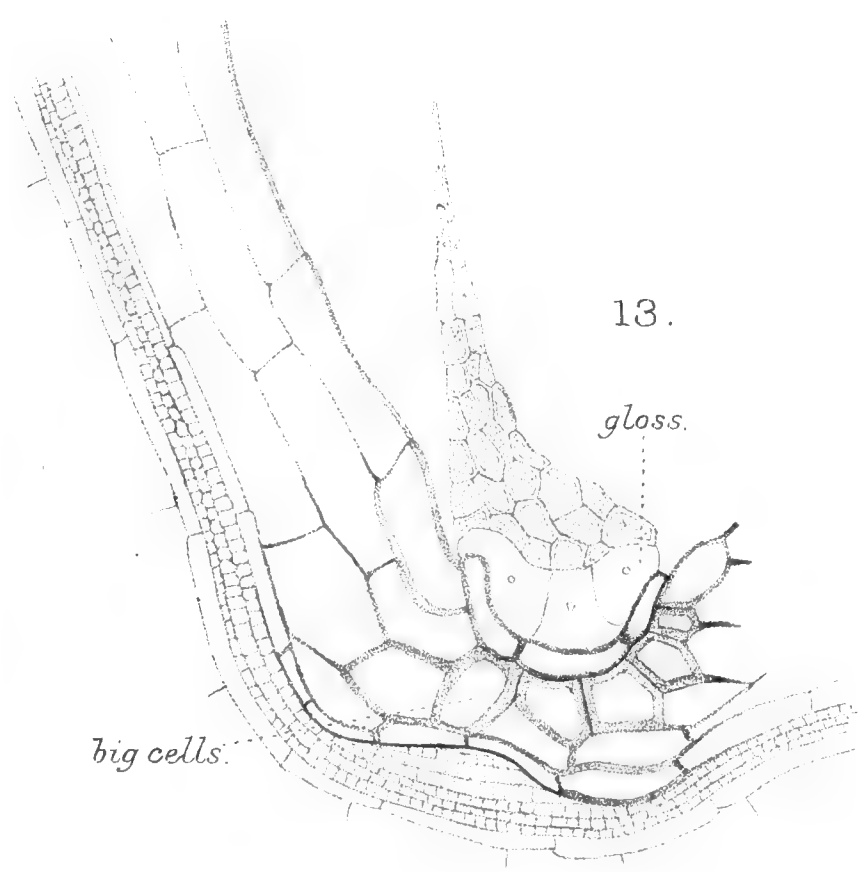
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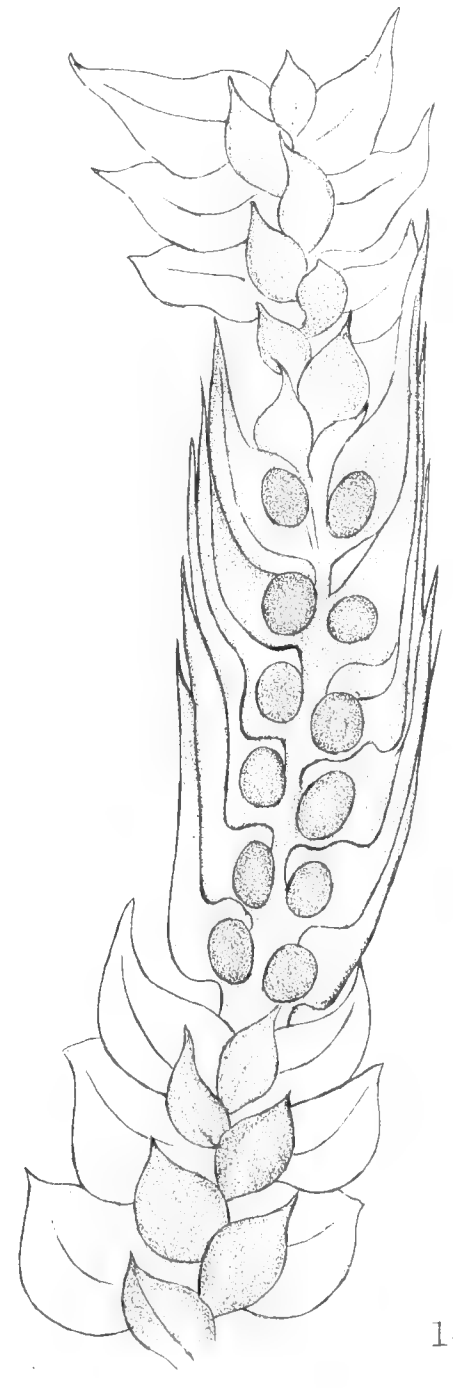
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14.





# Some Observations on the Tumours on *Veronica Chamaedrys* caused by *Sorosphaera Veronicae*.

BY

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AND

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With Plate V.

SOME time ago one of us found in his garden some irregular green swellings on the shoots of *Veronica Chamaedrys*; a microscopical examination of a section revealed the presence of a parasite, the cells being filled with amoebiform organisms in various stages of development, the terminal stage being a spherical body in which the thick-coated spores surround a central cavity.

The only reference that we could find to this organism was in Engler and Prantl's 'Die Natürlichen Pflanzenfamilien', I. Teil, 1 Abth., p. 6, in which Schröter, who wrote the article on Phytomyxineae, recognizes the four genera, viz.:

1. *Plasmodiophora* with free regularly formed spores;
2. *Phytomyxa* with free irregularly shaped rod-like or angular spores;
3. *Tetramyxa* with four spores enclosed in a delicate membrane;
4. *Sorosphaera* with many spores united in a hollow sphere.

Schröter states that *Sorosphaera* is a parasite in the parenchymatous cells of living plants, that the spores are elliptical, united in large numbers into spherical balls and covered by a common membrane, and that the diameter of these balls varies from  $15\mu$  to  $20\mu$ .

He recognizes three host plants on which *Sorosphaera* is parasitic, viz. *Veronica hederifolia*, *V. triphylla*, and *V. Chamaedrys*. In these plants the parasite causes swellings or tumours, in the enlarged parenchymatous cells of which are to be found the above-mentioned spherical balls. This being the only description we have been able to find of these tumours, we have made a further and more detailed study of them, the

result of which we embody in this communication. As to the distribution of these tumours, up to the present time we have only been able to find the disease on *V. Chamaedrys*, notwithstanding the fact that we have seen large crops of *V. hederifolia* growing in the immediate vicinity of diseased *Chamaedrys* plants. *V. Chamaedrys* is common in our district (Sevenoaks), but diseased plants are rare and mostly to be found in damp shady situations.

Nematode worms cause swellings and twistings of *Veronica* stems which often simulate, to some extent, in outward appearance those due to the attack of *Sorosphaera*.

The tumours themselves are to be found in various parts of the plant, their most common form being that of swollen stunted stems from which spring a few small deformed leaves; young shoots springing up from the procumbent stems of *Veronica* plants are especially liable to attack. An infected plant may have tumours in all stages of development; stems, petioles, and leaves may all show signs of the invasion of the parasite, in the case of the leaves the small swellings being found along the line of their mid-rib; occasionally a tumour may be found involving only half of a stem, in which case the latter becomes bent or curled. The tubercles vary in size from that of a pin's head to that of the last joint of the little finger; slugs are very fond of them, and possibly play a part in spreading the disease; those tumours which escape the attack of slugs finally become soft and rotten, the sorospheres are liberated by their decay, and the spores in all probability germinate in the soil, where the young amoebae live until they meet with fresh host plants to attack.

We have been successful in producing tumours by sowing *Veronica* seeds in a pot and sprinkling them with water containing the sorospheres from dried tumours pounded with a pestle in the water. There was no evidence of any disease in the roots, many of the young roots being examined microscopically with reference to this possibility; for this reason doubtless the parasite does little damage to the host plant, its effect is largely local, and we find no such destruction as that caused by *Plasmodiophora* in cabbage plants.

A drawing of a diseased plant is shown in Pl. V, Fig. 1.

It is obvious that the parasite with which we are concerned is closely allied to *P. Brassicae* in its structure, its chief point of difference being in the terminal phase of its life-history; accordingly we have availed ourselves of the most recent communications on that parasite by Nawaschin and Prowazek, the paper of the former being in 'Flora', 86. Band, 1899, and of the latter in 'Arbeiten aus dem kaiserlichen Gesundheitsamte'.<sup>1</sup>

The material used in our investigation was all collected in the neighbourhood of Sevenoaks; the larger tumours were cut into small pieces and

<sup>1</sup> Twenty-second Volume, second half. Berlin, 1905.

fixed in Bouin's fixing solution, consisting of formal 10 c.c., saturated aqueous solution of picric acid 30 c.c., and crystallizable acetic acid 2 c.c. This fixing agent we found rather better than Flemming's solutions, its action being quick and its penetrating power better. Benda's iron haematoxylin and Flemming's triple stains were used chiefly for staining purposes.

The life-history of *Sorosphaera* consists of a vegetative and a reproductive stage of development characterized by a difference in the nuclei. The former commences with the presence in the procambial plant-cells of an amoeba with one or a few nuclei; the protoplasm has the usual spongy granular appearance, in which is situated the nucleus, which consists of a spherical cavity, in the centre of which is the large deeply stained nucleolus or karyosome, from which achromatic strands radiate to the peripheral granules of chromatin on the inner side of the nuclear membrane; at times under the highest powers appearances suggest that these filaments pass through the nuclear membrane. This small amoebiform organism grows, and later on is found surrounded by starch grains; the nuclei divide in a way shortly to be described till comparatively large flattened or more or less spherical bodies are formed constituting a plasmodium, although this is not the true plasmodium of Cienkowsky, for it is not formed by the aggregation of amoebae from many spores, but rather from the growth of a single spore. Portions of this may be constricted off so that as many as six or more plasmodia may be found in one enormously hypertrophied plant-cell.

*The Nuclear Division of the Vegetative Phase.* The most easily recognized stage in the division of the nucleus is a cruciform figure (as shown in Fig. 5c) in which the karyosome has elongated and is surrounded by a ring, the whole resembling a miniature of the planet Saturn when viewed from the side. Viewed from above it is seen that this ring is of the nature of a plate, at the periphery of which nuclear granules can be recognized. The whole is included in the nuclear membrane, which now has a quadrilateral in place of its spherical outline. It resembles to some extent the figure of the nucleus of *Amoeba limax*. As to how this nuclear figure is produced it is not easy to say, but it would appear to arise from an elongation of the karyosome and the collection of the peripheral chromatin granules into a plate; this is shown in Fig. 5b. This stage has been observed and drawn by Nawaschin in his article.

Another stage which is fairly frequently met with is that drawn in Figs. 5e and f. It resembles a dumb-bell in shape, and is apparently produced by further elongation of the karyosome and a splitting of the equatorial plate, the two halves of which go to form a flattened cap at each extremity of the elongated karyosome. From this stage the future procedure is easy to trace until two daughter-nuclei are produced; these

are characterized by their elongated oval shape, and in them fine linin fibrils can be seen. From the difficulty with which these fibrils stain it is not easy to say what part they play in the process of nuclear division.

In a given amoeba all these stages take place simultaneously and are followed by a considerable increase of its size; they no doubt take place rapidly, as it is only after the examination of a number of sections that they are to be found at all. It should be noted that this mode of nuclear division takes place within the nuclear membrane. In this way, by the multiplication of nuclei, growth and germination take place and the small amoebae, as seen in Fig. 2, reach the size and number of those shown in Fig. 3.

*The Chromidial or Akaryote Stage.* The next stage in the development of the parasite is the disappearance of the nucleus we have described above—the ergoplasmic or vegetative nucleus—and the appearance of the nucleus of reproduction. The organism assumes a more definitely spherical shape in place of its former plasmodial appearance, the plasma stains more deeply with haematoxylin, the nuclear membrane disappears, and the clearly defined karyosome becomes more irregular in shape and smaller in size, whilst large granules of chromatin are to be seen at the periphery of the nucleus, often arranged at opposite poles. It seems probable that the chromatin of the karyosome is conducted to the periphery along the achromatic linin filaments. This stage is shown in Figs. 6 and 6*a*. The continuation of this process results finally in the complete disappearance of the karyosome and the chromatin granules, and we get, as is shown in Fig. 7, a spherical mass with vague and irregular vacuoles in its plasma, which are too badly defined for exact observation. It seems to us to be uncertain whether the vacuoles seen at this stage are the remains of the vegetative nuclei or whether they are freshly produced in the plasma.

In the next stage, Figs. 8 and 9, we see collections of chromatin granules and rods arranging themselves in these vacuoles into the reticular form familiar in the majority of resting nuclei.

When this is complete there is a separation of the plasma around each nucleus, forming what we may term amoebulae, which tend to separate but are held together in loose combination by the plasma. This is shown in Fig. 10. In the vegetative stage the multiplication of the organism takes place in a mechanical way by the separation of portions of it during its movements in the cell, but in the reproductive stage the relation of the nucleus to its surrounding plasma becomes more intimate, and from this time the plasma in immediate relation to the new nucleus separates from the plasma of neighbouring nuclei, and though the amoebulae are loosely aggregated together there is no longer any resemblance to a plasmodium.

The nuclei of the amoebulae next undergo two divisions, which take

place in the ordinary mitotic way, as shown in Figs. 11, 12, and 13. The result of these two mitoses is a reduction in the size of the amoebulae and of the karyokinetic figures, which are markedly smaller in the second division than in the first. At the close of the second division two adjacent amoebulae are often to be seen in contact, suggestive of a conjugation, but this appearance is more probably due to the fact that they are not separated from each other.

During these mitotic divisions, at the pole of each spindle, radii may be seen which probably proceed from a centrosome, though we have been unable to see this body. The amoebulae are now found in the cell in loosely aggregated masses, near the margins of which are often to be seen a few individuals independent of the general mass, as seen in Fig. 14. These aggregations become more intimate till the amoebulae are fused into spheroidal masses (Fig. 16); each amoebula, however, becomes by degrees more distinct as it secretes for itself a wall presumably of fungal cellulose (Fig. 17). This wall slowly thickens, and the arrangement of the amoebulae is then such that a hollow sphere results, in which the central cavity is surrounded by the spores, which are wedge-like in shape; this is the sorosphere, and is to be seen figured in Fig. 4.

The size of the sorosphere and the number of spores may vary considerably; occasionally two sorospheres become fused together, forming a compound sorosphere of an oblong or ellipsoidal shape. There is a reticulate vesicular nucleus at the broader end of each wedge-shaped spore.

When this stage is reached the tumour commences to rot, and is invaded by moulds and bacteria, liberating the sorospheres. What interval of time elapses before the spores are liberated from the sorosphere we have not determined, as observations of hanging-drop cultivations have shown no definite liberation. We have also not succeeded in cultivation experiments to a sufficient extent to be able to give an account of the germination and subsequent behaviour of the spore; the nearest approach to success was gained by inoculating a sterile infusion of *Veronica* leaves with boiled water in which portions of dead tumours had been placed. In this mass, which of course was no longer sterile, bacteria and moulds made their appearance, and, after an interval of some fourteen days, numbers of amoebae were found in a state of activity in a drop taken from the débris at the bottom of the tube. These amoebae, which each contained only a single nucleus, were later on found in an encysted condition.

*Mode of Infection of Veronica Plant.* The earliest tumours are to be found in the region of the growing point of the stems. This point comes out clearly in longitudinal sections, and it is possible to trace a series of infected areas getting larger and larger as one works down the stem. When the invasion is extensive the whole future of the shoot is modified,



and it remains a stump-like pyramid bearing only dwarfed and deformed leaves, but where the invasion is less the growing point may free itself from the disease and leave behind a tumour on one side of the stem, causing it to twist or bend. It is this multiple origin of the tumour which gives to it the lobulated appearance seen in sections of mature tumours. We have failed to find any evidence of the wandering of the parasite from cell to cell, thus confirming Nawaschin's opinion, expressed in his paper on *Plasmodiophora*, that the infected area is only increased by the division of already infected cells, and that neither the amoebae nor their nuclei have any power of penetrating through the cell wall into a healthy from an adjacent infected cell.

We are of opinion that infection takes place in the neighbourhood of the growing point; this is the case when healthy plants growing in pots under observation are brought into contact with infected material. Though not having observed an actual infection, we have, as stated above, observed the infected areas getting smaller as we approach the growing point, and have seen single isolated infected cells close up to growing points; by repeated division of these isolated cells larger infected areas or groups of cells would be formed.

*Structure of the Tumour.* The microscopical appearance of transverse sections of tumours varies, depending on how much of the stem has been involved in the early infection and on the stage of development of the parasite; where there has been extensive infection of the tissues in the vicinity of the growing point the whole stem and leaf system is reduced to a pyramidal mass, in which the cortex, pith, &c., are not to be distinguished, but in less extensive infections the tumour may involve only a portion of the stem and interfere but slightly with its growth, which may continue in the normal manner.

The tumour consists of masses of infected and modified cells, surrounded by cambial cells showing here and there a few spiral and annular vessels. In a section of a slightly infected stem the tumour consists of a small infected area or group of cells situated in the cambial ring, showing in its earliest stage but little interference with the general structure of the stem, except perhaps a slight bulging due to mechanical pressure.

The appearance of sections of tumours in various stages of development will be understood from Figs. 2, 3, and 4, which have all been drawn under a magnification of about 300 diameters by means of the camera lucida. These figures represent stages in the development of the tumour and parasite which may be fitly defined by the terms early, middle, and mature respectively. Fig. 2 shows a portion of an early tumour in longitudinal section; on the right is the cortex, between which and the infected cells are to be seen some layers of cambial cells. The infected area itself is seen to consist of infected and modified cells, all of which

display a large deeply staining nucleus and nucleolus surrounded by granular protoplasm; the diseased cells contain amoeboid parasites in close connexion with their nuclei; these organisms are small and usually contain several nuclei. Occasionally mononucleate ones are to be seen, but it is impossible to be sure whether they represent the earliest stage or whether they are in reality portions cut in section from a larger body.

In Fig. 3, the middle stage, we notice a considerable increase in the size of the infected cells, and to some extent of the healthy ones as well; these latter are crowded with starch grains embedded in the plasma. The amoebae have grown enormously in size, forming an irregular plasmodium in the cells, portions of which separate from the original mass and continue their development independently. Starch grains are also to be seen in the infected cells, in which, however, they are not nearly as plentiful as in the neighbouring healthy cells.

Fig. 4 shows the final stage of development. Here the infected cells are mostly filled with the spherical sorospheres; their protoplasm is scanty and their nuclei enlarged and degenerate.

*The Plant Nucleus.* The changes which the plant nuclei undergo during the life of the parasite are interesting. At the time that infection takes place the early cambial cell is hardly differentiated from the other cells of the growing point; its nucleus and nucleolus are large and stain deeply. In the normal course of events this nucleus would become elongated, and would consist of a fine reticulate network of linin with two or more large collections of chromatin (nucleoli), but in the case of infected cells the nucleus and nucleolus retain their spherical shape. It is a matter of considerable interest as to how this minute parasite is able to select a procambial cell for its development and avoid those other cells which do not retain their power of division as does the procambial cell. The varied grouping of infected and healthy cells in the tumours may be explained by the fact that the organisms may or may not be divided when cell division occurs; if division of the cell always included division of the organism we should get masses of infected cells surrounded by modified healthy cells; this, however, is rarely the case.

The cell nucleus shares in the hypertrophy of the infected cell, and after a time there is a failure in the formation of a dividing cell-way, and though division of the nucleus takes place there is no actual cell division. This is shown in Figs. 18 and 19, which represent the nuclear division or mitosis taking place; as is seen from these drawings, the size of these nuclei is enormous compared with those of normal cambial cells. As our drawings show, it is at the close of the vegetative stage that the nuclear divisions are to be seen in their greatest development; with the commencement of the reproductive stage of the parasite the activity of the cell declines, and after the formation of the sorospheres

only a few atrophied and degenerate nuclei are to be seen in the infected cells. Thus it is here, as in other cases of plant and animal parasitism, that the activities of the cell are moulded to suit the requirements of the parasite. The action of the parasite on the cell in the early stages does not seem to be injurious, and one might imagine that if the parasite could be extracted from the cell in an early stage, the latter would resume its normal mode of life and fulfil its destiny as a cambial cell.

*Comparison with Plasmodiophora.* The life-history of our parasite shows great similarity to that of *Plasmodiophora*, to which it is evidently nearly related; the various stages in the nuclear division, as described by Nawaschin and Prowazek in their papers, are identical with those we have observed in *Sorosphaera*; this similarity, however, ends in the terminal phase, in which we get the spores enclosed in a membrane; this would perhaps correspond to the capillitium of non-parasitic Mycetozoa. The nuclei of *Sorosphaera* are somewhat larger than those of *Plasmodiophora*, and the vegetative stage of the former is more commonly met with than that of the latter, in which the reproductive stage is the more common.

We have not, however, been able to agree in all particulars with the account of *P. Brassicae* as given by Nawaschin and Prowazek, though in the main the life-histories of both parasites are similar. Thus we have failed to find any evidence of conjugation of the amoebulae, though, as we have stated, they may be seen in close proximity, but we see no reason to consider this as the commencement of a conjugation rather than the result of a division. We fail also to observe any extrusion of polar bodies.

In the article on Mycetozoa in Lankester's Treatise on Zoology there is a sub-class Sorophora, embracing those forms which have no true plasmodium; *Sorosphaera*, we think, might be assigned to this; *Plasmodiophora*, however, is classed among the Proteomyxa.

In Arthur Lister's work on the Mycetozoa there are figures which suggest that there are two forms of nuclear division, a vegetative and a reproductive; further investigation on the nuclear division of non-parasitic Mycetozoa may throw light on their relationship to the parasitic ones.

#### SUMMARY AND CONCLUSIONS.

1. The tumours found on the stems and leaves of *Veronica Chamaedrys* are caused by the invasion of a Mycetozoan parasite, *Sorosphaera Veronicae*.
2. The life-history of *Sorosphaera* may be divided into three stages, viz. the Vegetative, the Akaryote or Chromidial, and the Reproductive, each being characterized by a difference in the nuclei.
3. The parasite has no power to penetrate through the cell walls,

and the tumours are derived from the repeated division of one or more infected cells.

4. The primary infection takes place in the vicinity of the growing point of the stem.

5. The parasite is closely allied to *Plasmodiophora Brassicae*, the nuclear division taking place in a similar manner. The wedge-shaped spores of the former are, however, to be found in spheres enclosed in a common membrane, in contrast to those of the latter, which are free.

*Postscript.*—Since the above communication was sent to the Editors of the *Annals of Botany*, a paper by Maire and Tison has appeared in the 'Annales Mycologici', vol. vii, No. 3, 1909, on the same subject. It is satisfactory to find that their figures and interpretations are in the main in agreement with ours. The point of entry of the parasite is not noted by these observers, nor the observation of Nawaschin as to the method of formation of infected areas.

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## EXPLANATION OF PLATE V.

Illustrating the paper by Dr. Blomfield and Mr. Schwartz on Tumours of  
*Veronica Chamaedrys*.

Figs. 2, 3, and 4 were drawn with a Leitz No. 6 objective and No. 3 ocular.

The figures 6 to 21 were drawn with 1.40 homog. oil immersion (Zeiss), 6 to 17 with compensating ocular 18, and 18 to 21 with compensating ocular 12.

Fig. 1. Portion of diseased plant showing tumour natural size.

Fig. 2. Section of early or young tumour.

Fig. 3. Section of middle tumour.

Fig. 4. Section of mature tumour showing sorospheres and amoebulae.

Fig. 5 *a-m*. Various stages of nuclear division taking place during the vegetative period of the parasite. For description see text.

Figs. 6-17. Sequence of changes undergone by the organism in the formation of the sorosphere. Figs. 6 and 6 *a* show the chromatin moving to the periphery. Fig. 7 shows the complete disappearance of the nuclei and the formation of the vacuoles. Figs. 8, 9, and 10 illustrate the formation of fresh nuclei; Fig. 11 the first nuclear mitotic division, which is completed in 12; Fig. 13 the second nuclear division; Figs. 14, 15, and 16 the three last stages in the formation of the sorosphere. Fig. 17, portion of a sorosphere in surface view.

Fig. 18. Hypertrophied plant-cell with four nuclei in process of division.

Fig. 19. The same, showing two nuclei in the spindle stage.

Figs. 20 and 21. Enlarged plant nuclei.

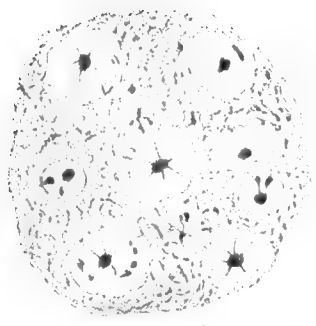








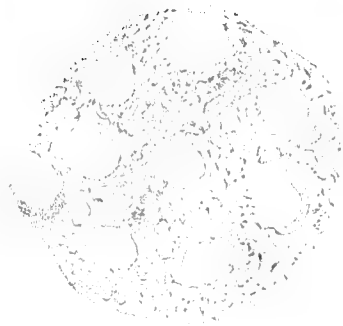
Fig. 5.



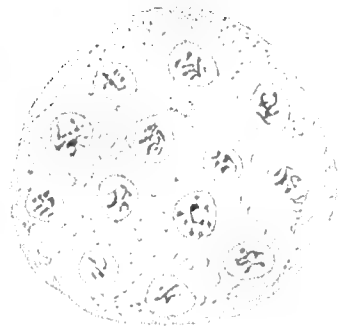
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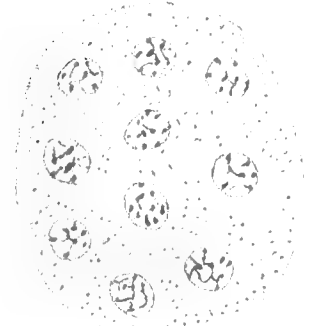
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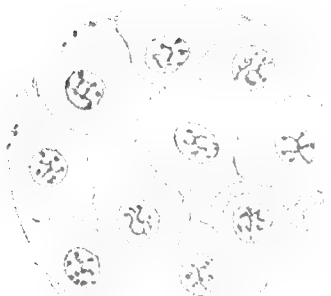
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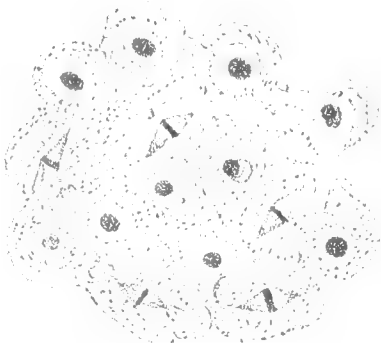
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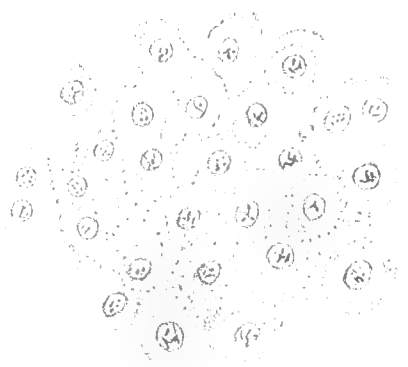
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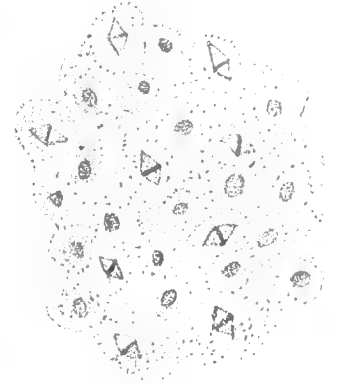
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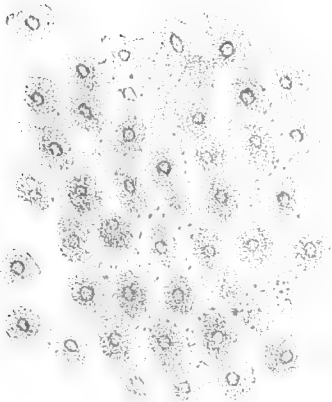
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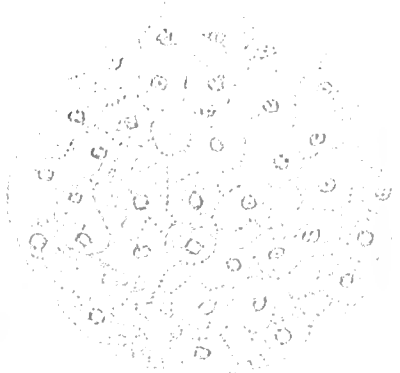
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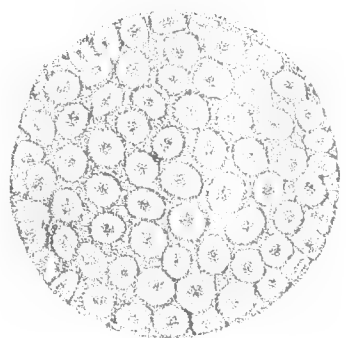
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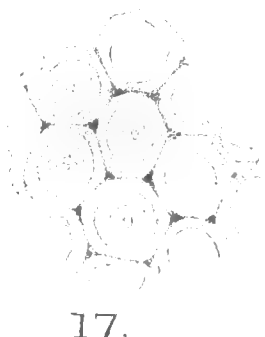
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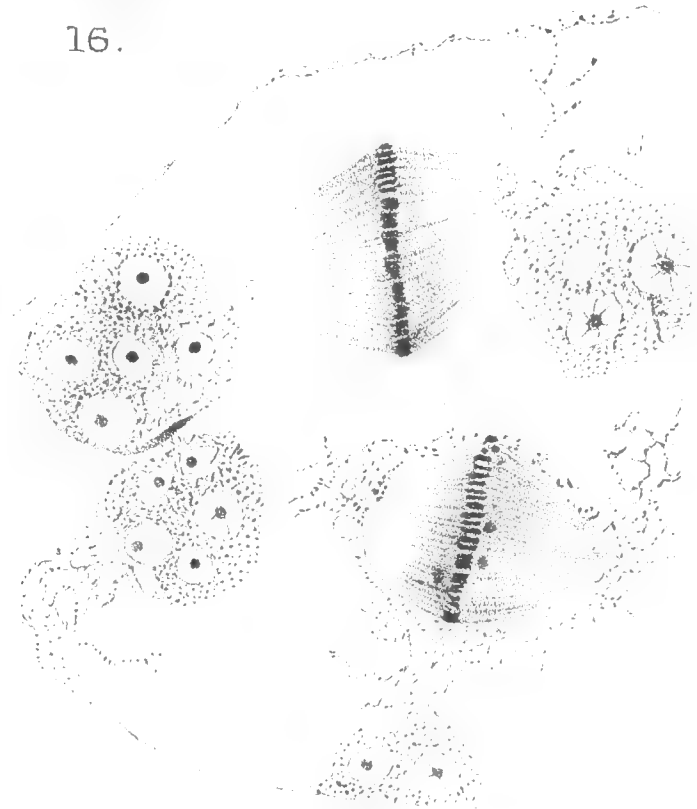
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17.



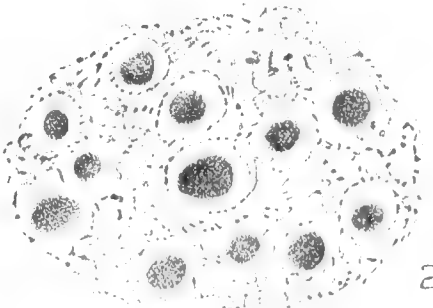
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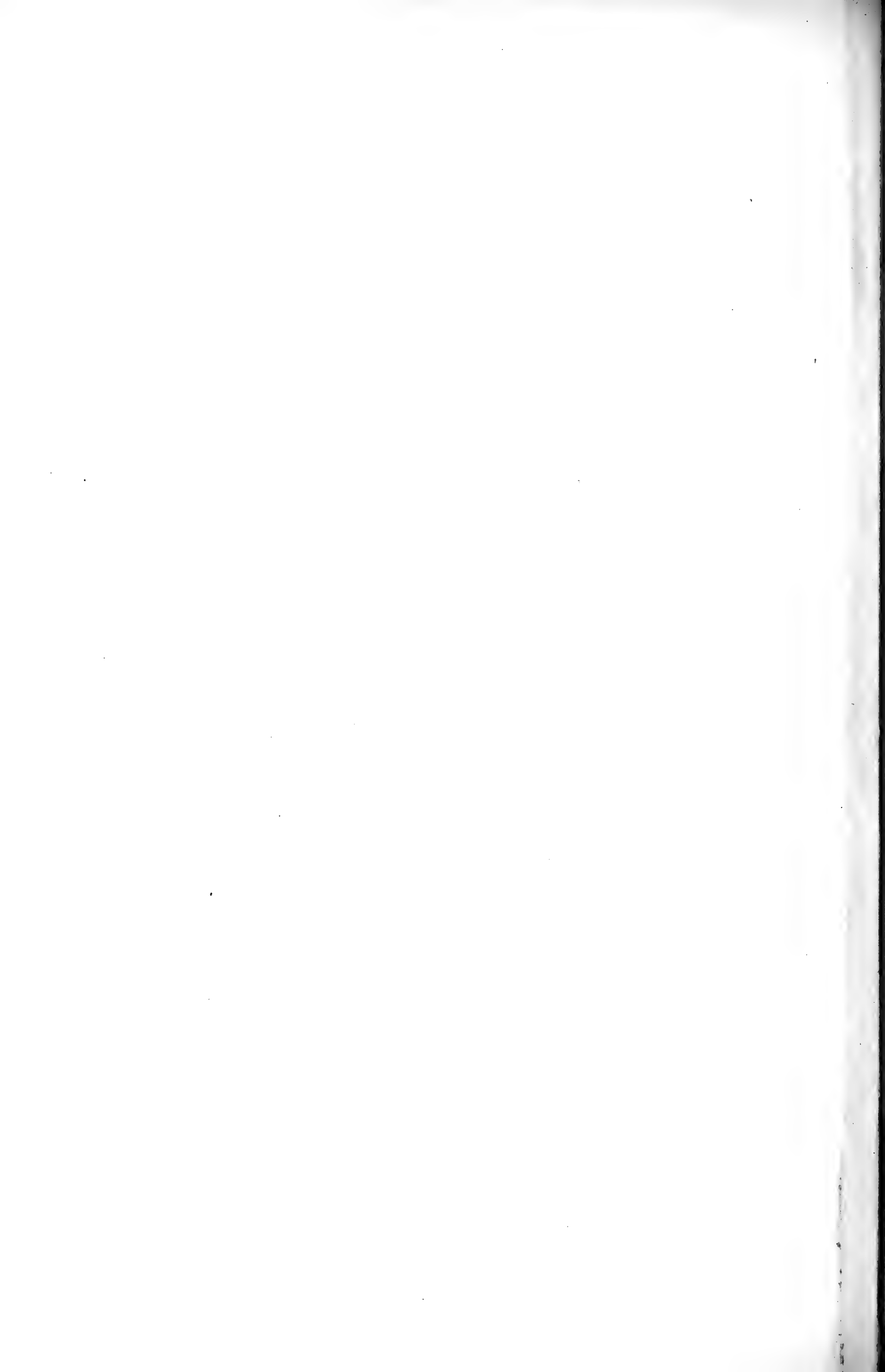
19.



20.



21.



# Cytological Observations on the Yeast Plant.

BY

HAROLD WAGER, F.R.S.,

AND

ANNIE PENISTON.

With Plates VI—X, and a Figure in the Text.

IN a former paper by one of us (Wager, '98) an interpretation of the various structures observed in the Yeast Plant was put forward, which reconciled many of the diverse opinions then held, and placed the whole problem of the morphology of the nucleus and its behaviour during division and spore-formation on a more satisfactory and definite basis.

In that paper it was pointed out that the deeply stained granule described by most of the previous observers as a nucleus is a homogeneous body, often so closely surrounded by granules as to give it the appearance of a granular nucleus, and that in addition there is present in healthy yeast cells a more or less distinct vacuole, easily seen in the living condition, which contains a granular substance. It was suggested that as these two organs of the cell are so closely bound up with each other, we were justified in regarding them as representing the nucleus of the higher plants.

This interpretation has been criticized by various observers in a number of interesting memoirs, but so many different views have been put forward, that instead of giving a more coherent and precise knowledge of the structure of the nucleus, they further enhance the pre-existing confusion.

Macallum, in a very interesting paper ('99), found that the yeast cell contains, at the beginning of fermentation, granules and vacuoles grouped irregularly and varying in number and character, but as a rule only one large vacuole. The basis of these granules seems to be a proteid substance, which may vary in its character during the various stages of fermentation. In the earlier stages they appear to have a fatty nature, although the reaction for fat is not intense enough to suggest a purely fatty composition.

At a later stage they react slightly, or not at all with the osmic acid of Flemming's fluid, and seem at this stage to be of a purely proteid character. The cytoplasm takes a diffuse stain with haematoxylin, due to a chromatin-like substance diffused through it, with a very deep stain at one or more points in the cell. Frequently a deeply staining, homogeneous corpuscle is present, which is neither a nucleus nor a nucleolus. Several of these, of small size, may be present in a cell, but cells are found without a trace of them. In some cells one may find a vacuole whose wall is rich in stainable material at one side of the corpuscle. In the cells of *Saccharomyces Ludwigii* cultivated in the sap of the Ironwood tree, fixed with Flemming's fluid and stained with iron-alum haematoxylin and eosin, the corpuscle was frequently demonstrated as a reddish body, having at times a tint of blue violet and surrounded by granules and elongated masses of chromatin. Sometimes, in corrosive sublimate preparations stained with very dilute solutions of haematoxylin, the corpuscles may be unstained, or stained no more deeply than the cytoplasm. He regards the garland-like chromatin structure as quite the same as the membrane of fine granules in contact with the nucleolus described by Wager ('98), though it does not appear to be as uniform as Wager figures it. The micro-chemical reactions for iron and phosphorus demonstrated the presence of masked iron and organic phosphorus in the corpuscle, in the wall of the vacuole in contact with it, and sometimes in the granules found in it and in the cytoplasm generally. The reaction is usually intense in the corpuscles, less strong in the cytoplasm. In the process of budding the corpuscle becomes elongated and constricted in its middle portion. One or both parts may pass into the bud or both may remain in the mother-cell. In sporulation, the cytoplasmic chromatin collects in the immediate neighbourhood of the corpuscle, which also undergoes certain granular changes, then elongates with constriction in its middle part. It may be compared to the division of the nucleolus in *Euglena viridis*, but is not of the nature of true karyokinesis.

Hoffmeister ('00), whilst agreeing that the nucleolus is a spherical homogeneous body, considers that it must be regarded as a true nucleus.

Guilliermond ('02, '04) found all the structures described by Wager, but disagreed with his interpretation. He states that:—(1) the nucleolar body (nucleus of the older observers) is not always attached to the vacuole, (2) it possesses in itself the ordinary structure of a nucleus, consisting of a nuclear membrane, nucleoplasm, and network with sometimes an indication of a nucleolus, and (3) the nuclear vacuole does not contain anything of the nature of a chromatin granular network, but that the granules contained in it correspond to the red granules of Bütschli and are composed of meta-chromatin, which may be regarded as a kind of reserve material. He agrees, however, that the nucleus (nucleolus) divides directly, and that,

in the process of budding, one portion of the divided nucleus passes into the bud as well as a portion of the vacuole and the metachromatin granules.

Barker ('02) found in isolated well-developed cells a large, uniformly stained, spherical body which stained very deeply, and was in most cases well differentiated from the rest of the cell contents. In haematoxylin stained preparations in which the stain was almost washed away, it showed occasionally signs of possessing a structure similar to that claimed for it by Janssens and Leblanc and Guilliermond. Numerous deeply stained granules were also found, chiefly in one vacuole, or sometimes in numerous small vacuoles, or distributed through the protoplasm, especially in the neighbourhood of the deeply stainable body.

According to Feinberg ('02) a mixture of methylene blue and eosin differentiates the structure best. The protoplasm stains blue and is homogeneous, and the nucleus, which has no regular position in the cell, stains red. He states that, in the resting stage, the nucleus is a round compact body consisting of chromatin, that it sometimes shows a looser structure in which separate granules can be seen, but with no chromatin network. He calls it the 'Kernpunkt', and compares it with the nucleoli of certain unicellular organisms such as *Amoeba* and Sporozoa, in which the nucleus consists of a nucleolus containing chromatin surrounded by a clear zone which is unstainable. There is, however, no clear area surrounding the yeast nucleus, and he suggests that it may be a chromoblast.

Hirschbruch's ('02) method of preparation is to dry the yeast on a slide, stain in an alkaline solution of fuchsin, then stain in methylene blue and allow the yeast to dry, and mount in Canada balsam. Janssens has already called attention to this 'fixation brutale' and the consequent deformation of structure which it probably entails. Hirschbruch's figures show a homogeneous red stained body which in some cases is surrounded by a clear zone. He disagrees with the interpretation put forward by Janssens and Leblanc, as to the nuclear structure of the vacuole and granules, and considers that the homogeneous red body must be regarded as the nucleus. He states that the clear space which sometimes surrounds this nuclear body has no membrane, and cannot be regarded as part of the nucleus. He describes a peculiar form of sexuality in which a blue stained granule, the male element, combines with the red stained nucleus, but the evidence for this is not very convincing.

Marpmann ('02), after fixing in Rolli's solution for twenty-four hours, staining in Heidenhain's haematoxylin, and subsequently staining in methylene blue or iodine green, obtains a black or blue-black body, which he regards as a nucleus, and a plasma with granules stained by the contrast stain.

Janssens ('03) believes that the nucleolus is always inside the vacuole when the latter is present; its appearance by the side of the vacuole he considers is due to the process of fixation. In following the fixation



of a cell under the microscope, he states that the vacuole rapidly contracts, and that the central moving granule which he regards as the nucleolus becomes lodged in one of the folds.

Swellengrebel ('05) investigated nuclear division in compressed yeast. He describes the structure of the nucleus as similar to that given by Guilliermond, but in opposition to Guilliermond he states that the nuclear division is mitotic. Four chromosomes are formed which arrange themselves in the form of a band at the equator of a spindle-shaped figure. After probable division they separate into two daughter groups which pass to opposite poles, forming a diaster stage. This division commences before the cell buds, and the metaphase is reached before there is any sign of budding. The division appears to Swellengrebel to resemble that of the micro-nucleus in *Paramoecium*.

Fuhrmann ('06) confirms in the main the results obtained by Swellengrebel. The various stages of mitotic nuclear division were found, including the formation of four chromosomes, an achromatic spindle, and perhaps centrosomes. The resting nucleus is small, and in well differentiated preparations exhibits a delicate structure. The chromatic substance appears as an aggregation of fine granules, among which a larger granule is sometimes seen, which may be a nucleolus. The presence of a nuclear membrane is by no means certain, and the clear space round the larger granule is nothing more than a vacuole either above it or below it.

Kohl ('08) states that the nucleus consists of a distinct nuclear membrane, with nuclear sap, a large crystalloid, and a fine nuclear network. The figures given by the author seem to have been drawn from badly stained or over-stained specimens. The crystalloid is nothing more than the nucleolus, nucleus, or corpuscle of various authors. It appears to be homogeneous, and presents none of the characteristics of a nucleus ascribed to it by Guilliermond. The nuclear membrane which is figured around it probably represents the outline of the vacuole either above or below it, but no details are given as to the stage of development of the yeast from which the preparations were made. The author states that the nucleus divides by fragmentation both in budding and spore-formation.

The conflicting accounts thus given by different observers are probably due not entirely to errors of observation, but to the remarkable variability in the behaviour of the nucleolus towards stains and reagents, to the variation in the nuclear vacuole and its chromatin contents, and to the varying amounts of volutin, glycogen, and phosphorus found in the yeast at different stages in its development. These variations may be connected with the conditions under which the yeast cell lives, with its periods of great activity alternating with great depression in an environment which is constantly changing. How far this may be the case is, however, a question for further investigation.

Our recent observations show that there is a closer connexion between the nuclear vacuole and the nucleolus than was formerly supposed, and we have come to the conclusion, as the result of a careful study of their structure and position in the cell and their reactions towards nuclear reagents and stains, that they definitely constitute the nucleus of the Yeast Plant.

#### EXAMINATION OF LIVING CELLS.

The microscopic examination of healthy yeast cells in a resting condition shows that they contain a central well-marked vacuole with hyaline contents, and usually one large or two or three smaller refractive granules, which exhibit the characteristic Brownian movement (Pl. VI, Figs. 1-3). The vacuole varies very much in size, in some cases filling the cell almost completely, in others being much smaller and sometimes hardly visible. The cytoplasm forms a more or less hyaline layer round the vacuole, and there are present in it a varying number of highly refractive granules, which are commonly arranged round the outside of the vacuole or vacuoles, if more than one is present, but are sometimes irregularly disposed in groups (Pl. VI). The uniform presence of the vacuole, its distinct appearance, and the constant presence of bright, refractive granules in it, indicate that it plays some important part in the cell economy. The nucleolar body (nucleolus) is invisible in perfectly healthy cells, though some indication of its presence is occasionally given by the position of the refractive granules (Fig. 2). If the cells lose some of their turgidity through the pressure of the cover-glass, the position of the nucleolus is often indicated by a depression in the vacuole; occasionally under these conditions it can be distinguished as a slightly refringent spherical body in contact with the central vacuole.

On the addition of a dilute aqueous solution of methylene blue or gentian violet, the nucleolus immediately shows as a lightly stained homogeneous body lying between the vacuole and the cell-wall, and in close contact with the former (Fig. 5). A number of the refractive granules in the cytoplasm, together with the moving granules in the vacuole, stain deeply with both methylene blue and gentian violet, and are therefore probably volutin granules (Figs. 5-7). Others stain black with osmic acid, and are doubtless globules of oil; an occasional granule may give the glycogen reaction with iodine, but there still remains a number of these highly refractive granules which are not accounted for.

If a culture of such a yeast is started in Pasteur's solution, it is found that after a few minutes the vacuole contracts (Figs. 8, 9). In the course of 20-30 minutes it regains its turgid appearance and is particularly distinct, although it has become somewhat smaller (Fig. 10). The vacuole then becomes less visible (Figs. 11, 12), till at the end of an hour it has apparently

disappeared in most of the cells (Fig. 13). Its presence is, however, indicated by the position of the refractive granules, and it is immediately visible on the addition of iodine solution (Fig. 16). In the course of two hours the cytoplasm becomes perfectly hyaline, and the cell appears to contain nothing but a number of refractive granules suggesting the possible limits of the vacuole (Figs. 14, 15), which is brought out clearly by iodine (Fig. 16).

If some of these cells are placed in water, the vacuole becomes visible in from thirty minutes to an hour. It then continues to increase in size for about four hours, at the end of which time the cytoplasm has apparently become vacuolated; in some of the cells two, three, or four large vacuoles occur, and there is some difficulty in determining which is the nuclear vacuole (Figs. 23-6). All these vacuoles except one (the nuclear vacuole) disappear on fixing. They must not be confused with the vacuoles which occur in connexion with the formation of glycogen during fermentation. After from 5-8 hours in Pasteur's solution the vacuole is again visible in many of the cells, but it is not clearly defined in the majority of the cells until some hours later (Figs. 17, 18).

The apparent disappearance of the vacuole is possibly a plasmolytic phenomenon brought about by transferring the yeast from the beer wort to the more concentrated Pasteur's solution. The refractive index of the cytoplasm is raised by the abstraction of water to much the same as that of the vacuole, so that the contents of the cell appear homogeneous. In all such cases, however, the vacuole is rendered visible in iodine solution.

Fermentation begins slowly; its rate of progress during the first few hours depends upon the condition of the cell when the culture is started. Yeast in the condition described, containing little glycogen, becomes distributed throughout the fermenting medium almost at once, none of the cells settling to the bottom, and a slight foam makes its appearance in the course of 1-2 hours. After about five hours the fermentation increases markedly up to 15-20 hours; during this period the cells bud rapidly, and form a thick foam with small air-bubbles. Fermentation continues vigorously, and at a normal temperature reaches its maximum in 30-45 hours. The budding activity is much reduced after 15-20 hours, and from that stage onwards the foam becomes frothy owing to the appearance in it of large air-bubbles. Shortly after the height of fermentation is reached, cells settle to the bottom in large quantities, fermentation rapidly decreases, and the foam disappears. After fifteen hours' fermentation, the central vacuole increases in size; it remains very large during the most active period, and shortly before the height of fermentation almost fills the cell. It then decreases in size (Figs. 20, 21), and when the cells sink is frequently smaller than in the early stages of fermentation. In many cells it occupies a very small proportion of the cell space, and in some has apparently completely

disappeared, leaving nothing but a number of refractive granules at one side (Fig. 22).

Soon after the beginning of fermentation other vacuoles may make their appearance in the peripheral cytoplasm. This is very observable in the cells of a compressed yeast, D.C.L., upon which some experiments have been made. The nuclear vacuole remains distinct in close connexion with the nucleolar body. On fixing and subsequently staining the cells carefully with fuchsin and methyl green, or Heidenhain's haematoxylin, it is found that the nuclear vacuole is the only one differentiated, the others having apparently become lost in the process of fixing. They may possibly be due to the ordinary vacuolation which takes place when active metabolism is set up, or they may be glycogen vacuoles. It is interesting to note that their appearance is coincident with the formation of patches of glycogen, and their disappearance coincides with the gradual fusion of these glycogen patches into a single large mass, which nearly fills the cell.

#### METHODS OF FIXING.

Since it is possible that the many conflicting results obtained may have arisen in part from the different methods of fixing followed by various observers, the action of a number of fixing fluids on the yeast cell has been carefully noted, and the results are here briefly summarized. The yeast used for this purpose was obtained from a brewery in a perfectly healthy condition. It had been fermenting about forty-eight hours, and presented the characteristic microscopic appearance already described, each cell containing a large well-defined vacuole with usually one large conspicuous, quickly moving granule towards its centre, and surrounded by numerous highly refractive granules (Figs. 1-3).

1. *Formalin*. A 1.6 per cent. solution of formaldehyde was used. The general appearance of the cell in this solution is much the same as in the living condition; the nucleolus is invisible, and the large refractive granule in the vacuole still remains moving vigorously. In the case of every other fixing agent tried, the movement of the granule ceased as soon as the fluid penetrated the cell.

2. *Iodine* in potassium iodide, Gram's solution (Figs. 29-31). The nucleolus becomes immediately visible, and after twenty-four hours the vacuole remains sharply defined and is not at all contracted. The refractive granules are conspicuous. In a few cases where one or two small vacuoles are present in addition to the central vacuole, these show the characteristic colour associated with glycogen.

3. *Chromo-aceto-osmic Acid*, Flemming's weak solution (Figs. 32-4). The nucleus becomes visible in a few minutes. After complete fixation, the cells remain much the same size as in the living condition, the nucleolus shows clearly, the vacuole in the majority of cells is sharply defined, although

in some cases it may be somewhat contorted. Some of the refractive granules in the cytoplasm are stained black, the others are very conspicuous, as is also the granule in the vacuole. The latter is commonly found towards one side of the vacuole, and is sometimes lodged in a fold; occasionally threads connecting it with the side of the vacuole show clearly. In some cells the vacuole is not very clear; in a few cases it is invisible, but a number of slightly refringent granules, which are obviously not the refractive granules seen in the living condition, can be seen in the position usually occupied by the vacuole; these sometimes show indications of a reticular arrangement.

4. *Perenyi's fluid*. In from 5–15 minutes the nucleolus is clearly defined in intimate connexion with the vacuole, which remains clear, although somewhat contorted (Figs. 35, 36). The nucleolus then tends to become less visible. When thoroughly fixed, the cells are smaller than in the living condition, and the granular contents are accentuated, although the refringent granules appear individually smaller; the most conspicuous object in the cell is the granule in the centre of the vacuole. The nucleolus is difficult to distinguish; it is visible as a lighter area in the cytoplasm and looks very much like an extension of the vacuole. The latter is smaller, but not contorted, and is rarely sharply outlined. In many cells the vacuole is represented by a slightly paler area in the cytoplasm surrounded by slightly refringent granules.

5. *Corrosive Sublimate*. (a) Concentrated solution in 5 per cent. acetic acid alcohol (70 per cent.) (Figs. 39–41). The cytoplasm becomes granular, obscuring the refringent granules. The nucleolus is defined as a paler area in the cytoplasm closely connected with the vacuole; the latter shows clearly in contrast to the granular cytoplasm; it is not contracted, but does not exhibit that peculiarly definite outline associated with iodine fixation. The refringent granule in the centre of the vacuole is conspicuous. (b) Concentrated aqueous solution (Figs. 37, 38). The nucleolus is invisible; the cytoplasm is homogeneous as in the living cell, but more refractive. The vacuole is clearly defined, but somewhat smaller than in the living cell, and is not contorted. The refractive granules are not so conspicuous as in the living cell, but are distinctly visible. Cells placed in this solution strongly resemble those that have been 5–10 minutes in Pasteur's solution.

6. *Picric Acid*. (a) Concentrated solution in 70 per cent. alcohol (Figs. 50–2). The granular contents of the cell are strongly accentuated; the nucleolus and the refringent granules, with the exception of the large central one, are completely obscured by the dense, granular cytoplasm. The vacuole is practically invisible in most of the cells, although in some it is indicated as a less granular area irregularly outlined, sometimes associated with a similar smaller oval area corresponding to the nucleolus (Figs. 50, 51). The only clearly differentiated object in the cell is the large



granule which in the living condition is observed in the vacuole. (*b*) Concentrated solution in 5 per cent. alcohol (Figs. 48, 49). The nucleolus is sharply differentiated; the peripheral cytoplasm is homogeneous. The vacuole is not visible, but its position is indicated by a number of refringent granules suggestive of a reticulum; among these one or more larger granules are to be distinguished.

7. *Alcohol*, 30 per cent. (Figs. 42-4). The nucleolus shows clearly, but the vacuole is invisible, the cytoplasm being completely hyaline. The refringent granules appear to be scattered throughout the cell; the large granule, which in the living cell is situated towards the centre of the vacuole, is at one side of the cell (Fig. 42). In addition there are visible a number of smaller and much less refringent granules.

8. *Absolute Alcohol* (Figs. 45-7). The nucleolus and vacuole are clearly visible; the latter is often slightly contracted (Fig. 45). The cytoplasm is hyaline with refringent granules, and the large granule in the vacuole is prominent.

#### METHODS OF STAINING.

As some of the existing confusion with regard to the various structures found in the yeast cell is undoubtedly to be attributed to the many different methods of staining that have been used to differentiate them, and to the tendency of certain observers towards the exclusive use of one method, it may be useful to give here some indication of the relative value of a number of the more important stains that have been found useful. We have found it best to make paraffin sections and stain them on the slide. The tube method of staining is not productive of good results, owing to the difficulty of controlling the staining and subsequent washing-out processes with precision.

A useful method of manipulating a small quantity of yeast is that described by Wager ('98). The *fixed* yeast is spread on a slide in a little water, and allowed to dry up completely. It can then be manipulated with as much ease as paraffin sections. If the cells are well fixed, no contortion or displacement is brought about by drying up, and excellent preparations are obtained.

The most reliable and effective results have been obtained with Heidenhain's method of haematoxylin staining. We stain for eight to thirty-six hours in a 5 per cent. solution of haematoxylin in distilled water, after mordanting for half an hour to three hours in a  $2\frac{1}{2}$  per cent. solution in distilled water of either ferric ammonium sulphate or potassium aluminium sulphate. The preparations are finally differentiated in the mordanting solution. When the first of these mordants is used, a sharp differentiation of the nuclein-containing elements of the cell is obtained, while the second



produces a beautiful general cytological stain. Useful results similar to those obtained with potash alum have been obtained by mordanting for half an hour in a 3 per cent. solution of ferrous ammonium sulphate, staining in the haematoxylin for two hours, and then decolouring in a solution of ammonium alum.

We do not find that contrast stains add to the clearness of preparations stained with Heidenhain's haematoxylin, and in many cases they tend to obscure the finer cytological details. Some excellent results have, however, been obtained by first staining in safranin (1 per cent. solution in 70 per cent. alcohol), and then proceeding as usual with the haematoxylin staining. Useful results have also been obtained by staining for a few minutes in methylene blue, or the methyl green-fuchsin mixture, after the final washing of the differentiated haematoxylin preparations.

Brazilin has been substituted for haematoxylin and gives similar results. It has, however, the disadvantage of requiring a much longer time to stain. Good preparations have been obtained by using 70 per cent. alcoholic solutions throughout.

Delafield's haematoxylin and Mayer's haemalum are useful general stains, but tend to be diffuse. Haemalum has the advantage of differentiating the volutin granules. The best results in both cases are obtained by overstaining, and subsequently differentiating in acid alcohol.

Methyl green and acid fuchsin is very useful for temporary preparations; 2-5 minutes in a mixture of equal parts of 5 per cent. solution of methyl-green and acid-fuchsin produce a beautiful differential stain, which stains the volutin as well as the chromatin granules. Permanent preparations can be obtained by staining 3-10 minutes in the mixture, then immediately washing in 90 per cent. alcohol and dehydrating rapidly. Clearing in clove oil enhances the brilliance of the stain, and extracts any excess of methyl green, but the clove oil must be washed out with xylol, or, better still, replaced by cedar oil. Such preparations mounted in neutral Canada balsam will keep well, if not unduly exposed to sunlight.

Methylene blue and acid fuchsin produce an even more beautiful stain than methyl green and fuchsin, when mixed in the right proportions.

Methylene blue has been used for the detection of volutin. We have found a 1 per cent. solution most useful. To obtain permanent preparations, it is necessary to stain for about two hours in this solution.

Safranin alone does not give good results, but in combination with gentian violet produces useful and brilliant staining. The preparations are stained eight hours or overnight in a 1 per cent. solution of safranin in 70 per cent. alcohol. They are then washed in alcohol and water and transferred to a 1 per cent. aqueous solution of gentian violet for 3-10 minutes. Dehydration must be carried through quickly, and any excess of gentian violet extracted by clove oil, which must be replaced by cedar oil.

A very delicate differentiation is possible with a 1 per cent. aqueous solution of gentian violet, but it is difficult to manipulate owing to the rapidity with which it washes out in alcohol. It is necessary to stain from 12-24 hours.

Gram's aniline violet is a useful stain for volutin.

Hanstein's aniline violet (safranin and acid-fuchsin) is a useful cytological stain which has the advantage of acting rapidly (5-10 minutes).

Jenner's stain, made by dissolving Grubler's water-soluble eosin, yellow shade, and Grubler's medicinal methylene blue separately in methylic alcohol, and mixing them in the proportion of 125 c.c. of a 0.5 per cent. solution of eosin and 100 c.c. of a 0.5 per cent. solution of methylene blue, has also been used, and has given satisfactory and interesting preparations.

The methods employed for the detection of organic phosphorus and masked iron are described in detail in the sections dealing with phosphorus and iron.

## THE NUCLEUS.

### a. The Vacuole.

The structure of the vacuole can be most easily observed in yeast which has been fermenting from 5-15 hours, fixed in iodine and stained in a 0.5 per cent. watery solution of haematoxylin after previously mordanting in a 2.5 per cent. solution of common alum. It contains, as was pointed out in 1898, a loose network consisting of a faintly stainable substance resembling linin with numerous deeply stained chromatin granules on the threads (Figs. 96-8 and 101-8). The threads of the network are peripherally disposed, lining the interior of the vacuole (Figs. 96, 102-5). This is in agreement with Macallum's statement that chromatin occurs at the periphery of the vacuole. The network is continuous with the plastin substance forming the basis of the nucleolus (Figs. 95, 102, 104, 105, 112), and the coarser threads and their connexion with the nucleolus can be clearly seen. The nucleolus and chromatin network bear the same morphological relation to each other as in such typical nuclei as are found in *Spirogyra*, in the root tips of *Allium* and *Phaseolus*, and in the pollen mother-cells of *Lilium*, except that the nucleolus is attached laterally to the network (Figs. 102, 111, etc.), and is thus in direct contact with the cytoplasm.<sup>1</sup>

The large size of the nuclear vacuole (Figs. in Pl. VI) is so disproportionate to the size of the cell, and contains such a large quantity of clear

<sup>1</sup> Janssens and Leblanc ('98) were the first to recognize that the prominent vacuole of the Yeast Plant is the nucleus. They unfortunately confused the real nucleolus with the moving granule inside the vacuole, which they regarded as the nucleolus. In a later paper Janssens ('03) emphasizes this erroneous interpretation by stating that this central granule comes to lie laterally apparently on the outside of the nucleus, through the contraction of the nucleus on fixing and the retreat of the granule into one of the folds of the membrane.

sap in proportion to the very diffuse nuclear network, that failure to recognize it as a nucleus is perhaps not astonishing. But the extraordinary activity of the yeast cell at certain stages in its development explains the unusual size of the nuclear vacuole, and in the light of the large vacuolar nuclei usually associated with gland or other cells which exhibit very active metabolism, affords an additional proof of its nuclear nature.

It is only under favourable conditions that the nuclear network can be clearly seen. Its appearance under the microscope varies with the number, size, and staining capacity of the granules present on the network, and the density and staining capacity of the cytoplasm. The most cursory examination of yeast cells shows that the chromatin granules vary considerably in size and arrangement. They are sometimes comparatively few in number (Fig. 105) and large (Figs. 59, 116), at other times smaller and more numerous (Fig. 109). They are sometimes arranged with remarkable regularity (Figs. 102-4), but usually they are unevenly distributed on the network. From time to time both the granules and the cytoplasm show marked differences in their behaviour to nuclear stains according to the conditions which obtain in the cell. In the early stages of fermentation the chromatin network (vacuole) occupies about a third of the space in the cell. Granules are present on the threads, but are not particularly conspicuous, and are rendered less so by the surrounding cytoplasm, which under these conditions is deeply stainable, as is also the nucleolus. The interior of the vacuole is apparently occupied by watery contents, which remain unstained, and in sharp contrast to the rest of the cell. It is clear that in this condition the chromatin network will not be readily seen, owing to its peripheral arrangement and consequent close proximity to the deeply stained cytoplasm, and at first sight the cell will appear to contain merely the deeply stainable body and simple vacuole surrounded by cytoplasm, as described by the majority of observers, who have apparently based their conclusions on cells in the early stages of fermentation. As fermentation continues the cytoplasm loses its capacity for nuclear stains, and the network becomes conspicuous, partly because of the increased transparency of the cytoplasm and partly because of the increase in the number of chromatin granules. The vacuole continues to occupy more and more space until, near the height of fermentation, it practically fills the entire cell (Figs. 17-19, 104). At this time the nucleolus stains feebly, but the granular substance at its periphery stains intensely. Immediately after the period of highest fermentation is passed, the chromatin network decreases until it occupies a comparatively small space, frequently much smaller than in the early stages of fermentation (Figs. 110, 140-5). This decrease precedes the sinking of the cells in the liquid, and, as we shall see later, is coincident with the increase of glycogen. In some cells, and

particularly in those from the bottom, the network is much reduced, and the greater part of the cell is occupied by the large glycogen vacuole (Fig. 145). On the disappearance of the glycogen the chromatin network again expands until it occupies about a third of the cell space (Figs. 146, 147).

The cytoplasm usually stains in inverse proportion to the size and staining capacity of the chromatin network, so that when we have a comparatively small vacuole, as in the early stages of fermentation, the cytoplasm is commonly deeply stained, and when a large conspicuous chromatin network is present during the most active period of fermentation, the cytoplasm remains almost completely unstained. In cells supplied with insufficient nutriment, the vacuole is much reduced in size and is often no larger than the nucleolus. As under these conditions the amount of stainable material in the nucleus is very small, both nucleolus and vacuole appear as faintly stained, pale areas in the cytoplasm, but at the periphery of the nucleolus deeply stained granules of chromatin are visible (Figs. 57, 58).

Generally, it may be stated that in healthy cells a faintly stained nucleolus corresponds with a large well-stained chromatin network; deeply stained nucleoli occur when the chromatin network is small, namely, in the early stages of fermentation and in cells containing a large amount of glycogen (Figs. 110, 112); and again, immediately preceding spore formation, when the chromatin network has disappeared.

The prominence of the nuclear vacuole during the active period of fermentation suggests that it may play an important part in the metabolism of the cell. At certain stages the chromatin-like substance is abundant at the periphery of the vacuole, but at other times the vacuole is masked by the cytoplasm, which stains deeply and gives a diffuse reaction for phosphorus (as Macallum has shown). These facts seem to indicate that the chromatin may pass into the cytoplasm, and that the nuclear vacuole contains and possibly elaborates chromatin material for the use of the cell during its active growth and development. The yeast nucleus does not possess a definite nuclear membrane, so that the passage of the chromatin from the vacuole into the cytoplasm or *vice versa* is not impeded, as might be the case in the nuclei of higher plants. But in these, as is well known, the nuclear membrane disappears at certain stages, and it has been suggested that this is to make possible a free interchange of nuclear and cytoplasmic material and a renewal of the vital activity of the cell. It is possible that in the yeast cell there is a constant interchange of stainable material (chromatin) between the nucleus and the cytoplasm.

It is impossible to give any satisfactory account of the elaboration of chromatin and its function in the metabolism of the cell. The sequence

of changes observable is, however, fairly definite, and the explanation may be offered that food material absorbed into the nuclear vacuole is there elaborated into chromatin by means of the nuclear network and possibly the nucleolus; and may then be extruded into the cytoplasm, either as chromatin or in a slightly different form, to take part in the various formative and other activities which are associated with the cytoplasm, and more especially perhaps in the production of enzymes.

*b. The Nucleolus.*

The nucleolus is a homogeneous, spherical or oval body in close contact with the nuclear vacuole.<sup>1</sup> It consists of a substance closely resembling the plastin of the nuclei of higher organisms and has very little affinity for nuclear stains. Under certain conditions which may obtain in the yeast cell, it becomes more or less impregnated with a chromatin-like substance which stains deeply.

There is also present at the periphery of the nucleolus a granular substance which stains intensely with Heidenhain's iron-alum haematoxylin, and remains strongly differentiated when every other part of the cell is decolorized. This substance, which resembles chromatin, often occurs in the form of a single large granule or a number of small ones (Figs. 49-80), and is so closely associated with the periphery of the nucleolus that it is difficult to determine from an ordinary preparation whether it is actually contained within the nucleolar substance or is merely in close contact with its surface. Instructive results with regard to the structure of the nucleolus and its morphological relation to this deeply-stained chromatin were obtained by squeezing out the contents of a number of yeast cells.

The yeast used for this purpose was stained *en masse* in Heidenhain's haematoxylin, the times for mordanting and staining being proportionally prolonged to ensure more precision in the subsequent washing-out processes. When the cells were sufficiently differentiated, they were well washed in water and transferred to dilute glycerine. A few cells were then placed on a slide and the contents expressed by means of sharp taps on the cover-glass. The nucleolus was thus, in many cases, separated from the other cell contents, and was clearly visible as a homogeneous body and not, as many observers state, a nucleus and enclosed nuclear sap.<sup>2</sup> The peripheral chromatin was clearly shown in many cases around the nucleolus,

<sup>1</sup> Guilliermond ('02) states that the nucleus (nucleolus) possesses the normal structure of a nucleus, with membrane, network, etc., and that it appears homogeneous only in badly fixed and stained or anomalous specimens of *S. cerevisiae*. But in the cells figured in his coloured Plate X, all the nuclei (nucleoli) are shown as homogeneous bodies, just as figured by Wager ('98). It is not clear to us why the author should have chosen badly stained or anomalous specimens for this purpose.

<sup>2</sup> We are not prepared to say that this homogeneous body (nucleolus) does not under some conditions become more or less vacuolated. Certain appearances observed in compressed yeast lead us to suspect that this may take place occasionally.



and in some cases was partially separated from it. The outline of the nucleolus in these cases remained intact, showing conclusively that the granules are not contained within it, but are closely associated with its surface (Figs. 81-93).

In perfectly normal cells, in the resting condition, and in the early stages of fermentation, the peripheral deeply stainable chromatin is present in the form of a single large granule situated on one side of the nucleolus (Figs. 53, 54, 106). As fermentation proceeds it increases in size, forming a cap surmounting the nucleolus (Figs. 67, 72, 116), and, under favourable conditions during the period of highest fermentative activity, it may extend over the greater part of the surface of the nucleolus. In preparations of such yeast stained with a general stain such as Mayer's haemalum, no indication of this encrustation is obtained, but the nucleolus appears to be very large, and doubtless the marked variation in the quantity of chromatin present on the surface of the nucleolus accounts to some extent for the variation in the size of this body, which has been noted by Guilliermond and Macallum.

New centres for the formation of the deeply stainable chromatin may occur on the periphery of the nucleolus, giving rise to some peculiar appearances that might be mistaken sometimes for the reticulate structure of a nucleus possessing a well-marked nucleolus (Fig. 73), sometimes for mitotic figures (Figs. 66, 69, 71); and Feinberg ('02) is possibly referring to one of these appearances when he says that 'the nucleolus sometimes shows a looser structure in which the separate pieces can be distinguished' (Figs. 56, 68, 70, 75, etc.). It is not uncommon for a new centre to arise at the side of the nucleus opposite to the original patch, bringing about an appearance which strongly resembles the anaphase of a typical mitotic division (Figs. 55, 60), and has evidently been mistaken for such by Swellengrebel ('05) and Fuhrmann ('06). The amount of chromatin present is at a maximum between thirty and forty-eight hours after the commencement of fermentation. After forty-eight hours it diminishes, first disappearing in the neighbourhood of the vacuole and gradually decreasing until there only remains a granule similar to that present in the early stages of fermentation.

In some cells the single granule is replaced by smaller granules, which may be few in number and comparatively large (Fig. 56), or numerous and more or less minute, when they may occur in a group, a double row, or a single row which may extend half-way round the nucleolus (Figs. 61-4, 70, 74). Sometimes we get a nucleolus completely surrounded by minute granules (Wager ('98), Fig. 4). Occasionally, in surface view, they appear in the form of a band across the nucleolus (Figs. 64, 71). Sometimes we get one large granule and a number of smaller ones (Fig. 70).

There can be little doubt that these granules correspond to the



chromatin elements described by Guilliermond ('02) as occurring in the nuclear hyaloplasm in favourable cases, pressed close to the membrane of the nucleus (nucleolus), or in the form of two or three large masses or of small filaments radiating from the centre. In cells which have been living for some time under bad nutritive conditions, it is found that in practically every case the dense granular mass is replaced by more or less numerous small granules, whereas in cells from healthy cultures this condition is only occasionally met with. It is also found that such a condition can be induced by simply placing the yeast under adverse nutritive conditions, either in a very dilute sugar solution, or on pieces of carrot, or in water.

In cells which have been living for a short time—about two hours—under these conditions, four or five granules are present at the periphery of the nucleolus. In twelve hours the granules are more numerous, but smaller and farther apart. Still later the nucleoli in most of the cells are wholly or partially surrounded by minute granules more or less evenly distributed over the surface of the nucleolus. But, although they increase in number, they become individually smaller, and there seems to be a gradual diminution in the actual quantity of stainable substance. This takes place so slowly that it is only perceptible when the cells are examined at long intervals.

The general appearance of the yeast cell under these conditions, as seen in preparations stained according to Heidenhain's methods, throws some light on the observations of Swellengrebel and Fuhrmann. The cytoplasm is deeply stained, the nucleolus is almost invisible and is often somewhat paler than the cytoplasm (Figs. 51, 57). The vacuole is small and is frequently no larger than the nucleolus; it contains no stainable granules and, as in the case of the nucleolus, is visible only as a faintly outlined somewhat paler area in the cytoplasm. The only part of the cell that remains strongly differentiated is the deeply stainable chromatin at the periphery of the nucleolus (Figs. 56-8, 61-76). When only four to six granules are present, they are very conspicuous, and are commonly situated between the nucleolus and the vacuole, in which position they look very much like chromosomes at the equator of a spindle-shaped figure (Figs. 58, 61), the faintly outlined nucleolus and vacuole corresponding to the halves of the spindle.

It is significant that the only stages in this mitotic division that Swellengrebel ('05) appears to have observed clearly should be the metaphase and disaster stages, and that appearances strongly resembling these should be of frequent occurrence in the yeast cell.

We have examined a large number of cells in all stages and under all conditions of development, and we feel convinced that all these various appearances which are so strongly suggestive of mitotic figures are accidental

only and of no morphological significance. They occur at all periods of fermentation, and badly nourished cells with these appearances are commonly met with in preparations of healthy yeast.

The deeply stainable chromatin at the periphery of the nucleolus is well marked in some compressed yeasts, especially D.C.L. It is found in various positions on the surface of the nucleolus, thereby bringing about appearances strongly suggestive of nuclear structure. In one example of D.C.L. which we examined it was disposed in the form of a curiously regular network, which showed in sharp contrast to the faintly stained nucleolus beneath, presenting the characteristic appearance associated with typical reticulate nuclei (Fig. 65).

The occurrence of chromatin or nuclein in this form on the surface of the nucleolus is not unique in our experience. It occurs in a similar position in *Polyphagus Euglenae*. We have also observed nucleoli in *Closterium* with varying amounts of chromatin on or at their surface (Fig. 100); and there are indications of a similar distribution of chromatin in the nucleoli of the root tips of *Allium* and *Hyacinthus*.

There are some indications that a division of the chromatin precedes that of the nucleolus in budding yeast cells. Nucleoli are frequently met with possessing two distinct deeply stained hemispherical patches which in some cases lie close together (Fig. 78), in others farther apart (Figs. 79, 94), and it is not uncommon to see an elongate nucleolus with one of these patches at each end (Figs. 55, 60, 77, 99). Further, in some cells we see two nucleoli each with a deeply stained patch (Fig. 80), and however small the portion of the nucleolus may be which enters the daughter-cell, it is invariably furnished with much the same amount of deeply stainable substance as the part retained in the mother-cell.

#### GLYCOGEN.

Glycogen makes its first appearance in the cell in the early stages of fermentation in the form of minute refractive granules which give the characteristic reaction with iodine (Figs. 131-3). In the course of three or four hours these are replaced by larger masses of glycogen, which appear in the living cell as distinct vacuoles in the cytoplasm around the nuclear vacuole (Figs. 134, 135). This explains the vacuolated appearance of the cytoplasm at this stage. At a later stage, these separate vacuoles disappear and the nuclear vacuole becomes surrounded by a single mass of glycogen (Figs. 136-40, 146). This gradually increases in size; the nucleus becomes pushed to one side; the nuclear vacuole seems to disappear, and finally the greater part of the cell space becomes occupied by glycogen (Errera, '98; Wager, '98). This is not visible in the living cell, owing to the high

refractive index of the glycogen, which gives the cell contents a hyaline appearance at this stage, but in iodine it appears as a large vacuole with dark brown contents (Figs. 141-5).

Harden and Rowland ('01), in studying the changes which take place in the liquefaction of yeast, pointed out that freshly preserved yeast consists of large cells with a small vacuole and granular cytoplasm which stains deep brown with iodine. As the evolution of  $\text{CO}_2$  proceeds the vacuole increases in size and the glycogen diminishes and finally disappears. They consider that the progressive increase in the size of the vacuole may possibly result from the accumulation of some substance produced, along with the  $\text{CO}_2$  from the glycogen.

According to Harden and Young ('02) the glycogen from yeast has the same chemical composition as that from other sources. It is probably a transitory food reserve, and from Meissner's observations ('00) it would appear that the yeast makes use of it by means of diastatic non-diffusible ferments. The late Professor Errera found ('98) that it disappears or accumulates very rapidly according to the conditions of nutrition and growth (cf. Meissner, '00; Will, '02; Lindner, '02). We have made a number of observations which fully confirm Errera's conclusion and show further that the presence or absence of glycogen coincides with the fluctuations in fermentative activity which are well known to occur but are not clearly understood.

We noticed when starting new cultures that the time which elapsed before active fermentation commenced was very variable. This was especially noticeable in brewery yeasts and is well known to bakers in connexion with compressed yeasts. In some cases the cells immediately sink to the bottom of the fermenting fluid and it is some time before fermentation begins to take place to any appreciable extent, and this is accompanied by the rising of the yeast cells into the upper layers of the fluid. In an ordinary brewery yeast this may not take place for one to five hours or even longer. In other cases the cells, having been thoroughly mixed with the fermenting fluid, do not sink to the bottom and fermentation at once becomes visible.

We find that the yeasts which ferment at once without sinking contain very little glycogen, whilst those which remain at the bottom of the jar for a considerable time contain a more or less large amount of glycogen.

In yeasts which contain a large quantity of glycogen when the culture is started, the fermentation, although slow at first, becomes in the course of ten to fifteen hours much more vigorous than in the culture of yeast which at the beginning contained very little glycogen, and the budding activity is much more marked.

When fermentation is started with a healthy brewery yeast which contains a large quantity of glycogen and which in consequence sinks to the bottom,

we find, in the course of one to three hours, that some of the cells come to the surface and that, although glycogen is still present in them, a marked decrease in the amount has taken place. In the cells which remain at the bottom glycogen is still abundant, but in these also a considerable decrease in amount is observable. After four or five hours most of the cells become distributed throughout the fluid and fermentation is more active.

During the next 5-15 hours the glycogen practically disappears in all the healthy cells, and, as we have already noted, this is the period of greatest vegetative activity. Between 10-20 hours the glycogen may reappear in small quantities. At first the increase is very slow and for some time almost imperceptible. It then increases rapidly, and in two hours a considerable amount may appear, but not enough to interfere with the nuclear vacuole. It is significant that this sudden increase is coincident with the decrease in budding activity.

From 20-30 hours the glycogen continues to increase but slowly; the cells are still budding but less vigorously; fermentation is increasing, and the nuclear vacuole during this period is very large. Immediately after or near the height of fermentation the glycogen increases rapidly, and this coincides with the decrease of the nuclear vacuole.

All the cells now contain a large quantity of glycogen, and a number of cells are found which apparently contain little else. So far very few cells have settled to the bottom, but about this time a large number of cells sink.

On examining these we find that practically every cell is filled with glycogen. Fermentation may be carried on for some time by the yeast which remains at the top, the cells gradually sinking as they become filled with glycogen, but fermentation is finished for the time being when they sink to the bottom.

If the fermenting fluid is not exhausted, after some time air-bubbles begin to rise from the bottom, and in the course of 5-10 hours a large number of the cells again come to the surface. This is attended by the disappearance of the greater part of their glycogen. They attain a maximum and again sink to the bottom, containing a large amount of glycogen.

This process may be repeated two or three times, but each time as the fluid becomes less concentrated the height of fermentation is reached sooner, and the cells sink with much less glycogen than in the first fermentation. This explains an apparent periodicity in fermentative activity which occurs when yeast is grown in a relatively large quantity of Pasteur's solution.

A slight foam may remain at the surface for a considerable time after the complete cessation of activity. Such a foam invariably consists of chains of cells which entangle the air-bubbles, and are thus enabled to remain at the surface although they may contain a large amount of glycogen.

In less vigorous fermentations the cells sink before a maximum amount of glycogen occurs. If the foam from a vigorous culture containing a considerable quantity of glycogen is stirred into fresh Pasteur's solution so that the air-bubbles escape, the cells at once sink.

The glycogen-filled cells are apparently capable of retaining their vitality for some time. It was found that after remaining quiescent in exhausted Pasteur's solution for ten days no perceptible decrease had taken place in the amount of glycogen present, and most of the cells were still capable of a vigorous fermentation when placed in Pasteur's solution, although the time that elapsed before their activity became obvious was much longer than when the transference to the fresh solution took place earlier.

#### VOLUTIN.

There are present in the yeast cell, under certain conditions, granules giving a characteristic reaction with methylene blue. These have been described at some length by Guilliermond ('02) and Arthur Meyer ('04). Guilliermond ('02) considers that they are identical with the metachromatic granules of Babes ('89, '95) and the red granules of Bütschli ('90, '96), that they consist of two substances, an outer deeply stainable one, and a less deeply stainable inner one, and that they possess none of the microchemical reactions of nuclein. Meyer ('04) states that they are of the same nature as the granules discovered by Ernst ('88), and he suggests for them ('03) the name *Volutin*. He points out that in their behaviour to stains and reagents volutin granules certainly bear some resemblance to nucleic acid. He does not agree with Guilliermond's view that they consist of two substances, but is inclined to think that they are a combination of nucleic acid with an unknown base. With this view Guilliermond has later apparently come to agree ('06). Among the principal reactions of volutin granules given by Meyer may be mentioned the intense staining in methylene blue or carbol fuchsin and the persistence of the colour when washed in 1 per cent. solution of sulphuric acid, their solubility in boiling water, in eau de javelle, and in mineral acids, and their insolubility in boiling water after treatment for thirty minutes or longer with formol. A full account of various other reactions is given in Meyer's paper ('04). According to Guilliermond ('02) these granules are contained chiefly in the vacuole (nuclear vacuole, Wager, '98), and upon this ground he assumes that they are the granules described by Wager ('98) as chromatin. He takes considerable pains to show that they cannot be regarded as chromatin, and that they have no connexion with the nucleus. Meyer, however, points out that the volutin granules are found chiefly outside the main vacuole in the cytoplasm. In the numerous cells which have recently come under our observation, we find, in conformity with the conclusion of Wager ('98)



and of A. Meyer ('04), and contrary to Guilliermond's emphatic statement, that the volutin granules lie for the most part in the cytoplasm outside the vacuole, though, as Meyer points out, often in close proximity to the vacuole wall. One large granule, or two, more rarely three, smaller granules which have some of the characteristics of volutin commonly occur within the vacuole, and these usually exhibit the characteristic Brownian movements. Guilliermond himself seems to be in some doubt as to their exact location. On p. 105 of his *Memoir* ('92) he says, 'Dans les premiers stades, la levure est constituée d'une vacuole renfermant des corpuscules méta-chromatiques.' On p. 261 he says, 'Ces corpuscules, qui correspondent aux granulations chromatiques décrites par certains auteurs (Raum, Eisen-schitz, Curtis, Wager), sont presque toujours localisés dans l'intérieur des vacuoles. On peut en rencontrer cependant dans le protoplasme et ils paraissent tous être d'origine protoplasmatique.'

Kohl's account ('09, p. 37) is that the granules lie in greater or less number in the cytoplasm, often crowded together on the walls of the vacuoles. In healthy cultures he found only one or two granules in each vacuole, and a few small ones in the rest of the cytoplasm, which might possibly be situated in small vacuoles. He considers that this would accord with most of the figures given by Guilliermond ('02). It is, however, not easy to see on what grounds Kohl makes this statement, for an examination of Guilliermond's figures (Plate X, Figs. 1-25) shows the volutin granules almost exclusively in the nuclear vacuole.

The amount of volutin present varies according to the stage of development and the metabolic activity of the cell (Figs. 5-7 and 163-82). In yeast obtained fresh from the Albion Brewery we usually found but little volutin (Figs. 163-4). In some cases the granule in the centre of the vacuole and a few small granules in the cytoplasm gave the volutin reactions, but in others no reaction was obtained, although the central granule was present and clearly visible in the living cell and in the unstained fixed specimens.

During the first five hours' fermentation in Pasteur's solution or in a strong sugar solution, we find that a slight but perceptible increase takes place (Figs. 167-8), which usually becomes marked after about ten hours, and under good conditions continues progressively till a maximum is reached in 40-50 hours (Figs. 179-80). After this the volutin decreases, and with the other cell contents becomes limited to a small space in the cell by the encroaching glycogen (Fig. 182). A considerable quantity of volutin is usually present at the end of fermentation, and so long as the cells remain apparently inactive this volutin decreases with extreme slowness.

Guilliermond states that these granules may persist for a considerable time, and that he found them still present in yeast which had been kept for three months in distilled water. We find in the case of old cultures



which have been kept in a quiescent condition until volutin has disappeared from most of the cells (about three weeks), that in some cells a large quantity is still present. Such cells, however, present the well-known characteristic microscopic appearance of disorganized cells. Further, in preparations of such yeast we find a number of granules which give the volutin reaction with methylene blue lying outside the cells. It seems probable that these are volutin granules which have escaped from disorganized cells.

Under active metabolic conditions volutin disappears and reappears with remarkable ease, and for this reason is extremely difficult of observation. If the temperature is lowered so as to impede but not stop fermentation, when a large quantity of volutin is present, it may disappear completely from every cell within 1–5 hours and reappear in the same time when the original activity is resumed.

When the volutin granules first make their appearance they are extremely small, and occur in the immediate neighbourhood of the chromatin network, but on the outside (Pl. X). Each granule appears to originate independently, and in the early stages of their formation it is common to see several minute granules at remote intervals immediately outside the chromatin network. It is significant that in these early stages they are never found in the vicinity of the nucleolus.

As soon as the granules become numerous they tend to aggregate, as Guilliermond points out, and apparently fuse to form large granules, often forming a single granule of enormous size (Figs. 171–3). They may then take up a position in the neighbourhood of the nucleolus, and it is common to find a number of comparatively large granules or a single enormous granule resting on the nucleolus, frequently completely obscuring it (Figs. 171, 180). Sometimes they increase in number without aggregating, and remain round the outside of the chromatic network, where they often exhibit a very regular arrangement, resembling a reticulum (Fig. 175), especially when stained in haemalum, and occasionally in methylene blue preparations. It seems possible that this is what Hieronymus saw, and regarded as similar to a structure described by him for the Cyanophyceae. (See Wager, '98, p. 503.)

When the volutin granules begin to disappear, they first break up into smaller granules, and then, as Guilliermond states, there can be no doubt that they become dissolved in the cytoplasm, which becomes intensely stained with methylene blue just at this stage. It is interesting to note in this connexion, that in preparations stained with methylene blue, or the fuchsin-methyl green mixture, or similar combinations, these granules are often seen, each surrounded by a less deeply stained area in the cytoplasm, which gives them the appearance of being contained within small vacuoles.

It may here be pointed out that the intense reaction of the cytoplasm

to methylene blue does not coincide with a strong reaction to Heidenhain's iron haematoxylin. On the contrary, when the cytoplasm stains strongly in this stain (i. e. during the first few hours of fermentation, and under starvation conditions) it stains very slightly with methylene blue, and at the height of fermentation, when it often stains deeply with methylene blue, it remains clear and unstained by the haematoxylin.

We have already drawn attention to the fact that the granule usually present in the centre of the vacuole does not always give the volutin reaction; in these cases it is usually obvious in preparations stained with iron haematoxylin as a reddish granule of characteristic appearance. In those cases where the central granule gives a strong reaction with methylene blue, in the fresh condition and also after fixing in alcohol and iodine, we find there is no reaction after fixing with Perenyi's fluid. This is what we should expect, since Meyer states that volutin is soluble in 25 per cent. nitric acid, which is the percentage contained in Perenyi's mixture. But although it gives no reaction with methylene blue, the central granule is still present and can be clearly seen in the unstained cells. Further, it now stains faintly but unmistakably with iron haematoxylin (Fig. 169). We have previously noticed in dealing with phosphorus and iron that this central granule sometimes gives reactions for both elements. This behaviour, taken in connexion with its position in the nucleus, and the fact that it is sometimes apparently attached to the chromatin network by delicate threads (Fig. 169), would lead us to believe that it differs from the volutin granules in the cytoplasm, both in its more intimate relation to the nucleus and in its chemical composition.

How far all the granules described as volutin may be regarded as similar, and what rôle they play in the cell economy, are difficult questions. All we can say at present is, that they are probably some kind of reserve substance produced under conditions which we are not able to determine definitely; some of them may be derivatives of the nucleus, some of them derivatives of the cytoplasm. It is possible that, both in yeast and in the Cyanophyceae, they may be a product of the nuclear activity. In the case of the Cyanophyceae they are found in connexion with the central body, mainly at its periphery, and Bütschli ('90, '96) regarded them as representing the chromatin of the nucleus.

Wager ('98) mentions two distinct kinds of granules in the yeast cell: chromatin granules which are invisible in the living cell and stain strongly with nuclear stains, and bright refractive granules visible in the living cell, some of which are of a proteid nature. There can be no doubt from his account of their appearance and staining reactions that the latter are what Guilliermond describes as metachromatin. Guilliermond disposes of these two kinds of granules by saying that the stains used by Wager do not give the characteristic red colour to the 'metachromatin' (volutin) granules,

that they are not sufficient to establish the identity of two kinds of granules, and, furthermore, that the stains used by himself, namely, methylene blue and haemalum, indicate clearly that there is only one kind of granule present.

As most of Guilliermond's conclusions appear to have been based on preparations stained with methylene blue and haemalum, it might be well to emphasize the fact that methylene blue is not a good nuclear stain, and one would not expect to find the chromatin granules differentiated by it. Guilliermond himself points out that the nucleolus is only faintly stained and barely differentiated from the cytoplasm. Haemalum, on the other hand, stains everything in the cell, the chromatin network, nucleolus, and 'metachromatin' granules. The differentiation in this stain is not as clear as in preparations stained with Heidenhain's haematoxylin, but in good preparations the 'metachromatin' granules stain black and the chromatin reddish blue, so that it is difficult to understand how Guilliermond failed to recognize the two kinds of granules.

It is possible that Guilliermond's inability to recognize the presence of the chromatin network was partly due to a fixing agent extensively used (picric acid), which accentuates the granular contents of the cell but does not sharply differentiate the threads of the network or the nucleolus from the cytoplasm.

The 'metachromatin' (volutin) granules differ from the chromatin granules in the following respects:—(1) They are visible in the living cell as bright refractive granules, whereas the chromatin granules and nucleolus are invisible. (2) They stain deep red or black with methylene blue. The chromatin granules and nucleolus stain a pale blue like the cytoplasm, and the former are commonly invisible. (3) They remain unstained by Heidenhain's haematoxylin, when the chromatin granules and nucleolus are strongly differentiated by it. (4) They are characteristically different in shape from the chromatin granules, being peculiarly angular, and they may attain a size incompatible with the description given of chromatin granules. (5) They lie individually free in the cytoplasm, whereas the chromatin granules are arranged on a network or in close contact with the nucleolus. (6) They sometimes exhibit Brownian movements in the living cell. (7) Finally, with the exception of the one or two granules clearly visible in the vacuole, they are always situated *outside* the nuclear vacuole.

Both the 'metachromatin' and chromatin granules are stained with methyl green and fuchsin, methylene blue and fuchsin, and safranin and gentian violet after fixing in iodine. Although at times these staining methods give a beautiful differentiation of both sets of granules, they cannot be relied upon, owing to the varying reaction of the chromatin network. Aniline gentian violet stains both sets of granules but does not differentiate them. Gram's aniline violet method, if carefully manipulated,

stains the volutin granules black and the nucleolus and chromatin network violet. Haemalum is the only stain which invariably differentiates both sets of granules, the chromatin granules being reddish blue and the 'meta-chromatin' black.

#### IRON.

Macallum ('95) has shown that various parts of the vegetable cell contain iron in organic combination. It may occur in any part of the cell, but is most abundant in the nucleus and nuclein constituents and in such organs as the pyrenoid and in the chromatophore. It is found also in various species of *Saccharomyces*. If cells of *S. cerevisiae* are treated with glycerine-ammonium sulphide solution for several days at a temperature of 60° C., their cytoplasm acquires a greenish tint due to the iron. Sometimes, however, the reaction may appear only in a few granules scattered through the cytoplasm. In cells subjected to the action of sulphuric acid alcohol and subsequently treated with acid potassium ferrocyanide solution, the presence of iron is shown by a faint blue colour in their cytoplasm with sometimes blue granules. In *S. Ludwigii* treated in the same way, the stainable substance is found chiefly at the periphery of the vesicles and in a substance which constitutes corpuscles of a nucleolar character, large spherical elements which in the glycerine-ammonium sulphide mixture appear darker green than the surrounding cytoplasm.

In a later paper ('99) the author states that after ten days at the latest in the glycerine-ammonium sulphide solution, the presence of 'masked' iron is distinctly demonstrated in the one or more corpuscles which may be present in each cell (*S. Ludwigii* and *S. cerevisiae*), in the walls of the vacuoles, and in the cytoplasm generally.

We have found the distribution of organic iron in yeast cells, when examined with the acid ferrocyanide solution after the action of sulphuric or nitric acid alcohol, very much as described by Macallum ('95), but the coloration was very slight. When the cells were treated with 5 per cent. solution of haematoxylin (Macallum, '97), however, instead of the ferrocyanide solution, the staining was much stronger, and, although the coloration was diffuse, it was possible to distinguish the nucleolus clearly in nearly all cells under high powers of the microscope. Specimens hardened in alcohol were used. These were placed in an alcoholic solution of nitric or sulphuric acid (4 parts  $H_2SO_4$  and 100 parts methylated spirit, or 3 parts strong  $HNO_3$  to 100 parts spirit) for twenty-four to thirty hours at a temperature of 35° C. to unmask the iron. They were then washed in alcohol and placed for a short time in the haematoxylin solution. After this they were washed several times in distilled water, then in alcohol, and finally mounted in balsam or dilute glycerine. Wherever iron exists in the cell, the coloration is slaty blue, the yellowish coloration in other parts of the cell which comes out on washing in the water and alcohol being due



to the haematoxylin only. The colour both of the cytoplasm, granules, and nucleolus was light slaty blue. The nucleolus was, however, slightly more deeply stained than the surrounding cytoplasm. In compressed yeast the granular substance at the periphery of the nucleolus was more deeply stained than the nucleolus (Figs. 152-6). The chromatin network in the vacuole was only slightly stained, a few granules here and there on the network showing a deeper coloration. In some cases the central volutin granule or granules in the vacuole gave a distinct reaction for iron (Figs. 154-6).

These observations confirm Macallum's conclusion, therefore, that in *Saccharomyces* the cytoplasm gives a diffuse reaction for 'masked' iron, and that in addition to this there is a distinct reaction for it in the homogeneous nucleolus, nucleus, nuclein body, or corpuscule of various observers.

#### PHOSPHORUS.

The chemical constitution of the various elements of the living cell can only be inferred indirectly from micro-chemical reactions upon cells killed in various ways and from the chemical analysis of dead cells. The conclusions obtained from analysis of dead cells are complicated by the fact that we cannot isolate the separate elements of the cell for examination. It has been shown, however, that digestive fluids dissolve certain portions of the cell contents, and that among those that are least acted upon is the nucleus, and especially the deeply stainable portion of it known as the chromatin. Kossel, taking advantage of this, found that the part of the cell which remains undigested contains a certain substance which gives on analysis about 6 per cent. of phosphorus. Meischer investigated the spermatozoa of the salmon, which contain large nuclei and very little protoplasm, and succeeded in isolating an albumin containing phosphorus which formed 50 per cent. of the whole, the phosphorus present being about 10 per cent. He came to the conclusion that phosphorus was present in the nucleus, and called the substance containing it nuclein.

The localization of phosphorus in the cell is of great importance in cytological investigation, for although other substances such as lecithin and zymogen are found to contain it, nuclein is the substance chiefly rich in phosphorus. It is evident, therefore, that in small cells in which the details of structure are not well marked, the presence of phosphorus, taken in conjunction with the results obtained from various reagents and stains, may be of great value in the identification of the nucleus or of nuclear elements. The methods of gross analysis adopted by Meischer, Kossel, and others are not suitable for this purpose; it is necessary to obtain some micro-chemical method in order to determine exactly which parts of the cell contain phosphorus. The most delicate test for phosphorus is the yellow precipitate which is produced by ammonium molybdate in the presence of nitric acid. This precipitate is very abundant even when there is only

a small amount of phosphorus present, owing to the fact that it is composed largely of the molybdic oxide and contains only 3.1 per cent. of  $P_2O_5$ . It is sufficiently abundant in a cell to be distinctly visible under the microscope, but the yellow precipitate is often masked by the yellow coloration produced in the tissues by the action of the nitric acid, and consequently no differentiation is visible. Macallum ('98) has, however, devised a method by which this difficulty is overcome. Molybdic oxide, when acted upon by reducing agents, such as pyrogallol, stannous chloride, and zinc chloride, is changed in colour from white to green, blue or black, according to the reagent employed. The subsequent treatment of cells, which have been acted upon by the nitric ammonium molybdate solution, with one of these, therefore, produces a dark coloration in those parts in which the precipitate has been formed. As, however, both pyrogallol and stannous chloride produce a similar effect upon ammonium molybdate itself, neither of these is suitable, since, if the nitric ammonium molybdate solution is not properly washed out, which appears to be difficult to accomplish in many cases, they cause a coloration in the tissues as well as in the phosphorus-containing elements. Zinc chloride does not give any colour to the ammonium molybdate, but its action upon the precipitate is too slow for practical purposes. Macallum ('98, '99) found, however, that phenylhydrazin hydrochloride in 1-4 per cent. solution in water gives a deep green coloration of the phospho-molybdate precipitate, but does not cause any coloration of the ammonium molybdate. The nuclein-containing elements are in consequence clearly differentiated. Macallum ('99) used this method to demonstrate the presence of organic phosphorus in yeast cells, and found that the corpuscle (nucleolus) is rich in it, that the wall of the vacuole in contact with the corpuscle gives a distinct reaction for it, and especially at times the granules contained within the vacuole. We have found the method extremely useful in determining the distribution of the nuclear constituents of the cell, and our observations confirm those of Macallum, and afford some additional evidence for our views as to the nature of the yeast nucleus.

The best results were obtained with fixed cells (alcohol or iodine), the distortion in the case of fresh material being so great as to preclude the recognition of definite reactions in particular parts of the cell. The cells are treated for ten minutes to forty-eight hours with a nitric acid solution of ammonium molybdate, which produces the yellow coloration in those parts which contain phosphorus. They are then washed and placed in a freshly made 1-4 per cent. solution of phenylhydrazin hydrochloride in water. In the course of two or three minutes the phospho-molybdate becomes reduced to the green oxide, and those parts of the cell in which it is contained can be easily differentiated by their colour, which varies from light to dark green (Figs. 117-30), from the other parts of the cell, which



are slightly yellow. The preparations, after washing, can be dehydrated and mounted in balsamin the usual way. Good results were obtained from cells mounted whole, but the best differentiation was seen in sections cut in paraffin by means of a microtome.

We found that many photographic reducing agents, such as eikonogen, amidol, quinol, etc., can be used to bring about the change in colour of the precipitate, but they are not very satisfactory, as the results are confused by a brownish-yellow coloration of the cell contents. Nevertheless, when the distribution of phosphorus has been generally determined by means of phenyl-hydrazin, they are very useful.

The phosphorus reactions at various stages in the growth of the yeast cells have been carefully followed in *S. cerevisiae*, I. Hansen, which was obtained in a pure culture from Mr. Murphy. It was grown in wort and fixed in absolute alcohol at definite intervals throughout fermentation. We find that when a small nuclear vacuole is present, during the early stages of fermentation in young cells, the phosphorus is diffused throughout the cytoplasm and nucleolus, the granules at the periphery of the latter showing a marked reaction.

As the vacuole increases in size during the progress of fermentation the phosphorus disappears almost entirely from the cytoplasm, but a more pronounced reaction for it becomes visible in the nucleolus and nuclear vacuole (Fig. 119, 128, 129), and in the granular substance at the periphery of the nucleolus (Figs. 123, 127). The volutin granule or granules near the centre of the vacuole are also found occasionally to give the phosphorus reaction (Figs. 129, 130).

The evidence thus obtained of the distribution of the phosphorus-containing element supports the conclusion, already arrived at on the grounds of their structure and staining reactions, that the vacuole and homogeneous body attached to it constitute the nucleus of the yeast cell.

We find that the phosphorus reaction corresponds with the intensity of the haematoxylin staining, and both being more strongly marked in the substance at the periphery of the nucleolus, this must therefore be considered to contain the greatest proportion of nuclein.

Phosphorus begins to be prominent about fourteen hours after the commencement of fermentation, and appears to increase steadily up to from thirty-three to forty-eight hours, after which it decreases.<sup>1</sup>

There appears to be no inorganic phosphorus in the yeast cell. Specimens were placed in ammonium molybdate solution for ten or fifteen minutes, at 30–35° C., and after washing in water were placed in the phenyl-

<sup>1</sup> These observations were made upon a series of pure yeast cultures set up and preserved by Mr. J. H. Murphy of Leeds. We hope that we may be able jointly with him to make a more complete investigation of the variation in the amount of phosphorus present in the nucleus under different conditions.

hydrazine. No reduction was obtained. Specimens kept in the molybdate solution for half an hour gave a very slight green reaction in the nucleolus and surrounding granules.

#### BEHAVIOUR OF THE NUCLEUS DURING BUDDING AND SPORE-FORMATION.

In the process of bud-formation the nucleolus becomes elongated and constricted and divides into two equal or unequal masses, one of which, together with a part of the nuclear vacuole and chromatin granules, passes into the young bud. The division of the nucleus may take place either entirely in the mother-cell, or the final separation may be accomplished in the neck joining the bud to the mother-cell. The granular chromatin mass round the nucleolus is also more or less equally divided along with the nucleolus. The division is a direct one, and there is no trace of anything in the nature of a mitotic figure with chromosomes and nuclear spindle.

In the early stages of spore-formation, a large number of bright refractive granules appear in the cell. From their reactions towards stains Wager ('98) regarded them as of a proteid nature, but Guilliermond found that they possess the characteristic properties of the metachromatic granules discovered by Babes in the Bacteria and Cyanophyceae, which Bütschli called red granules and which have been more recently named volutin by A. Meyer. In order to get rid of these granules it is quite sufficient to fix the cells in Perenyi's fluid. Cells thus fixed show clearly, when treated with appropriate nuclear stains, the granular chromatin network in contact with the nucleolus. The bright refractive volutin granules are easily seen (Wager, '98) in the living cell; the chromatin granules only after fixing and staining. Guilliermond has apparently confused these two sets of granules, taking it for granted that the bright refractive granules observed in the living cell are the same as the chromatin granules which are visible in the dead stained cells. As a matter of fact, the two sets of granules are entirely different in their appearance and staining reaction.

The changes which take place in the nucleus immediately preceding its division into the spore nuclei are difficult to follow, and we have very little to add to what has already been given by Wager ('98) and other observers. The chromatin network first of all contracts round the nucleolus (Fig. 113), and the nuclear vacuole disappears (Figs. 114, 115). The nucleolus at this stage is frequently obscured by the chromatin granules and the remains of the chromatin network around it. The whole mass often presents the appearance of a deeply stained irregular body. The actual structure can, however, be brought out by staining in safranin and

gentian violet, by which the nucleolus, surrounded by the irregular mass of chromatin granules, can be differentiated. An appearance commonly observed at this stage is a group of strongly stained chromatin granules, more or less radially arranged around the nucleolus. Sometimes a nucleolus is seen with a single strand only of the chromatin network attached to it. Then the chromatin granules begin to disappear. The cytoplasm in the immediate neighbourhood of the nucleolus becomes more deeply stained, and, together with the less deeply stained peripheral portion, exhibits a beautiful alveolar structure.

We are unable to confirm the account given by Wager ('98) of the division of the nuclear vacuole first of all into two, 'then probably by further division into numerous smaller portions, until finally a delicate foam structure of the protoplasm is produced.' Repeated careful examination both of living and of stained preparations failed to reveal any satisfactory evidence for this, and we are inclined to agree with Guilliermond ('02) that the foam structure is due to the increase in the number of volutin (metachromatin) granules which appear in large numbers round the nucleolus, and to glycogen vacuoles which occur more especially at the periphery. Whether the foam structure is due to the repeated subdivision of pre-existing volutin and glycogen vacuoles we cannot say, but we are inclined to think that in the case of the volutin it is largely due to the formation of granules *de novo* from material which is suddenly made available in the cell.

The presence of volutin granules in the nuclear vacuole, and their appearance often in close contact with it and the nucleolus, indicates that they may be products of the nuclear activity. In the Cyanophyceae they also occur in the nucleus (central body). It is quite possible therefore that the large increase in the volutin at the time the nuclear vacuole disappears may be the result of the formative activity of the chromatin granules then set free. At a later stage the volutin grains seem to dissolve, and there is reason to believe that they are finally used up in the formation of the spores, and especially of the spore walls.

The nucleolus has meanwhile increased in size, but shows a less marked staining capacity, and a deeply stained granular mass in contact with it now becomes visible. This appears to be similar to that already described and figured (Figs. 53, 54, 56, &c.), but is larger and seems to be more deeply embedded in the nucleolus. This differentiation, however, is observed only when the stain has been well washed out. In normally stained specimens which have not been so well washed out, it can be seen that at the periphery of the nucleolus there is a granular chromatin layer in close contact with it. Immediately around this is the deeply stainable sporogenous cytoplasm which takes part in the formation of the spores.

The nucleolus and the granular chromatin mass are all that remain of the original nucleus. Division now takes place by elongation of the nucleolus into an hour-glass shape with a long drawn-out middle portion, and the separation of the chromatin into two equal or nearly equal portions, as described and figured by Wager ('98, Figs. 61-78) and by Guilliermond ('98, Pl. III, Figs. 21-30). Guilliermond appears, however, to have mistaken the chromatin granular mass for the nucleolus, and the nucleolus for the sporogenous cytoplasm. It is certainly not easy to differentiate the two, especially in the later stages.

We are not now inclined to lay any stress upon the suggestion (Wager, '98) that the chromatin granules are chromosomes. The whole process appears to be one of direct division or fragmentation, in which nothing in the nature of a spindle figure or definite chromosomes can be observed. At the same time, we must not forget that a nucleolar substance and a deeply stained chromatin granular mass take part in the process, which suggests that it may be of a rudimentary mitotic character, recalling that observed in *Euglena* (cf. Macallum, '99). 'The difficulty of observing all the details of the division is, however, so great that one must be very cautious in attempting an explanation of the facts observed.'

The disappearance of the nuclear vacuole and the formation of daughter-nuclei by division of the nucleolus appears not to be peculiar to the Yeast Plant. Some interesting observations of Ikeno ('03) upon the genus of simple Ascomycetes, *Taphrina*, show that comparable phenomena take place here. The ascogenous cell-nucleus produced by the fusion of two nuclei contains a nucleolus-like chromatin body. During the later stages of development the nuclear vacuole, which usually contains a network, undergoes disorganization, leaving the homogeneous nucleolus or chromatin body free in the cytoplasm. The chromatin body then divides, either by a process of fragmentation or budding as in the *T. Johansonii* type, or by a rudimentary mitosis as in the *T. Cerasi* type, into the nuclei of the spores. The chromatin of the nuclear vacuole escapes into the cytoplasm either before or during disorganization. It is probably nutritive in character and serves for the growth of the cytoplasm of the ascus. He compares this breaking down of the nuclear vacuole to the disappearance of the nuclear vacuole of the Yeast Plant as described by Wager ('98). Whether the nucleolus lies inside, as in *Taphrina*, or outside the nuclear vacuole, as in yeast, is quite irrelevant. What is important is that the vacuole has a temporary existence in both cases, and that it disappears entirely in both cases as a preliminary to the formation of the spores. May we not, therefore, regard the nuclear vacuole in both these cases as simply a storehouse for the temporary accumulation, or perhaps elaboration, of a chromatin substance for the use of the cell?

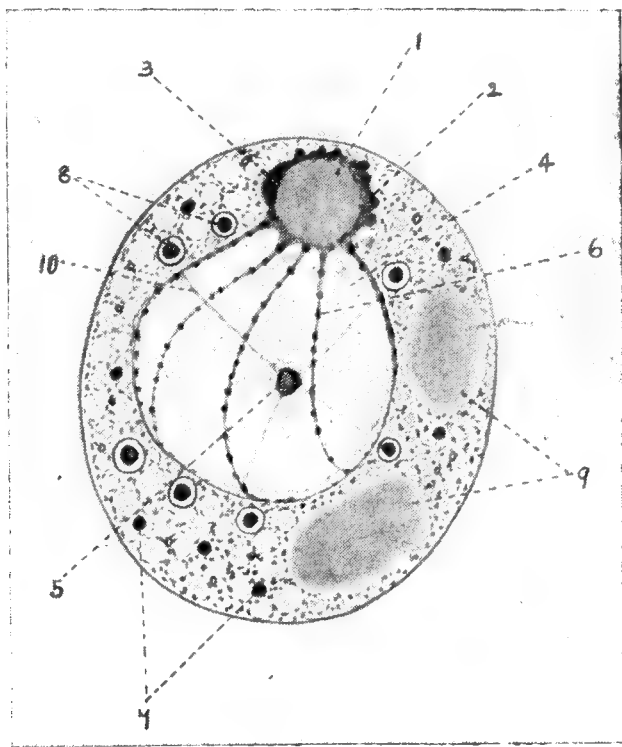
Numerous cases are known in which nuclear substance is extruded into

the cytoplasm for special purposes, particularly in reproductive cells. But we know of very few cases in which the nucleus as such disappears, the nuclear network becoming dissolved in the cytoplasm, leaving only the nucleolus as the representative of the nucleus out of which new nuclei are to be developed (cf. Griggs, '09).

#### SUMMARY.

A drawing of the yeast cell is here given (Text-figure), in which all the various structures described in this paper are diagrammatically represented.

The nucleus consists of a nucleolus and a nuclear vacuole. The vacuole contains a clear nuclear sap, a peripheral network, and one or two, rarely more, bright refringent granules, which exhibit a Brownian movement. The nucleolus is attached laterally to the network, with which it



TEXT-FIGURE. Diagram of Yeast Cell. 1. Nucleolus. 2. Peripheral layer of chromatin. 3. Chromatin patch on one side of nucleolus. 4. Nuclear vacuole. 5. Central volutin granule in the vacuole. 6. Chromatin network. 7. Granules of fatty substance. 8. Volutin granules. 9. Glycogen vacuoles. 10. Delicate suspending threads for the central volutin granule.

is continuous, in such a way that a large part of its more or less spherical surface is in direct contact with the cytoplasm. There seems to be no definite limiting membrane around the nucleus, and the apparent disappearance of the vacuole in certain fixing fluids may be due to the contraction of the vacuole and the intrusion of the surrounding cytoplasm into it, in such a way that the delimitation between the two is no longer clearly visible.

In its essentials the nucleus of the Yeast Plant possesses the normal structure of a nucleus, except that a well-marked nuclear membrane cannot be seen, and that the nucleolus is placed on one side of the chromatin network in direct contact with the cytoplasm.

The nuclear vacuole varies much in size, according to the state of activity of the cell. At certain stages it becomes much contracted, and the



nuclear network in consequence shows up more clearly, but it seems not to disappear entirely except during spore formation.

The strands of the nuclear network occur mainly at the periphery of the vacuole, a few delicate threads only being visible in the central region of the vacuole. They are thicker and more prominent where they join the nucleolus than elsewhere.

Both nucleolus and network may become more or less impregnated with chromatin, and granules of chromatin are found under conditions of great fermentative activity on the nuclear network and in a mass around the nucleolus and partly embedded in its substance. There it forms a dense peripheral layer, which gives the nucleolus its strong capacity for stains at certain stages.

The variation in the amount of the strongly stainable chromatin substance present both in the nucleolus and the network probably depends upon the variation in metabolic activity in the life of the yeast cell. It is most abundant at the period of highest fermentative activity. In the early stages of fermentative activity, and during the preliminary stages of spore formation, the cytoplasm stains very deeply with nuclear stains, and gives a reaction for phosphorus and iron. This appears to be due, as Macallum first pointed out ('95, '98, and '99), to chromatin diffused through it. It soon disappears as fermentation proceeds, and the deeply stainable granular chromatin around the nucleolus then becomes more distinctly visible.

The basis of the nucleolus and nuclear network appears to be a homogeneous substance, which does not possess a strong affinity for stains, and gives the reaction of the plastin network of the nuclei of higher plants.

At certain stages the chromatin granules disappear almost entirely from both nucleolus and network, with the exception of a single deeply stainable patch on one side of the nucleolus. Even when a large quantity of chromatin is present, this black patch can be brought into prominence by prolonged washing out of the stain. Sometimes the amount of chromatin present is so small that the nucleolus appears as a small colourless or nearly colourless body with a dark granule (or more than one) on one side of it. It is probably this that has given rise to the erroneous interpretation of many observers that the nucleolus itself is a nucleus with a nucleolus, chromatin granules, and nuclear sap.

The prominence of the nuclear vacuole at the height of fermentation, the appearance of chromatin on the network at its periphery, and the disappearance of the diffuse chromatin from the cytoplasm, suggest that possibly the vacuole plays an important part in the elaboration of chromatin material which is excreted into the cytoplasm to be used up in the metabolism of the cell during its active fermentation.

The nucleolus and the chromatin network give a distinct reaction for phosphorus and iron. The cytoplasm, during the period when it stains



deeply in nuclear stains, does the same, but at other times the reaction is very slight. We have obtained some evidence that the amount of phosphorus in the nucleus increases up to a certain stage and then diminishes again. During the height of fermentation, the granular mass around the nucleolus gives a strong reaction for it, but from the forty-eighth hour onwards it steadily decreases.

The cytoplasm often contains bright refractive granules which are visible in the living cell. Some of these are composed of a fatty substance, others are similar to the metachromatin granules of Babes or the red granules of Bütschli, which are now called volutin granules. They vary very considerably in number at various stages. The volutin granules appear and disappear with remarkable ease, and are associated with active metabolic conditions. They occur either directly in the cytoplasm or in what may be called volutin vacuoles, and from one to three granules, possessing somewhat similar characteristics, are usually found in the nuclear vacuole.

Glycogen is very abundant at certain stages. It is visible in the living cell in the form of clear bright refractive vacuoles or vacuolar spaces. It accumulates or disappears very rapidly according to the conditions of nutrition, and sometimes almost completely fills the cell. In healthy cells, the accumulation of a large quantity of glycogen seems to bring about a decrease in fermentative activity, which is only recovered as the glycogen gradually becomes used up again or disappears.

In the process of bud formation, the nucleus divides amitotically into two equal or unequal portions, one of which passes into the daughter-cell together with a portion of the vacuole and chromatin. In spore formation, the nuclear vacuole and network disappear, the nucleolus becomes closely surrounded by chromatin granules and then divides by elongation and constriction into two equal or nearly equal daughter-nuclei, each of which consists of a portion of the nucleolus with its surrounding granular chromatin. These two nuclei again divide to form the spore nuclei around which the sporogenous cytoplasm accumulates.

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## EXPLANATION OF FIGURES IN PLATES VI-X.

Illustrating the Paper on Yeast by Mr. Harold Wager and Miss A. Peniston.

Figs. 1-52 and 145-51 were drawn from one sample of yeast, obtained from the Albion Brewery. Figs. 101-12 are also from brewery yeast.

Figs. 1-3, 8-15, 17, 18, 20-28 were made from living cells; Figs. 29-52 from fixed but unstained cells.

Figs. 54-80, 94, 96-103, 105-8, 110, 112-16 have been drawn from preparations stained with Heidenhain's haematoxylin (mordanting and subsequently reducing with ferric ammonium sulphate); Figs. 81-93 from preparations stained with haematoxylin after mordanting in potash alum; Figs. 163-8 and 170-2 from preparations stained with Mayer's haemalum stain, and Figs. 173-82 from preparations stained with methylene blue.

In all other cases special reference is made to the staining method.

Except where otherwise stated, the drawings have been made from yeast fixed in Gram's iodine solution.

### PLATE VI.

Figs. 1-3. Yeast cells fresh from the brewery (24 hours' fermentation).

Fig. 4. The same yeast, after 5-10 minutes in Perenyi's fluid, to bring out the nucleolus.

Figs. 5-7. The same yeast, after running methylene blue under the cover-glass, showing the amount of volutin present.

Figs. 8-15. The same yeast in Pasteur's solution. Figs. 8 and 9, ten minutes in Pasteur's solution; vacuole slightly contorted. Fig. 10, thirty minutes in Pasteur's solution; vacuole sharply defined, turgid but somewhat smaller. Figs. 11-13, one hour in Pasteur's solution; vacuole very indistinct in 11 and 12; in 13 it has apparently disappeared. Figs. 14 and 15, one and a half hours in Pasteur's solution; the cytoplasm is hyaline: the vacuole is invisible, but its presence is indicated by the position of the refractive granules.

Fig. 16. The same yeast after the addition of Gram's iodine solution; the vacuole now shows clearly.

Figs. 17 and 18. The same yeast, twenty-nine hours in Pasteur's solution; vacuole again clearly visible and very large.

Fig. 19. The same cell as Fig. 17, after the addition of iodine, showing position of nucleolus.

Figs. 20 and 21. The same yeast, 40-45 hours in Pasteur's solution, foam, showing a much smaller vacuole.

Fig. 22. The same yeast, 40-45 hours in Pasteur's solution, bottom; the vacuole has disappeared, and the cell apparently contains nothing but a number of refractive granules at one side.

Figs. 23-6. Fresh yeast cells, similar to those figured in Figs. 1-3, after 1-4 hours in distilled water.

Figs. 27 and 28. The same after twenty-four hours in distilled water.

Figs. 29-56 show the effect of a number of fixing fluids on the fresh yeast figured in Figs. 1, 2, and 3. Figs. 29-31, Gram's iodine solution. Figs. 32, 33, and 34, Flemming's weaker fluid. Figs. 35 and 36, Perenyi's fluid. Figs. 37 and 38, corrosive sublimate, concentrated aqueous solution. Figs. 39, 40, and 41, corrosive sublimate, concentrated aqueous solution in 70 per cent. alcohol with the addition of 5 per cent. glacial acetic acid.

PLATE VII.

Fig. 41. Corrosive sublimate. See above.

Figs. 42-4. Alcohol 30 per cent.

Figs. 45-7. Absolute alcohol.

Figs. 48, 49. Picric acid; concentrated solution in 5 per cent. alcohol.

Figs. 50-2. Picric acid; concentrated solution in 70 per cent. alcohol.

Fig. 53. Brewery yeast, after five hours in Pasteur's solution, stained with Heidenhain's haematoxylin and methylene blue. The nucleolus has a deeply stained patch in surface view.

Fig. 54. D.C.L. compressed yeast after four hours in sugar solution (5 per cent.); nucleolus with deeply stained patch.

Fig. 55. Brewery yeast, after twenty-three hours in Pasteur's solution, fixed in Perenyi's fluid. The nucleolus is seen in surface view, with two deeply stained patches. Here, as in the two preceding figures, the nucleolus substance appears to be drawn out into the threads of the chromatin network; the nuclear vacuole in each case is pressed out behind the nucleolus and appears to surround it.

Fig. 56. 'Town Hall' compressed yeast, fresh, showing nucleolus with irregular deeply stained patches. The chromatin network is obscured by the densely stained cytoplasm.

Figs. 57 and 58. Cells which have maintained a feeble fermentation for four hours in an insufficient supply of wort. In each case, the nucleolus is faintly stained and the cytoplasm deeply stained; the deeply stained patch on the surface of the nucleolus is the only part of the cell strongly differentiated.

Fig. 59. Brewery yeast, fresh, showing a curious appearance of the nucleolus, caused by the deeply stainable substance on its surface. The nucleolar substance appears to be drawn out into the threads of the chromatin network.

Fig. 60. Brewery yeast, after twelve hours in a limited supply of wort; shows a faintly stained elongate nucleolus with a deeply stained patch at each end, strongly resembling a mitotic figure.

Figs. 61-4. Brewery yeast, fixed in 30 per cent. alcohol after feebly fermenting for four hours in an insufficient quantity of wort. The stain was reduced to differentiate the granules on the surface of the nucleolus.

Figs. 65-76. D.C.L. compressed yeast. The figures show appearances most strongly suggestive of nuclear structure and mitotic phenomena. They were selected from a single example of D.C.L. and are remarkable for the peculiar distribution of a large amount of deeply stainable substance on the surface of the nucleolus. The cytoplasm in most cases was densely stained, obscuring the structure of the vacuole.

Figs. 77-80. Brewer's yeast, after two hours in an insufficient quantity of wort.

Fig. 77. Cell showing an elongate nucleolus with a deeply stained patch at each end.

Fig. 78. Cell containing a nucleolus with two deeply stained patches, farther apart than in the preceding figure. Fig. 80, cell containing two nucleoli each possessing a deeply stained patch.

PLATE VIII.

Fig. 81. Cell showing nucleolus dividing.

Figs. 82-93. Nucleoli which have been pressed out of the cells. Fig. 82, the dividing nucleolus actually pressed out of the cell figured in Fig. 81. Fig. 83 shows the deeply stainable substance

partially separated from the nucleolus, and portions of the chromatin network are shown still attached to the nucleolus in Figs. 90 and 91.

Fig. 94. Brewery yeast, after four hours in Pasteur's solution, foam, fixed in Perenyi's fluid. The granular structure of the chromatin shows clearly; the nucleolus presents a curious appearance, resembling the telophase of mitotic division. The volutin granules inside the vacuole are stained (haematoxylin).

Fig. 95. Brewery yeast, after five hours in 5 per cent. sugar solution, stained with brazilin. The nucleolar substance appears to be drawn out into the threads of the chromatin network.

Figs. 96-8. Brewery yeast, forty-eight hours in Pasteur's solution, foam, fixed in a mixture of equal parts of Flemming's and Perenyi's solutions. The structure of the nucleus shows clearly; the contents of the nuclear vacuole are more deeply stained than the cytoplasm.

Fig. 99. D.C.L. compressed yeast after four hours in 5 per cent. sugar solution. The chromatin network shows clearly attached to an elongate nucleolus. The latter with a group of deeply stained granules at each end strongly resembles a mitotic figure.

Fig. 100. Transverse section of the cell of *Closterium*, showing the nucleolus with chromatin patches on the surface.

Fig. 101. Budding cell showing the structure of the chromatin network and its morphological relation to the nucleolus.

Fig. 102. Yeast cell, after two hours in 5 per cent. sugar solution, mounted in glycerine; showing the structure of the nucleus.

Fig. 103. Structure of nucleus; the nucleolus is seen through the chromatin network.

Fig. 104. Yeast cell, after fifteen hours in Pasteur's solution, stained with safranin and gentian violet; shows the structure of the nucleus. The granules are small and evenly distributed on the threads of a complicated network.

Fig. 105. Cell with nucleus. The chromatin network is simple, consisting of few threads bearing few granules; the origin of these threads from the nucleolus is clearly shown.

Figs. 106, 107, and 108. Budding cells, showing typical stages in the division of the nucleus.

Fig. 109. Yeast cell, after five hours in 5 per cent. sugar solution, stained with brazilin. The chromatin network is compressed, probably by the glycogen present.

Fig. 110. After thirty-seven hours in Pasteur's solution, bottom, showing a cell almost full of glycogen.

Figs. 111 and 112. Old cells; Fig. 111 is from a five hours' culture in 5 per cent. sugar solution, stained in brazilin.

Figs. 113, 114, and 115 represent the initial stages of spore formation in 'Town Hall' compressed yeast.

Fig. 113. The contracted chromatin network.

Fig. 114. A slightly later stage; the nucleolus is seen through the contracted network and appears as if surrounded by granules.

Fig. 115. A still later stage, showing granules in close proximity to the nucleolus. One strand of the network remains.

Fig. 116. Drawn from a preparation of brewery yeast, fixed in Perenyi's fluid after forty-eight hours in Pasteur's solution (foam), to show the volutin granule inside the vacuole.

#### PLATE IX.

Figs. 117-30. Cells in which the phosphorus reaction is shown; the colour is slightly deeper than is visible under the microscope.

Figs. 117-22. Cells from bottom after two hours' fermentation.

Figs. 123-7. Cells from bottom after ten hours' fermentation.

Figs. 128, 129, and 130. Cells after five hours in 5 per cent. sugar solution.

Figs. 131-50. Glycogen.

Figs. 131-9. 'Town Hall' compressed yeast stained with iodine; Figs. 131-5, after five hours in 15 per cent. sugar solution, showing early stages in appearance of glycogen.

Figs. 136-9. After eight hours in 15 per cent. sugar solution, showing later stages in the formation of glycogen.

Figs. 140-3. Brewery yeast from bottom, after 409½ hours' fermentation, showing cells containing a large quantity of glycogen. Stained in methyl green, followed by iodine and examined in water.



Fig. 144. Brewery yeast, from foam after 156 hours' fermentation, showing glycogen inside nuclear vacuole. Methyl green followed by iodine and examined in water.

Fig. 145. Brewery yeast, cell containing a maximum of glycogen, from bottom after 191 hours' fermentation.

Figs. 146 and 147. Stages in the disappearance of glycogen. Yeast in the condition figured in Fig. 145 was placed in fresh Pasteur's solution; after twenty-nine hours most of the cells presented the appearance figured (Figs. 146, 147).

Fig. 148. Yeast fresh from brewery.

Figs. 149 and 150. Brewery yeast after 10-15 hours' fermentation; first appearance of glycogen.

Fig. 151. The same yeast after twenty-nine hours' fermentation.

Figs. 152-6. Cells showing the reaction for masked iron; fresh D.C.L. compressed yeast; fixed in 30 per cent. alcohol: stained by Macallum's haematoxylin method.

Figs. 157-2. Some of the colour effects obtained by temporary staining with methyl green and fuchsin.

Figs. 157-9. Brewery yeast from foam after 156 hours' fermentation.

Figs. 160-2. Brewery yeast from bottom after three hours' fermentation. In this case the cells were previously stained in Heidenhain's haematoxylin and differentiated in Perenyi's fluid.

#### PLATE X.

Figs. 163-82. Volutin in brewery yeast. The volutin granules are stained intensely in methylene blue and are represented in the figures as black granules.

Figs. 163, 164, and 165. Early stages in the appearance of volutin. In Fig. 165 the granules are outside the vacuole, in close contact with it.

Fig. 166. Granule in nuclear vacuole, five hours in Pasteur's solution. This is the only volutin granule in the cell.

Figs. 167 and 168. Volutin granules after five hours in Pasteur's solution.

Fig. 169. After thirty-seven hours in Pasteur's solution, fixed in Perenyi's fluid and stained with Heidenhain's haematoxylin, shows a volutin granule in the nuclear vacuole.

Fig. 170. Cell from the same specimen of yeast fixed in iodine. Contains a large volutin granule in the vacuole.

Fig. 171. The same fixed in iodine solution and showing an enormous volutin granule resting on the nucleolus.

Fig. 172. Cell showing a number of volutin granules on the nucleolus.

Figs. 173 and 174. After forty-four hours in a 5 per cent. sugar solution.

Figs. 175 and 176. After ten and a half hours in Pasteur's solution.

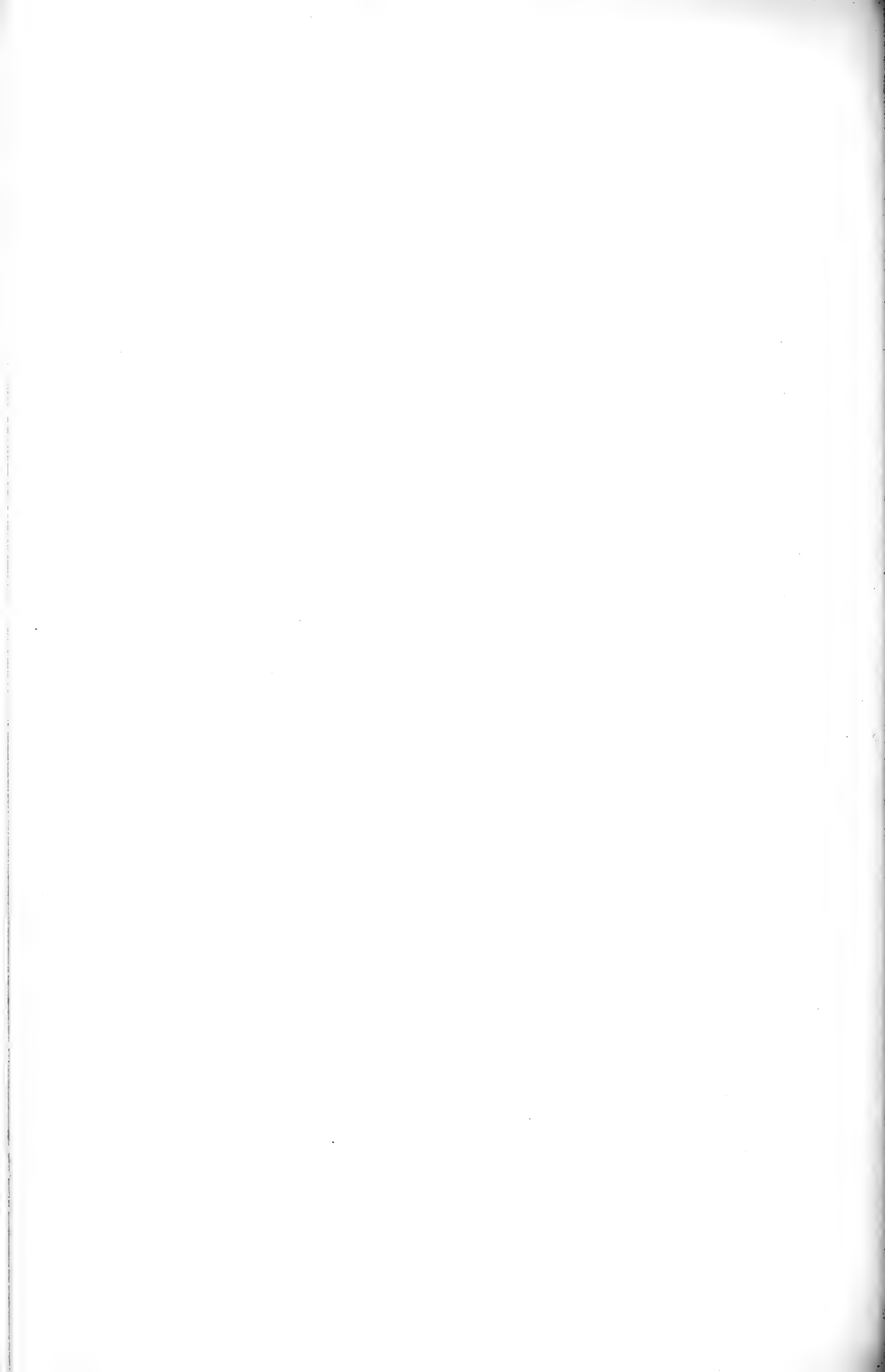
Figs. 177 and 178. After thirty-one hours in Pasteur's solution.

Figs. 179 and 180. After forty-four hours in Pasteur's solution; a maximum amount of volutin is present. In Fig. 180 there is a dense, granular mass of volutin around the nucleolus.

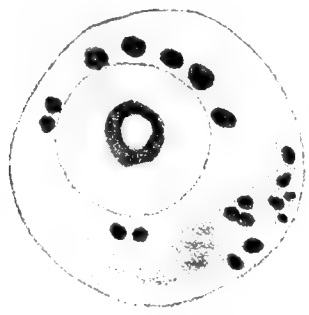
Fig. 181. After ninety-five and a half hours in Pasteur's solution, foam. A large number of volutin granules around the vacuole.

Fig. 182. After ninety-five and a half hours in Pasteur's solution, bottom; the greater part of the cell is occupied by glycogen.

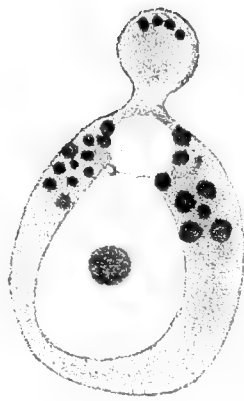




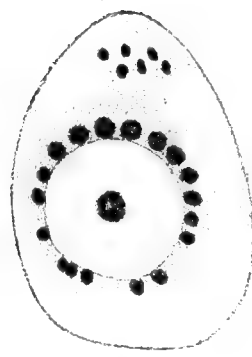




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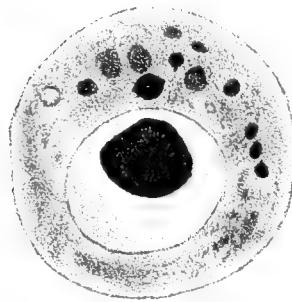
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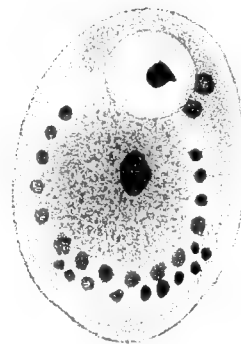
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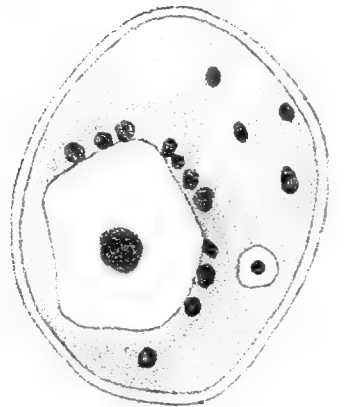
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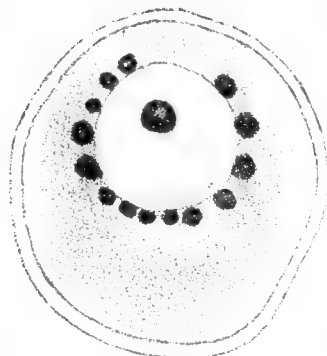
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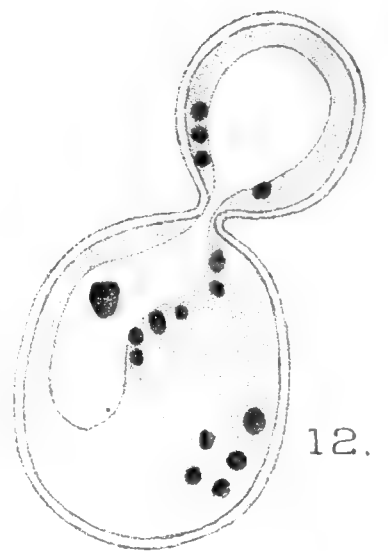
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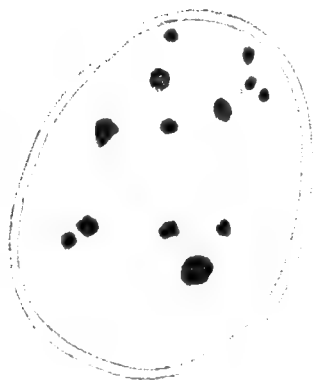
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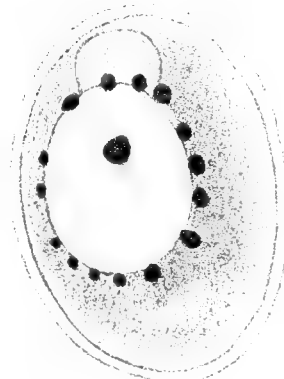
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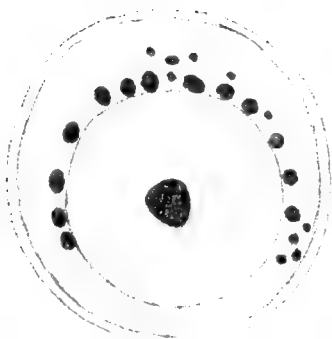
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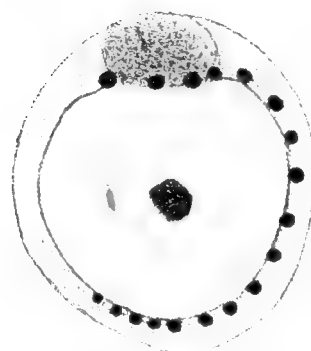
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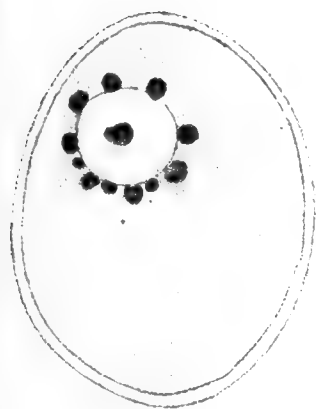


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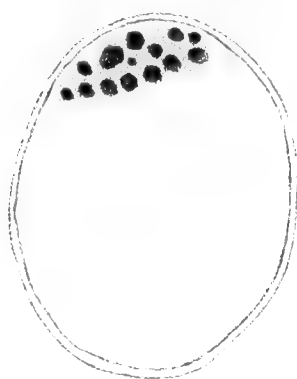


20.

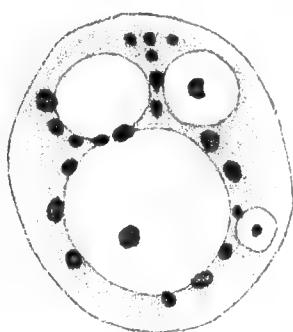
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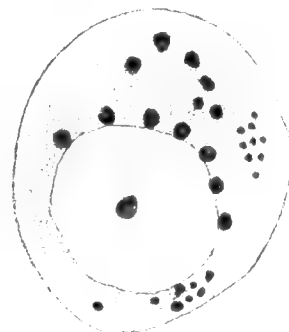
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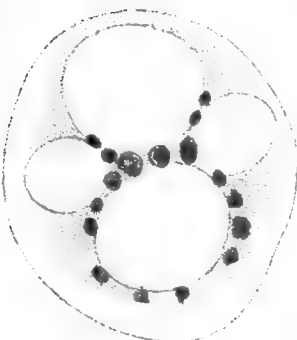
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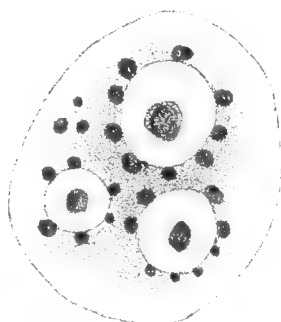
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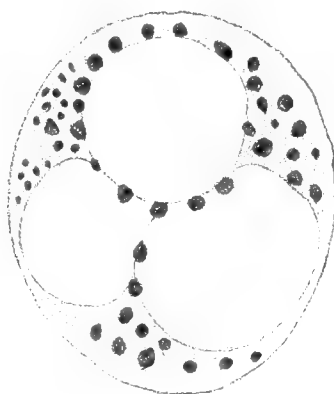
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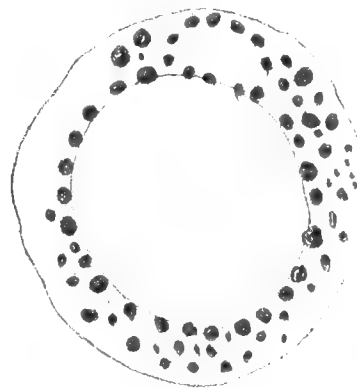
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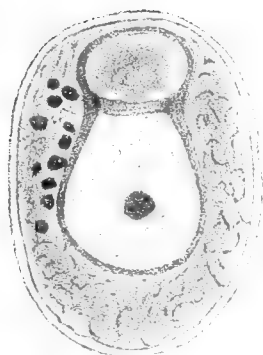
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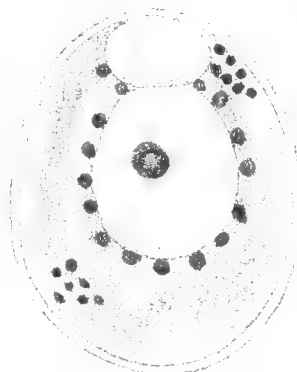
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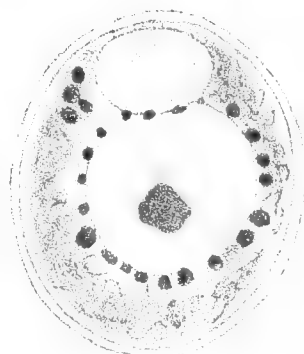
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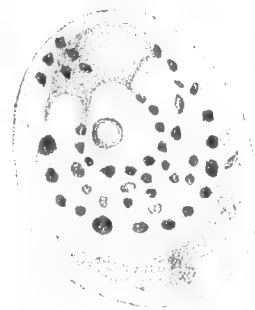
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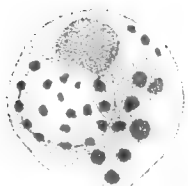
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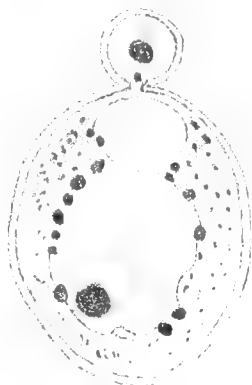
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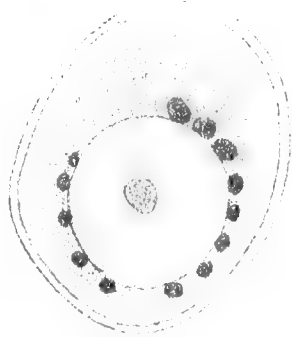
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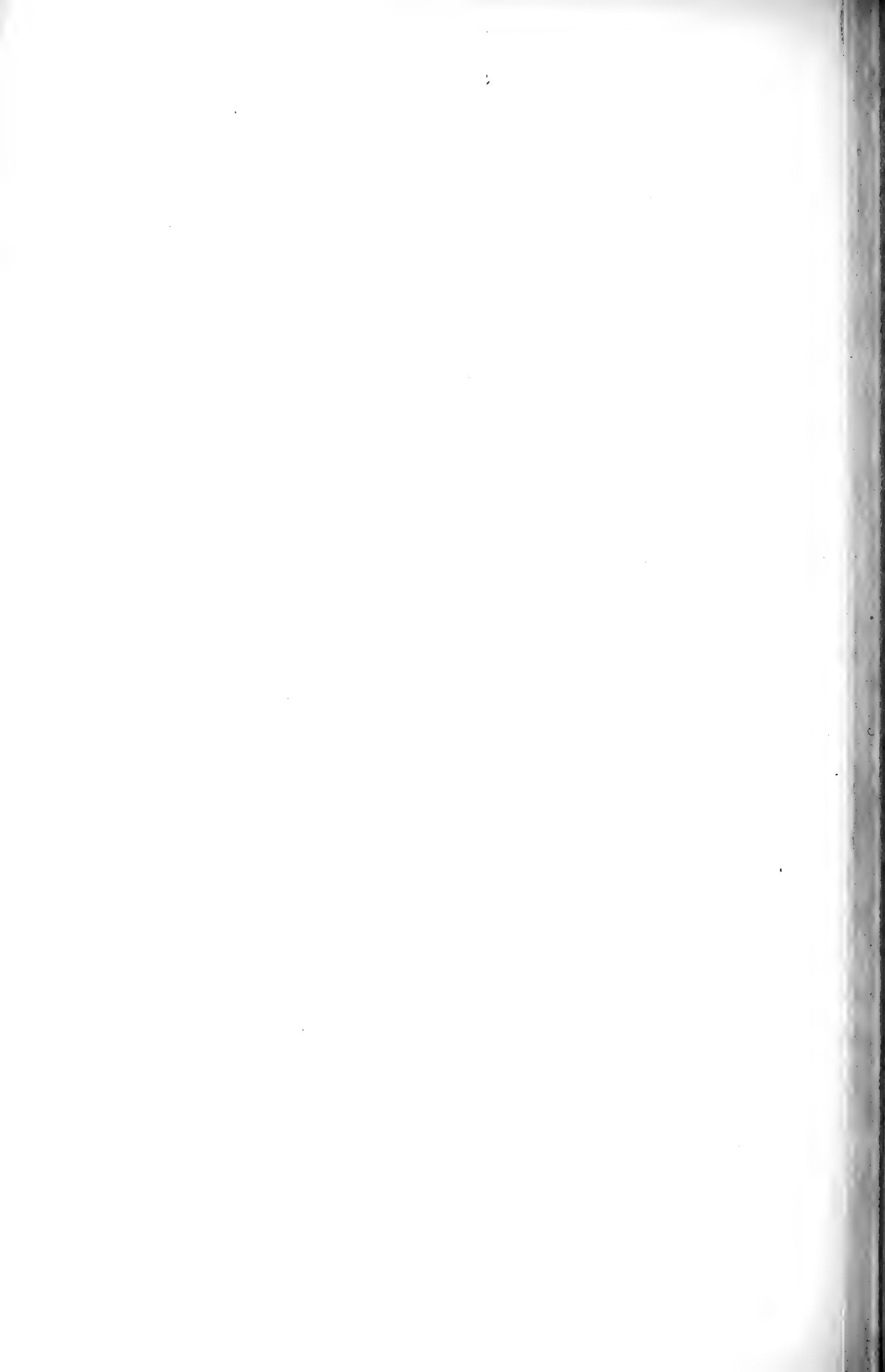
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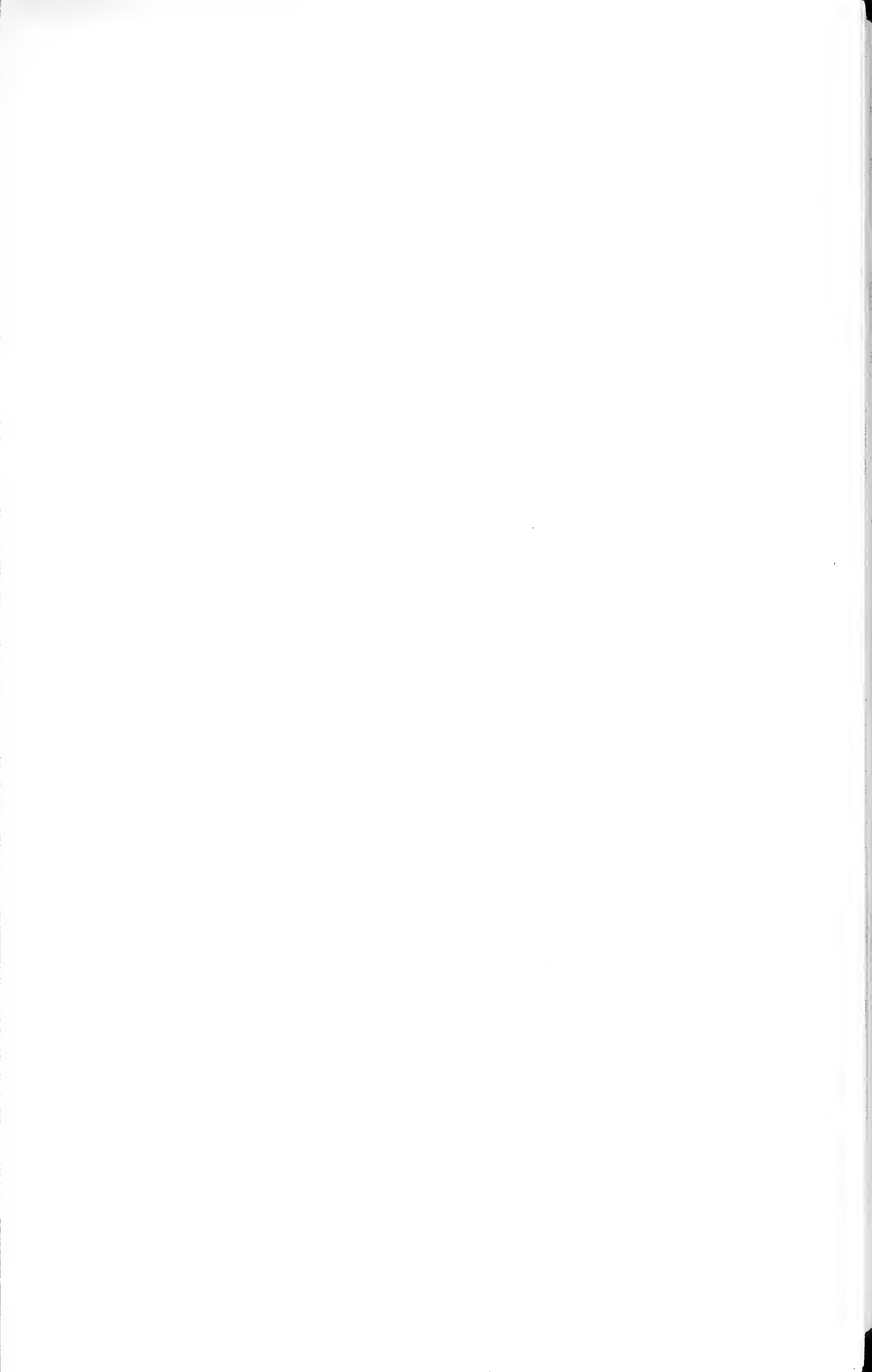


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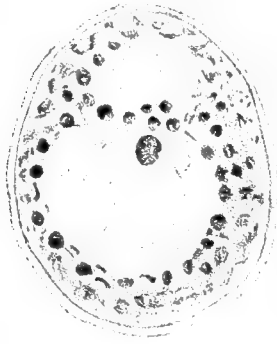


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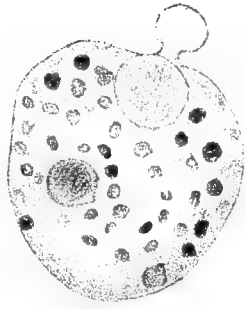








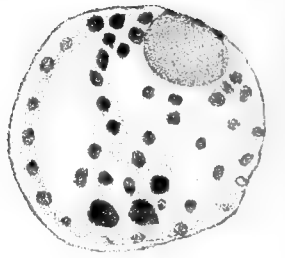
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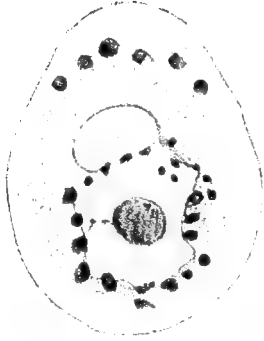
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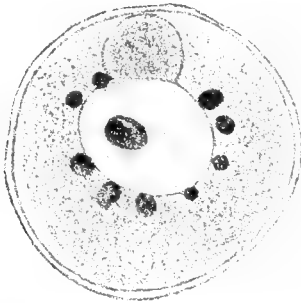
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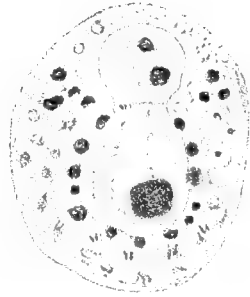
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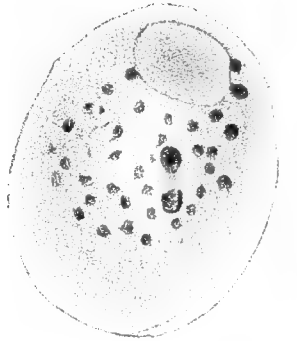
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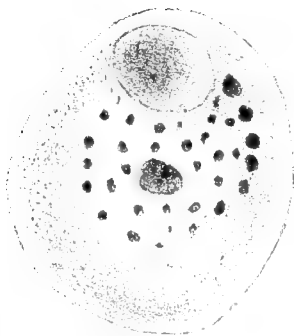
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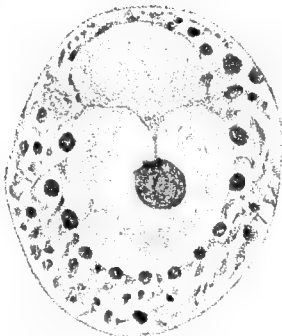
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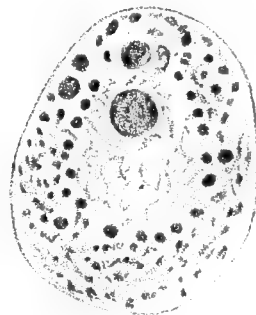
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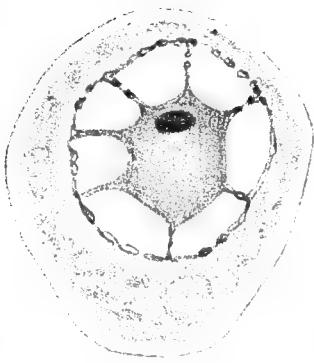
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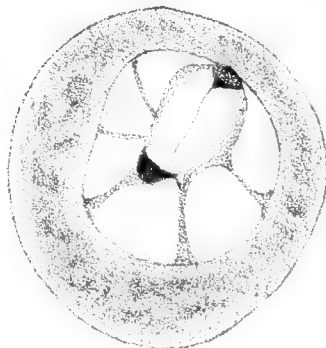
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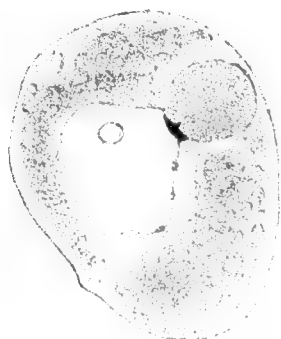
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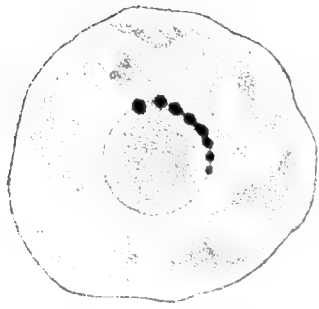
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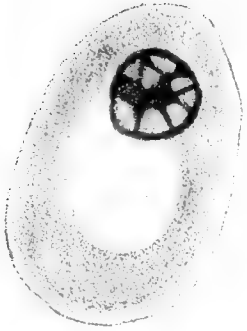
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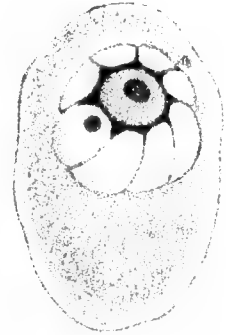
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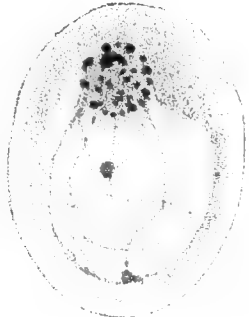
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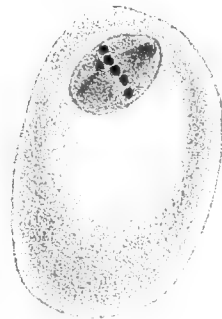
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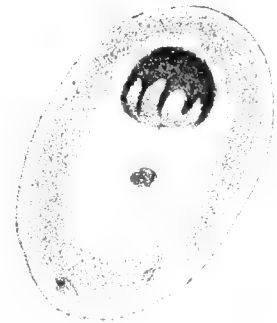
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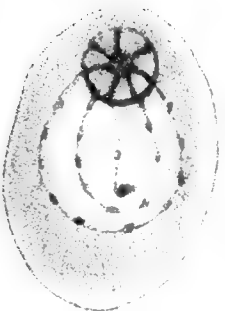
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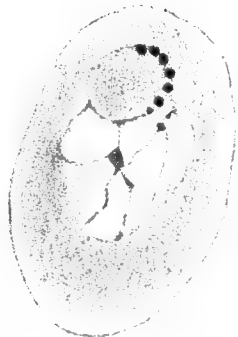
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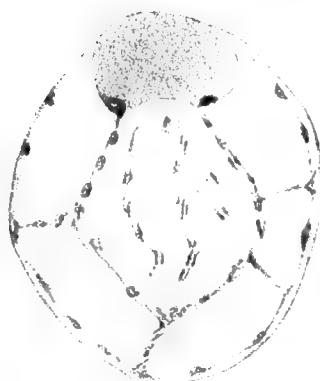
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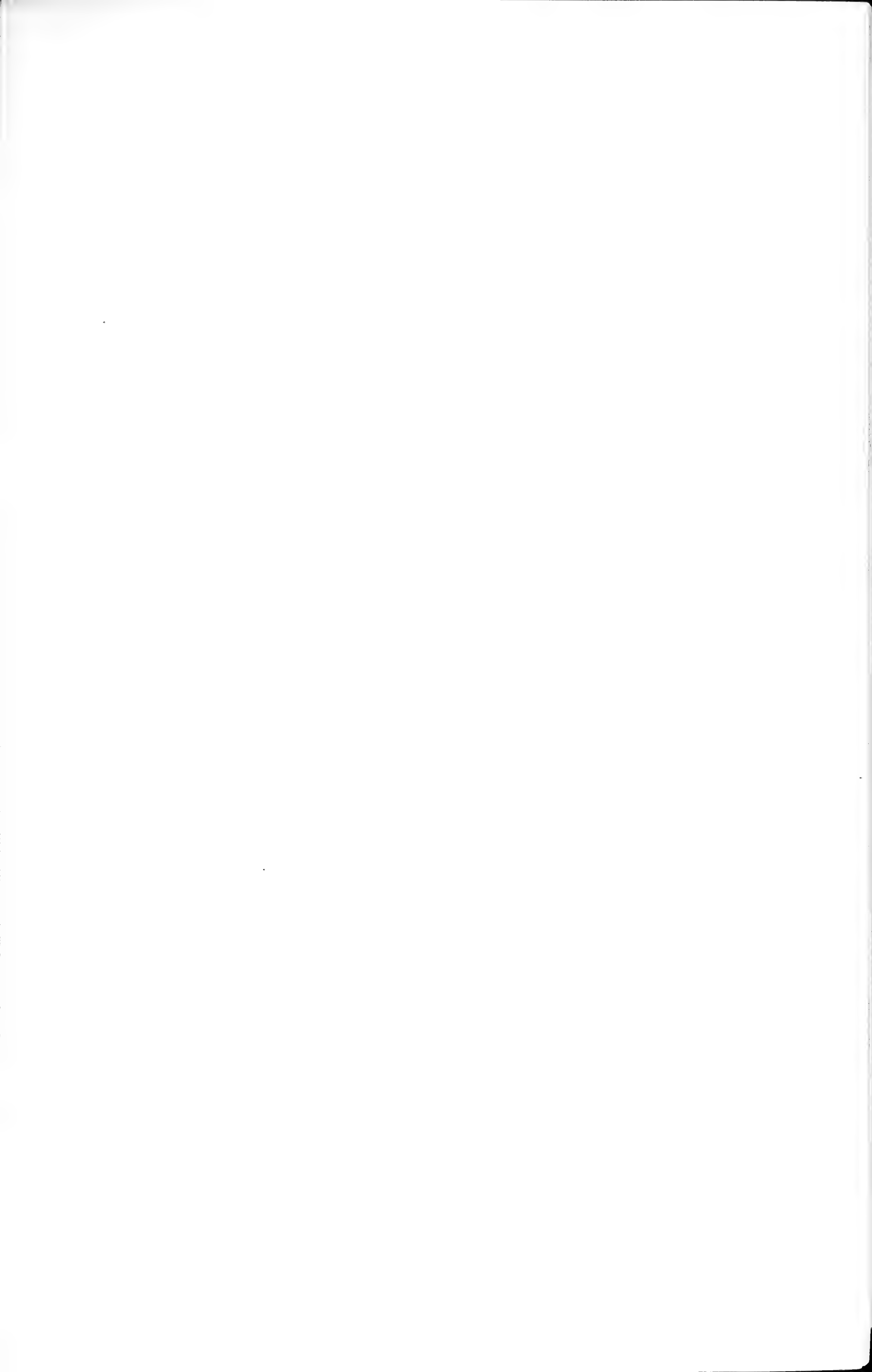


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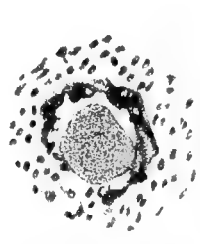
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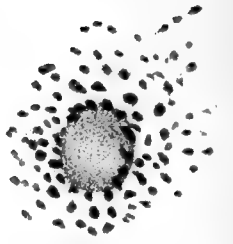
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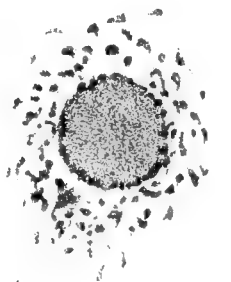
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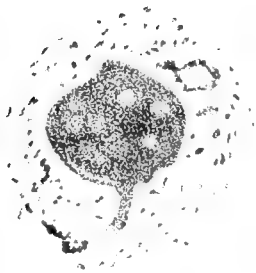
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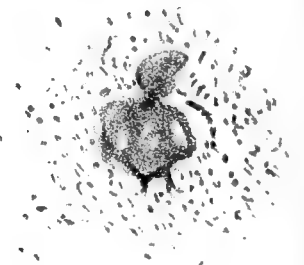
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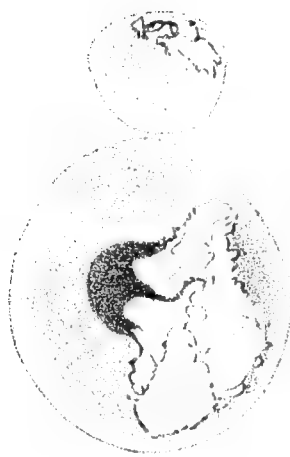
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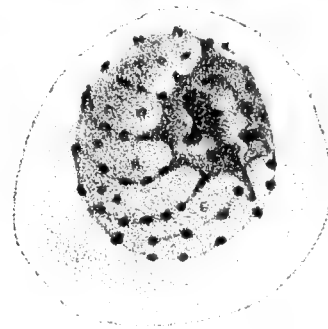
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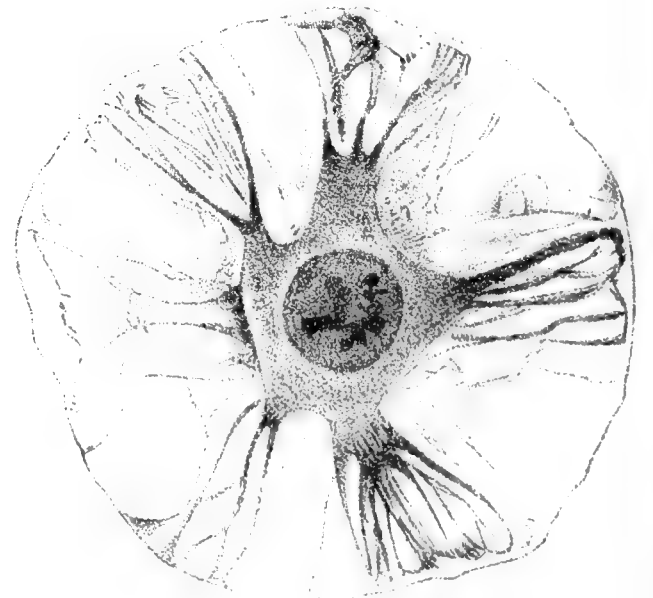
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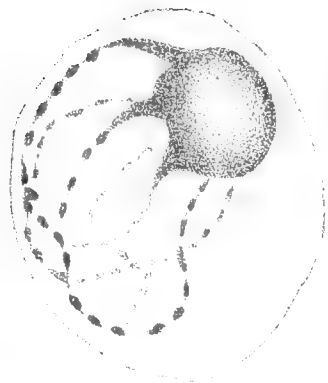
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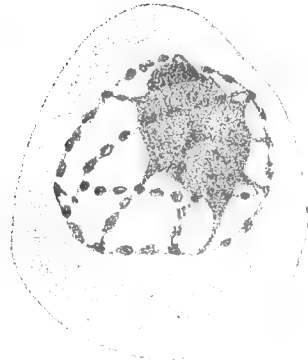
WAGER AND PENISTON — YEAST PLANT.



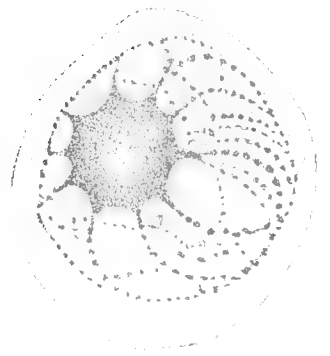
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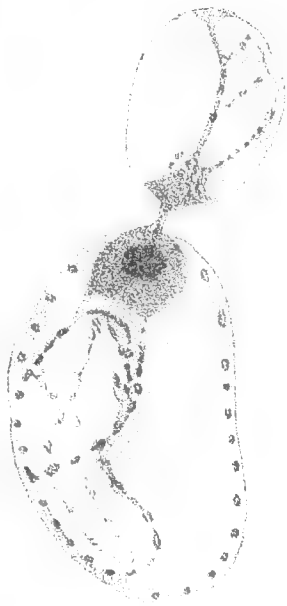
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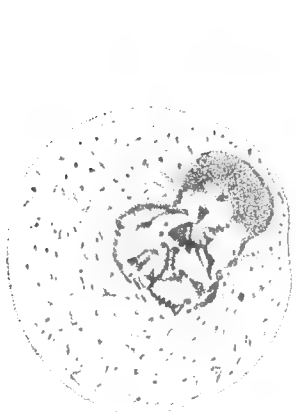
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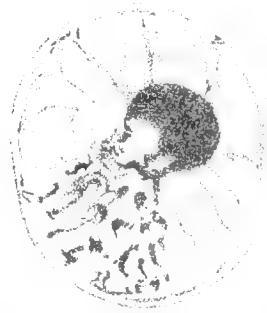
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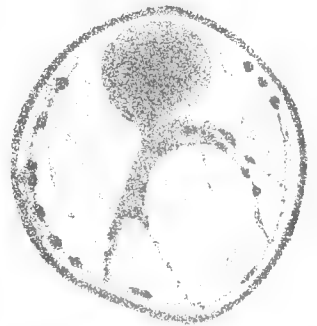
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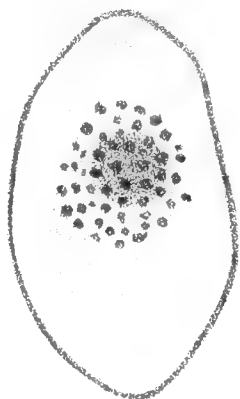
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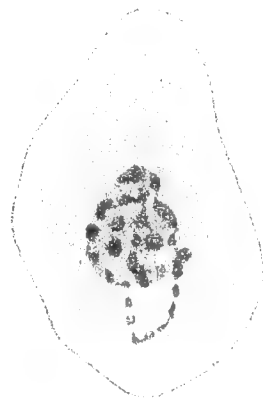
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113.



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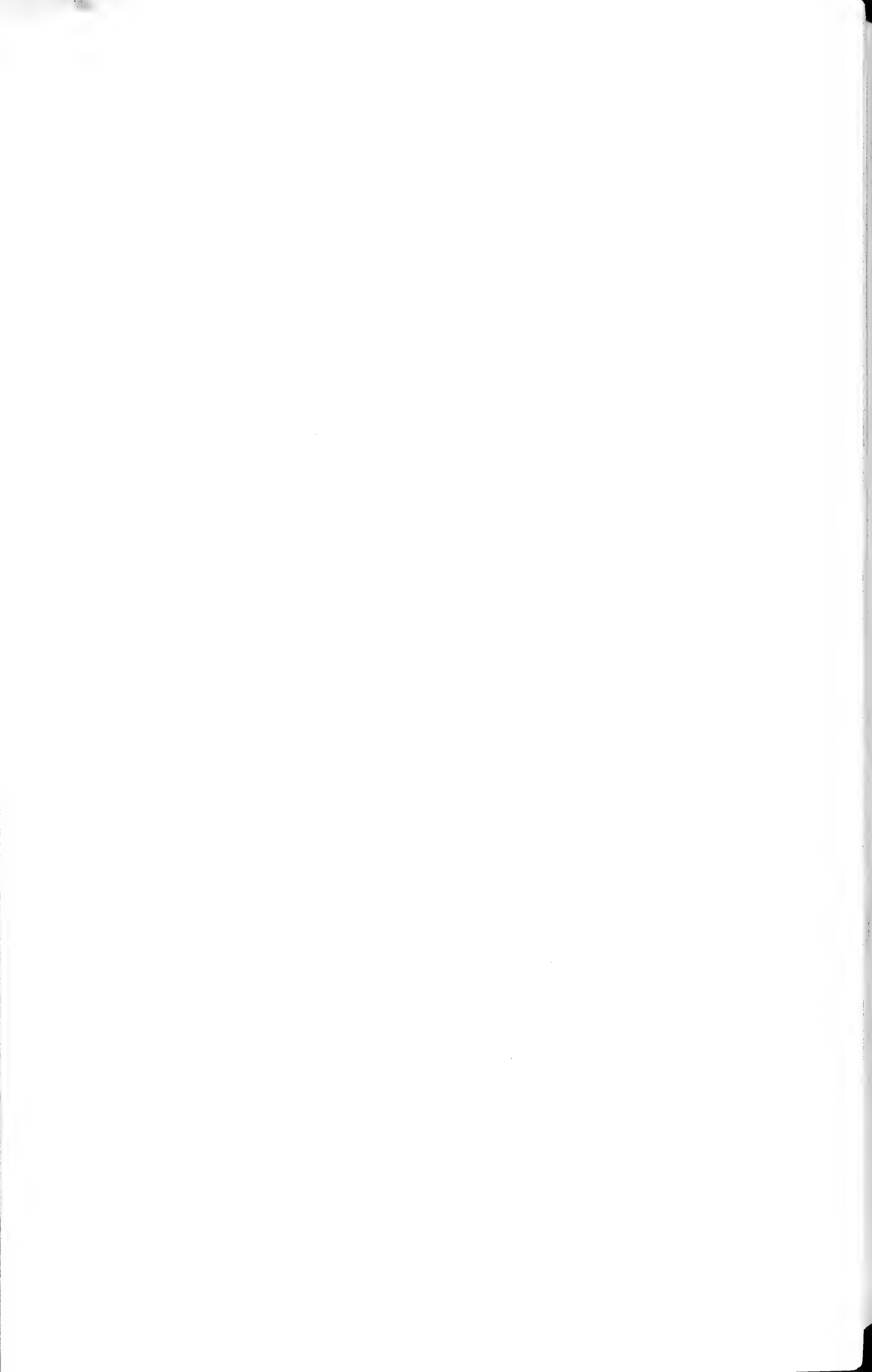
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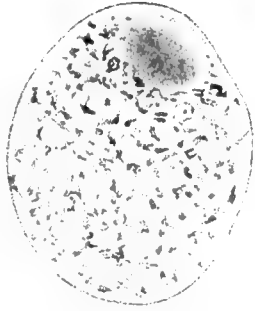




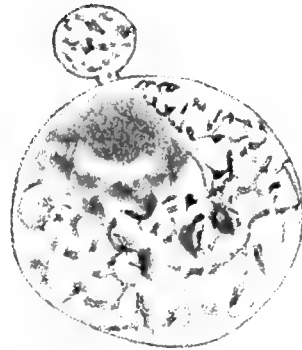




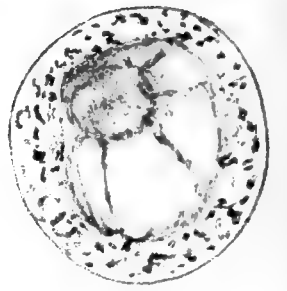
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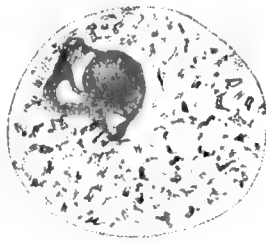
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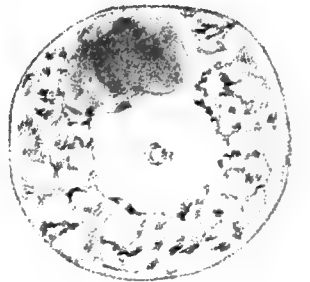
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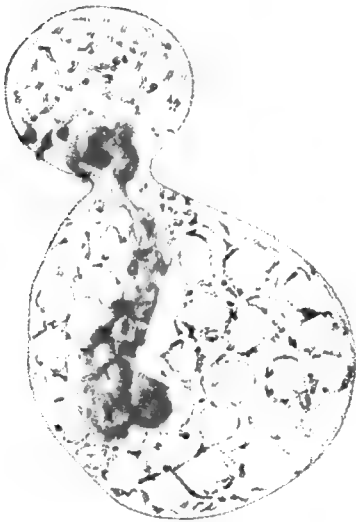
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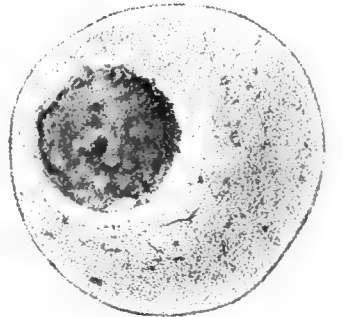
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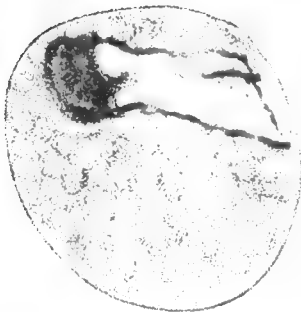
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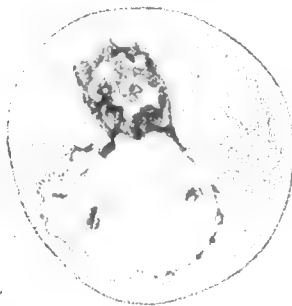
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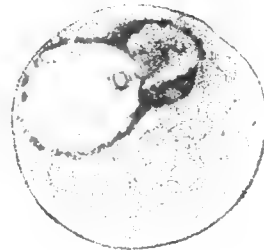
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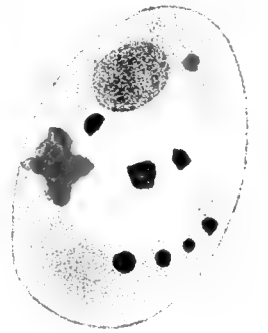
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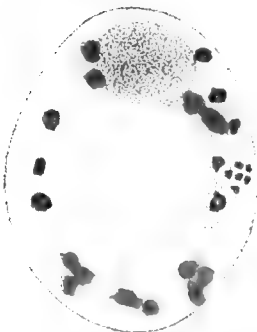
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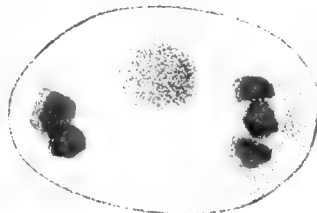
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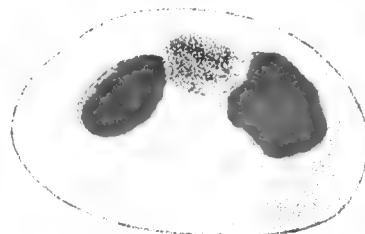
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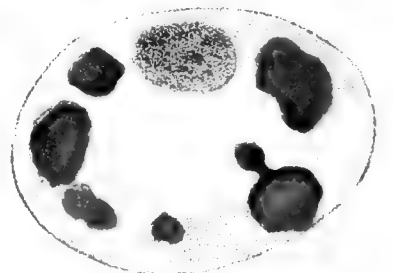
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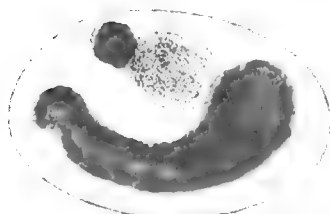
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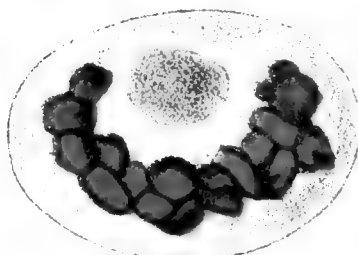
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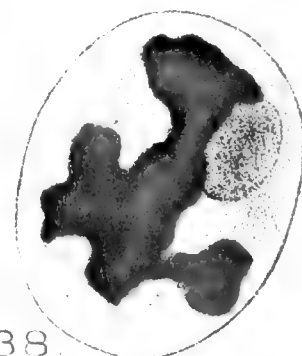
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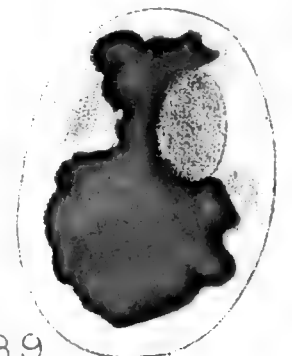
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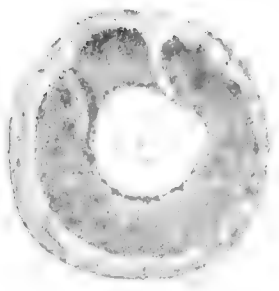


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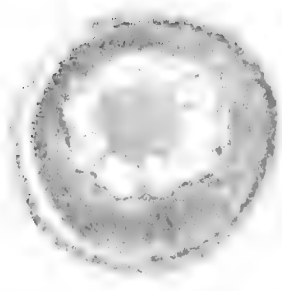


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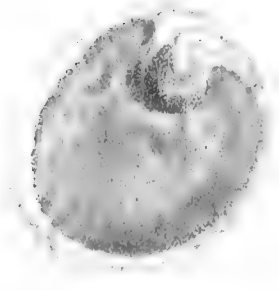
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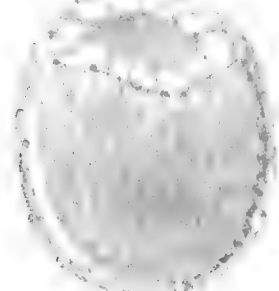
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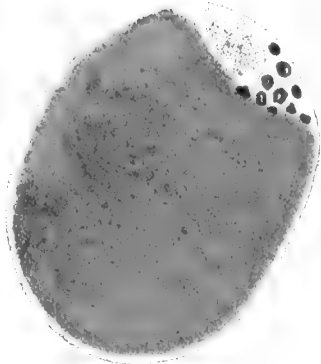
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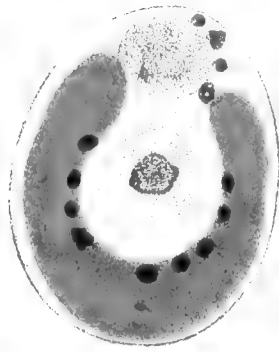
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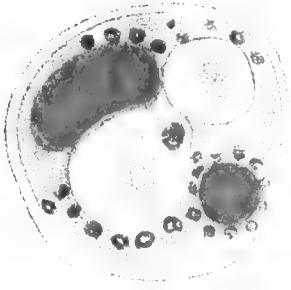
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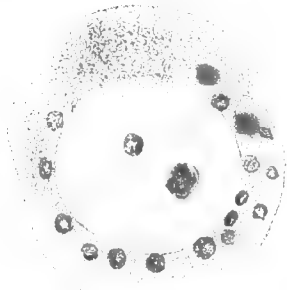
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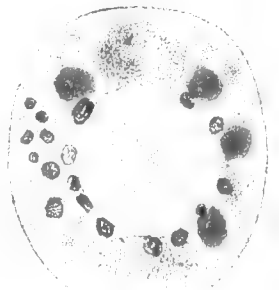
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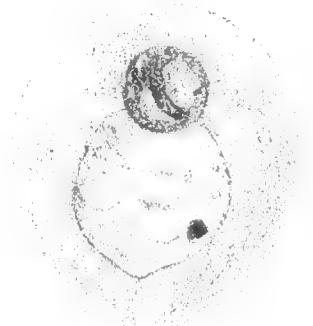
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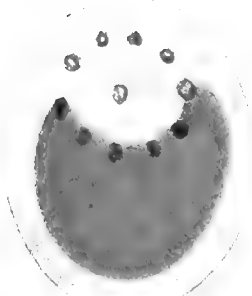
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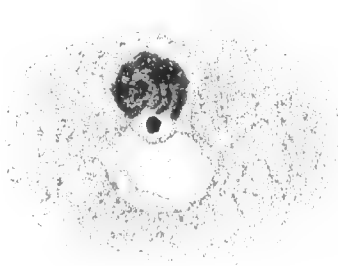
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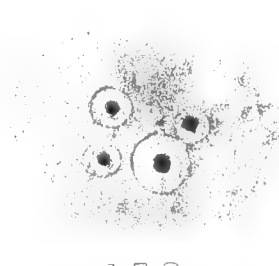
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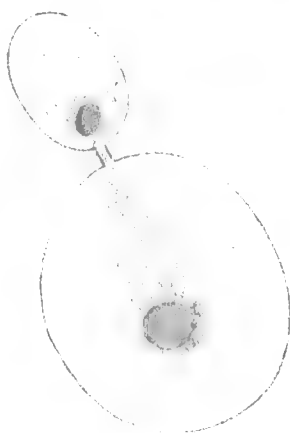
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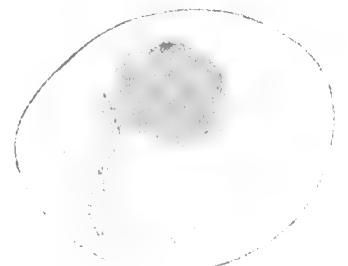
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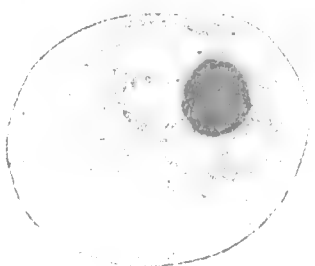
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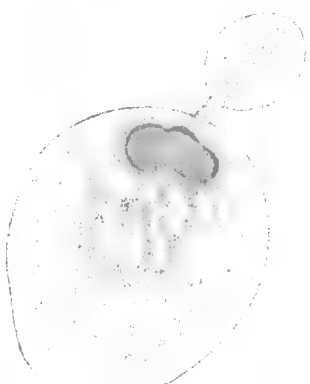
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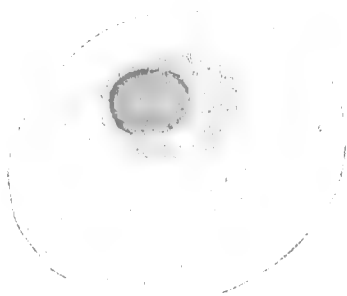
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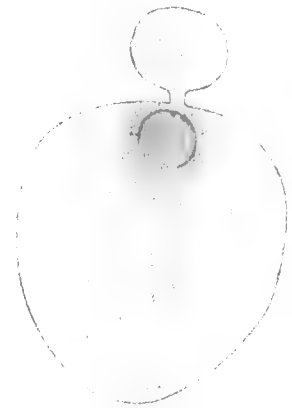
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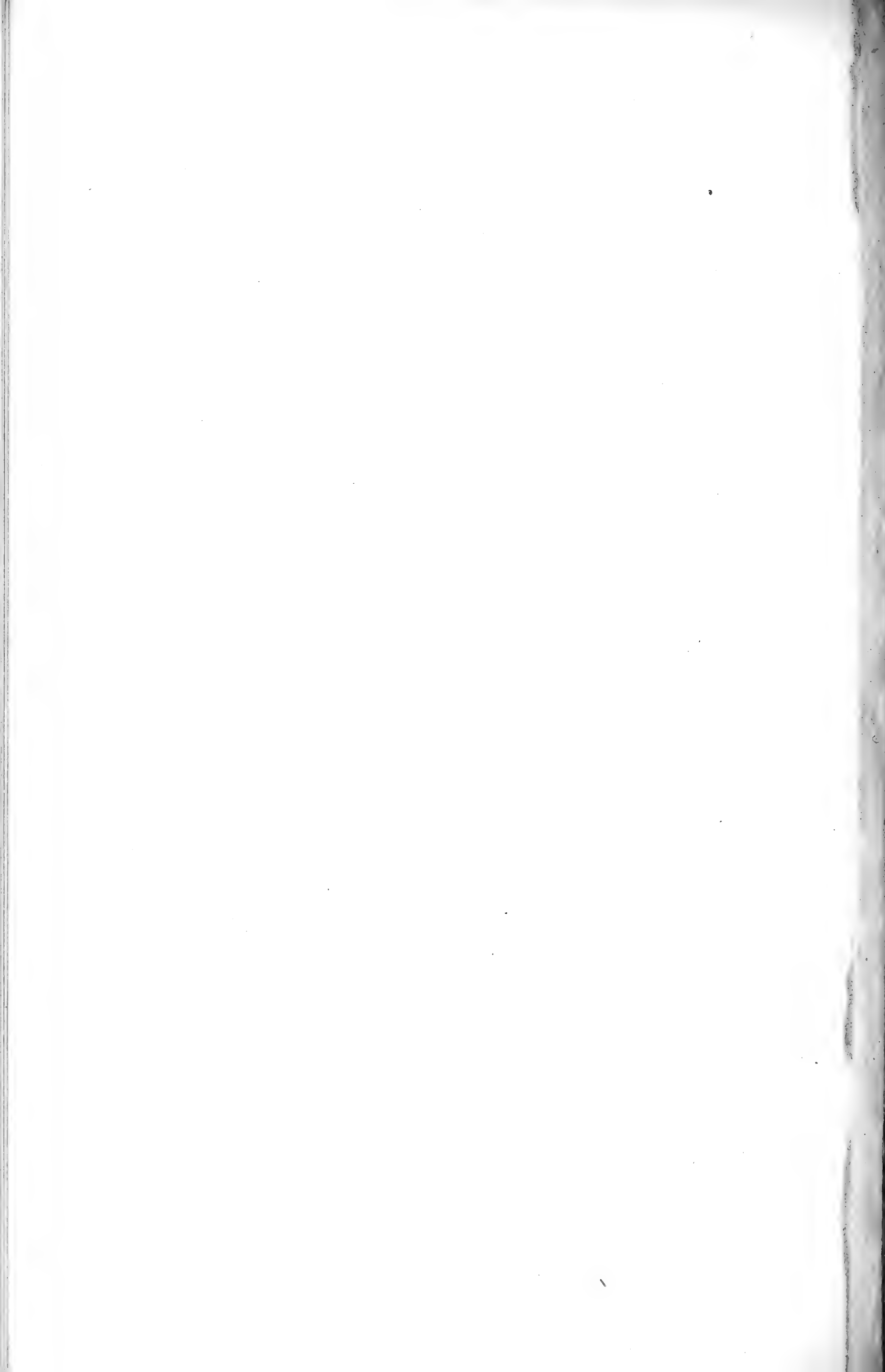
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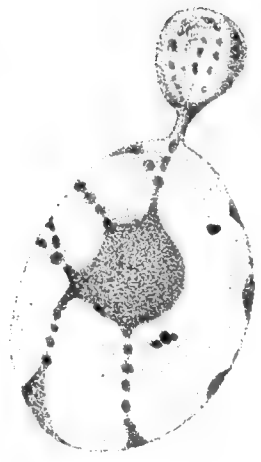


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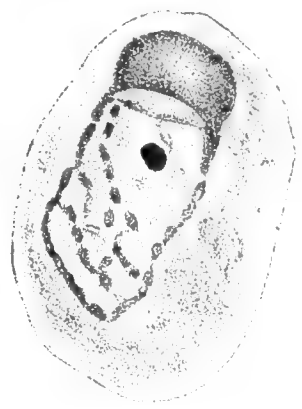
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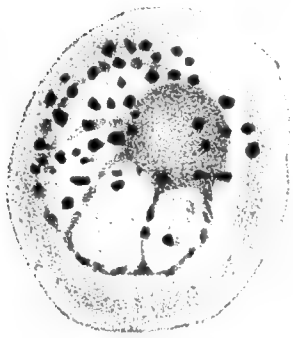
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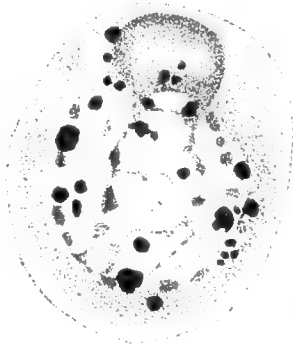
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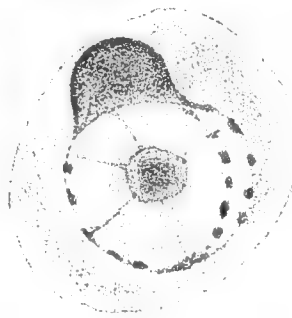
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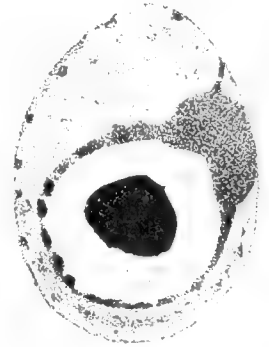
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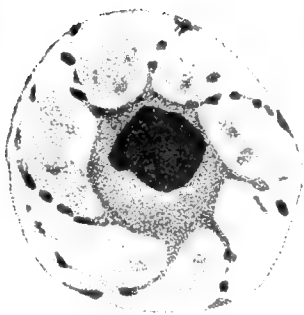
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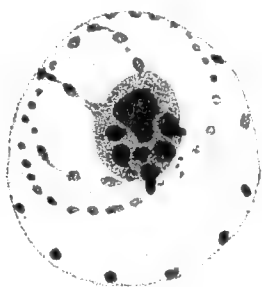
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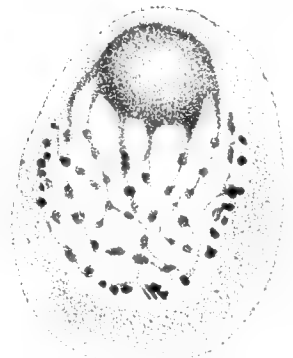
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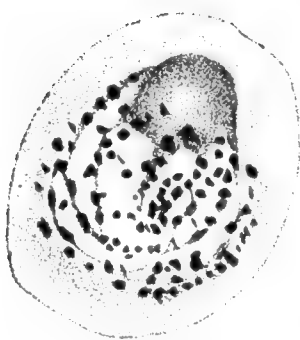
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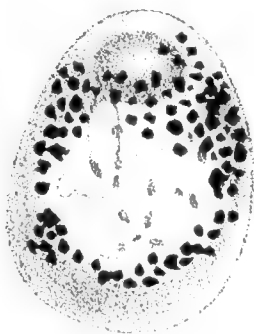
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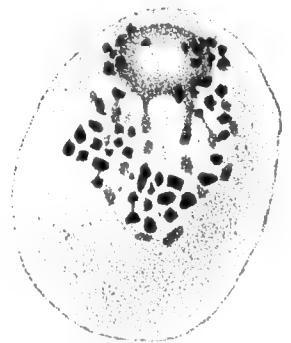
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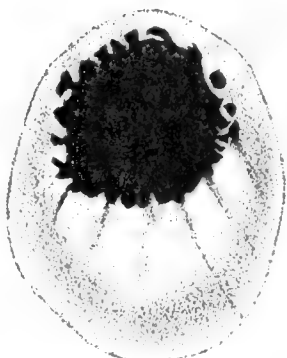
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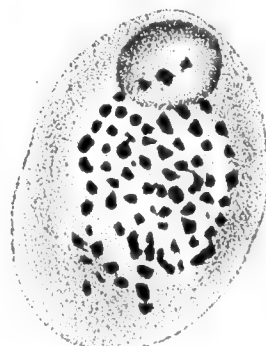
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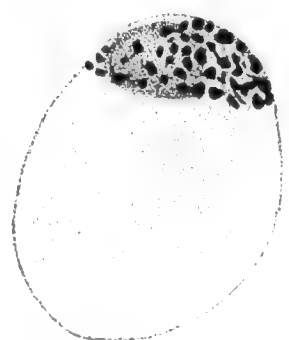
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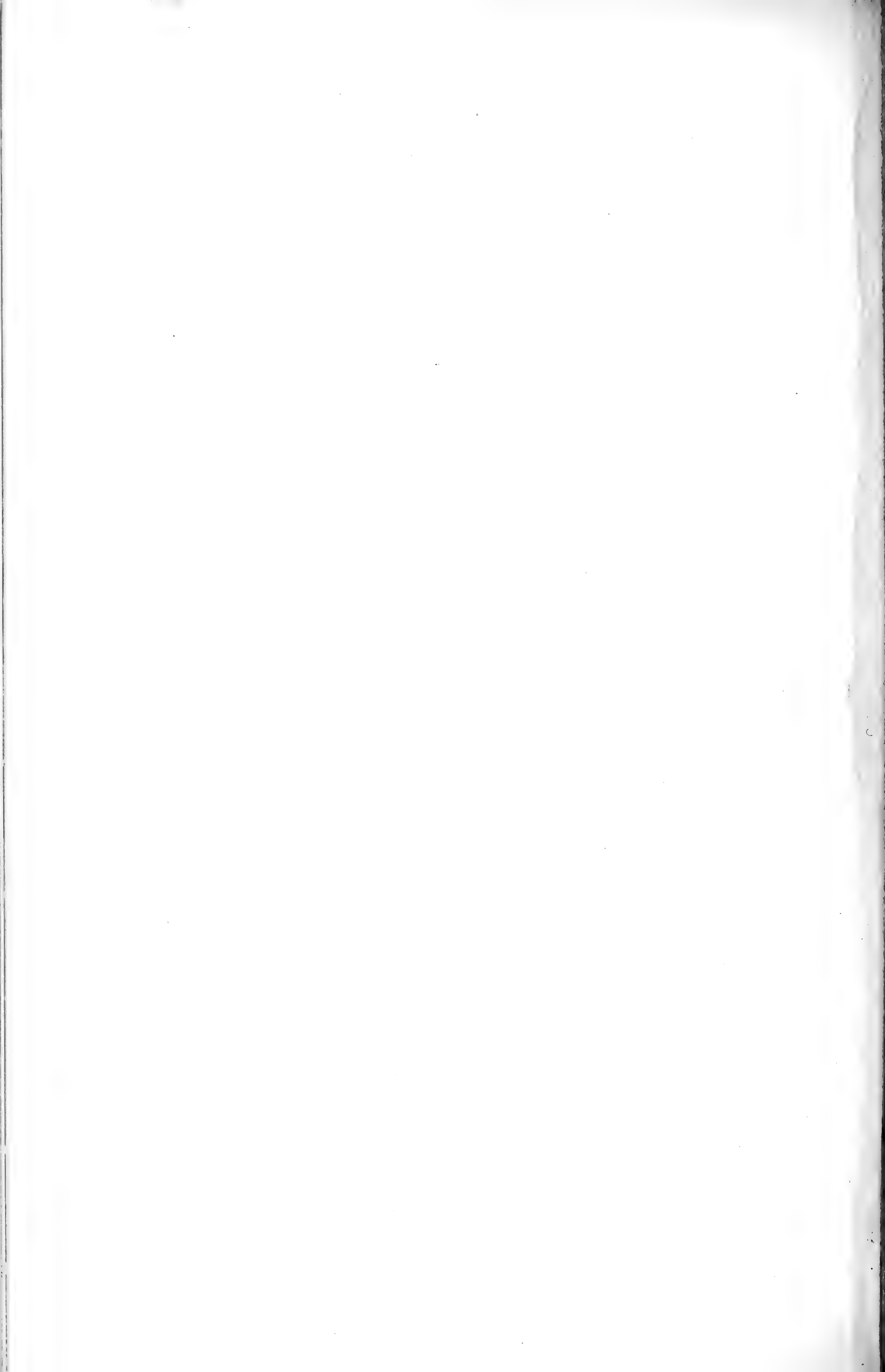


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# Transpiration and the Ascent of Water in Trees under Australian Conditions.

BY

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**With five Figures in the Text.**

IN two previous papers<sup>1</sup> on the ascent of water in trees, investigations were made in regard to the physics of the ascent of sap, and from the data thus obtained experiments were performed to test whether any of the existing theories afforded a complete explanation of all the phenomena associated with the ascent of sap in tall trees. The net result of the previous work has been to show that the problem is more a kinetic than a static one, the actual resistance to flow during active transpiration being much greater than might be imagined from purely theoretical considerations. In addition it was shown that no evidence could be obtained of any pressure or suction at a given point sufficient to explain the total ascent in a tall tree during active transpiration, and that in the wood of a tree killed by formalin the sap appeared to turn to the older, still living wood, which normally, owing to its higher resistance and lesser activity, takes little or no part in the ascent of sap. The mere fact that dead wood soon loses its conductivity and cannot retain it permanently under transpiration conditions, even after injection with water, is sufficient to show that in this respect at least the ascent of sap is a vital problem, that is to say, one which cannot at present be wholly explained by reference to known physical and chemical agencies. All the data on which these conclusions were based were, however, obtained in England with English or English-grown trees, and naturally the opportunity of checking all the conditions in Australia, where the tallest trees grow, and where the

<sup>1</sup> Phil. Trans. R.S. of London, Series B, vol. cxcviii, 1905, p. 41; Ibid., vol. cxcix, 1908, p. 341.

conditions are in some respects almost unique, and with abundant material close at hand, was of great value. The work was carried out with the aid of a grant by the Royal Society from the Home Government's research fund.

The points of special importance to determine were: the rate of transpiration under Australian conditions, the rate of ascent of sap, more especially in *Eucalyptus* trees, the length and diameter of the vessels, the condition of the conducting tissue during active transpiration, and the maximal and average resistances to flow in functioning stems. The total resistance to flow naturally will depend on the height of the tree, and the tallest trees known only slightly exceed 300 feet in height. In the previous paper the greatest height given for *Eucalyptus amygdalina* was 303 feet. I have since found an authentic properly measured record of 330 feet by Mr. Clement Hodgkinson, a former head of the Lands Department, taken nearly half a century ago, and although the tree does not now appear to be in existence, there is no reason to doubt the record.<sup>1</sup> As regards this genus, *Eucalyptus diversicolor* of W. Australia appears to come second, and the *Eucalyptus microcorys* of N.S. Wales third in height, but many species are often found to attain heights of 100 to 200 feet in the heavily timbered districts of Victoria, although the trunks are usually slender as compared with the height. A tree, however, commonly continues to grow in diameter after it has attained its maximal height, and hence in aged specimens the disparity between the diameter and height is reduced. The next tallest tree recorded is presumably the 'Keystone State' *Sequoia* of Calaveras with 325 feet in height and 45 feet in diameter. The Sequoias appear in general to have bulkier stems for a given height than *Eucalyptus*, and this, coupled with the slower rate of transpiration, makes up for the comparatively high resistance to flow in their non-vascular wood. In fact, as has already been shown,<sup>2</sup> the development of wood on a tree is primarily due to the necessity of maintaining a sufficient sectional area of wood containing living cells, and hence capable of active conduction, the purely mechanical functions of the stem being secondary in character; the main stem of a tree which has attained its maximal height will continue to increase in girth and strength even although the upper half may be decreasing in weight.

#### TRANSPIRATION UNDER AUSTRALIAN CONDITIONS.

In at least one respect the Australian conditions as represented in the Melbourne district are comparatively unique, for at certain times hot dry winds blow from the interior and cause rapid rises of temperature up to 100°

<sup>1</sup> Dr. Howitt, in the Proc. Roy. Soc. of Vict., New Series, vol. iii, 1891, p. 128, gives a measurement of 350 feet on a fallen Eucalypt, but without details and without mentioning whether the measurement included any portion of the root system.

<sup>2</sup> Phil. Trans. R.S., Series B, vol. cxviii, 1905, p. 69.

or even 120° F. The curves appended<sup>1</sup> (Fig. 1) give some idea of the character of these changes, which usually end suddenly when the cool south wind sets in, the temperature often falling a degree a minute for a quarter to half an hour or at a less rate over a somewhat longer period. The rate of evaporation from a free surface of water is exceedingly rapid when the hot dry north wind is blowing from the interior, and since these hot spells often come when the ground is still quite moist, we should expect to find the rate of transpiration extremely high at such times. The hot spell rarely lasts long and is usually followed by a cool change often accompanied by rain.

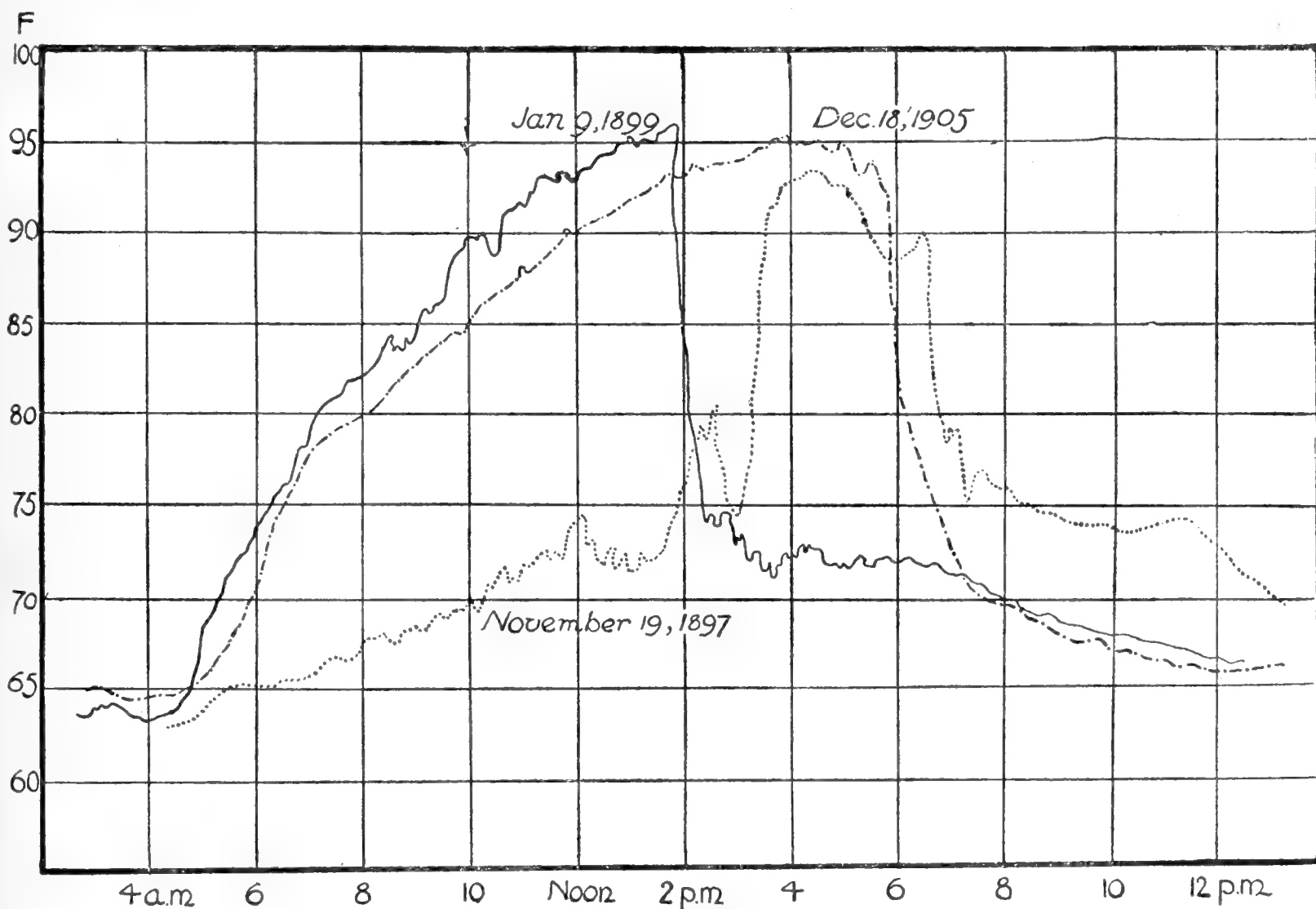


Fig. 1. Thermographs from Melbourne Observatory

The fall of temperature is usually more rapid than the rise, but in the curve for November 19, 1897, the reverse is the case. In neither case does the suddenness of the change appear to operate injuriously upon the vegetation, while its rapidity and irregular occurrence would be sufficient to prevent slowly responding plants specially adapting themselves to it.

Nevertheless a prolonged hot spell results in a regulatory diminution of the rate of transpiration even when the soil is kept moist. Cut branches supplied with water show the same peculiarity, although, as might be expected, they always transpire less actively under otherwise similar conditions than plants rooted in the soil. The amount of water passing

<sup>1</sup> Extracted from unpublished Observatory records with Mr. Baracchi's kind consent.

through a similar branch taken from an equally actively transpiring plant and subjected to its own head of water was also measured, and it will be seen that assuming the flow to be proportional to the head, it would require heads several times the length of the stem to produce the transpiratory rate of flow. As a matter of fact, the head required in a transpiring plant to maintain the transpiration current increases more rapidly than the rate of flow as the velocity of flow increases. If the stems are previously specially prepared and fully saturated, the resistance is of course greatly decreased, but we are concerned not with theoretical conditions, but with the actual ones existing in living trees.

#### A. CUT BRANCHES.

##### I. *Eucalyptus maculata var. citriodora.*

Branch, cut end placed in vessel of eosin. Evaporation from vessel prevented by enveloping in rubber sheeting.

In direct sunlight, temp. (shade) 24° C.

*Diameter of stem*, 3.6 cm.

*Duration of exp.*, 2 hours.

*Loss by transp.*, 99.386 grams (49.693 grams per hr.).

No. of leaves, 7,600.

Average area, 10.4 sq. cm.

Total area of leaves, 79,040 sq. cm. (stomata on both surfaces).

Total area of stomatal surface, 158,080 sq. cm.

Total number of stomata, 1,011,712,000 (64 per sq. mm.).

Loss per hour per sq. metre, 3.1 grams.

Loss per hour per stoma, .000048 milligram.

Rate of ascent, 128.5 cm. per hour.

*Amount passed through similar branch under its own head of water* = 12.5 grams per hour.

Hence head required to maintain transpiratory rate of flow should be not less than seven to eight times the length of the stem.

##### II. *Nerium Oleander.*

Stem set up as in I. ; shade temp. 30° C.

Duration of exp., 1 hour.

No. of leaves, 131.

Average area, 15.6 sq. cm.

Total area of leaves, 2,013.6 sq. cm. (stomata on both surfaces).

Total area of stomatal surface, 4,087.2 sq. cm.

*Loss by transpiration*, 16 grams.

Average no. of stomata, 318,801,601 (78 per sq. mm.).<sup>1</sup>

<sup>1</sup> The fact that the stomata are sunk in pits below the surface makes exact counting difficult.

Loss per hour per sq. metre, 3.9 grams.

Loss per hour per stoma, .00005 milligram.

Rate of ascent, 25.5 cm. per hour.

Amount passed through similar branch under its own head = 0.4 gram per hour. Hence head of water required to produce transpiratory rate of flow would be at least forty times length of stem.

The observations on rooted plants in pots were prolonged throughout the year, but only those data are given which represent various conditions at the different seasons. The appended curves give the relation between the temperature and the amount of evaporation from the plant and from a free surface of water. The hot wind is also a very dry one. Hence the enormous rise in the rate of evaporation, whereas the hot north wind about 27° C. causes the transpiration to undergo a regulatory decrease.

### B. TREES IN POT.

*Eucalyptus corynocalyx* (young tree with oval dorsiventral leaves, stomata on under surface only), area of transpiring surface, 5,120 sq. cm.

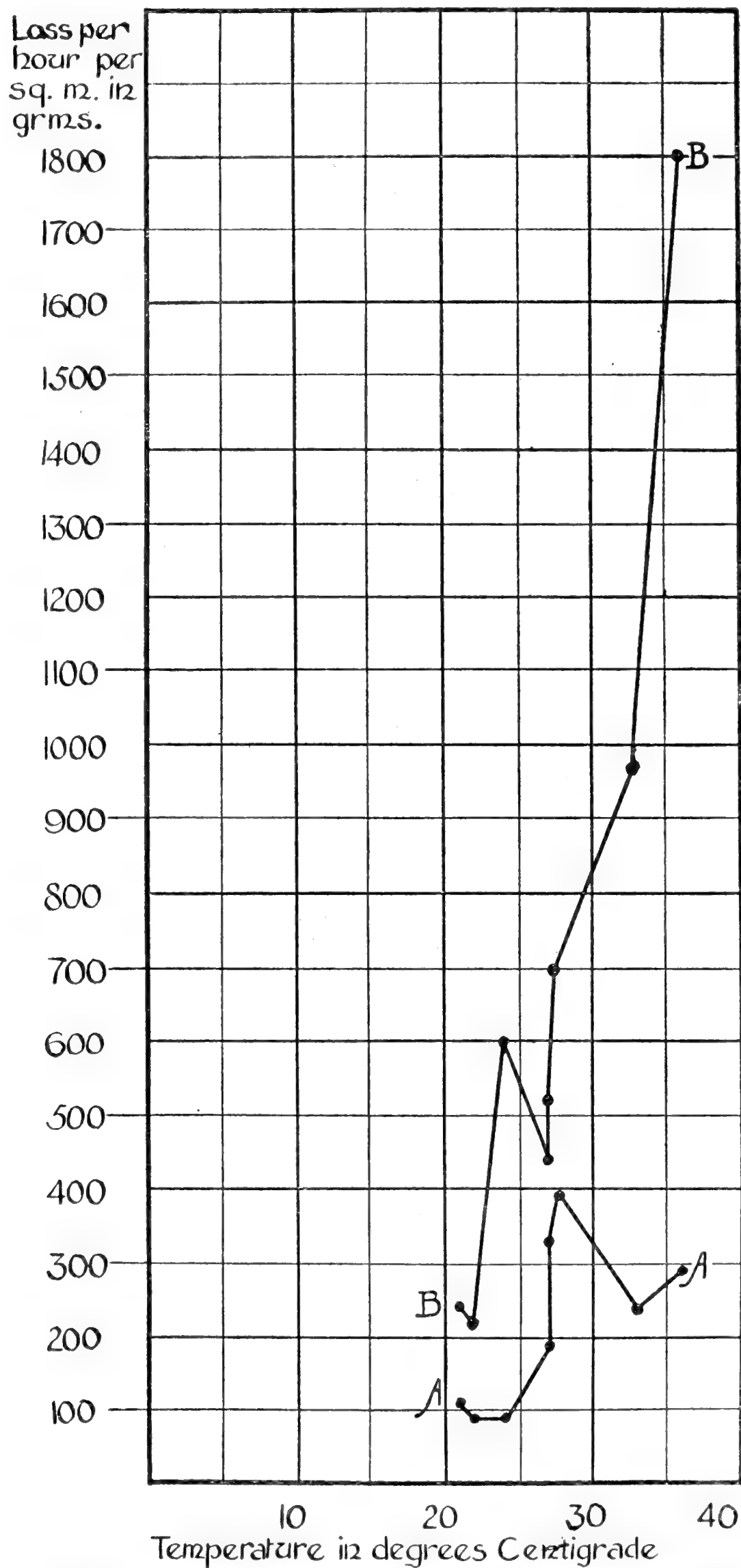
#### *Winter.*

<i>Date and Temperature.</i>	<i>Conditions.</i>	<i>Loss per hour per sq. m.</i>	<i>Loss per hour per sq. m. from free surface of water.</i>
18. 6. '08 16.5°	N. wind, dry	33.2 grams Loss per stoma = .00016 milligram	293 grams
15. 7. '08 13°	Clear sky, no wind	22.2 grams Loss per stoma = .00011 milligram	268 grams

#### *Summer.*

14. 10. '08 27.5°	Clear, sun, slight N. wind	395.8 grams Loss per hour per stoma = .00198 milligram	707 grams
26. 10. '08 24°	Clear, sun, slight S. wind	97.9 grams Loss per hour per stoma = .000489 milligram	597.8 grams
28. 10. '08 22.5°	Dull, no wind, little sun	97.9 grams	219.5 grams
2. 11. '08 27°	Hot sun, little wind, atmosphere moist	325 grams	536.6 grams
5. 11. '08 21°	Dull, slight wind	118 grams	243.9 grams
23. 11. '08 27°	Hot sun, no wind, air moist	195 grams	439 grams
25. 11. '08 33°	Hot sun, no wind, atmosphere dry	263.5 grams	975.6 grams
30. 11. '08 36°	Hot sun, no wind, atmosphere dry	295.8 grams	1,805 grams





A. Loss from leaves. B. Loss from free surface of water.  
*Eucalyptus corynocalyx*. Tree in pot

Figure 2

*Dracaena Draco.*

Area of stomatal surface = 3.2256 sq. m.

Stomata on both surfaces, most plentiful on upper surface along midrib.

This plant was kept under observation from November 13 to 30 inclusive, excepting 16th, 17th, and 24th. During this period the total loss amounted to 2,754.412 grams or an average of 172.15 per day.

The average loss per hour per sq. m. = 11.7 grams.

Average loss per hour per stoma = .00078 milligram.

The following table shows a few of the individual results under as divergent conditions as possible :—

<i>Date and Temperature.</i>	<i>Conditions.</i>	<i>Loss per hour per sq. m.</i>
13. 11. '07 16.5°	Shady, no wind	1.8 grams
14. 11. '07 24°	Clear, sun, no wind	10.6 grams
19. 11. '07 25°	Clear, sun, slight wind	13.2 grams
	per stoma = .00088 milligram	
21. 11. '07 30°	Cloudy, wind	6.6 grams
	per stoma = .0004 milligram	
22. 11. '07 33°	Clear, sun, strong N. wind	6.6 grams
	(Stomata partly closed?)	
23. 11. '07 30°	Clear, sun and wind	15.4 grams
	per stoma = .001 milligram	
25. 11. '07 30°	Cloudy, strong N. wind	17.6 grams
	per stoma = .00117 milligram	
26. 11. '07 19.5°	Cloudy, S. wind	6.6 grams
29. 11. '07 20.5°	Clear sky, slight wind (plant in shade)	4.4 grams
	per stoma = .00029 milligram	

Apparently in this case with a hot dry north wind, transpiration attains a maximum at or near 30° C. of 17 to 18 grams per hour per sq. metre. If the temperature rises beyond this the stomata begin to close and the rate of transpiration falls.

THE RATE OF ASCENT OF SAP IN EUCALYPTUS TREES.

A few orientating experiments were performed with some trees of *Acacia mollissima* which happened to be available. The best method is to cut the trees with a very sharp axe, keeping the cut surface under a jet of water. The cut end is then placed in a bucket of filtered water for a few minutes, and then rapidly transferred to a vessel containing a measured

quantity of eosin solution. However carefully the work is done a little air always enters, and by blocking some vessels might cause the liquid to rise more rapidly in the others than it does in the intact tree.

Hence one tree, A, was sawn through with a cut sloping slightly downwards and an inch jet of water assured that no air entered. The sawn end was then cut afresh with a wood chisel under water and trimmed with a sharp razor. The second one, B, was cut with a sharp axe and the cut end exposed to air for thirty seconds, with the following results after each tree had been in eosin for two hours:—

	<i>Height.</i>	<i>Rate of ascent of eosin.</i>	<i>Amount absorbed.</i>	<i>Temperature.</i>	<i>Condition.</i>
Tree A	3.65 metres	0.972 metre per hour	53.8 c.c. per hour	18–20° C.	clear
Tree B	5.30 metres	0.625 metre per hour	98.6 c.c. per hour	25–28° C.	slightly cloudy

It is evident, therefore, that any blocking with air lowers the rate of ascent of sap in the whole stem, and that the highest value obtained in any experiment will approach most closely to the rate of ascent in the intact tree. This is also shown by the fact that the rate of transpiration per sq. metre of leaf surface is always less under otherwise similar conditions from a cut tree with its base in water than from the intact rooted tree, and is still less when a cut branch is suspended in dry air. When transpiration is active the leaflets on a cut *Acacia* tree with its base in water begin to fold together in half an hour and are nearly or completely folded in two to three hours, while the leaves themselves droop downwards. Using every precaution and with a short exposure the maximum rate of ascent for a cut transpiring tree of *Acacia* was found to be 2.46 metres per hour, as in the following experiment.

*Acacia mollissima.*

Tree 3.8 metres high, lower branches close to ground removed.

Cut under stream of water with sharp axe.

Shade temp. 27° C. North wind. Time, 12–1 p.m. Dec. 7, 1908.

Eosin ascended 2.46 metres in one hour.

Amount absorbed, 552.8 c.c. per hour.

Leaflets at angle of 30–90° at commencement.

After half an hour angles began to lessen (deficiency of water?).

At close of experiment distinct signs of drooping in main petioles.

By noting the number of leaves and hence obtaining the transpiration rates per sq. metre from the cut tree and from a separated branch a control of the results can be obtained. Thus in a preliminary two-hour test with a two-year-old shoot of *Eucalyptus cornuta* 3 metres high, the eosin ascended 60 cm. per hour (shade temp. 19–21° C.) and the stem absorbed 34 c.c. of water per hour.

The total number of leaves was 3,323 with a total area of 7.665 sq. metres. The leaves, being almost all vertical, had stomata on both surfaces and the water absorbed per hour per sq. metre of transpiring surface was therefore 2.22 c.c. A cut branch in air, however, lost 68.5 c.c. per sq. metre per hour, and on examination of the cut stem of the tree it was found that a gummy mass beneath the bark had been carried by the axe over the cut surface, blocking very many of the vessels.

In a similar three-hour experiment with a young tree of *Eucalyptus viminalis* 4.6 metres high at 23–24° C. it was found that the eosin rose 93 cm. per hour, and 108.6 c.c. were absorbed, which was only 3.84 c.c. per hour per sq. metre of surface, whereas a cut branch with 805 leaves lost 70.15 c.c. per sq. metre during the first hour of the experiment, and another with 880 leaves lost 60.9 c.c. of water per sq. metre per hour during the latter two hours. In this case the blocking was due to the notching of the axe edge and imperfect trimming, but it suffices to show that partial blocking at the cut surface affects the amount absorbed much more than it does the maximal rate of ascent and never causes the latter to become higher than it would be in the intact tree.

In addition, the amount absorbed by a cut tree, as well as that exhaled by a cut branch suspended in air, steadily decreases, even if the external conditions remain constant, or even if they change so as to favour transpiration. Thus in a two-hour experiment with a cut branch of *Eucalyptus viminalis*, while the temperature rose from 23° to 26° C., the amount of water transpired per sq. metre was 40.8 c.c. in the first half-hour, 30 c.c. in the second, 27 c.c. in the third, and 23.5 c.c. in the fourth.

Taking every possible precaution, the following data were obtained in November, 1908, between 11 and 2 p.m. with two young trees of *E. viminalis* (A and B) and one of *E. amygdalina* (C):—

	<i>Eucalyptus viminalis</i> .		<i>Eucalyptus amygdalina</i> .
	A.	B.	C.
Height of tree	4.3 metres	3.95 metres	5.1 metres
Temperature	26° C.	27° C.	26° C.
Rate of ascent	12.3 metres per hour	10.33 metres per hour	6.5 metres per hour
No. of leaves	7,460	6,200	3,520
Total transpiring surface	23.11 sq. metres	20.92 sq. metres	28.8 sq. metres?
Water absorbed per sq. metre of total leaf surface	116.6 c.c. per hour	146.8 c.c. per hour	202 c.c. per hour
Water transpired per sq. metre from a cut branch suspended in air	80.95 c.c. per hour	118.3 c.c. per hour	113.6 c.c. per hour

None of the observations exceeded half an hour's duration, but even then the rate of absorption is much less than the maximal transpiration values for rooted plants of *Eucalyptus corynocalyx* (300–400 c.c. per hour per sq. metre of transpiring surface). It is evident, therefore, that the sap may rise in *Eucalyptus* trees<sup>1</sup> at rates of 30 or 40 feet an hour under

<sup>1</sup> Vertical leaves, stomata on both surfaces.

optimal conditions. In the case of the tree A the area of the stem at the narrowest point below the main branches was 1,256 sq. mm. and the vessels averaged 29 per sq. mm., so that the total number in the stem at that point was 36,440. The diameter of the largest was 0.13 mm., and the average diameter 0.083 mm. Hence the total area of cross-section of the vessels would be 1.93 sq. cm. At a rate of 1,230 cm. per hour the vessels if filled with water would discharge nearly 2,361 c.c. per hour, whereas the actual amount of water absorbed by the tree was 920 c.c. in twenty minutes. At the base of the stem, however, there were approximately 55,800 vessels with a discharging rate of over 3,600 c.c. per hour. The eosin was fairly evenly distributed throughout the wood from the base to half-way up, and hence not only were the whole of the vessels conducting, but their effective conducting area was little or not at all reduced by the presence of air in them, and the tracheides also may have played some part in the conveyance of water.

Even in the case of a capillary vessel occupied by a continuous column of water and with perfectly smooth internal walls a rate of flow of 10–12 metres per hour entails considerable friction and resistance. Thus, in a tube 0.008 cm. diam. and 10 metres long the frictional resistance would be in the case of water at 20° C. equivalent to a head of 1,250 cm. of water approximately, or slightly over an atmosphere. Hence in such a case the viscosity factor alone more than doubles the head due to the height of the tree, and to this must be added the resistance due to the transverse partitions, to the irregularities on the internal walls, and to the usual presence of more or less air.

#### THE DIAMETER AND LENGTH OF THE VESSELS IN BRANCHES AND IN THE MAIN TRUNK.

The experiments detailed below were performed by driving mercury into the stem under pressure and noting when it escaped from one or more vessels as pieces were cut from the distal end. For the most part each experiment meant the sacrifice of an entire young tree, and the quantity of mercury used was naturally great. For demonstration purposes, however, it is sufficient to use air driven into the stem under pressure from a cylinder of compressed air, and if the wood is fresh and the trunk quite sound this gives as accurate results as the mercury method for determining the length of the longest vessels (Exp. II with *Acacia mollissima*), since air only escapes freely from vessels uninterrupted by partition walls, even when a pressure of two or three atmospheres is used. The mercury method, however, enables the open vessels to be counted until the number increases considerably, whereas with air, as soon as more than three or four are opened, it is difficult to count the number further back with any accuracy.

The following observations on *Acacia* show that a single vessel may run nearly the whole length of the tree, while less than 1 per cent. ran for one-third, and not more than 5 per cent. for one-fifth the length of the main stem. The vessels in the branches are usually smaller and shorter than in the main axis, and they are more numerous per sq. centimetre in the wood on young stems than in that on older ones. Not only are the longest vessels found in the main axis but they do not appear to run into branches to any extent, and the same applies to the branches themselves; the longest vessels on a main axis do not as a rule run into secondary branches. Hence the number of partition walls in the path of a particular line of sap-flow will always be greater than the number of branchings it passes, and as regards the resistance to flow, this will put a young branch at the top of a tree on a greater equality with the twigs on a basal branch than would otherwise be the case.

Mercury Method.

*Acacia mollissima* :

I.		Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
(a)	Full tree Height 2.44 m.	6 yrs.	3.5 cm.	New wood	200 cm., 5 vessels at 134 cm., also one in protoxylem at 170 cm. (exceptional)	.131 mm.	.101 mm.
(b)	Root and hypocotyl	6 yrs.		3rd yr's. wood	33 cm., 3 vessels at 25 cm.		
(c)	Branch of same	3 yrs.	1.3 cm.	New wood	102.5, 5 vessels at 70 cm.	.104 mm.	.065 mm.
II.	Full tree	3 yrs.	6.2 cm.	New wood	240 cm., 60 vessels at 95 cm.	.13 mm.	.09 mm.
Fork (a)	Forked trunk Height 4.5 m. Mercury method						
					Average no. of vessels at tip <sup>1</sup> (21 per sq. mm.), 1336		
Fork (b)	Height 3.94 m. (Air pressure)	3 yrs.	6 cm.	In new wood, one in spring and one in autumn wood	213 cm. (2 vessels), 8 vessels at 125 cm.	.09 mm.	.07 mm.
					Average no. of vessels at tip (30 per sq. mm.), 1500		
(c)	Branch of same Length 94 cms.	2 yrs.	1.4 cm.	New wood	3 vessels at 94 cm., 6 vessels at 6 cm.	.08 mm.	.05 mm.
					Average no. of vessels at tip (38 per sq. mm.), 665		
(d)	Branch of same Length 1.48 m.	3 yrs.	2.1 cm.	New wood	115 cm., 38 vessels at 28 cm.	.1 mm.	.07 mm.
					Average no. of vessels at tip (26 per sq. mm.), 735		
(e)	Branch of same Length 1.21 m.	2 yrs.	1.3 cm.		119 cm., 18 vessels at 37 cm.	.1 mm.	.06 mm.
					Average no. of vessels at tip (42 per sq. mm.), 1188		

<sup>1</sup> Tip = where the longest vessel ends.



*Acacia mollissima* (continued):—

	Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
(f) Branch of same Length 1.25 m.	2 yrs.	1.6 cm.	New wood	121 cm., 17 vessels at 74 cm.	.098 mm.	.07 mm.
Average no. of vessels at tip (24 per sq. mm.), 831						

*Acacia Lophantha*:

Branch	1 yr.	1 cm.		40 cm., 29 vessels at 22 cm.	.12 mm.	.08 mm.
Average no. of vessels at base (13 per sq. mm.), 1021						

Very similar results were given by young trees of *Eucalyptus*, except that there was an even more marked difference in the appearance of the wood from the trunk and branches, the vessels of the latter being much smaller in diameter and much more numerous per sq. centimetre. The fact that one or two vessels out of several thousand may run nearly the whole length of the tree, and only a small percentage one-third to half the length of the tree, the great majority being very much shorter than this, is somewhat suggestive. It may be that these few very long vessels are specially adapted to convey continuous columns of water with ease and rapidity, but that since a single bubble or plug of gum may throw the whole tube out of action more or less completely, the plant prefers to rely for everyday use on the more numerous much shorter vessels, where the blocking of single vessels is less serious. On the other hand, the formation of these single vessels of great length may be more or less the result of accident, or at least not a fact of any special functional significance.

## Mercury Method.

I. *Eucalyptus maculata* var. *citriodora*:

	Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
(a) Branch	6-8 yrs.	2.1 cm.	Newest wood	25 at 96 cm.	.145 mm.	.093 mm.
(b) Branch	6 yrs.	2.5 cm.	New wood	119 cm., 19 vessels at 78 cm.	.076 mm.	.054 mm.
Average no. of vessels in stem, 9,500						
(c) Branch	4 yrs.	1.5 cm.	3rd yr's. wood	105.5 cm., 22 vessels at 85 cm.	.084 mm.	.065 mm.
Average no. of vessels in stem, 8,835						

II. *Eucalyptus viminalis*:

Forked tree.  
Height 2.51 m.

Fork A.	6 yrs.	4.5 cm.	In new wood	198 cm., 9 vessels at 147 cm.	.091 mm.	.062 mm.
Fork B.	6 yrs.	4.4 cm.	In new wood	199.3 cm., 4 vessels at 195 cm.	.091 mm.	.062 mm.
Branch of same Root and hypocotyl	3 yrs. 6 yrs.	1.4 cm. Root, 2.5 cm., Hypo., 12 cm.	In new wood	5 vessels at 90 cm. 17 vessels at 62 cm.	.118 mm.	.082 mm.

Judging from its position, the longest vessel probably extended from root through main axis. Length about 316 cm.

*Eucalyptus globosa*:

	Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
(a) Full tree, height 6.7 m. Mercury applied at tip of trunk.	8 yrs.	8.9 cm.; diam. at cut tip, 2 cm.	7th and 8th years' wood	6.11 m. 2 vessels, 34 vessels at 2.9 m.	.137 mm.	.087 mm.
Average no. of vessels in stem at tip, 8 per sq. mm.; at base, 9 per sq. mm.						
(b) Branch of same Length 94 cm.	3 yrs.	1 cm.	New wood	87 cm., 10 vessels at 80 cm.	.069 mm.	.053 mm.
Average no. of vessels in stem, 60 per sq. mm.						

A few data are appended upon the dimensions of the vessels in the *Oleander*, *Ficus*, *Deeringia*, and *Tecoma*, but in none of these did the vessels attain anything like the length shown by single vessels of *Eucalyptus* and *Acacia*. Experiments have still to be performed on adult trees of *Eucalyptus* 100 feet high or more, but these have been postponed for the present, since the work must be done on the spot where the trees grow and entails considerable expense.

## Mercury Method.

*Nerium Oleander*:

	Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
(a) Stem (exposed tree)	2 yrs.	1 cm.		52 cm.	.057 mm.	.038 mm.
(b) Stem (shaded tree)	6 yrs.	1.2 cm.	4th yr's. wood	75.5 cm. (shaded, therefore less active transpiration), 26 vessels at 39 cm.	.035 mm.	.021 mm.
Average no. of vessels in stem, 108,500						
(c) Stem (shaded tree)	4 yrs.	.9 cm.	3rd yr's. wood	74 cm., 17 vessels at 40 cm.	.046 mm.	.026 mm.
Average no. of vessels in stem, 61,236						

As far as these results go they show that the length of the vessels is more affected by the age of the stem than by the activity of transpiration, whereas the degree of functional use appears to affect their diameters to a marked extent, active transpiration favouring an increase in diameter.

## Mercury Method.

*Ficus elastica*:

	Age.	Diameter at base.	Position of longest vessel.	Length of longest vessel.	Diameter of largest vessel.	Average Diameter of vessels.
Branch	10 yrs.	1.8 cm.	New wood	47 cm., 20 vessels at 38 cm.	.137 mm.	.085 mm.
Branch	2 yrs.	1 cm.	New wood	53 cm., 25 vessels at 32 cm.	.092 mm.	.072 mm.

*Deeringia altissima*:

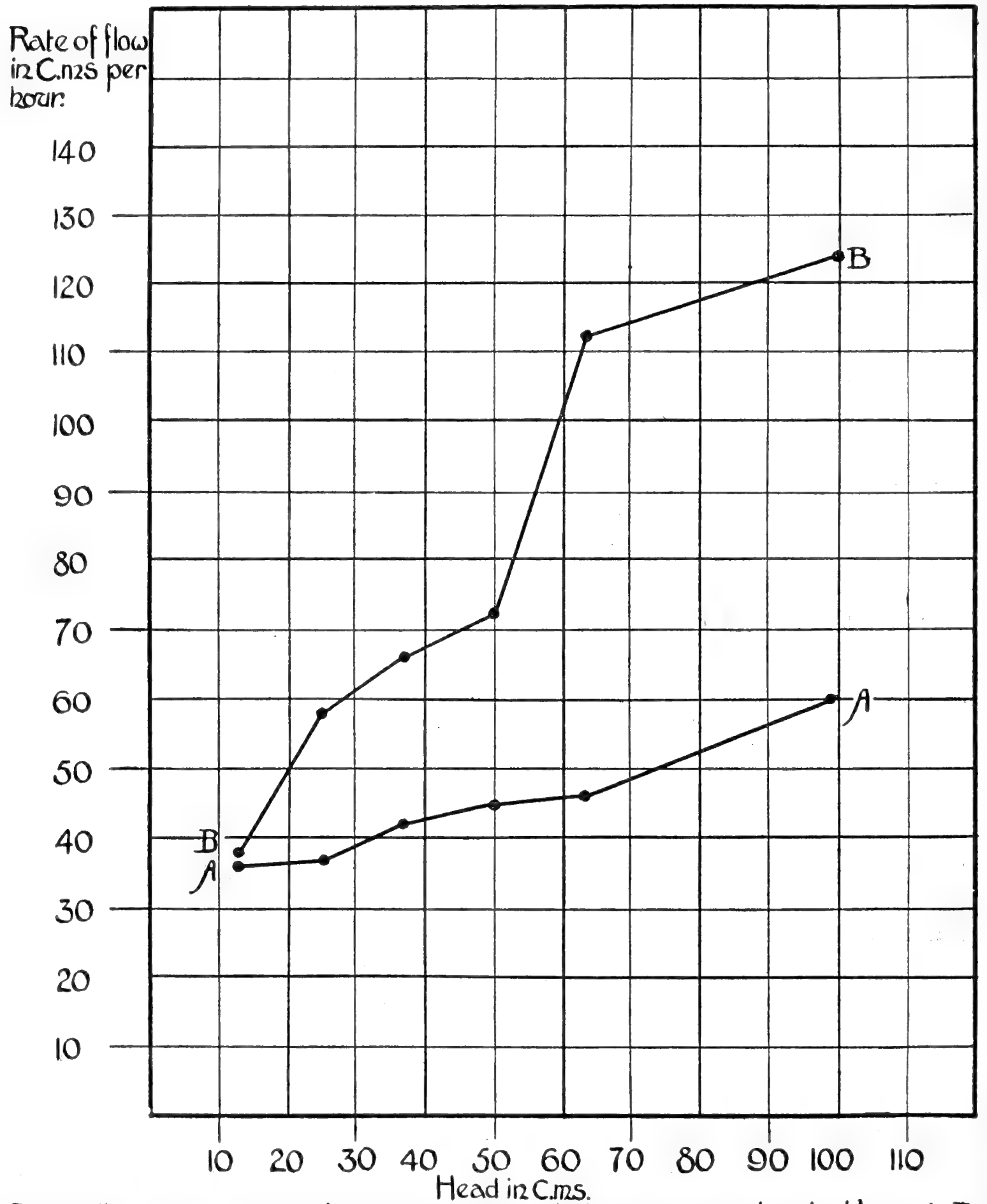
Branch	3 yrs.	.7 cm.	New wood	74 cm., 12 vessels at 43 cm.	.047 mm.	.032 mm.
Branch	3 yrs.	1 cm.	2nd yr's. wood	97 cm., 15 vessels at 69 cm.	.059 mm.	.004 mm.

*Tecoma*:

Branch	3 yrs.	6 cm.		48 cm., 8 vessels at 28 cm.	.076 mm.	.05 mm.
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THE RESISTANCE TO FLOW UNDER VARYING PRESSURES.

The resistance to flow naturally depends upon a variety of factors, and from the data already given as to the dimensions of the vessels, it is



Rate of flow under varying heads in non-saturated-A, and in saturated branch-B.  
Figure 3. *Eucalyptus viminalis*.

evident that, other things being equal, it will be more rapid in large branches and main stems than in equal lengths of the smaller branches under similar heads. In the following experiments the branches for each

set of experiments were all of equal length, and as far as possible of equal diameter and similar shape. In each double series the first set, A, consists of branches taken directly from actively transpiring trees; the second set, B, consists of branches saturated as far as possible with water. The branches were laid horizontally and eosin driven through under varying pressures, noting the time of flow in each case.

I. *Eucalyptus viminalis.*

A.

Length of stem 50 cm.

<i>Heads in cm. of water.</i>	<i>Rate of flow per hour.</i>
1. 12.5 cm. ( $\frac{1}{4}$ head)	36 cm.
2. 25 cm. ( $\frac{1}{2}$ head)	37 cm.
3. 37.5 cm. ( $\frac{3}{4}$ head)	42 cm.
4. 50 cm. (1 head)	45 cm.
5. 63 cm. ( $1\frac{1}{4}$ heads)	46 cm.
6. 100 cm. (2 heads)	60 cm. (older branch)

B.

<i>Heads in cm. of water.</i>	<i>Rate of flow per hour.</i>
1. 12.5 cm.	38 cm.
2. 25 cm.	58 cm.
3. 37.5 cm.	66 cm.
4. 50 cm.	72 cm.
5. 63 cm.	112 cm.
6. 100 cm.	124 cm.

*Acacia mollissima.*

A.

Length of stem 50 cm. Temp. 25° C.

<i>Heads in cm. of water.</i>	<i>Rate of flow per hour.</i>
1. 12.5 cm.	28 cm.
2. 25 cm.	59 cm.
3. 37.5 cm.	68 cm.
4. 50 cm.	80 cm.
5. 63 cm.	88 cm.
6. 75 cm.	99 cm.
7. 100 cm.	187 cm.

B.

<i>Heads in cm. of water.</i>	<i>Rate of flow per hour.</i>
1. 12.5 cm.	32 cm.
2. 25 cm.	64 cm.
3. 37.5 cm.	118 cm.
4. 50 cm.	172 cm.
5. 63 cm.	375 cm.
6. 75 cm.	388 cm.
7. 100 cm.	460 cm.

These results fluctuate considerably, but so also does the material, and it is quite useless by careful selection to obtain consistent results

which do not represent any such consistency in the plant. The resistance to flow in transpiring plants varies in fact along the path of the transpiration current, and all we need to determine is the maximal total resistance and the approximate limits of variation. In any case the results suffice to show that the presence of air not only lowers the conductivity but causes increasing heads to produce less and less of the expected flow. The discrepancy may become remarkable. Thus in the following series of experiments with *Eucalyptus amygdalina* the segments 1-7 were taken

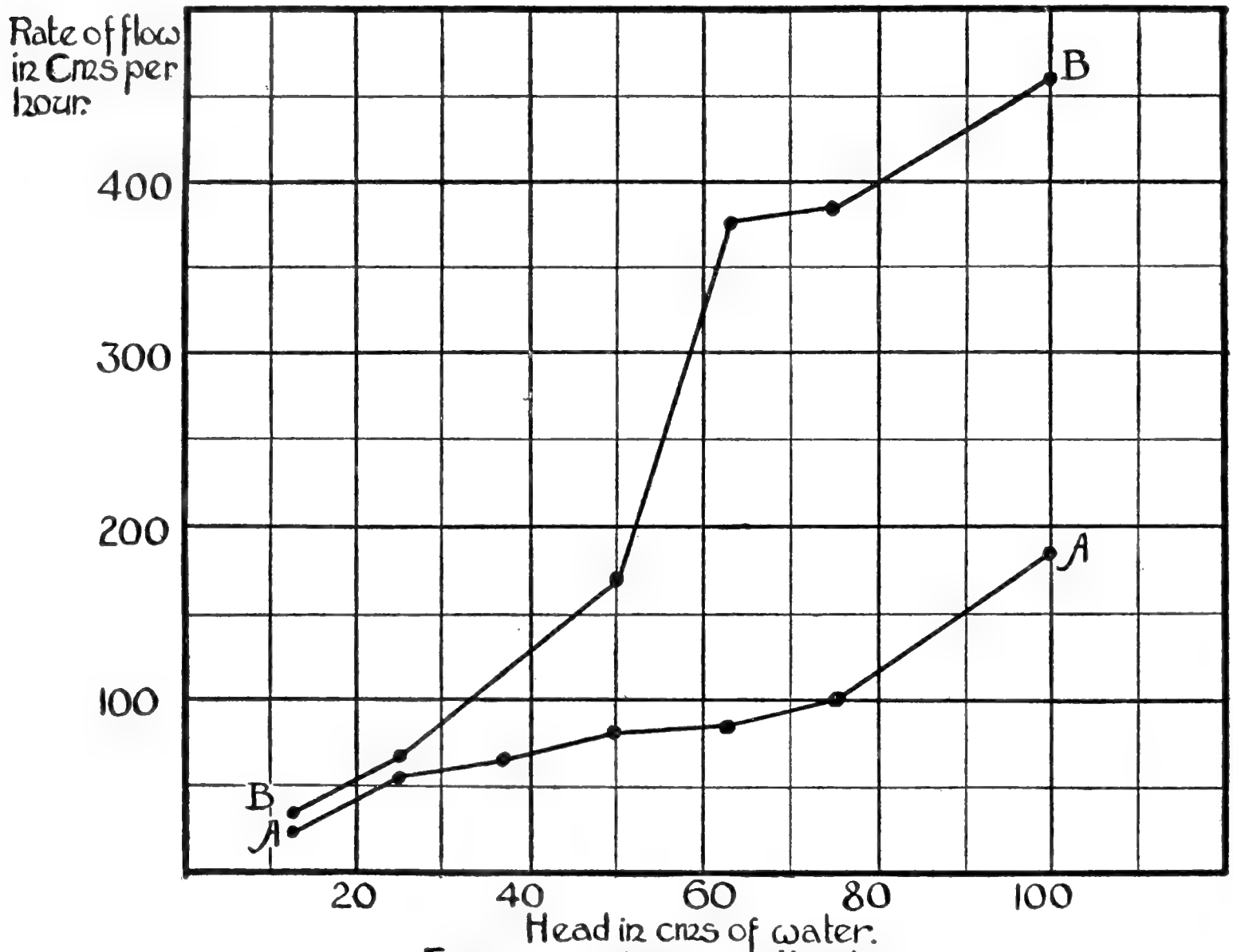


Figure 4. *Acacia mollissima*.

from two slender transpiring shoots with short and narrow vessels, whereas No. 8 was a large main stem of a tree and was saturated with water. Even in this case, in which the resistance to flow is probably nearly minimal, it requires a head of twice the length of stem to produce a rate of flow of 3 metres an hour, which is a fair average rate for the active transpiration current in *Eucalyptus*, and to produce the maximal rate observed of 12 metres per hour would need a correspondingly increased head.

*Eucalyptus amygdalina.*

Length of stem 50 cm. Temperature 20°.

Heads in cm. of water.	Rate of flow per hour.
1. 12.5 cm.	13.6 cm.
2. 25 cm.	15.8 cm.
3. 37.5 cm.	18.6 cm.
4. 50 cm.	20.2 cm.
5. 63 cm.	25.4 cm.
6. 75 cm.	32.2 cm.
7. 100 cm.	41.8 cm.
8. 100 cm.	324 cm. (branch of tree saturated with water).

With exceptionally favourable material of *Acacia mollissima*, Miss Rees obtained a maximal rate of flow of 1,080 cm. per hour for a 50 cm. length of stem under a head of 50 cm. of water at 25° C. Assuming that this was

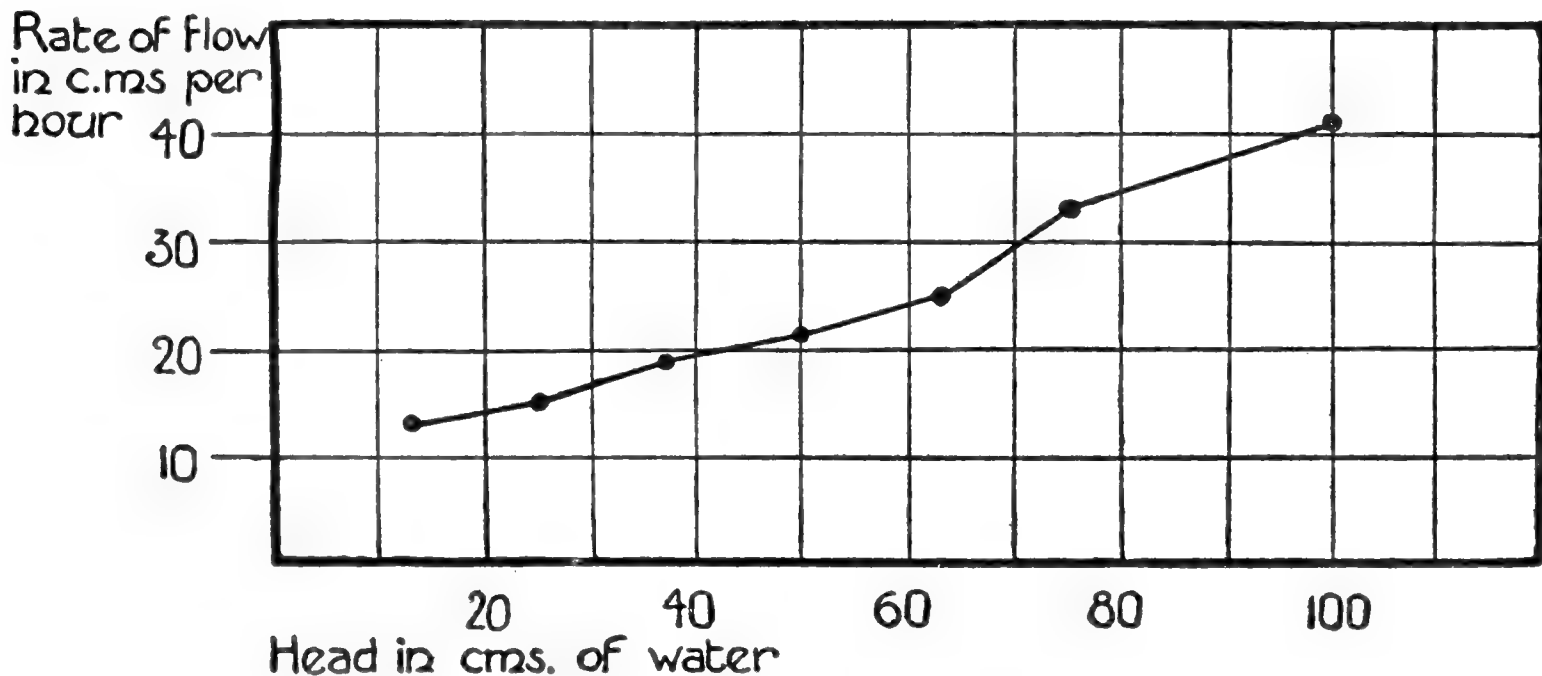


Fig 5 *Eucalyptus amygdalina*

through an open vessel of 0.01 cm. diameter, the rate of flow would, according to a theoretical calculation from Poiseuille's formula, have been thirty times greater, and in a vessel of 0.05 cm. diameter seven times greater. Similarly, with a head of 100 cm., the rate of flow was only 1,560 cm. per hour, or less than one-thirtieth the theoretical value. The difference is due to the facts that the vessels are not perfectly circular, that their walls are not smooth, that they are longer than the stem bearing them, and that at every sharp bend with a high rate of flow, eddy currents will form which increase the resistance to flow, and finally that whenever one vessel is conducting under pressure more rapidly than neighbouring ones, lateral exudation through the permeable walls will tend to level up, or rather level down, the rate of flow more or less to the average one for



the whole wood cylinder. The influence of the eddy currents and lateral exudation will increase as the pressure and resultant velocity of flow increase.

EXPERIMENTS ON RATE OF ASCENT OF SAP IN DEAD AND LIVING CUT BRANCHES IN DRY AND SATURATED AIR.

*Method.* Living and dead stems devoid of leaves were saturated with water and placed with their basal cut ends in a vessel of eosin in a saturated atmosphere or exposed to air. The stems were all saturated by driving water in under pressure, since it was found impossible to saturate them fully by drawing in water by means of a suction pump. In injecting the stems no blocking occurred if a fresh surface was cut under water before attaching to the pump and distilled or filtered water was used. After a certain length of time the height to which the eosin had ascended in the different stems was noted.

I. *Acacia mollissima.*

(a) Living stems saturated with water by means of high-pressure pump.

		Temperature 22°.
(i) <i>In dry air.</i>		
<i>Duration of exp.</i>		<i>Rate of ascent per hour.</i>
1.	2 hours	14.1 cm.
2.	2 hours	11.25 cm.
(ii) <i>In saturated atmosphere.</i>		
1.	2 hours	4.65 cm.
2.	2 hours.	8.5 cm.

(b) Stems killed by injecting mercuric chloride and then saturated with water by means of high-pressure pump.

(i) <i>In dry air.</i>		
1.	2 hours	5 cm.
2.	2 hours	5 cm.
(ii) <i>In saturated atmosphere.</i>		
1.	2 hours	1.5 cm.
2.	2 hours	3.5 cm.

II. *Eucalyptus amygdalina.*

(a) Living stems saturated with water by means of high-pressure pump.

		Temperature 21°.
(i) <i>In dry air.</i>		
<i>Duration of exp.</i>		<i>Rate of ascent per hour.</i>
1.	1 hour	27 cm.
2.	1 hour	15 cm.
(ii) <i>In saturated atmosphere.</i>		
1.	1 hour	12 cm.
2.	1 hour	15 cm.

(b) Stems killed by injecting with mercuric chloride and then saturated with water by means of high-pressure pump.

(i) <i>In dry air.</i>	
1. 1¼ hours	3.2 cm.
2. 1¼ hours	4 cm.
(ii) <i>In saturated atmosphere.</i>	
1. 1¼ hours	2.4 cm.
2. 1¼ hours	.8 cm.

The rate of ascent decreases in geometric progression as the experiment progresses and the liquid rises in the stem, as can be seen from the following and preceding data:—

*Nerium Oleander.*

(a) Saturated stem in dry atmosphere:

<i>Duration of exp.</i>	<i>Rate of ascent per hour.</i>
1. 3 hours	7.6 cm.
2. 7 hours	4.3 cm.

(b) Saturated stem in saturated atmosphere:

1. 24 hours	.42 cm.
2. 4 hours	1.1 cm.
3. 4 hours	1.2 cm.

(c) Saturated stem killed with mercuric chloride in saturated atmosphere:

1. 4 hours	.4 cm.
2. 24 hours	.3 cm.

Although the results show a certain amount of variation, they agree in so far as the most rapid ascent occurs in cut saturated branches in dry air, a less rapid ascent in a saturated atmosphere, and still less in the case of stems killed by means of mercury chloride. At first sight we have what seems a conclusive proof of an upward pumping action exerted by living stems, for it is difficult to see how any ascent could be produced by capillarity and evaporation in a saturated stem in a saturated atmosphere. The action does not seem, however, to be appreciably influenced by whether the stem is in the normal or inverted position provided both ends are equal in diameter, and further the fact that any rise at all is shown in a dead saturated stem in a saturated atmosphere is sufficient proof that the ascent is physical in origin. Its exact nature needs, however, further investigation. An ascent of liquid in a saturated stem in a saturated atmosphere at a rate of 12–15 cm. in an hour is too rapid to be explained by ordinary diffusion or imbibition.

The rate of ascent decreases in geometric progression as the experiment progresses and the sap rises up the cut stem. Exactly the same applies to the capillary ascent in a glass tube, for, as the column rises, less and less force is available to overcome the gravitational acceleration and

frictional resistance of the ascending stream. In the case of the *Oleander* the maximal height reached in a saturated atmosphere (10 cm.) was attained before the end of the day, and when once equilibrium has been reached, any further prolongation of the experiment simply lowers the apparent rate of flow. With experiments of equal and not too prolonged duration the liquid rises most rapidly in the saturated stem in dry air, less rapidly in the living saturated stem in saturated air, and still more slowly in the stem killed by mercuric chloride.

Suggestive as these results are, they must only for the present be taken as indicating a possible mode of demonstrating the existence of a pumping action in the wood of transpiring trees.

If such a pumping action exists we should expect to find the sap ascending in the stem of a tree after the leaves had been removed, especially if the tree was previously short of water.

Accordingly the whole of the branches and leaves were removed from a tree of *Araucaria excelsa* 8½ metres high. After attaching the trunk to scaffolding it was sawn across at the base under a jet of water, trimmed under water and lowered into a drum of eosin solution. After two months, no eosin having appeared at the top, it was sawn up and examined.

The eosin had risen on one side in the second and fourth rings to a height of 134 cm. The wood was uniformly tinged only below 50 cm., but was moist and living at all points. The experiment was begun near the close of summer after a two months' spell of dry weather, and yet the rate of ascent barely exceeded 2 cm. per day on the average for the whole time. This fact negatives the conclusions that might have been drawn from short lengths of stems, but leaves it still an open question as to whether or no a pumping action may exist but only act under the stimulus of demand, and be of such character as to allow a moderate suction of nearly equal intensity at all points to extend to the ground, each region in the path of the transpiring current maintaining its water in a labile condition so as to neither appreciably add to nor diminish the suction exerted by the leaves, which is transmitted by cohering columns of water as far as their tensile strength will allow and as far as they exist.

#### SUMMARY.

The rate of evaporation per sq. metre of leaf-surface from cut branches, whether placed in water or not, is always less than from a plant rooted in the soil, under otherwise similar conditions.

When the air is hot and dry the evaporation from a free surface of water undergoes an enormous increase, but that from a living plant undergoes a regulatory decrease, and may be only one-sixth as active as the former. Under optimal conditions a rooted plant of *Eucalyptus corynocalyx*

may lose 396 grams of water per sq. metre of transpiring leaf-surface per hour, whereas the maximum rate for *Dracaena Draco* was 17.6 grams.

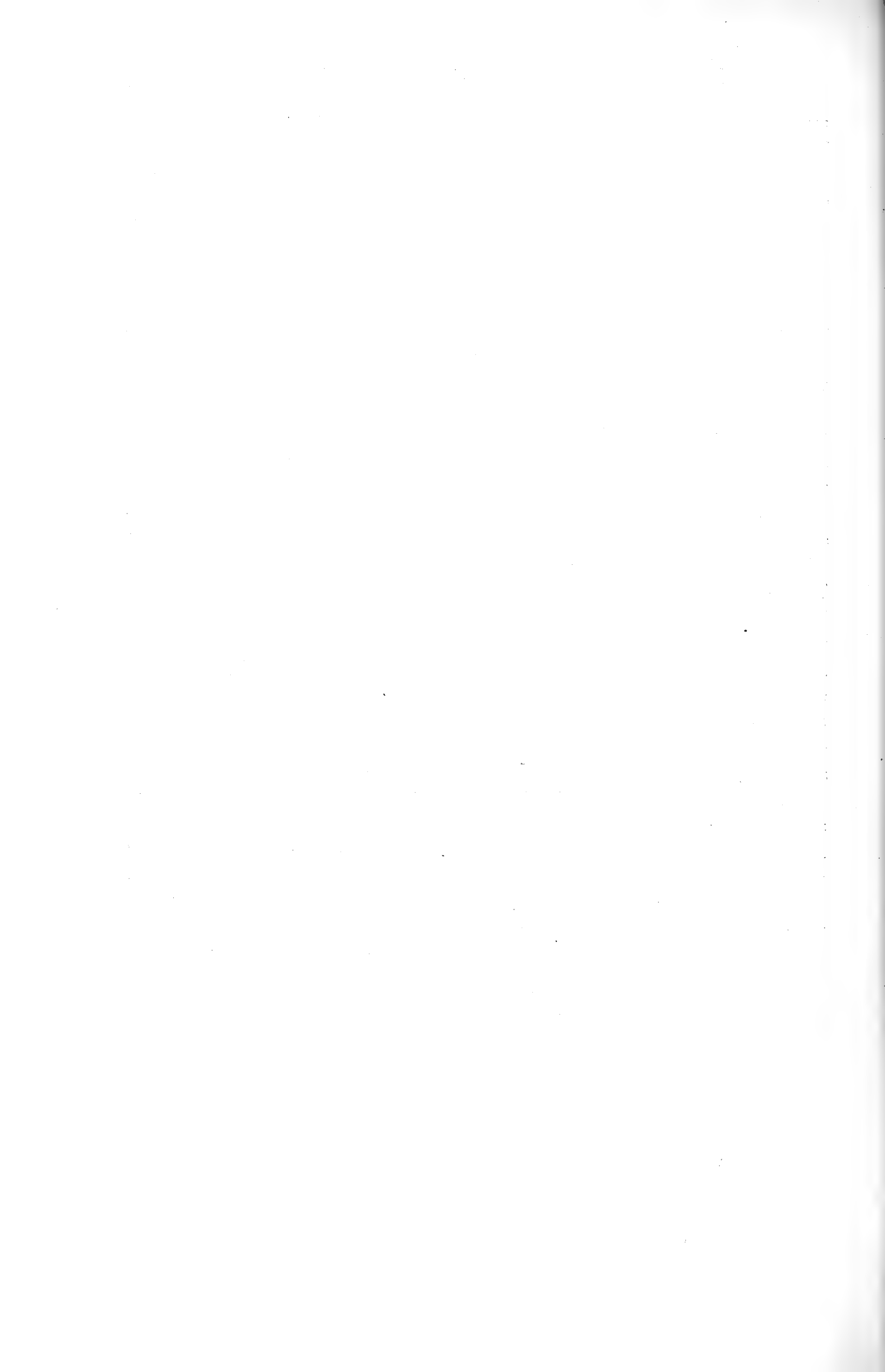
Cut trees always absorb water at a less rate than rooted ones evaporate it. The maximum rate of ascent of sap noted was 12.3 metres per hour (*Eucalyptus viminalis*) and 6.5 metres per hour (*E. amygdalina*), whereas in cut branches of *Eucalyptus* and in cut trees of *Acacia mollissima* it rarely exceeds 1 to 2 metres an hour, and is often less than 1 metre.

Single vessels may run nearly from end to end of the main trunk in young *Eucalyptus* and *Acacia* trees several metres high, but only a very small fraction exceed half the main trunk in length. In the branches the vessels are shorter and narrower, but the sap will usually not pass any more transverse partitions in the wood-vessels than it does in the process of passing from root-hair to wood, and from the wood to the transpiring surface. The existence of a rapid transpiration current appears to favour the development of broad vessels, but not to affect their length.

Branches containing air taken from actively transpiring trees show a much greater resistance to flow than when saturated with water; and with increasing heads the rate of flow does not increase proportionately. To produce the transpiration rate of flow heads of two to ten times the length of stem may be required, but in unblocked fully saturated stems with large long vessels a head of one-fifth the length of stem may be sufficient. During prolonged active transpiration, however, the total resistance to the upward flow of sap in a *Eucalyptus* tree may amount to a head of two to ten times the height of the tree, which would therefore equal in the tallest trees a maximal pressure lying between 20 and 100 atmospheres.

A coloured liquid will rise slowly in a saturated stem kept in a saturated atmosphere, but a somewhat slower ascent is shown after the stem has been killed, so that the phenomenon is not the result of any vital pumping action, and must be capable of a physical explanation, although in a saturated stem it cannot be due to capillarity or imbibition, and is too rapid to be the result of diffusion.

No appreciable rise of sap took place in a tree deprived of its leaves, but a pumping action may only be excited when the leaves are exerting suction on the water in the wood.



# Foliar Gaps in the Osmundaceae.<sup>1</sup>

BY

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With Plates **XI** and **XII**.

IN 1900 Jeffrey (1) proposed the division of all vascular plants into two great phyla: the *Lycopsida*, comprising the Lycopodiales and Equisetales, and characterized by ventral sporangia, small leaves, and the absence of foliar gaps in the central cylinder; and the *Pteropsida*, including the Filicales, Gymnosperms, and Angiosperms, all of which have dorsal sporangia, large leaves (at least primitively), and foliar gaps. That these two phyla differ in the position of their sporangia and in the size of their leaves, as far back as we have geological record, is now generally agreed. That they are clearly divided in the manner of departure of the leaf-trace is not, however, universally admitted. The question as to whether there are foliar gaps in the Lycopsida has been thoroughly discussed by Jeffrey in a recent paper (2). In the other main group, the Pteropsida, gaps are unquestionably present in all the families save one, the Osmundaceae. That there are numerous cases of the absence of foliar gaps, both in the fossil and the living members of this group, has recently been strongly urged by several writers, notably Kidston and Gwynne-Vaughan (3). The present investigation was undertaken to clear up the relation of leaf-trace to central cylinder in this interesting family.

More or less complete material of seven members of the group was obtained. These are the three common American species of *Osmunda*, *O. regalis*, L., *O. cinnamomea*, L., and *O. Claytoniana*, L.; *Todea barbara*, Moore; and the three filmy *Todeas*, *T. superba*, Colenso, *T. hymenophylloides*, Rich., and *T. Frazeri*, Hook. and Grev. Only portions of the mature leaves could be secured in the last species.

The central cylinder of the stem in the Osmundaceae consists, as is well known, of a ring of xylem strands, separated by parenchymatous 'rays', and surrounding a pith. Outside the xylem is a more or less continuous ring of phloem. The method of departure of the leaf-trace from the cylinder has been worked out by several investigators, among the foremost of whom is Zenetti (4). The first indication of the formation of a foliar

<sup>1</sup> Contributions from the Phanerogamic Laboratories of Harvard University, No. 20.



strand is the appearance, usually near the outer margin of one of the xylem bundles, of a group of small protoxylem elements. Just inside this, a cluster of parenchyma-cells soon arises, which gradually increases in size towards the centre of the stem until it unites with the parenchyma of the pith, leaving the xylem-bundle in the shape of a horseshoe, with the protoxylem group near the middle of its inner face. The outer part, or curve, of the horseshoe now separates itself from the rest of the bundle and departs (with the phloem, which now surrounds it) as the leaf-trace, leaving the original bundle divided into two by a 'ray' of parenchyma. This ray is a foliar gap in the xylem caused by the departure of a leaf-trace. The two bundles eventually unite, closing up the gap thus formed.

This mode of separation of the leaf-trace from the cylinder was found to be by far the most common one in the group. Certain exceptions to it, however, were noted. In the stem of *O. cinnamomea* numerous instances were observed in which the cluster of parenchyma, after its appearance in the xylem-strand, instead of becoming continuous with the pith, broke first through the *outer* part of the xylem, on either side of the departing leaf-trace, and thus separated the trace from the original bundle. A transverse section cut through this region seems to show clearly a leaf-trace leaving the cylinder without causing a break in the continuity of the xylem. Continuous serial sections were cut through such places as this, however, and it was found, in every case where a complete series could be obtained, that the xylem-bundle was soon much constricted and finally broken into two by a 'ray' of parenchyma opposite the outgoing trace. This 'ray' is often only a few cells wide, but is nevertheless a true foliar gap, differing from the others only in its relatively late appearance. It soon closes up, as do the other gaps. Pl. XI, Fig. 1, shows a leaf-trace departing from the cylinder of *O. cinnamomea*, apparently without making a break in the xylem. Fig. 2 shows the same trace at a slightly higher level. The gap opposite to it is now plainly discernible.

*O. regalis* shows much the same condition of affairs. With most of the traces the gap in the cylinder is formed when the foliar strand separates from the stem-bundle, but in a number of instances observed the parenchyma of the pericycle and of the pith came into communication only after the trace had progressed a considerable distance into the cortex. Fig. 3 exhibits a trace just departing from the cylinder. In Fig. 4 the very narrow gap is shown which eventually appears in the xylem opposite this bundle.

In *O. Claytoniana* these cases of apparent absence of gaps, though occurring, are not as frequent, and almost all the traces go off in the usual manner.

The mature stems of three species of the genus *Todea* were examined—those of *T. barbara*, *T. superba*, and *T. hymenophylloides*. In the first of these, well-marked gaps were found in every case, and no temporary

apparent absence of a gap was observed. Gwynne-Vaughan, however, claims to have found instances in this species where the leaf-trace departed in what he designates as a 'protostelic', or gapless, manner. The interruptions are all short, and the lateral continuity of the xylem is therefore relatively great, but no instances of such a gapless condition were observed by the present writer.

In *T. superba* the leaf-gap in almost every case is narrow, and there were numerous instances noted where the trace was well into the cortex before a break opposite to it appeared in the central cylinder. Fig. 5 shows a trace apparently departing in a so-called 'protostelic' manner. Fig. 6 exhibits the condition of affairs a little higher up, where a well-marked gap has been formed. Long series of sections were cut through the stem of this species and in no case was the absence of a leaf-gap observed. In their paper on the anatomy of *Todea*, Seward and Ford (5) figure a section of the stem of this species showing a leaf-trace departing apparently without leaving a gap. This figure is referred to by Kidston and Gwynne-Vaughan and by Tansley (6) as a probable example of the 'protostelic' exit of a leaf-strand. In view of the facts above described, however, it seems highly probable that a condition is present similar to that shown in Fig. 5, and that a gap in the xylem would subsequently appear. Seward and Ford do not call attention to the absence of leaf-gaps in this species in any other way than by the above-mentioned figure.

In *T. hymenophylloides*, also, the gaps, or 'rays', are narrow in proportion to the size of the bundles. The great majority of traces depart in the usual manner. A number of instances were observed, however, where the gap was apparently absent for a while, but later appeared. Figs. 7 and 8, two stages in the passing off of a leaf-trace from the cylinder, show such a condition. In this form, also, Kidston and Gwynne-Vaughan mention finding cases where gaps were absent. None of these were observed by the present writer.

It would appear, therefore, that as far as the living Osmundaceae are concerned, foliar gaps are characteristically present, for in all the species where the whole course of a trace could be followed till it was far out in the cortex a break in the xylem ring opposite to it invariably appeared. The question now arises as to whether this family is *primitively* phyllo-siphonic—whether, as Jeffrey maintains, their ancestors belonged to the phylum Pteropsida and had foliar gaps, or whether, in the ancient members of the group which possessed siphonostelic central cylinders, the leaf-trace departed without causing a break in the continuity of the xylem ring.

There are two general ways in which the answer to this question may be sought. The first, and by far the most satisfactory of the two if it can be successfully pursued, is to study the actual fossil progenitors of the

family. The second is to examine the structure of those portions of the living forms which are known to be retentive of ancestral features.

Without going into the vexed question of the origin of the Osmundaceae, and leaving aside all such protostelic members of the group as *Zalesskya* and *Thamnopteris*, in which, obviously, leaf-gaps cannot occur and which therefore have little bearing on the present question, we find in the genus *Osmundites*, which possesses a medullated cylinder with a parenchymatous pith, a close and suggestive parallel to the conditions in living forms. There are five species at present included in this genus, the structure of all of which is now well known. Of these, perhaps *O. Skidegatensis* is the most striking. This fossil was first described from the Lower Cretaceous of Canada by Penhallow (7), and has also been investigated by Kidston and Gwynne-Vaughan (3). It is very large, possessing a wide pith surrounded by a central cylinder which has internal as well as external phloem. The method of departure of the leaf-trace from the cylinder is particularly noteworthy. It differs from the condition found in any of the other Osmundaceae in that the continuity of the whole central cylinder is interrupted, and the tissue of the pith becomes directly continuous with that of the cortex, and not, as in the other forms, with the pericycle-parenchyma only. The leaf-trace thus goes off in a fashion very similar to that found in *Adiantum pedatum* or any other of the ordinary siphonostelic ferns.

*Osmundites Gibbiana* of Kidston and Gwynne-Vaughan, *O. Dowkeri* of Carruthers, and *O. chemnitzensis* of Unger closely resemble one another in stelar structure. The general type of cylinder is very similar to that found in *O. regalis* or *T. hymenophylloides*. Foliar gaps are always present, though they are usually rather narrow, and the stem-bundles are consequently close together.

In *O. Dunlopii* of Kidston and Gwynne-Vaughan, however, a nearly continuous and unbroken ring of xylem appears at first sight to be present around the pith. The figures presented by the authors show this ring to be deeply constricted at intervals, and it seems entirely possible that very narrow 'rays', such as we have described in several species of *Osmunda* and *Todea*, might have originally occurred at these constrictions but might be undemonstrable in the present indifferent state of preservation. There are, moreover, a number of actual breaks in the ring which, according to these investigators, are probably due to accident. Some of these may also possibly represent true foliar gaps, or 'rays'. Several figures are presented showing the departure of the leaf-trace from the cylinder. They strikingly suggest the condition in some of the forms which we have above described, where the gap does not appear for some time after the trace has broken away from the stele. It is noteworthy that where the trace is figured as just departing from the cylinder there are but slight indications of a gap, but that

where it is well out into the cortex a deep constriction appears opposite to it in the xylem ring. Thin serial sections were of course impossible to obtain, as in the living species. On the whole, it seems very probable that we have here to deal with a form somewhat resembling *T. superba*, where foliar gaps are always present, though sometimes so narrow that they are hard to make out. It would not be at all surprising if in the process of fossilization the structure of these rows of thin-walled cells should be entirely lost to view. The theory that such a form as *O. Dunlopii* has been derived from one with a stele of the type represented by *Thamnopteris Schlechtendalii*, by the gradual alteration of the central tracheides into pith-parenchyma, is at present supported by too little evidence to warrant its acceptance. Most probably, if this were the course of evolution, the transitional forms between the two types would show leaf-traces departing without the formation of gaps in the xylem. These transitional forms are lacking, however, and, as we have shown above, wherever there is a ring of xylem around a pith of parenchyma, a departing leaf-trace, either at or soon after its separation from the stele, subtends a foliar gap.

As far as it goes, therefore, the fossil evidence seems to support the view that foliar gaps are a primitive feature for the Osmundaceae. This testimony is not entirely sufficient, however, and we must turn to our other source of information, the structure of the conservative regions of the living members of the family.

Of these portions of a plant which retain ancestral features, the seedling has long been recognized, in connexion with the theory of recapitulation, to be one of the most important. The structure of the young plant in various members of the Osmundaceae has been investigated by Leclerc du Sablon, Faull, Seward and Ford, and Chandler. The first of these writers (8) worked out the structure and development of the vascular system in a young specimen of *O. regalis*. He found that there are originally two separate strands of xylem which fuse into a single protostele, surrounded by phloem. In the centre of this appears very soon a cluster of parenchyma-cells constituting a pith. Whenever a leaf-trace leaves the cylinder, it causes a break, which is soon repaired, in the continuity of the xylem ring. A little higher up in the stem, however, where the departing leaf-traces are numerous, gaps are formed more rapidly than they become closed, and the ring is consequently broken into a number of bundles, separated by 'rays' or foliar gaps. In no case, after the stele had assumed the tubular condition, was the absence of a foliar gap observed.

Faull (9), who investigated young plants of *O. cinnamomea* and *O. Claytoniana*, found a very similar state of affairs. The young stele possesses a continuous ring of xylem, which, however, is *always* broken at the departure of a leaf-trace. This break is subsequently closed, as in *O. regalis*.



Seward and Ford (5) observed conditions in a young plant of *T. hymenophylloides*. They remark that in its essential features it agrees with *O. regalis*, as described by Leclerc du Sablon. After the appearance of the young pith, however, these authors observed that the first leaf-traces did not necessarily cause a break in the xylem ring, though well-marked gaps were formed by all the subsequent traces. The figure which they present of such a gapless condition resembles very much the early stage in one of those cases of delayed gaps to which we have above called attention. Another section, through the very base, or youngest part, of a mature stem, shows a ring of xylem broken at one point by the departure of a leaf-trace, thus presenting a state of affairs identical with that found in the species of *Osmunda*. These observations of Seward and Ford have been rather widely cited by other authors as evidence that the primitive method of departure of the leaf-trace in the Osmundaceae is a gapless one. Careful serial sections should be made through the region in question, however, before we may feel sure of the actual state of affairs.

Chandler (10) studied young plants of *T. hymenophylloides* and *T. Frazeri*. He simply states that his results in the former species confirm those of Seward and Ford, but gives no detailed account of the anatomy of the stem. In *T. Frazeri* he investigated only the very young stem, before the appearance of the pith. The single leaf-trace whose departure he observed was simply constricted off from the protosteles. He examined the 'seedlings' of a large number of species of ferns and found that in every case, where the young stele is tubular, the departure of a leaf-trace causes a break in the continuity of the xylem ring.

The writer was able to examine the stems of young plants of *O. regalis* and of *O. cinnamomea*. They were too old, however, to show the continuous ring of xylem described by Leclerc du Sablon and Faull. Wherever the trace leaves the cylinder, in either of the two species observed, a wide gap results (Fig. 9).

The slender bases of mature stems of *T. hymenophylloides* and of *T. superba* were also examined, and in every case pronounced, though narrow, gaps were observed. Fig. 10 shows this young condition in the former species. Several gaps are visible, as well as two leaf-traces in different stages.

As far as the evidence from the young plant goes, therefore, it seems clearly to sustain the view that leaf-gaps are primitive structures in the Osmundaceae. Kidston and Gwynne-Vaughan, however, have used the structure of the 'seedling' to support the theory that the original condition in the family is a gapless ring of xylem. They cite the observations of Seward and Ford on *T. hymenophylloides*, which, as we have seen, would be much more reliable had they been made from a study of careful serial sections; those of Chandler on *T. Frazeri*, which could have nothing to do

with the question of leaf-gaps, as the plants he examined had not progressed beyond the protostelic stage; and of Faull on *O. cinnamomea* and *O. Claytoniana*, which distinctly show that, although the young condition is a tubular stele, the continuity of the xylem ring is *always* broken at the departure of a leaf-trace.

Another region of the plant which is rapidly coming to be recognized as very retentive of ancestral characters is the vascular supply of the leaf. The occurrence of centripetal wood in the leaves of *Cycas* and *Prepinus*, and in the cotyledon of *Ginkgo*, when it has almost, if not quite, disappeared from the rest of the plant, are good illustrations of this. A still more striking case was recently investigated in this laboratory by Mr. A. J. Eames (11), who has found centripetal xylem in the vegetative and reproductive leaves of species of *Equisetum*, though it disappeared ages ago from the stem of the ancestors of living *Equiseta*.

In the Osmundaceae the leaf-bundle is somewhat like a flattened arch in shape, with incurved ends, and consists of a band of xylem elements more or less completely surrounded by phloem. It thus presents a rough resemblance to a portion of a siphonostelic central cylinder. It was thought that the relations of the vascular supply of the pinnae to this leaf-bundle would be of interest as showing the probable primitive method of departure from the stele of a foliar trace. The structure of the bundle in the rachis was consequently investigated in the three species of *Osmunda*, and in the four species of *Todea* already mentioned in this paper.

In all the species, traces to the pinnae go off from the leaf-bundle near the incurved ends of the arch. In *O. regalis* the first indication of this separation is the bending out of the arch at either end. This progresses so far that the xylem is broken at the adaxial end of the young trace. Such a condition is shown in Fig. 11. The trace to the pinna remains connected at its other end with the foliar strand for a long time, however, even until the gap in the leaf-bundle becomes closed up again behind it. Fig. 12 shows this state of affairs, which would seem to indicate that the trace has gone off without leaving a break in the xylem. The gap is thus a very short one. Through it, however, the fundamental tissue inside the bundle, or 'pith', becomes continuous with that outside the bundle, or 'cortex'. This cannot be seen at any one height, however, for the ground-tissue between the arms of the pinna-trace is cut off by the closing stele from its connexion with the 'pith' before it becomes continuous with the 'cortex' by the complete separation from the leaf-bundle of the vascular tissue of the free end of the trace. At a certain level, therefore, there is apparently an island of fundamental tissue completely surrounded by vascular elements.

The rachis of a leaf of a very young plant of *O. regalis* was also examined. Though as yet very small, and composed of but few cells, the



leaf-bundle shows two well-marked gaps opposite the traces departing to the pinnae.

In *O. cinnamomea* the very small vascular supply of the pinna leaves the foliar bundle in a curious fashion. The arch first bends outward near its ends, as in *O. regalis*. In this case, however, the trace, consisting of the xylem-arch with its included phloem and stelar parenchyma, is constricted off, and the gap behind it closed, before the xylem of the two bundles separates. Fig. 13 shows the condition of affairs before the gap has entirely disappeared. The trace to the pinna now breaks away from the leaf-bundle, and when seen at this height apparently causes no interruption in the xylem of the leaf-stele. The fundamental tissues on the inside and on the outside of the main bundle do not become continuous at this point as they do in *O. regalis*, a gap being formed in the xylem-ring only, just as in the stem, without breaking through the entire cylinder. This gap is an oblique perforation in the wall, and the interruption in the ring of wood can be seen at no one level.

In *O. Claytoniana* the condition of affairs is somewhat more simple. The sides of the arch bend out as before, but in this case the trace breaks away quickly, and both its ends become free at about the same time. A gap is formed in the xylem only, the rest of the stelar tissue being merely constricted opposite the point of departure of the trace (Fig. 14). The xylem gap in this case is much longer than in the other two species, and does not close up for a considerable distance.

In *Todea barbara*, the rachis is much stouter than in the filmy members of the genus, and the foliar bundle is consequently large. The trace of the pinna arises near the sides of the arch, as before, and, as in *O. regalis*, becomes free at its adaxial or lower end much sooner than at its upper one. Since the gap does not speedily close, however, the internal and external fundamental tissue become freely continuous through it. Fig. 15 shows the stage in the departure of the trace before it becomes entirely free. The break in the vascular tissue of the leaf-stele is not repaired till long after the separation of the pinna from the rachis.

The leaves of the other three species of *Todea* are characterized by their so-called 'filmy' habit. The lamina is very thin, and possesses neither intercellular spaces nor stomata. The vascular supply to the leaf is consequently much reduced.

In *T. hymenophylloides* the strand departing to the pinna leaves a well-marked gap in the leaf-stele through which the 'pith' and 'cortex' become continuous (Fig. 16). This gap, like the one in *T. barbara*, remains open for a considerable distance up the rachis.

In the case of *T. Frazeri*, a clear break in the xylem is caused by the outgoing trace of the pinna, but the continuity of the vascular ring is not quite interrupted. A deep constriction occurs in it, however, along which

the internal and external endoderms almost meet (Fig. 17). The state of affairs here is somewhat intermediate between that found in *O. Claytoniana* and that in *T. hymenophylloides*.

In the last species, *T. superba*, the trace to the pinna is very small. Opposite to its point of departure, a gap is formed in the leaf-stele around the ends of which the internal and external endoderms become continuous. This gap is not wide enough, however, to permit the connexion through it of the 'pith' with the 'cortex', the two rows of endodermal cells lying side by side, as shown in Fig. 18.

In all the species examined, therefore, gaps in the leaf-stele at the point of departure of the vascular supply of a pinna were observed, though in *O. regalis* and *O. cinnamomea* they are very short, and at certain heights apparently absent.

There seems to be a general relation between the size of the trace to the pinna and the character of the gap which it subtends in the leaf-bundle. The pinnae of *O. regalis* are fewer, and consequently larger than those of the other two species in the genus, and the gaps subtended by their traces are consequently much wider. *T. barbara* and *T. hymenophylloides*, also, whose pinnae are rather large in proportion to the leaf, possess more prominent 'pinna-gaps' than does *T. superba*, where the primary divisions of the frond are much smaller. A leaf of *Dicksonia antarctica* was looked at in this connexion. Near the middle of the leaf the pinnae are large, but diminish in size towards the base till they become very small. It was found on examination that the traces to the large pinnae left wide gaps, through which the inner and the outer fundamental tissue became freely continuous, while in the case of the small pinnae the gap was minute and affected the xylem only, not breaking through the whole stele.

The evidence from the leaf-trace, therefore, distinctly confirms that derived from fossil forms and from the structure of the young plant, in pointing towards the primitive existence of the foliar gap in the Osmundaceae.

#### CONCLUSIONS.

The ancestry of the Osmundaceae, as we have above remarked, is much a matter of doubt. From such fossil evidence as we possess, however, that bears on the matter, and from what we have observed in the structure of the young plant and of the leaf, both very tenacious of primitive characters, it seems reasonably certain that in all those ancient members of the family which possessed a true parenchymatous pith, the leaf-trace, as it departed from the stele, always formed a gap in the xylem, and very probably, as indicated by the structure of *Osmundites Skidegatensis*, in the whole vascular ring. The evidence also seems to point towards the correctness of Jeffrey's and Faull's view of the Osmundaceae as a reduction series, for the occurrence, in so many cases where it can hardly be explained

on physiological grounds, of the strong tendency for a foliar bundle to cause a gap in the stele from whence it arises, seems most easily attributable to the persistence, in a reduced condition, of an ancestral feature. The Osmundaceae, therefore, must apparently be included in Jeffrey's phylum Pteropsida. They give strong support to the general principle proposed by him that in all the members of this great group, the departure from a tubular stele of a vascular strand, no matter how much reduced, which supplies a foliar organ, causes a break in the continuity of the xylem of the stele.

#### SUMMARY.

1. In the mature stem of the six species of the Osmundaceae which were studied, a foliar gap, or break in the continuity of the stelar ring of xylem, was formed at the departure of the leaf-trace from the cylinder. A number of cases were observed, however, in all the species but one, where the gap did not become complete for some time after the departure of the leaf-trace, which thus at first seemed to go off in a gapless manner. In no case which could be thoroughly investigated by complete serial sections, was there found to be the real absence of a foliar gap.

2. None of the fossil Osmundaceae, so far as we know them, which possessed a true parenchymatous pith, present a clear instance of the departure of a leaf-trace which does not cause a break in the xylem-ring. In *Osmundites Dunlopi*, which seems at first sight to be an exception to this statement, we probably have a form with very short and narrow gaps, which have been largely obliterated in the process of fossilization.

3. In all the young plants of the Osmundaceae which have been investigated by the writer and others, foliar gaps have been observed from the very youngest condition, with the barely possible exception of the very early stages in *Todea hymenophylloides*. Our evidence in this species, however, is as yet insufficient.

4. In the seven species studied, the departure of the trace to the pinna, or primary division of the frond, was always found to leave a gap in the arch-shaped leaf-bundle. In three species this gap affected only the xylem, but in the remaining four a complete break in the vascular tissue was made. In only three of the latter, however, was the gap wide enough to permit of the connexion through it of the fundamental tissue on the inside with that on the outside of the leaf-bundle. The width and character of the gap in all instances seemed to depend largely on the relative sizes of the pinna-trace and the main bundle.

5. From such fossil evidence as is available, therefore, and from the structure of the young plant and of the foliar strands, both of which are known to be conservative of ancestral characters, it seems quite clear that the presence of foliar gaps is a primitive feature in the Osmundaceae.

6. The Osmundaceae are therefore very properly placed in Jeffrey's phylum Pteropsida, the members of which are primitively phyllosiphonic.

I am under obligations to Mr. A. J. Eames and to Professor Jeffrey for their kindness in supplying material, and wish to express to the latter my sincere thanks for advice during the course of the work.

This investigation was carried on in the Phanerogamic Laboratories of Harvard University.

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### DESCRIPTION OF PLATES XI AND XII.

Illustrating Mr. Sinnott's paper on Leaf-gaps in Osmundaceae.

Fig. 1. *Osmunda cinnamomea*. Leaf-trace leaving the cylinder, apparently without making a complete gap. × 40.

Fig. 2. *O. cinnamomea*. The same trace as in Fig. 1 a little higher up in the stem, showing the well-marked gap subtended by it. × 40.

Fig. 3. *O. regalis*. Leaf-trace departing from the cylinder in an apparently gapless fashion. × 40.

Fig. 4. *O. regalis*. The narrow gap eventually subtended by the trace shown in Fig. 3. × 200.

Fig. 5. *Todea superba*. Leaf-trace departing without making a complete gap. × 60.

Fig. 6. *T. superba*. The same trace as in Fig. 5, showing the well-marked gap which finally appears. × 40.

Fig. 7. *T. hymenophylloides*. Apparently gapless condition at the departure of a leaf-trace.  $\times 40$ .

Fig. 8. *T. hymenophylloides*. The narrow gap which eventually appears opposite the trace shown in Fig. 7.  $\times 200$ .

Fig. 9. *Osmunda regalis*. Section of a young plant, showing two departing traces and their subtended gaps.  $\times 60$ .

Fig. 10. *Todea hymenophylloides*. Section through the slender base of a stem, showing two traces in the early stages of departure, and three foliar gaps.  $\times 40$ .

Fig. 11. *Osmunda regalis*. Showing the gap formed in the leaf-bundle by the trace departing to the pinna.  $\times 40$ .

Fig. 12. *O. regalis*. Condition of affairs slightly higher up than in Fig. 11, showing the apparent absence of a gap at the departure of a trace to a pinna.  $\times 40$ .

Fig. 13. *O. cinnamomea*. Trace to the pinna being constricted off from the leaf-bundle.  $\times 60$ .

Fig. 14. *O. Claytoniana*. 'Pinna-gap,' affecting only the xylem of the leaf-bundle.  $\times 40$ .

Fig. 15. *Todea barbara*. Early stage in the departure of the trace to the pinna, showing complete break in the leaf-stele.  $\times 40$ .

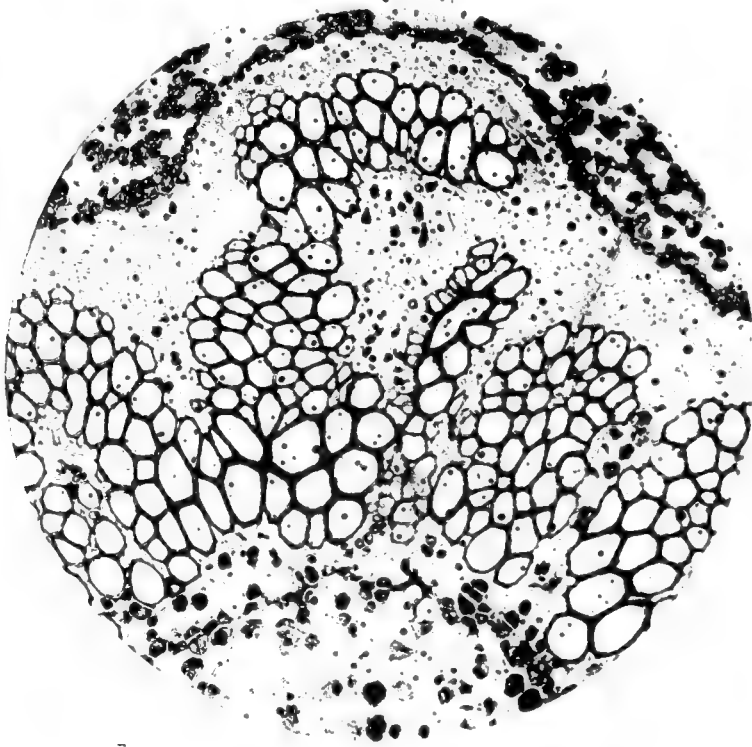
Fig. 16. *T. hymenophylloides*. Leaf-bundle, showing two 'pinna-gaps' and the trace subtending one of them.  $\times 40$ .

Fig. 17. *T. Frazeri*. Leaf-bundle with two 'pinna-gaps' which do not quite break through the stele.  $\times 40$ .

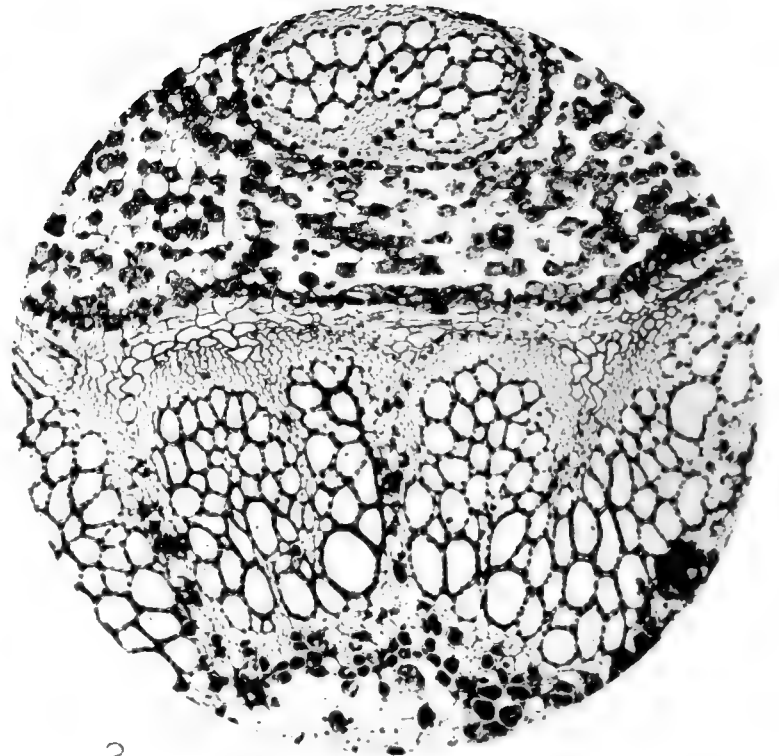
Fig. 18. *T. superba*. Leaf-bundle, with two traces departing to the pinnae, showing the complete gaps in the leaf-stele.  $\times 40$ .



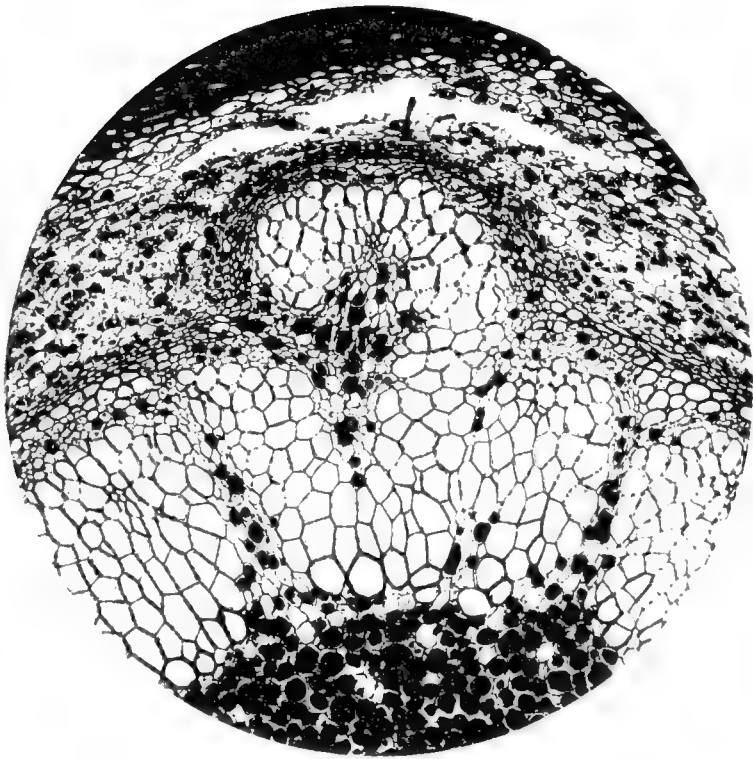




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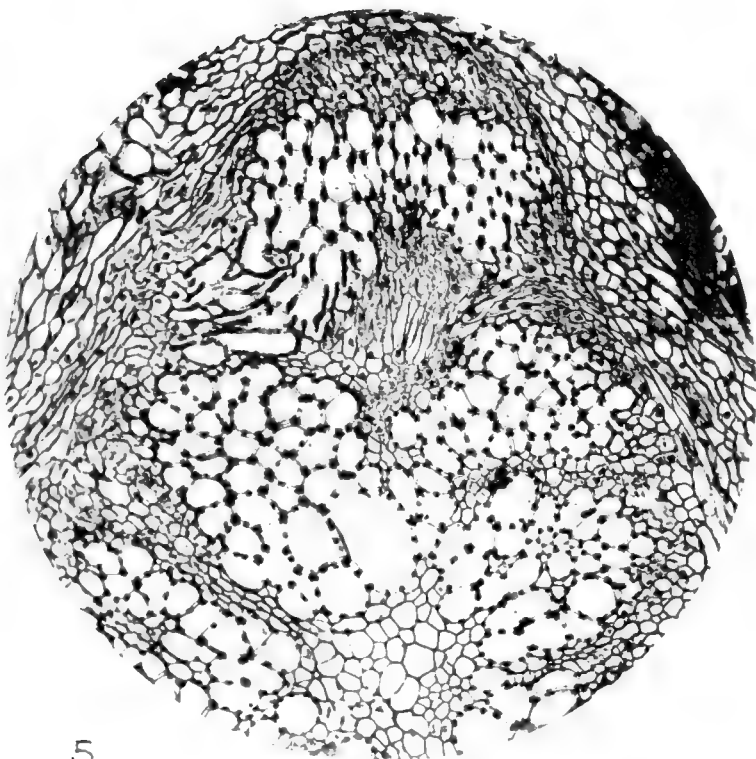
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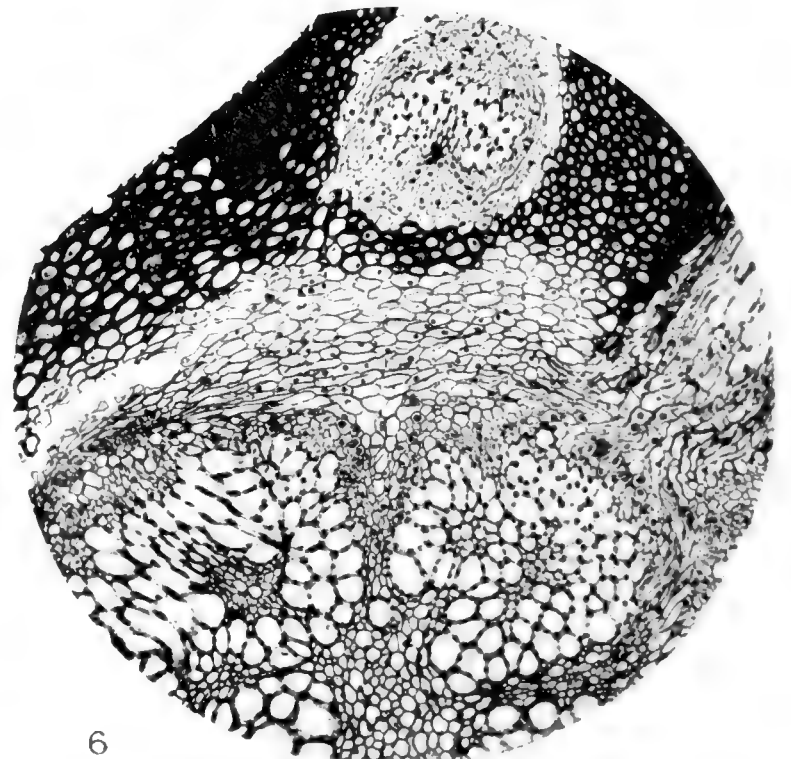
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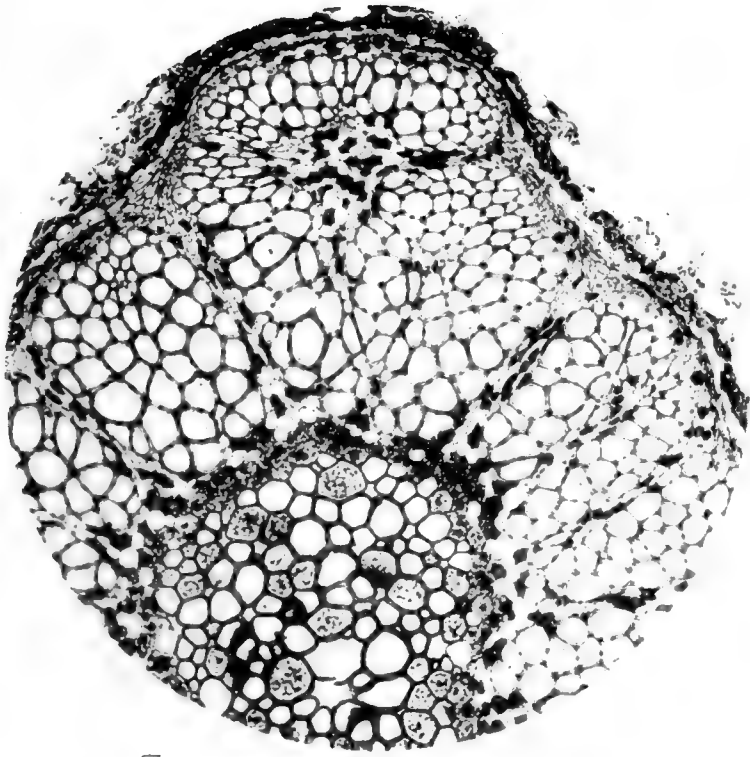
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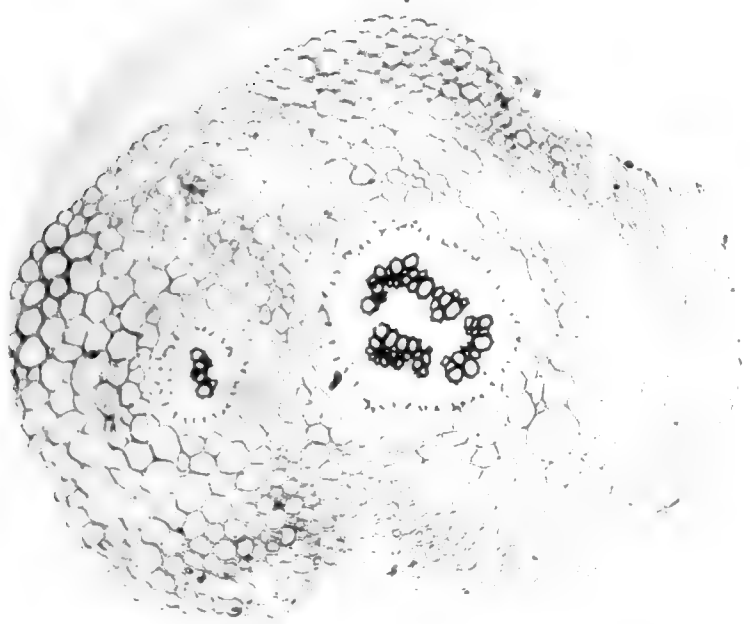
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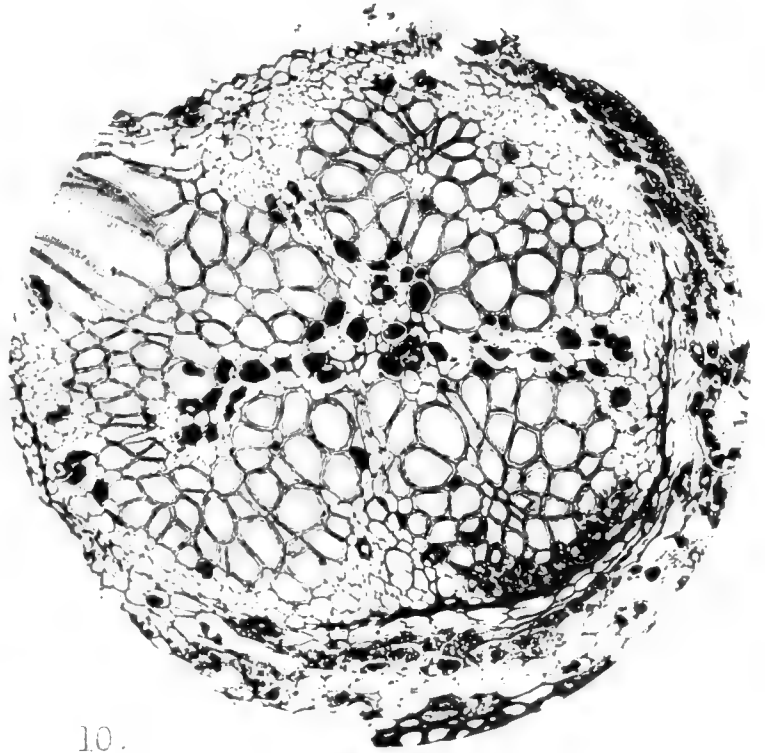
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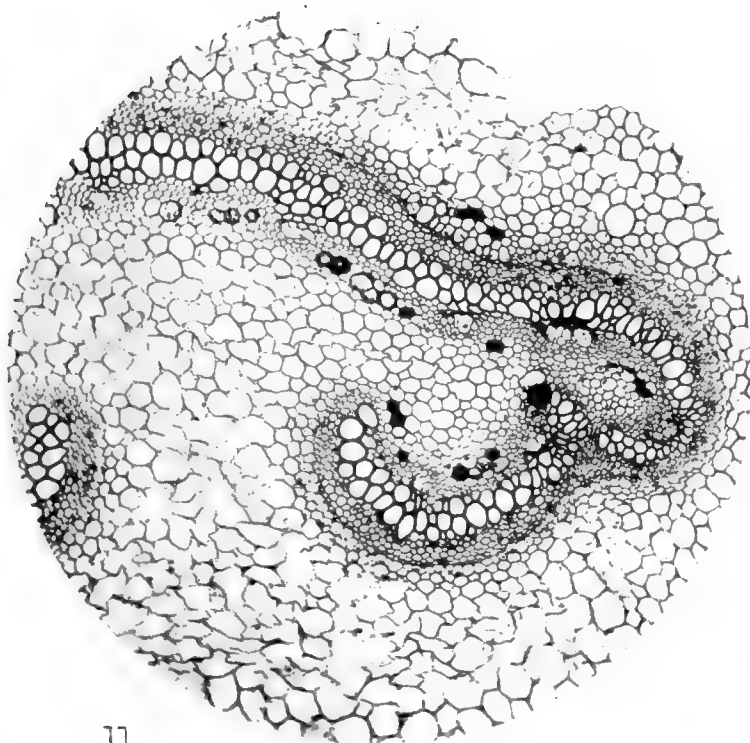
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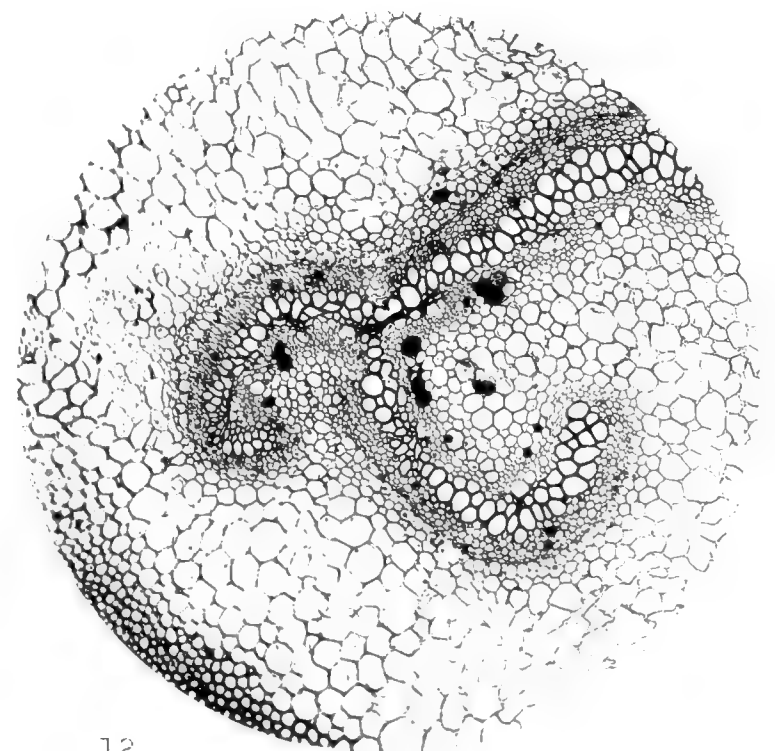
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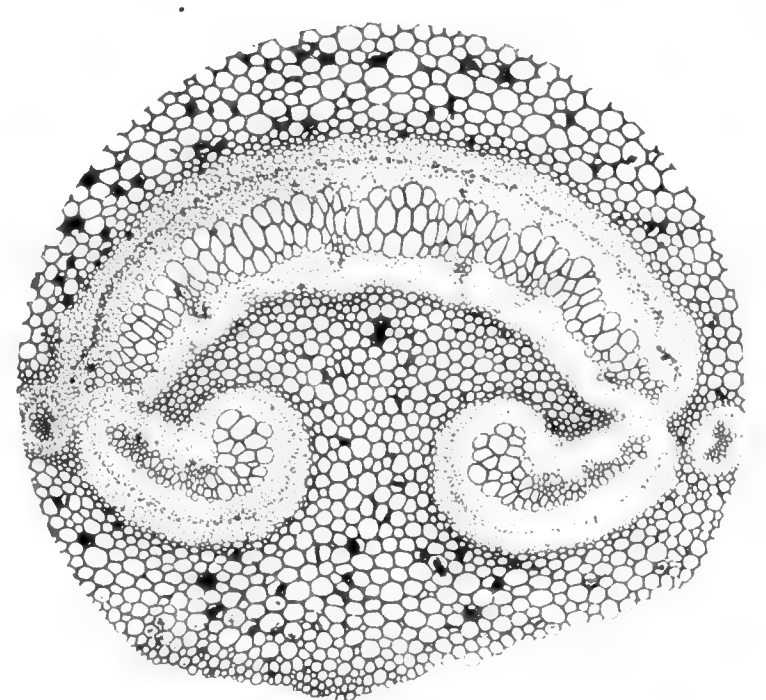
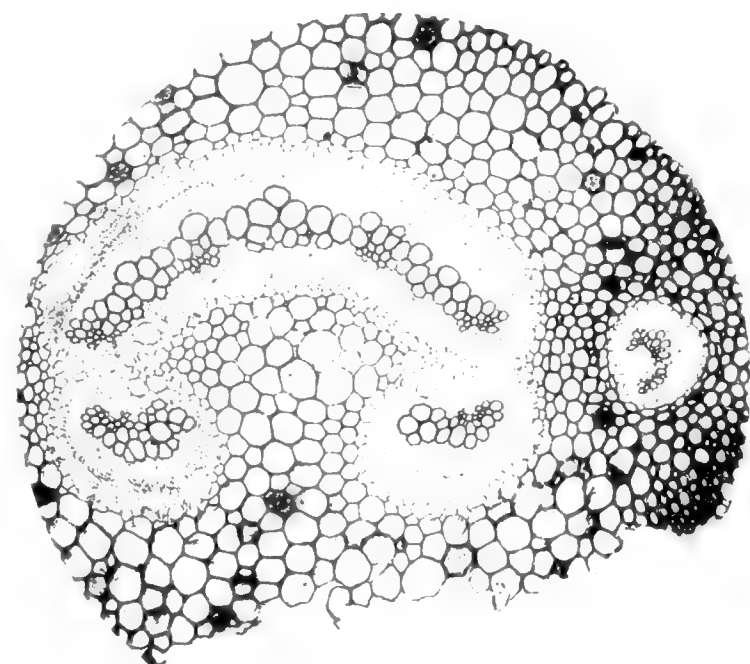
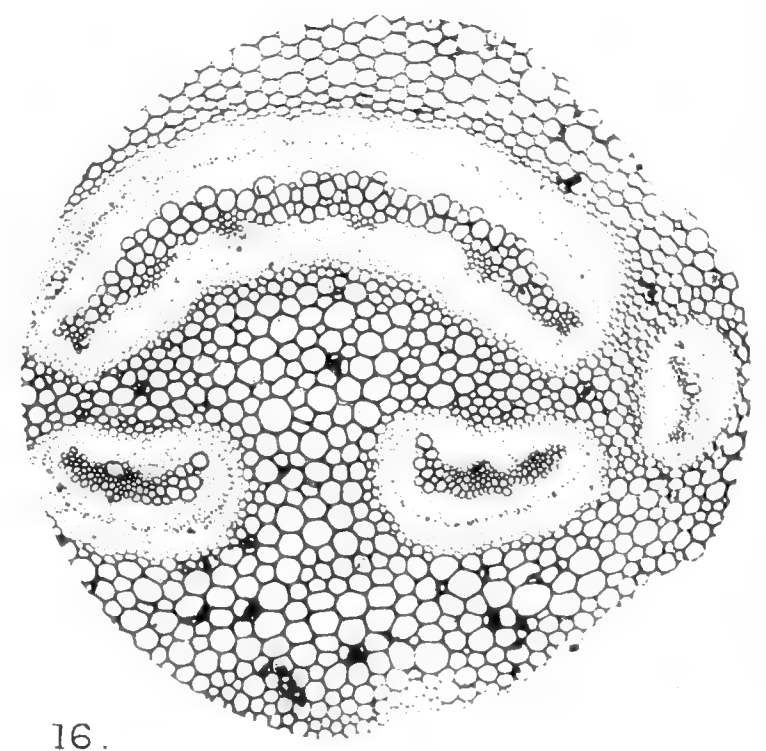
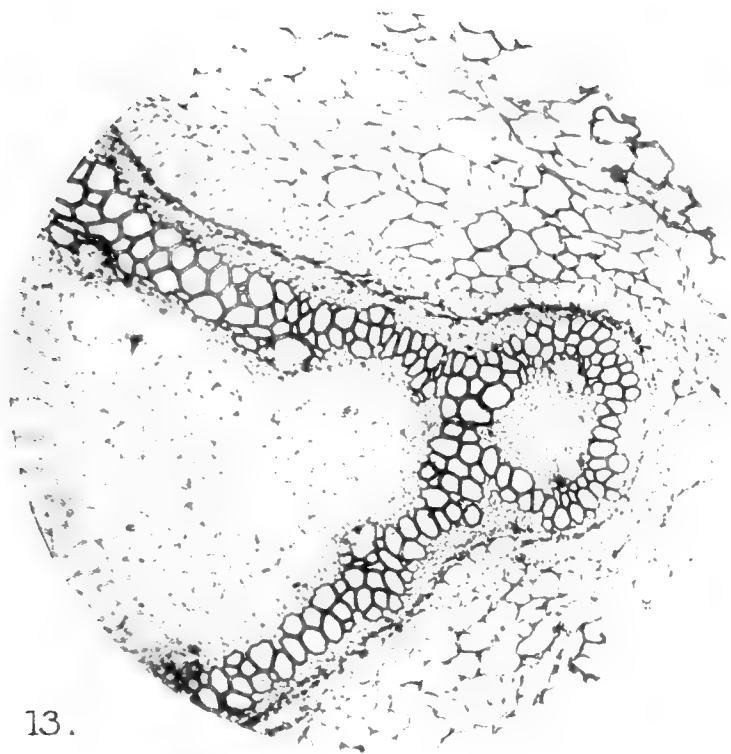


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12.









# The Distribution of the 'Bars of Sanio' in the Coniferales.<sup>1</sup>

BY

ELOISE GERRY, M.A.

With Plate XIII.

THE 'bars' or 'folds' of cellulose, which, when stained with haematoxylin, are especially obvious as horizontal or more or less semicircular markings in the tracheide walls of a radial section from such a Conifer as *Pinus silvestris*, L., were described by Sanio in 1872. Although these structures were named the 'Bars of Sanio' from him, and in spite of the fact that he declares that 'diese scheibenförmige Verdickung der Scheidewand ist bisher übersehen',<sup>2</sup> we find that in 1849 Göppert had indicated their presence in two drawings, one of *Cryptomeria japonica*, and the other of *Cupressinoxylon aequale*, which he used to illustrate his 'Monographie der fossilen Coniferen'.<sup>3</sup>

Little attention, however, has been directed towards the bars of Sanio, although their presence in *Pinus silvestris*, L., is indicated in the chapter concerning coniferous woods in Zittel's 'Handbuch der Palaeontologie'.<sup>4</sup> Again, in 1898, Dippel mentions them in his chapter entitled 'Entstehung der behöften Poren'.<sup>5</sup> But here, as before, *Pinus silvestris*, L., is the only species cited.

It is the purpose of this paper to discuss their distribution from observations made upon all the genera of living Conifers, together with some fossil forms. For this study radial sections 5  $\mu$  in thickness were used. The bars, since they are composed of cellulose, take a blue stain with haematoxylin, and therefore stand out clearly from the red background obtained by the general staining of the tracheide walls with safranin. In every case they appear most abundantly in the thin-walled tracheides in the spring-wood of the stem and in the root. Often when they are absent or difficult to discern in the stem they are found without difficulty in the root.

<sup>1</sup> Contributions from the Phanerogamic Laboratories of Harvard University, No. 21.

<sup>2</sup> Jahrbücher für wissenschaftliche Botanik, Bd. 9, S. 78, 1873-1874.

<sup>3</sup> Leiden, bei Arnz & Co., 1850.

<sup>4</sup> II. Abtheilung, München u. Leipzig.

<sup>5</sup> Das Mikroskop, zweiter Theil, Braunschweig, von F. Vieweg & Sohn.



The description of the abundance and modification of the bars of Sanio in each genus of the living Conifers, taken in the order of classification of Engler and Prantl, is as follows. Beginning with the Abietineae, we find in *Pinus* numerous straight bars between pits which are, for the most part, in single uncompressed rows. In *Picea canadensis* the pitting, although mainly uniseriate, shows a few opposite pits. The greater part of the bars are straight, but instances of curved and, rarely, even of double bars appear. In *Pseudotsuga taxifolia* the general uniseriate pitting, with exceptional cases of opposite pitting, is obscured by tertiary thickening. Nevertheless the straight or occasionally slightly curved or double bars are visible in abundance. *Cedrus Libani* has a closely packed uniseriate and opposite pitting with straight bars. *Larix occidentalis* likewise clearly shows numerous bars of Sanio. In *Tsuga canadensis* (root) abundant straight or curved and double bars are present with the, for the most part, opposite pitting. In *Keteleeria* species (root) we find both opposite and uniseriate pitting with a notable crowding and increase in the number of pits between the bars; here also the bars are straight, curved, and often double between the pits. In a similar way *Pseudolarix Kaempferi* has both uniseriate and opposite pitting with straight, as well as strongly curved, bars. *Abies balsamea*, the last of this group, with its generally uniseriate pitting and curved bars, is illustrated in Pl. XIII, Fig. 1.

In the Taxodineae also, the bars of Sanio are well developed. Although in the root of *Sciadopitys verticillata* pits without bars occur, yet, for the most part, curved bars, 'tilde' shaped bars, and even double bars are in good evidence. In *Cunninghamia sinensis* they are not so thick, but apparently have split and adhered to the edges of the pits. *Athrotaxus cupressoides* has single rows of pits and numerous bars. Likewise *Cryptomeria japonica* and *Glyptostrobus heterophyllus* show a sufficient number of bars to make their presence unquestionable. In *Sequoia washingtoniana* the pits are both uniseriate and opposite, often being somewhat oval in shape; the bars are generally straight. *Taxodium distichum* is characterized by a crowded, flattened, and often irregular pitting. The bars are straight, curved, and slanting. There are, moreover, some indications between the opposite pits of vertical bars perpendicular to the horizontal bars of Sanio.

Further evidence is derived from the Cupressineae. In *Chamaecyparis thuyoides* the pitting is uniseriate, and the straight, often double, bars are very abundant. *Callitris arborea* and *Biota orientalis* have uniserial pitting and numerous bars. In *Thuyopsis dolabrata* the uniseriate pits are not crowded, and the bars are not so numerous. *Libocedrus decurrens* has uniserial, rarely opposite, pits with both straight and curved bars. *Cupressus Goveniana*, with its single-rowed, open pitting, shows scattered bars—straight, curved, or double. *Fitsroya patagonica*, too, has uniseriate pitting

with curved, double bars. A photograph of a characteristic region of the stem of *Thuja plicata* is shown in Fig. 5.

The Podocarpaceae are an especially interesting group, for, although bars are present in all the members, there exists at the same time cases of a markedly compressed and crowded pitting without bars. In *Dacrydium cupressinum* (root) there are both uniseriate and opposite rows of pits; the bars—curved, straight, or double—are numerous. A photograph of a section of *Microcachrys tetragona* with its uniseriate pitting and numerous bars is shown in Fig. 3. *Saxegothea conspicua* (root), showing, from a characteristic region, its well-developed and unmistakable, although rather scattered, distribution of bars, appears in Fig. 4. *Podocarpus polystachya*, which has opposite as well as uniseriate pitting, manifests its prominently developed bars in the photograph shown in Fig. 2.

Lastly, the Taxineae give the concluding cases where bars of Sanio are present. Although in many cases the tertiary thickening obscures the bars, yet they are found as follows. In *Phyllocladus hymenophylloides* there are uniseriate and opposite pits, and curved, double bars. *Cephalotaxus drupacea* and *Taxus canadensis*, where the tertiary thickening is most highly developed, show, between the uniseriate pits, bars of Sanio, which, though not numerous, are unmistakable. *Torreya taxifolia*, with its opposite and uniseriate pits, shows a very marked development of straight, curved, and double bars.<sup>1</sup>

In definite contrast with the preceding are representatives of the two remaining genera of living Conifers, *Araucaria imbricata* and *Agathis australis*, which do not possess the bars of Sanio. *Araucaria imbricata* (stem) is shown in Fig. 6. Compressed pitting with a tendency to clusters of alternating pits is characteristic of the stems of both genera. In the root, on the other hand, the presence of uniseriate compressed rows of pits is rare in comparison with the general alternating and closely packed arrangement. The root appears to be one of the centres of the preservation of ancestral traits and is, in the preceding genera, the place of persistence and greatest development of bars, but in the roots of the Araucarineae even vestigial traces of bars are completely absent.

The conditions found in all the available fossil Conifers confirm this segregation of the genera with Araucarian affinities from the remaining tribes of Conifers which, as has been shown, all manifest the bars of Sanio. *Geinitzia Reichenbachi* with its uniseriate, open pitting shows no indication of bars. Neither does *Brachyoxylon*, which is shown in Fig. 7, nor a new undescribed genus closely related to it and very abundant in Mesozoic deposits. *Araucaryopitys americana*, illustrated in Fig. 8, with its rather compressed uniseriate pitting, also gives no evidence of bars. Even after special treatment such as bleaching with chlorine water fol-

<sup>1</sup> The bars of Sanio also appear in *Ginkgo biloba*, the only living member of the Ginkgoales.

lowed by staining with haematoxylin, although the pit membranes are stained blue, there is not the slightest indication of bars in any of the above-mentioned genera.

On the other hand, in *Prepinus statenensis*, where they were observed by Jeffrey,<sup>1</sup> they appear, as shown in Fig. 9, as prominent light bands. In an undescribed *Pityoxylon* from Martha's Vineyard the characteristic double bars are well illustrated by Fig. 10. In *Pityoxylon scituatense* they appear unmistakably as shown in Fig. 11. Again we find them in a fossil *Picea*, dug up with a mammoth in Alaska, where they stand out almost as well, after the usual staining, as they do in the living material. In *Sequoia Penhallowii*, Jeffrey, of the California Gold Gravels the bars are strikingly present as appears in Fig. 12. The fine state of preservation of all these fossil woods makes the presence of the bars perfectly obvious even in unstained sections such as those of *Prepinus statenensis*, Fig. 9, and of *Pityoxylon scituatense*, Fig. 11, or even in spite of the fact that, being cellulose, the bars have sometimes become decomposed and left transparent spaces such as appear in *Sequoia Penhallowii*, Fig. 12, and in *Prepinus statenensis*, Fig. 9.

#### CONCLUSIONS.

The distribution of the bars of Sanio as above described establishes a constant and useful diagnostic character in the determination of fossil woods. In woods with Abietineous affinities we always find bars of Sanio even though at the same time we may find more or less Araucarian-like pitting. But in the Araucarineae we never find bars, although in fossil forms such as the Araucariopityoideae and the Brachyphylloideae, we find Abietineous as well as Araucarian pitting.<sup>2</sup>

The presence of bars of Sanio in the Podocarpineae points with other evidence to the probability that they are more closely related to the Abietineae than to the Araucarineae. At least they may have sprung from a common branch, for the apparent relationship is strengthened by the presence of the tendency towards recapitulation and preservation in the root which indicates the primitive, rather than the recently acquired, character of the bars. The ancient character of the bars of Sanio is further emphasized by the fact that they occur in *Prepinus statenensis*, which lays an undoubted claim to primitiveness on the ground of its double foliar transfusion sheaths and centripetal wood. Therefore, judging from the fossil evidence and from the well-developed distribution at the present time, the bars of Sanio appear to be a definite and constant anatomical characteristic of all the Coniferales except the Araucarians.

<sup>1</sup> On the Structure of the Leaf in Cretaceous Pines. Ann. Bot., vol. xxii, April, 1908.

<sup>2</sup> Hollick and Jeffrey: Studies of Cretaceous Coniferous Remains from Kreischnersville, N.Y., p. 75. Mem. N. Y. Bot. Garden, No. III, May, 1909.

SUMMARY.

1. Bars of Sanio occur in thirty-five of the living genera of the Coniferales.

2. They do not occur in two genera of the living Conifers, namely, *Agathis* and *Araucaria*.

3. This distribution is confirmed by fossil evidence which shows the bars to be absent in Conifers of Araucarian affinities.

4. The presence of bars of Sanio is of practical use as a diagnostic character for fossil woods.

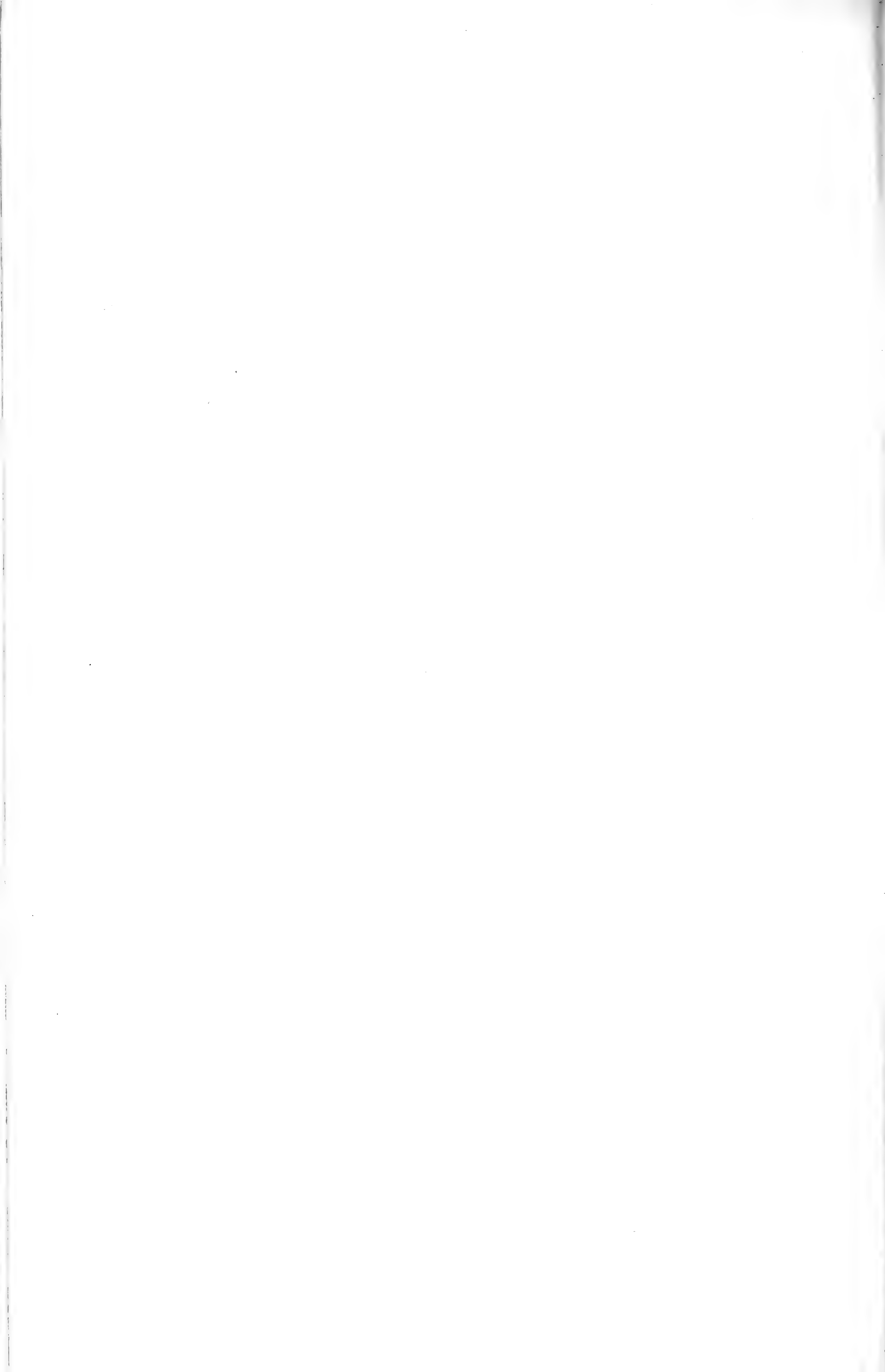
In closing the writer wishes to express her thanks to Prof. E. C. Jeffrey for his helpful advice, guidance, and generous provision of material.

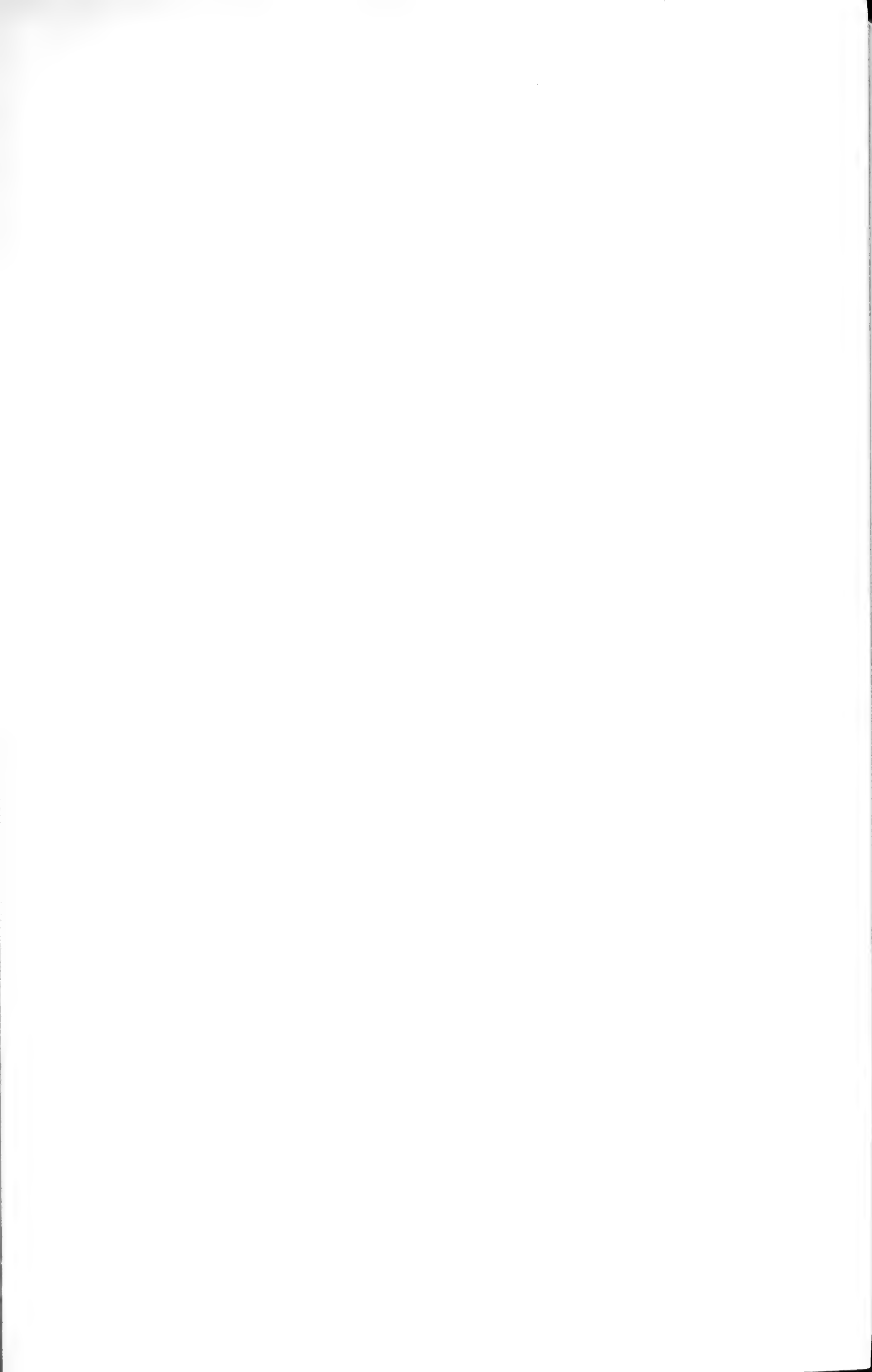
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DESCRIPTION OF PLATE XIII.

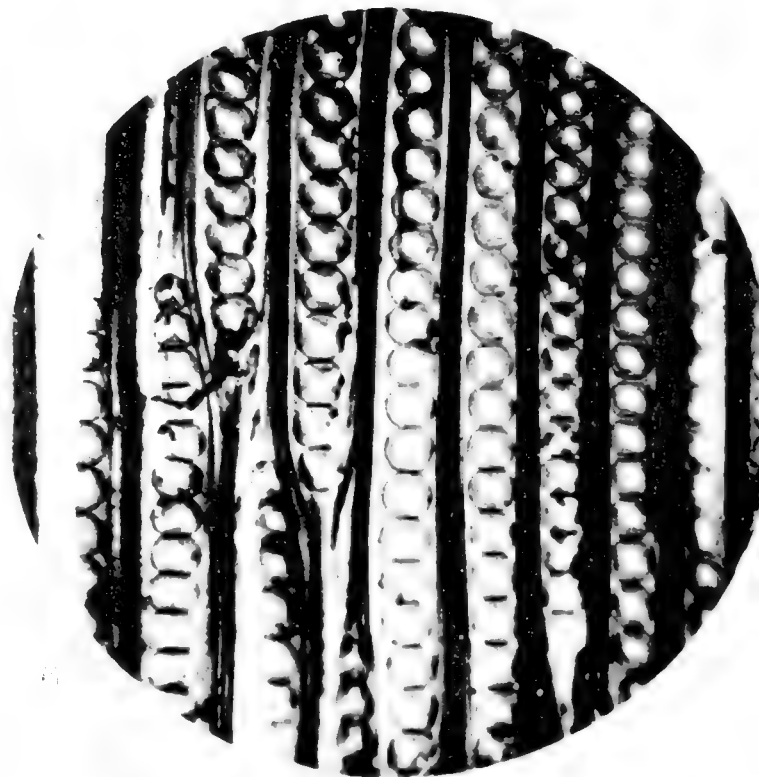
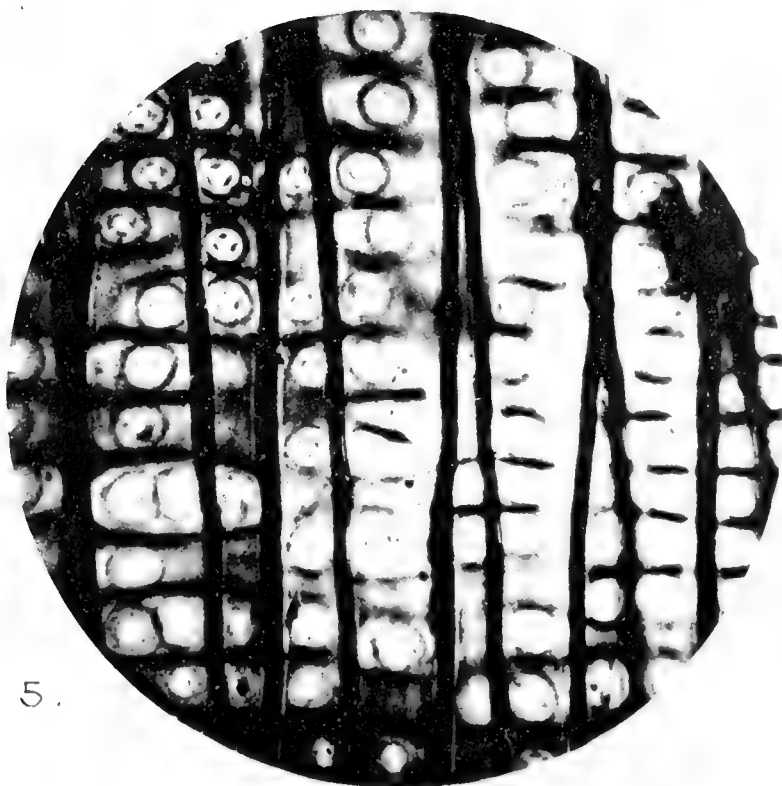
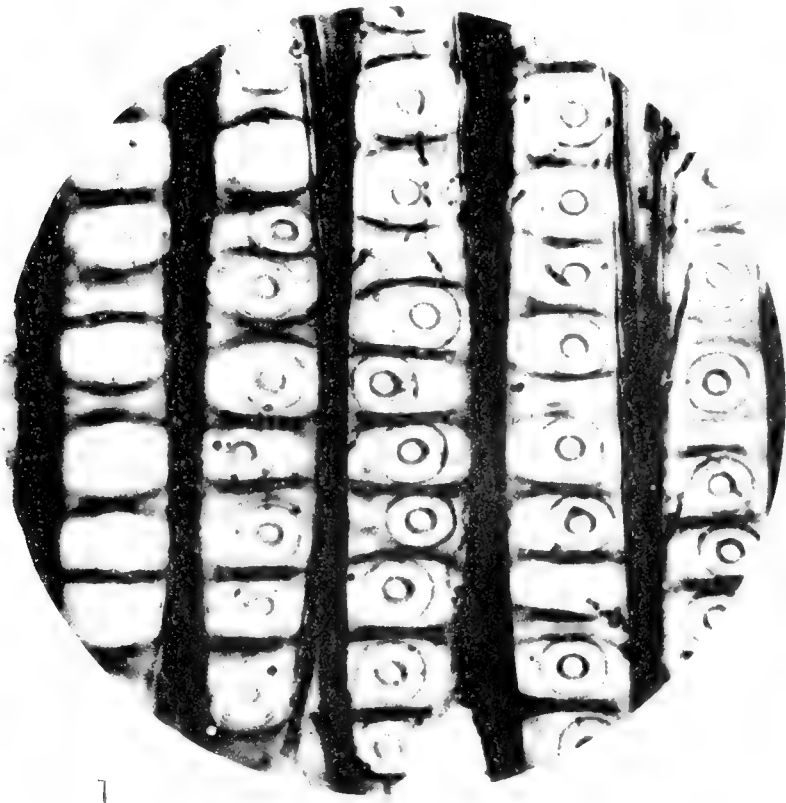
Illustrating Miss Gerry's paper on the 'Bars of Sanio'.

- Fig. 1. Radial section of the wood of *Abies balsamea*. × 180.  
Fig. 2. Radial section of the wood of *Podocarpus polystachya*. × 500.  
Fig. 3. Radial section of the wood of *Microcachrys tetragona*. × 500.  
Fig. 4. Radial section of the root of *Saxegothea conspicua*. × 500.  
Fig. 5. Radial section of the wood of *Thuja plicata*. × 180.  
Fig. 6. Radial section of the wood of *Araucaria imbricata*. × 180.  
Fig. 7. Radial section of the wood of *Brachyoxylon*. × 180.  
Fig. 8. Radial section of the wood of *Araucaryopitys americana*. × 180.  
Fig. 9. Radial section of the wood of *Prepinus statenensis*. × 500.  
Fig. 10. Radial section of the wood of *Pityoxylon* (undescribed). × 180.  
Fig. 11. Radial section of the wood of *Pityoxylon scituatense*. × 500.  
Fig. 12. Radial section of the wood of *Sequoia Penhallowii*. × 500.

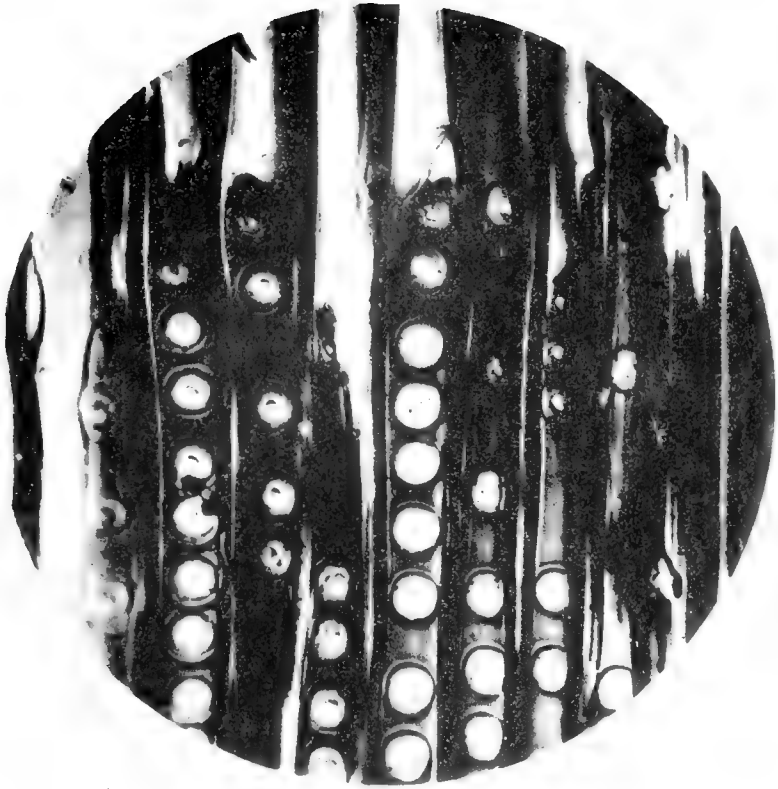








GERRY - BARS OF SANIO.



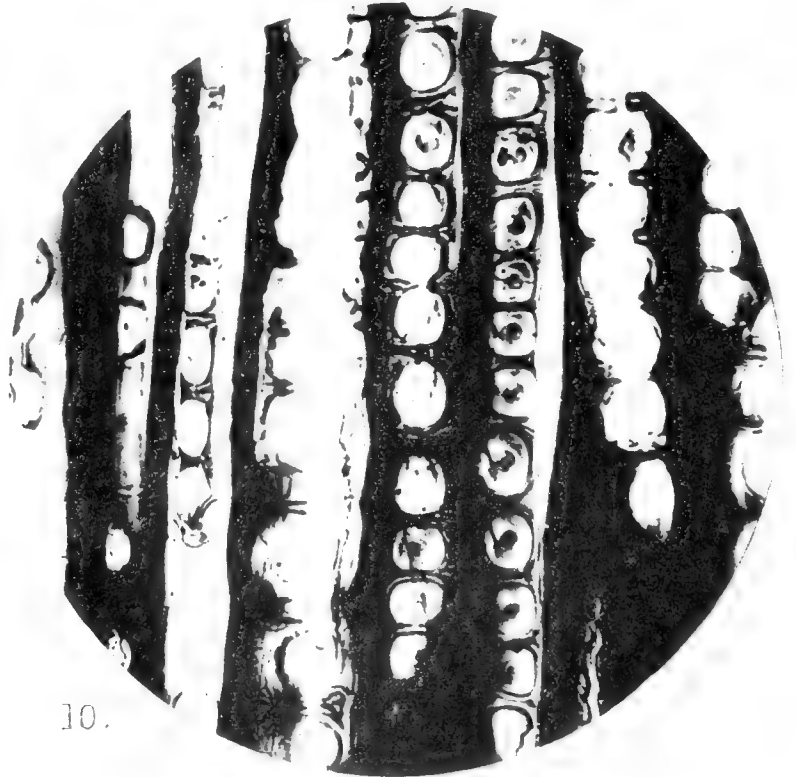
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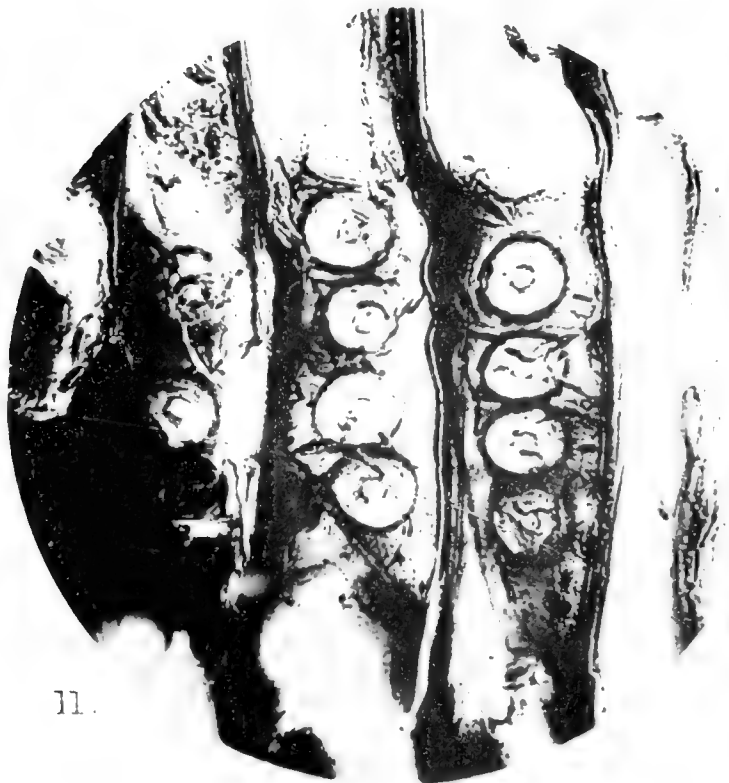
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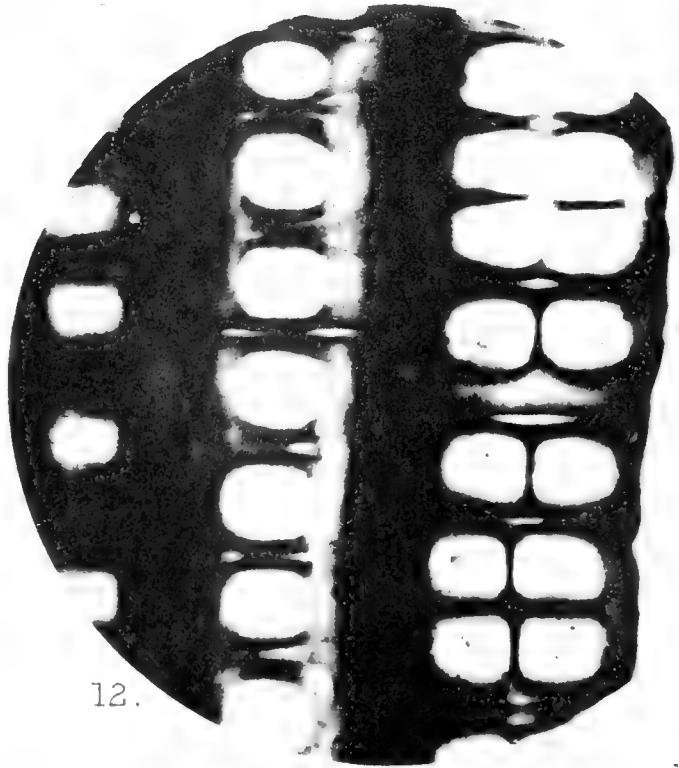
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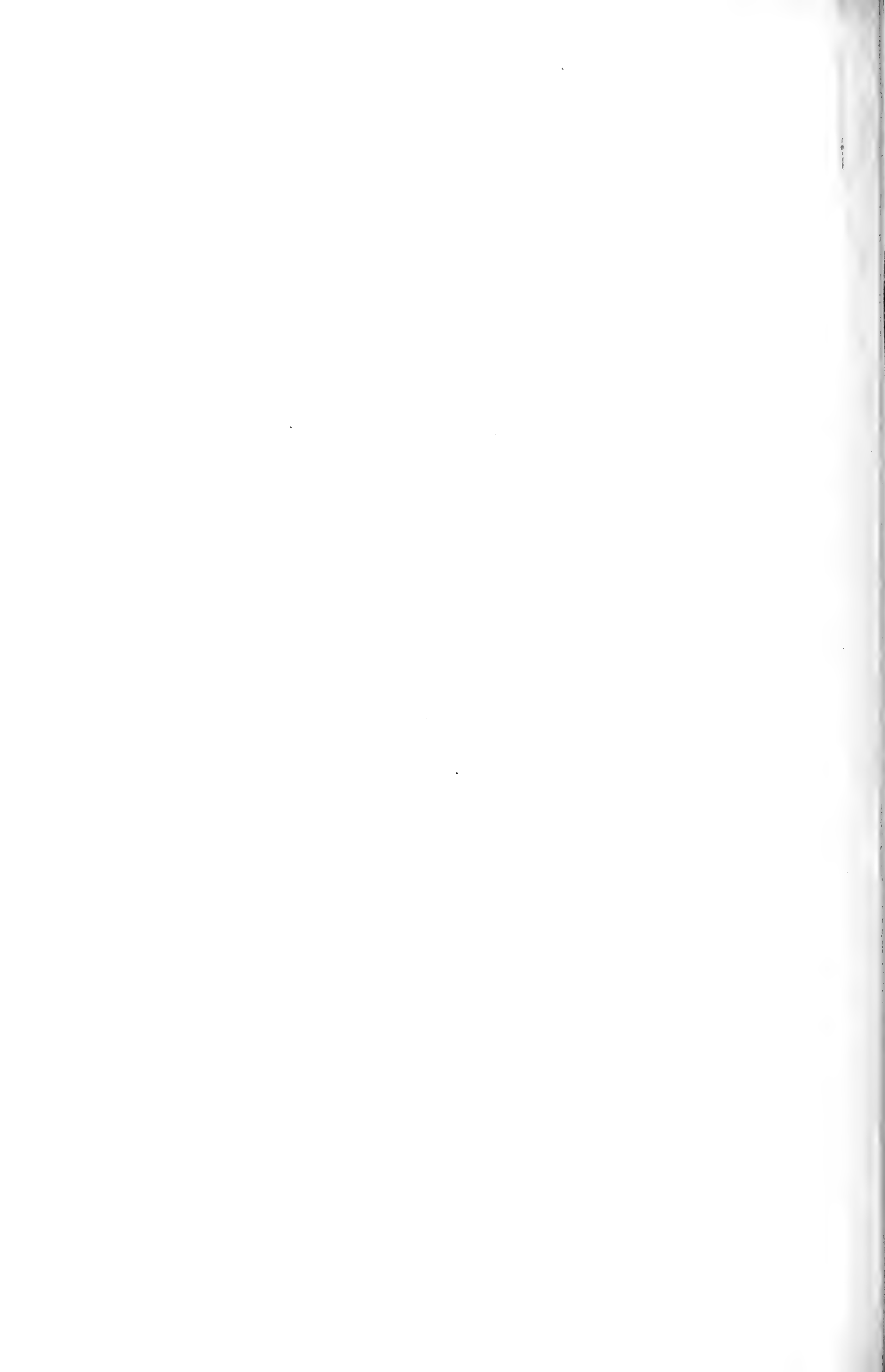
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11.



12.



# The Seedling Structure of certain Cactaceae.

BY

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*University of London, Goldsmiths' College.*

With eighteen Diagrams and nineteen Figures in the Text.

CONSIDERABLE importance has of late been attached by some observers<sup>1</sup> to the transition-phenomena in seedlings of Spermophyta, more especially with regard to the help given by them in the elucidation of phylogenetic problems. In view of the value thus assigned to seedling anatomy it seemed desirable to make as complete a study as possible of the seedlings of some well-defined families. The Cactaceae, with their marked adaptations to dry conditions, seemed to offer a profitable field for investigation, particularly as the form of the seedlings of this group bears so evident a relation to that characteristic of the adult.

Ganong,<sup>2</sup> in his comprehensive account of the morphology of Cactaceous seedlings, states that the progressive condensation of the embryos runs strictly parallel to the condensation in the adults—it has its lowest term in *Pereskia*, and reaches its highest in the almost globular Mamillarias; further, this condensation is due to adaptation to a habitat of increasing desert conditions. He suggests that the form of the adults, 'as it becomes more and more fixed and intensified, tends to work back into earlier and earlier stages in the ontogeny of the successive individuals; until, finally, a character adaptively acquired by the adults works back into the epicotyl . . . and finally into the embryo.' This 'working back into the epicotyl' may be comparatively rapid, but the impression of such features on the embryo seems very slow; this is 'due no doubt to the fact that the embryos have a set of activities of their own in their early life which keeps them from being too plastic to other influences working upon them'.

<sup>1</sup> Sargent, E.: Theory of the origin of Monocotyledons, founded on the structure of their seedlings. *Ann. Bot.*, xvii, 1903.

Tansley, A. G., and Thomas, E. N.: The Phylogenetic Value of the Vascular Structure of Spermophytic Hypocotyls. Report of Brit. Ass., Section K, 1906, p. 761.

<sup>2</sup> Ganong, W. F.: Contributions to a Knowledge of the Morphology and Ecology of the Cactaceae. II. The Comparative Morphology of the Embryos and Seedlings. *Ann. Bot.*, xii, 1898.



The present communication forms part of Mr. T. G. Hill's scheme of investigation of seedling-structure, and has been carried out with a view to discovering whether there is any relation between the external morphology of the seedlings and their anatomy; in other words, whether the adult adaptations to physiological conditions, which have impressed themselves on the form of the young seedling, have had a corresponding influence on its internal structure.

The forms of many of the seedling Cactaceae have been figured at various times, but as Ganong<sup>1</sup> has already given a full account of the work done in this direction no detailed reference is required here; so far as has been ascertained, however, no observations on the seedling structure of any member of the family have been recorded.

No details of the colour factors of the seedlings will be given, as this question also has been fully dealt with by Ganong.<sup>1</sup> Owing to the great difficulty experienced in obtaining seeds, and the failure of these in many cases, when procured, to germinate, it has been found impossible to examine representatives of all the genera; in all, forty-seven species belonging to eleven genera have been investigated. I wish, in this connexion, to express my thanks to Professor Trelease of the St. Louis Botanic Gardens and to Dr. Rose of the Smithsonian Institute, both of whom sent me seeds, and also to Mr. Hales, Curator of the Old Physic Gardens, Chelsea, who not only obtained many seeds for me, but kindly undertook the germination of them all.

I should also like to take this opportunity of expressing my thanks to Mr. T. G. Hill, at whose suggestion this investigation was begun, for the encouragement and advice which he has constantly given me throughout its progress.

The methods employed were a slight modification of those described for the Gymnosperm seedlings;<sup>2</sup> in all cases the seedlings used were microtomed, and longitudinal preparations were frequently made in addition to the transverse series.

#### THE SEEDLING STRUCTURE OF THE CACTACEAE.

##### PERESKIA.

*Pereskia n. sp.*, Rose. The seedlings are of an ordinary dicotyledonous type and show no sign of succulence; they have a long slender hypocotyl and two thin leaf-like cotyledons, one very much smaller than the other (Fig. 1).

This asymmetry of the seedling, caused by the difference in size of its



Fig. 1. *Pereskia n. sp.* × 1.

<sup>1</sup> Ganong, W. F. : loc. cit.

<sup>2</sup> Hill, T. G., and de Fraine, E. : On the Seedling Structure of Gymnosperms, I. Ann. Bot., xxii, 1908.

seed-leaves, is a characteristic feature of the Cactaceae, and may be due to the shape of the seed. The embryo is curved, and has a small quantity of endosperm lying against its concave side; the smaller cotyledon is on this side and is covered by the larger convex one. The seed-leaves have no true petiole, but towards their base they are narrowed off, and are almost oval in transverse section. Each cotyledon has, in its broadest part, from eleven to twelve small vascular strands; these either fuse among themselves or end blindly in the mesophyll, until towards the base of the seed-leaf only the median and two lateral bundles remain. Fairly high up in the seed-leaf the median bundle bifurcates, and the phloem groups rotate until they lie almost in line with the xylem, in which by this time the protoxylem has become exarch. The two lateral bundles (*l*) fuse with the bifurcated main bundle towards the base of the cotyledon in the manner indicated in Diagram 1, Fig. 1.

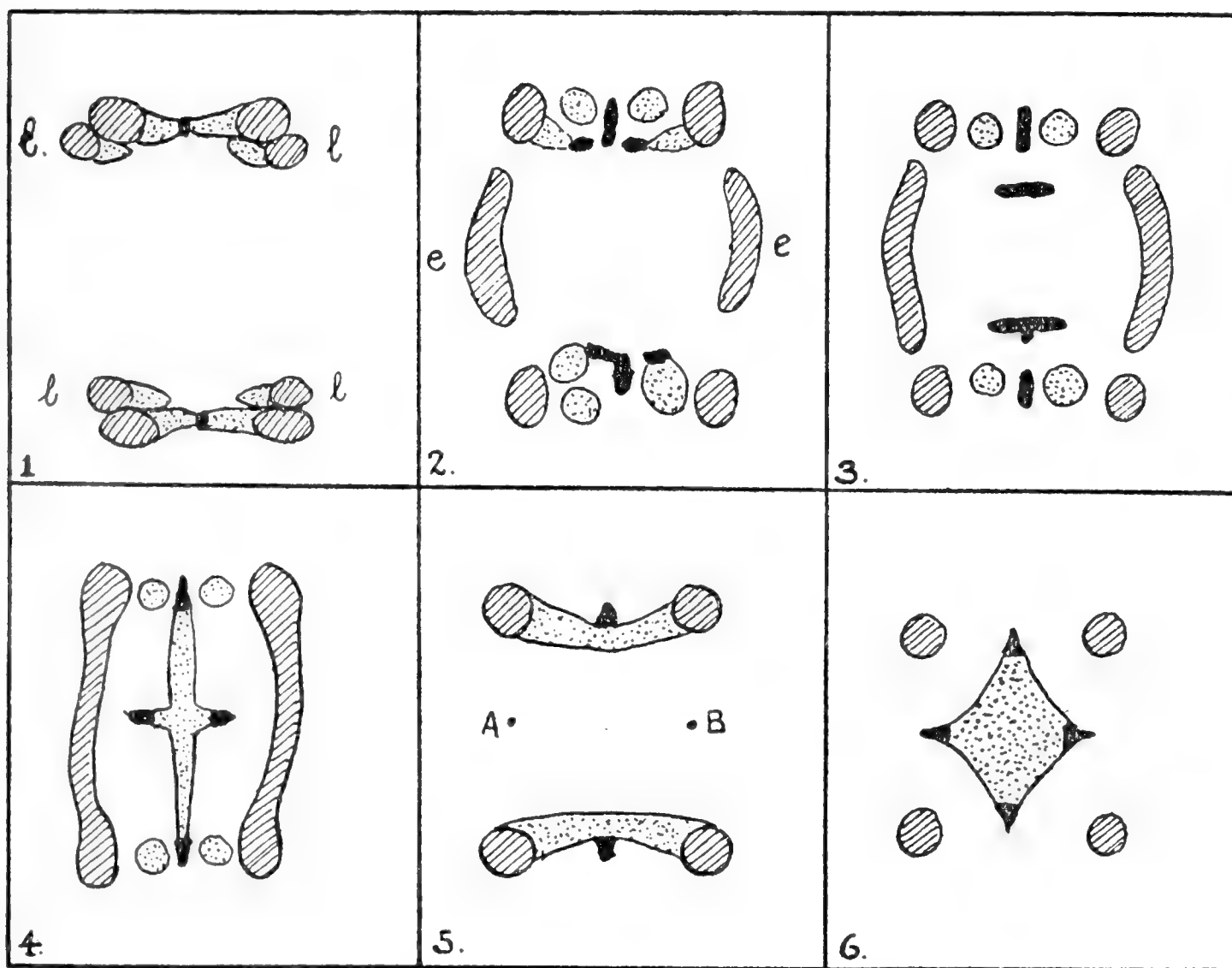


DIAGRAM 1. *Pereskia n. sp.* In this, and in all the following diagrams, the protoxylem is indicated by the black areas, the metaxylem by dots, the phloem by diagonal shading, and the cambium by a broken line.

The xylem elements are much scattered, and it is extremely difficult to make out the position of the protoxylem with any degree of certainty, but it is highly probable that during the passage into the hypocotyl the protoxylem branches in three directions (Diagram 1, Fig. 2). At this stage



a strand of epicotylar phloem bridges the space between the cotyledonary phloem groups. Soon after their separation the protoxylem elements arranged in the tangential position begin to move inwards, leaving the metaxylem and the radially placed protoxylem in an isolated position (Diagram 1, Fig. 3). Before reaching the centre of the axis these lateral branches pass outwards again, and the adjacent ones fuse; thus the two original protoxylem groups have, by branching into three and the subsequent fusion of adjacent lateral arms, given rise to four protoxylem strands in a manner which strongly recalls Miss Sargent's *Anemarrhena* type. The four groups of protoxylem formed in the manner just described very soon become connected, by the differentiation of tracheides, in such a way that a cross-shaped xylem plate is formed (Diagram 1, Fig. 4). This arrangement, however, does not long persist; the first indication of a change is given in the gradual breaking down of the phloem arcs opposite to the last-formed xylem groups; all the epicotylar phloem from this stage slowly dies out. Concurrently with these changes the intercotyledonary xylem arms gradually disappear, until finally only the two cotyledonary 'double' bundles with the protoxylem in the exarch position remain (Diagram 1, Fig. 5). All the details of the transition above described take place very rapidly and are restricted to the upper part of the long hypocotyl; no other changes occur until the root region is almost reached. At the base of the hypocotyl an isolated xylem element then arises in a position similar to that marked *A* (Diagram 1, Fig. 5); a little later this is followed by a second at *B*; new elements are then rapidly formed in the centripetal position until finally a solid core of xylem occupies the centre of the stele (Diagram 1, Fig. 6). The root is thus seen to be typically tetrarch, but two of its protoxylem groups are disconnected from the protoxylem of the higher regions of the hypocotyledonary axis.

The upper part of the root is, for a short distance, very thickly clothed with long root-hairs; below this region a cork cambium appears in the outer layers of the cortex, but at the age at which the seedlings were examined it had not been very active.

*Pereskia Pititache*, Karw. In general external characteristics the seedlings of this species are extremely like those of *Pereskia n. sp.*, except that the difference in size between the two seed-leaves is much less marked in them than in the latter. The structure of the cotyledons is also similar to that of *Pereskia n. sp.*, and the transition follows the course indicated in Diagram 1, Figs. 1-5. There is, however, no further change after the gradual disappearance of the intercotyledonary xylem arms, and the root structure is of the type which will be described later as characteristic of the genus *Cereus*, for it possesses two xylem-strands and four well-marked phloem-bundles.

In the seedlings of *P. Pititache* there is considerably less metaxylem

developed than in *Pereskia n. sp.*; and there is throughout the whole seedling a great abundance of cluster crystals.

OPUNTIA.

In external features the seedlings of this genus still maintain an almost normal dicotyledonous appearance (Fig. 2), but the hypocotyl has become somewhat more reduced in length as compared with *Pereskia*, while, at the same time, it is slightly more succulent, and the cotyledons though still leafy are distinctly more fleshy. The seed-leaves, as in the other genera, are markedly asymmetrical; they have a network of veins, as many as ten vascular strands being present in the transverse section of the cotyledon at some levels, but towards the base these are usually reduced to one, in which, by this time, bifurcation has generally occurred; there is never any suggestion of a petiole to the cotyledon, such as is indicated in *Pereskia*. The large cortical cells of the hypocotyl frequently contain crystals, e.g. *O. imbricata* and *O. Bergeriana*, and mucilage sacs are a characteristic feature of *O. stricta* and *O. Pseudo-tuna*.



FIG. 2. *Opuntia Tuna.* × 1.

Transition.

*Opuntia Ficus-indica*, Mill. In this species one 'double' bundle enters the hypocotyl from each seed-leaf; in it the bifurcation and subsequent separation of the phloem have proceeded so far that the xylem, which is composed of protoxylem elements only, is situated between two groups of phloem elements (Fig. 3, A).

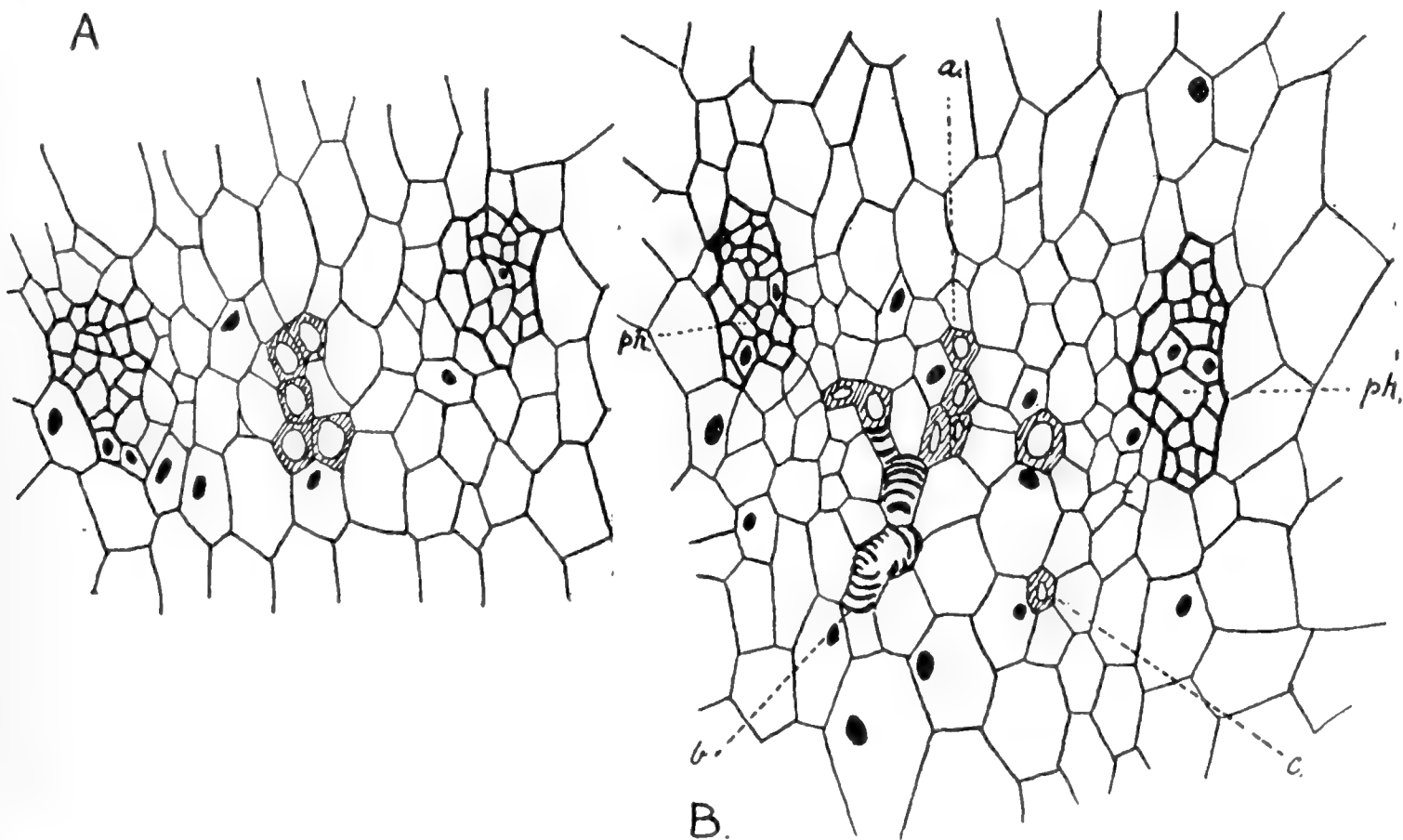


FIG. 3. *Opuntia Ficus-indica.* × 220.

Almost immediately after the entry into the hypocotyl has been made the protoxylem branches in three directions (*a*, *b*, *c*, and *a*<sub>1</sub>, *b*<sub>1</sub>, *c*<sub>1</sub>, in

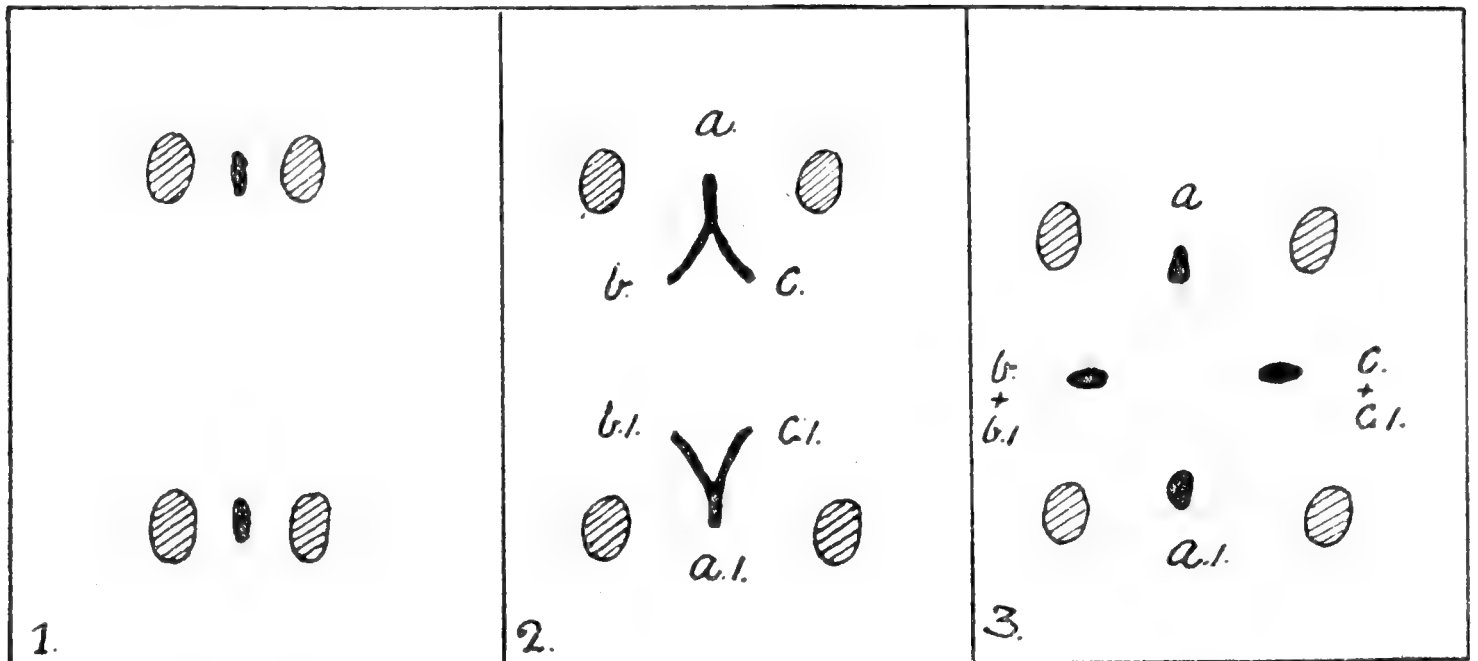


DIAGRAM 2. *Opuntia Ficus-indica.*

Diagram 2, Fig. 2, and Fig. 3, B), the adjacent arms (*b*, *b*<sub>1</sub>, and *c*, *c*<sub>1</sub>) fuse and give rise to the two intercotyledonary poles of the tetrarch root (Diagram 2,

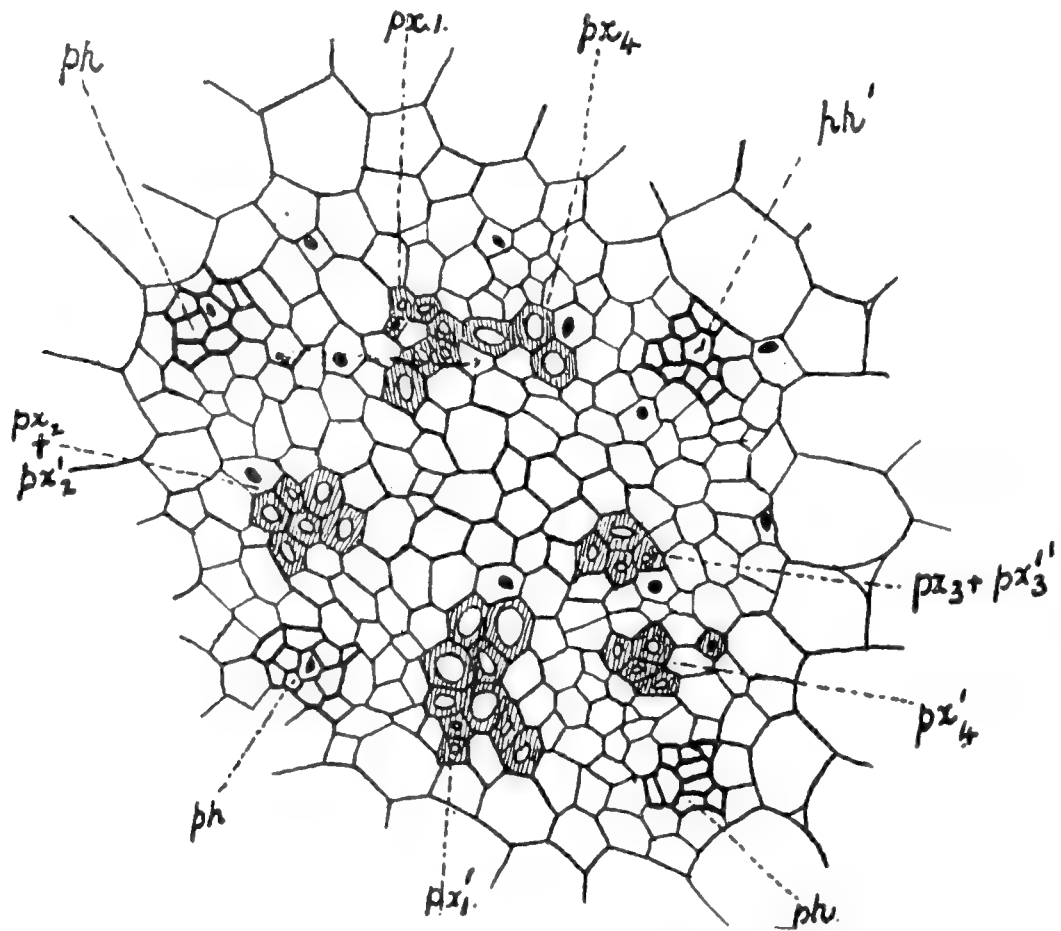


FIG. 4 A. *Opuntia Tuna.* Series B.  $\times 220$ .

Fig. 3). In this species, and in other of the *Opuntias*, there is thus the formation of a tetrarch root-structure from two cotyledonary traces by means of a transition which is of a type practically identical with *Anemarrhena*,<sup>1</sup> a type which, so far as it has been possible to ascertain, has

<sup>1</sup> Sargent, E. : loc. cit.

hitherto been undescribed among Dicotyledons, with the possible exception of *Eranthis* and *Podophyllum*.

*Opuntia imbricata*, D.C. In all essential features the transition in this seedling resembles that in *O. Ficus-indica*.

*Opuntia Tuna*, Mill.

Series A showed a type of transition such as is found in *O. Ficus-indica*.

Series B had at first a tetrarch root, produced in the same way as in Series A; but, at a lower level, this changed to a pentarch structure in the following manner.

The intercotyledonary protoxylem group ( $px_3 + px_3'$  in Fig. 4 A)

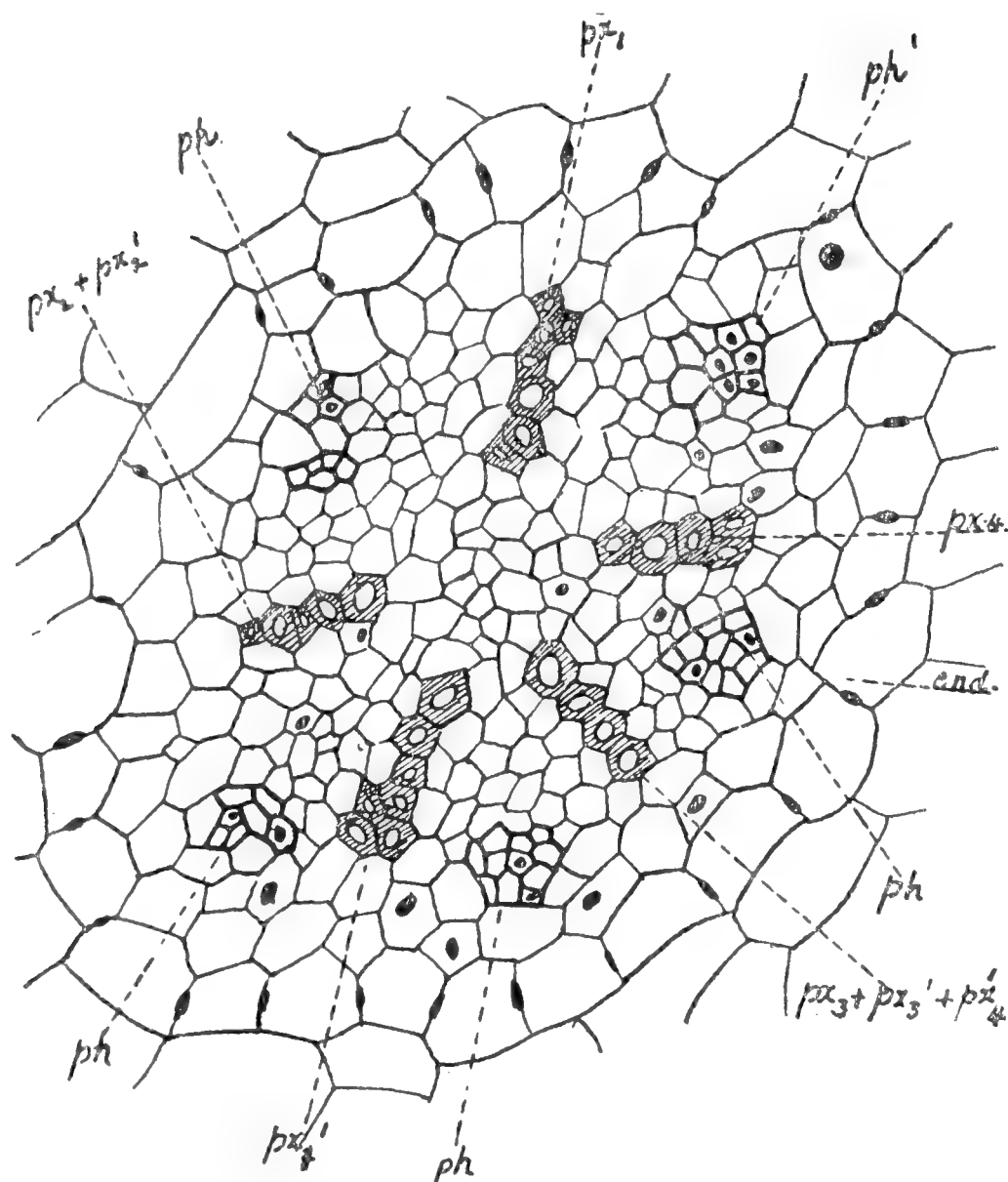


FIG. 4 B. *Opuntia Tuna*. Series B.  $\times 220$ .

appeared to be about to die out, when it was reinforced by a branch of xylem from  $px_1'$  ( $px_4'$ ); simultaneously  $px_1$  gave off another branch ( $px_4$ ), and finally a fifth phloem group ( $ph^1$ ) arose between  $px_1$  and  $px_4$  (Fig. 4 B); in this way the pentarch structure was completed.

Series C had a transition which was a slight modification of the *Anemarrhena* type. Two V-shaped bundles passed into the hypocotyl from the two cotyledons, the arms of the V very rapidly opened out, and the protoxylem which occupied its apex divided; half formed the exarch protoxylem group of the cotyledonary root-pole, the other half passed inwards

towards the centre of the axis, where it fused with the similar protoxylem group from the other cotyledonary bundle; metaxylem elements rapidly formed on either side of the plate thus produced. At a lower level one half of this intercotyledonary protoxylem plate died out, the other half moved rapidly outwards and formed the third root-pole (Fig. 5,  $px_3 + px'_3$ ) and soon became connected by metaxylem elements with the other two protoxylems. The cotyledonary xylem groups (Fig. 5,  $px_1$  and  $px'_1$ ) again branched ( $px_2$  and  $px'_2$ ) and the fourth protoxylem of the tetrarch root was established.

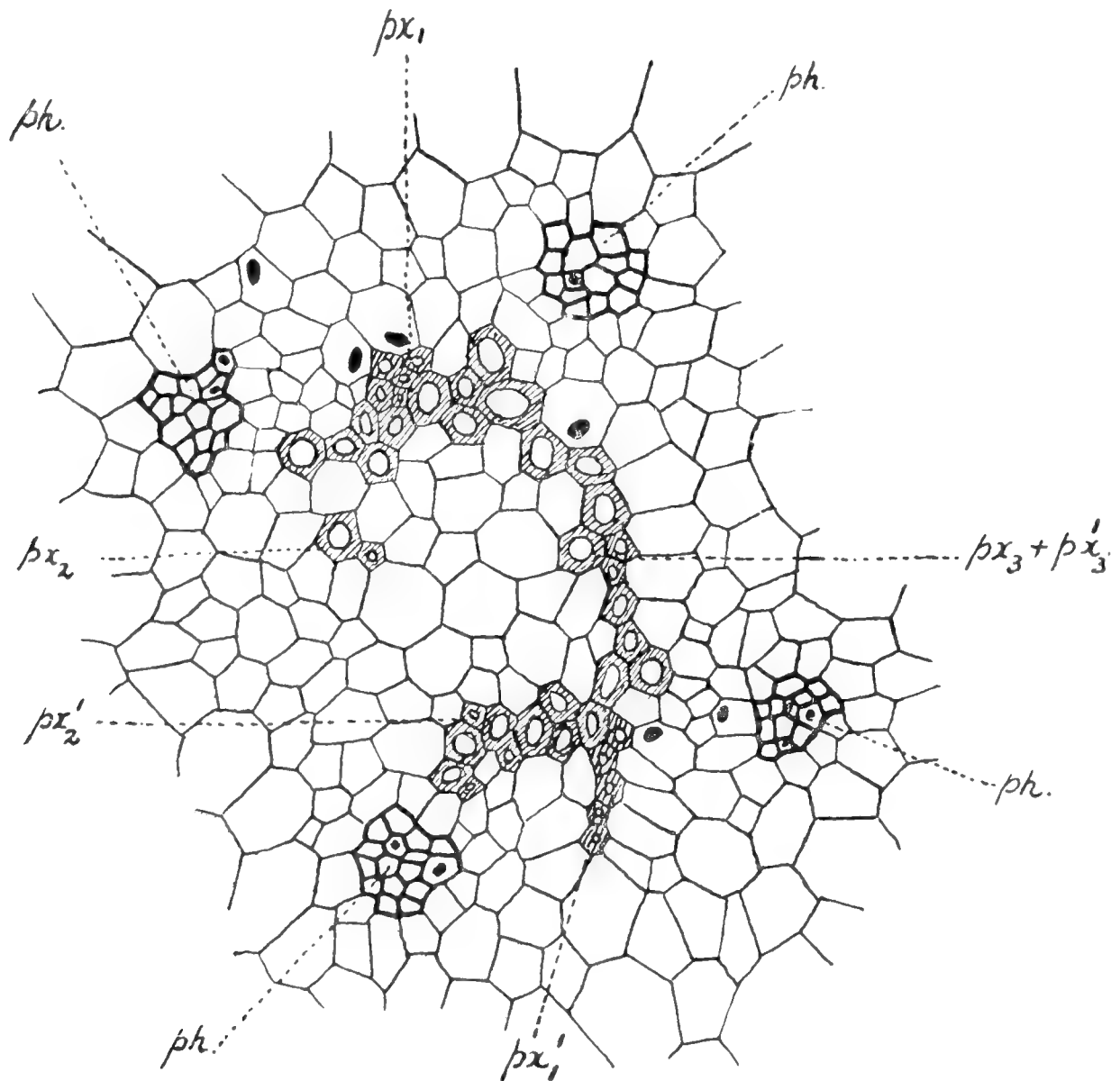


FIG. 5. *Opuntia Tuna*. Series C.  $\times 220$ .

Two epicotyledonary strands were well developed in the upper part of this seedling and, during the early stages of the transition, they appeared to be about to take part in the root formation; they did not do so, however, and finally gradually died out.

*Opuntia polyantha*, Haw. Each cotyledon supplies the hypocotyl with one 'double' bundle, in which the xylem is arranged in three groups; very few xylem elements are present and in all probability no metaxylem has as yet developed. The lateral xylem groups of the 'double' bundle pass outwards towards the intercotyledonary plane and there fuse with the similar groups from the other 'double' bundle; the median group remains

in position and forms one of the cotyledonary root-poles. These movements of the wood are somewhat masked by the rapid increase in the number of tracheides which arise in the centre of the axis; they do not long persist and have all disappeared by the time the tetrarch root has become established.

In another example of this same species a modification of this process took place. The cotyledonary bundles were of the normal V-shaped type, part of their protoxylem became exarch by rotation, while the rest passed towards the centre of the hypocotyl and gave rise to a diarch plate; a little later this branched right and left and completed the tetrarch root-structure.

*Opuntia Bergeriana*, Weber. The transition-phenomena of the single seedling of this species which was examined was of the type found in *O. Ficus-indica*. Epicotyledonary strands were present in the upper part of the hypocotyl; they were well differentiated, and at a lower level fused laterally with the cotyledonary bundles. Cambium was developed in the bundles and contributed a few secondary elements to the metaxylem, and in the tetrarch root secondary thickening had well begun.

*Opuntia stricta*, Haw.

Series A is in all essential features like *O. Bergeriana*.

Series B is in the first stages of the transition identical with Series A, but the lateral xylem branches gradually die out and the hypocotyl in its middle region possesses only two 'double' bundles. In these features the seedling strongly resembles *Pereskia* (Diagram 1, Fig. 5); further, as in this species, a tracheid arising in the intercotyledonary plane and reinforced by others developed centripetally, once more restores the tetrarch root-structure.

Series C is of interest in that the seedling possessed three cotyledons, each of which contributed one V-shaped bundle to the hypocotyl. Six epicotylar strands were also present, arranged in pairs between the 'double' bundles, with the nearest one of which they almost immediately fused. It was impossible to say which elements represented the protoxylem, for though a considerable quantity of wood was present the elements were practically indistinguishable from one another. Soon after the fusion of the epicotylar strands with the seed-leaf-traces, the xylem of the bundles separated into three parts, so that at this level there were in the hypocotyl six collateral strands of wood and bast and three isolated xylem groups. Towards the base of the hypocotyl the adjacent collateral strands rotated their xylem and fusion took place, new tracheides connected up these xylem groups with the three isolated strands of wood, and so a ring of xylem, with six protoxylems alternating with the six phloem bundles, resulted. 'Barrel' tracheides of the type which will be described later under the genus *Echinopsis*, gradually developed in the centre of the axis until a solid



core of xylem resulted. In this seedling three cotyledonary bundles, by a branching of the xylem into three, have given rise to a hexarch root.

*Opuntia albicans*, Salm-Dyck. Only two seedlings of this species were obtained for examination, and in all essential characters of the transition they resembled *O. stricta*, Series B.

*Opuntia maculacantha*, C. F. Foerst. A 'double' bundle passes into the hypocotyl from each cotyledon (Diagram 3, Fig. 1), and during the passage through the cortex the phloems, carrying with them the metaxylem, move further apart and leave the protoxylem more or less isolated. No sign of any branching of the protoxylem occurs, and the remaining two poles of the tetrarch root originate by the development of tracheides at *A* and *B* (Diagram 3, Fig. 2).

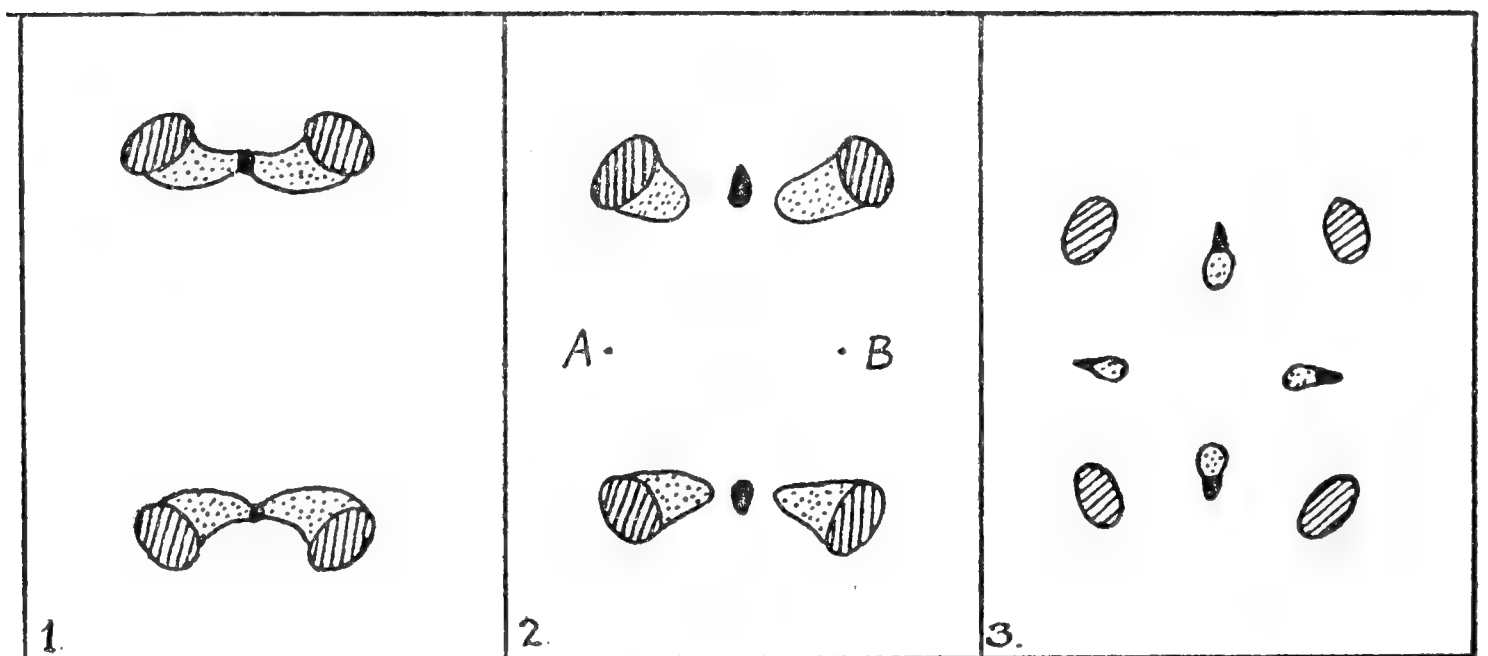


DIAGRAM 3. *Opuntia maculacantha*.

A few metaxylem elements are contributed to these two xylem groups by the cotyledonary metaxylem strands lying on either side. As in *O. stricta*, a few large barrel tracheides develop in the centre of the axis towards the hypocotylar base, but they do not long persist.

*Opuntia Pseudo-tuna*, Salm-Dyck. The transition in the only seedling of this species available for examination took place as in *O. maculacantha*; it is somewhat complicated, however, by the considerable number of secondary tracheides present, for much secondary thickening had taken place. It further differed from *O. maculacantha* in the complete absence of 'barrel' tracheides.

#### NOPALEA.

*Nopalea n. sp.*, Rose. Of this genus the seed of only one species germinated, and judging from this example the seedlings bear a very close resemblance to those of the genus *Opuntia*, with the exception that the difference in size of the two cotyledons is much more marked in *Nopalea* than in *Opuntia*, or indeed in any other genus; further, the seedlings show a slight advance in succulence (Fig. 6).

The seed-leaves possess one median and six or seven lateral bundles, which either fuse or end blindly in the tissues of the mesophyll. At the base of the cotyledon only one bundle remains, which, at this level, has bifurcated and rotated (Diagram 4, Fig. 1). Six epicotyledonary bundles (*e*) also enter the hypocotyl; they arrange themselves in two groups between the cotyledonary bundles, and a cambium soon connects them with the phloem of the seed-leaf-traces; no xylem is differentiated in them during any part of their course. The xylem of each cotyledonary strand soon begins to send off a branch towards the centre of the axis (Diagram 4, Fig. 2); this strand almost immediately loses its metaxylem, and then branches right and left; at a later stage, the adjacent arms from the two cotyledonary bundles fuse (Diagram 4, Fig. 3). During these movements of the xylem, the phloem-strands lying next to the cotyledonary phloem groups fuse with them, and the differentiation of the median epicotyledonary phloem-bundle ceases. Metaxylem tracheides develop centripetally and a typical tetrarch structure is attained.



FIG. 6.  
*Nopalea.*  
× 1.

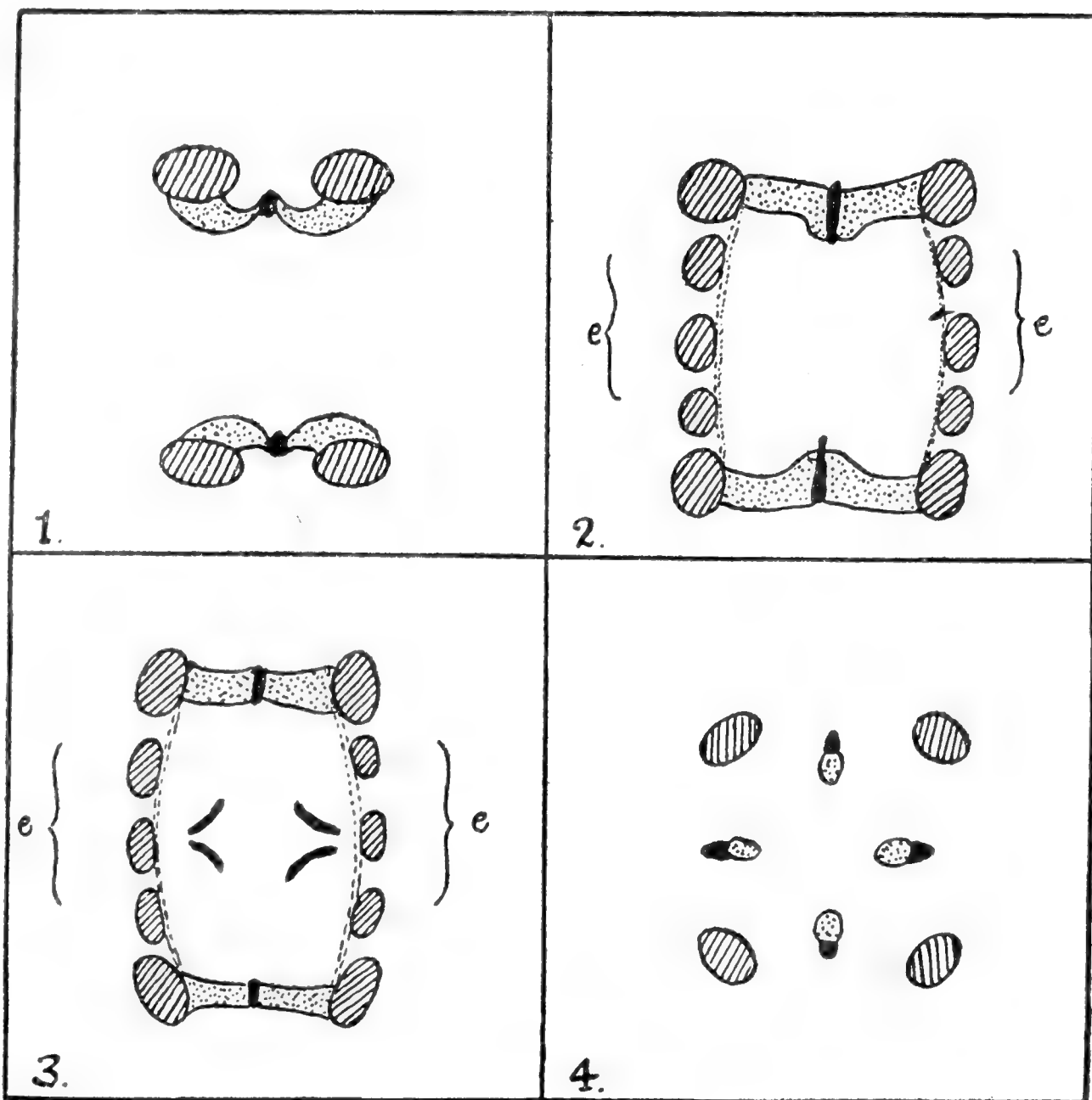


DIAGRAM 4. *Nopalea.*

The seedlings of this species thus show a method of transition from stem to root structure which is essentially of the *Anemarrhena* type, but in the one case the 'double' bundles are derived from two cotyledons, while in the latter they are furnished by a single one.

#### PHYLLOCACTUS.

Seeds of various species of *Phyllocactus* were planted, but only one set germinated.

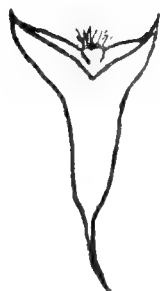


FIG. 7.  
*Phyllocactus*  
*Hookeri.* × 2.

*Phyllocactus Hookeri*, Walp. These seedlings showed a somewhat succulent hypocotyl, with two short cotyledons, which in form and in their degree of fleshiness resembled those of *Cereus tortuosus* (Fig. 7). In general characters the structure of the cotyledon and hypocotyl is very similar to that of species of *Cereus*; and in three of the four seedlings examined, a small cotyledonary bud occupied the base of the cotyledon, although in one case only did this bud possess a vascular supply.

#### *Transition.*

1. Series A, B, and C, in which the bundle of the cotyledonary bud was absent.

Each of the cotyledons possessed one small endarch bundle; in one seedling the bifurcation of the phloem took place before the entrance into the hypocotyl, in the others it was delayed for some time, and in no case did any division of the xylem occur. Four very small and ill-differentiated epicotylar strands were present in the upper part of the hypocotyl, but these played no part in the formation of the root, and their differentiation soon ceased. The half-phloems of the cotyledonary bundles separated and left the xylems isolated, the wood consisted of a few somewhat scattered elements, and the protoxylems attained the exarch position rather by a rearrangement of the elements than by a definite rotation. Towards the base of the hypocotyl a loose central strand of wood is produced by the centripetal development of tracheides; at the same time the opposite phloem groups gradually approach as though fusion were about to take place; a junction however is not effected, and the phloems separate again, so that the root-structure is of the type found in *Cereus*, in which there are four phloem- and two xylem-bundles.

2. Series D, in which the bundle of the cotyledonary bud was present.

The cotyledonary bud in this seedling was poorly developed and consisted of a tiny mass of parenchymatous tissue with a few multicellular hairs, but it was provided with a vascular supply. Besides the two cotyledonary bud-bundles and the two endarch cotyledon-strands, there were in the upper part of the hypocotyl four epicotylar traces. The

seed-leaf-bundles showed bifurcation and rotation of their phloem, and the cotyledonary bud-bundles rotated and moved outwards to meet the incoming cotyledon-traces and finally fused laterally with them. The four epicotylar strands played no essential part in the transition, they merely passed outwards and fused with the 'double' bundles. The behaviour of the vascular tissue is thus precisely similar to that illustrated for *Cereus tortuosus* (Diagram 5, Figs. 1 and 2). The phloems and the metaxylems of the 'double' bundles thus produced separate and rotate round the protoxylem, which they thus leave exposed in the exarch position, and the root is again of the *Cereus* type.

#### CEREUS.

The seedlings belonging to this genus show a considerable advance in succulence when compared with *Opuntia*, while at the same time they exhibit a marked diminution in size. The somewhat long and slender hypocotyl characteristic of *Pereskia* and *Opuntia* is replaced by a shorter, swollen structure in the species of *Cereus*. The cotyledons are no longer leaf-like, but are small, pointed and succulent, and are set slightly apart, with the inner line of their broad bases parallel. The two cotyledons of a seedling still show a difference in size, though this feature is not so well marked as in *Opuntia*, *Nopalea*, and *Pereskia*; the whole seedling is indeed slightly asymmetrical (Fig. 8). Towards the base of the cotyledons a small embryonic bud, bearing a few multicellular hairs, appears at the middle of their ventral surface. The structure of the seed-leaf is very simple. It consists of a mass of large, rounded, parenchymatous cells, with extremely small intercellular spaces, and it is traversed by a small vascular bundle, which, towards the base of the cotyledon, may be reinforced by two still smaller lateral strands, and by an inner bundle which formed the vascular supply of the cotyledonary bud. The epidermal cells in some species, such as *C. tortuosus*, *C. triangularis*, and *C. Jamacaru*, show a differentiation into small rectangular cells, which appear in groups consisting of one to six elements in transverse section, and much larger cells in which the outer wall is either convex or somewhat elongated. *C. peruvianus* and *C. Spachianus*, on the other hand, show no such differentiation, and the epidermal system in these species is composed of large cells with a distinctly convex outer wall. As in all the other genera examined, the stomata are of the usual Cactus type; they have subsidiary guard-cells, are on a level with the general epidermal surface, and, where a differentiated epidermis occurs, the stomata are restricted to the areas of the small rectangular elements. The hypocotyl is at first oval, but rapidly becomes circular in transverse section; it is made of very large, rounded, parenchymatous



FIG. 8.  
*Cereus*  
*tortuosus*.  
× 2.

cells, practically without intercellular spaces. Towards its base downward growth may occur in two or more regions, resulting in a curious cortical lobing of the upper part of the slender root; a fuller description of this phenomenon will be given later. In other cases the passage from hypocotyl to root is only accompanied by a great diminution in the size of the cortical cells. The short primary root usually possesses a slight development of cork in its outer cortical region.

The transition from stem- to root-structure is remarkably uniform throughout the genus; the phenomena will therefore be described for one species, and only the slight and usually unimportant differences exhibited by those other species which have been examined will be noted.

*Transition.*

*Cereus tortuosus*, Forbes. Each cotyledon of the seedlings of this species has one small endarch bundle throughout its length, but towards the base very small lateral strands appear, one on each side of the median

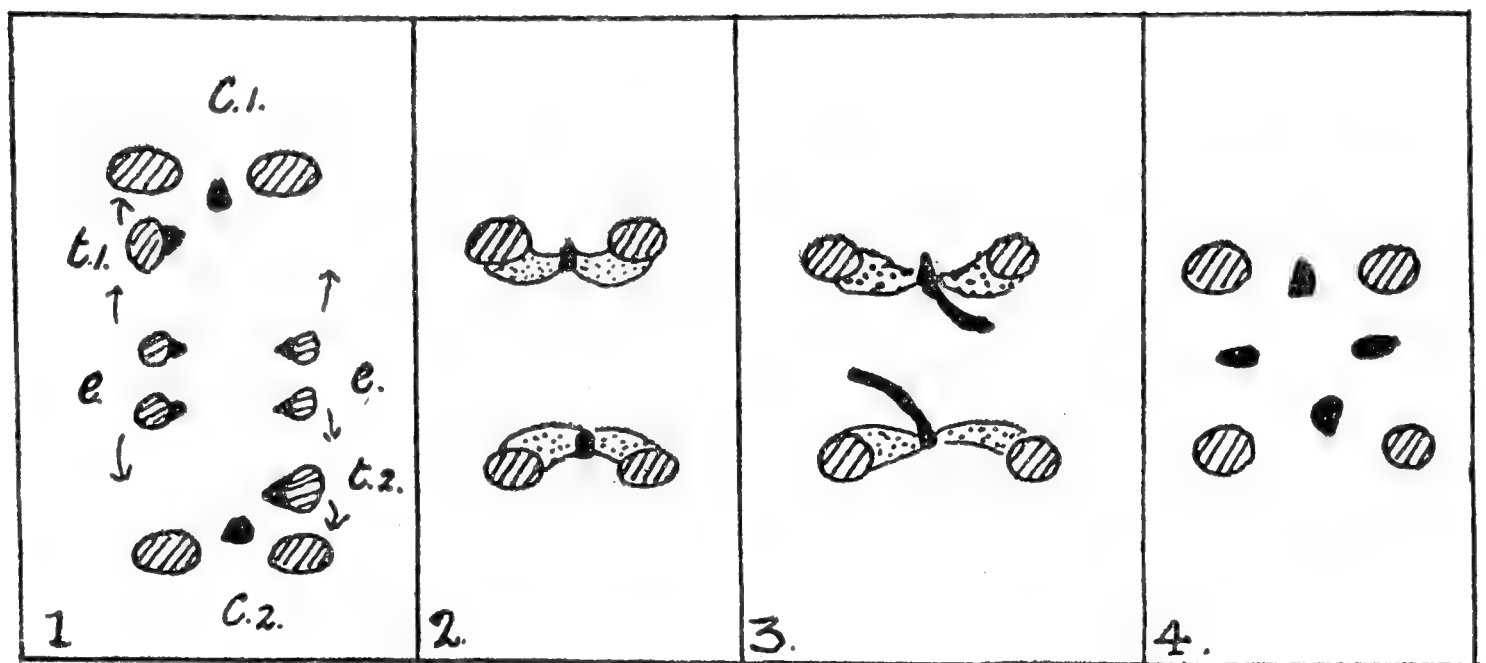


DIAGRAM 5. *Cereus tortuosus*.

one, in which, by this time, the phloem has bifurcated. The lateral bundles run obliquely through the mesophyll and fuse with the median strand. About this level a fourth bundle makes its appearance; it originates in the cotyledonary bud region and evidently provides its vascular supply. This cotyledonary bud bundle moves rapidly outwards, rotating as it does so, and finally fuses laterally with one portion of the cotyledonary bundle ( $t_1$ ,  $t_2$ , Diagram 5, Fig. 1).

During these movements the bundles have entered the hypocotyl, where four small epicotylar strands ( $e$ ) may or may not be present; if they are differentiated they pass outwards and fuse with the seed-leaf-traces (Diagram 5, Fig. 2). With the fusion of the cotyledonary bud-bundle with the seed-leaf-strand the transition is practically effected, for in general no further



change occurs, except for the slight and gradual passing in of the vascular elements towards the centre of the axis. In the usual case, the root possesses four well-marked, though small, phloem groups and only two bundles of xylem. This root arrangement is usually maintained so long as any differentiation of the tissues can be observed, but one seedling examined showed an interesting variation. Towards the base of the root each of the protoxylems sent off a branch, which finally resulted in the formation of a tetrarch root of the normal type (Diagram 5, Figs. 3 and 4).

In all the seedlings of *Cereus tortuosus* examined, the xylem almost entirely consisted of protoxylem; metaxylem tracheides appear just below the cotyledonary node and are situated on either side of the protoxylem group, between it and the phloem (Diagram 5, Fig. 2); occasionally a few elements may appear between the groups of protoxylem. The root, however, rarely possesses any wood except the protoxylem, in the stages examined.

The metaxylem elements are very large in transverse section, and the thickening of the wall projects far into the cavity. They are always sharply defined from the small protoxylem elements (compare with Fig. 19), and consist of the short, broad tracheides with the annular or spiral thickening ridge, which are described by Solereder<sup>1</sup> as occurring in some species of the Cactaceae. According to his observations these 'barrel' tracheides seem to be entirely wanting in the stems of the species of *Cereus*, but however that may be, they are certainly to be found in the seedlings. Van Tieghem's<sup>2</sup> studies of these cells led him to the conclusion that they were merely a peculiar form of parenchyma, but an examination of well lignified elements in this and in other genera has failed to reveal the presence of cytoplasm and nucleus. It seems probable, therefore, that as Darbishire<sup>3</sup> suggested, Van Tieghem's observations were made on very young tracheides, for at the stage when the thickening band is not completely lignified, cytoplasm is usually seen.

*Cereus peruvianus*, Mill. The seedlings of this species show rather more variation in their cotyledonary bundles than do those of *C. tortuosus*. In one series the small cotyledon had but one lateral strand present, while the larger cotyledon had two; another seedling developed no laterals, while a third had only a median bundle in the smaller cotyledon, but in the larger had two strands, slightly inclined towards one another, which during the transition behaved as a 'double' bundle. The transition phenomena are almost identical with those of *C. tortuosus*, with the single exception to be described.

<sup>1</sup> Solereder: *Systematic Anatomy of the Dicotyledons*, Vol. i. Trans. by Boodle and Fritsch. Oxford, 1908.

<sup>2</sup> Van Tieghem: *Cell. annelées et spiralées des Cact.* Bull. Soc. Bot. de France, 1885.

<sup>3</sup> Darbishire, O.: *Observations on Mamillaria elongata*, Ann. Bot., xviii, 1904.



The larger cotyledon ( $c_2$ ) had two strands which rotated and behaved as the 'double' bundle of the smaller cotyledon ( $c_1$ , Diagram 6, Fig. 1). Fusion of the cotyledonary bud-bundle with the seed-leaf-trace took place as in *C. tortuosus* ( $t_1$ ,  $t_2$ , Diagram 6, Figs. 2 and 3), and subsequent movements of the vascular elements resulted in a root structure similar to that described above for that genus. Towards the base of the root, however, changes take place, which result in the formation of a pentarch structure (Diagram 6, Figs. 4 and 5). The protoxylem group ( $x_2$ ) sends off a branch,

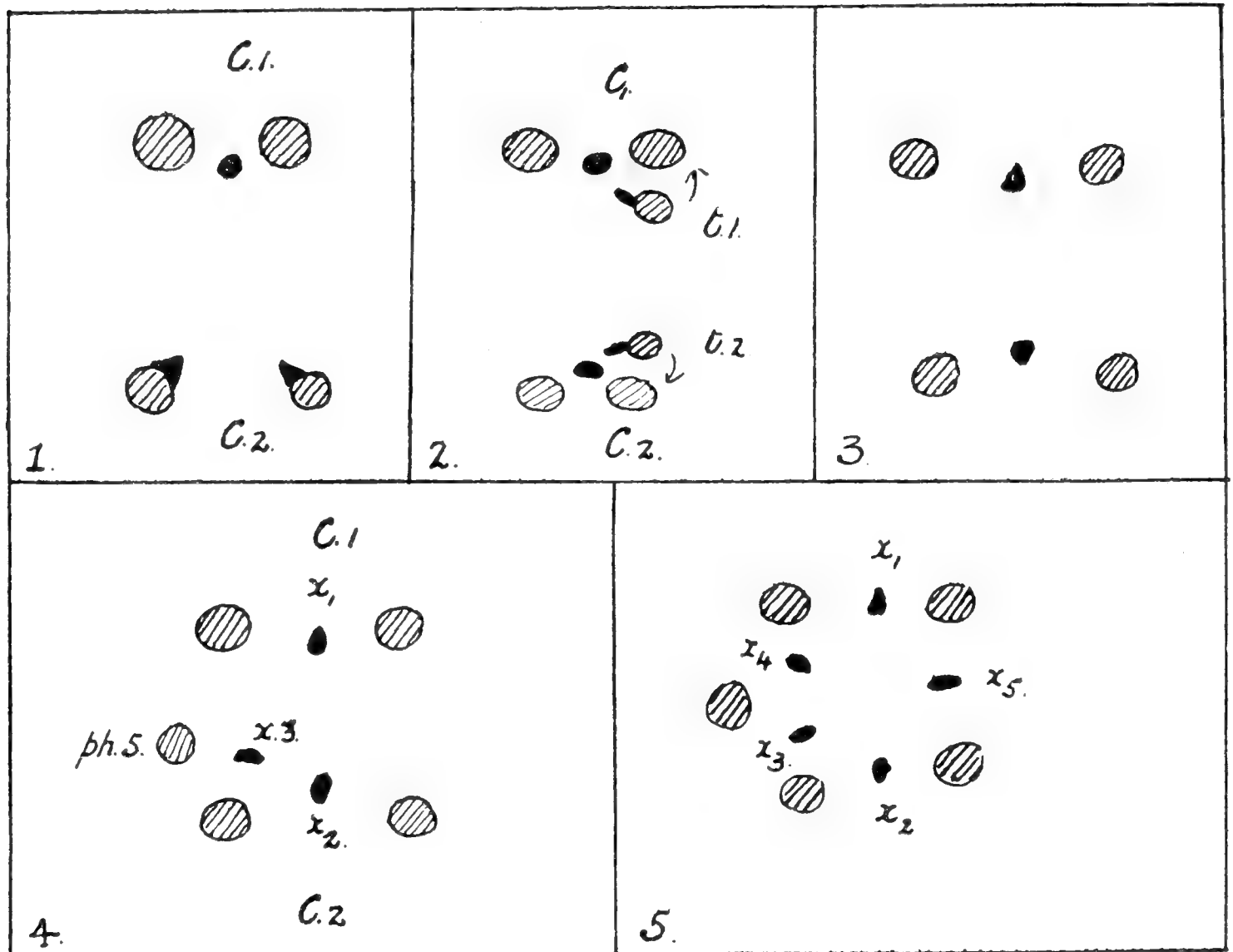


DIAGRAM 6. *Cereus peruvianus*.

consisting of a few elements, between the adjacent phloem groups to its left; very soon a phloem strand ( $ph_5$ ) arises to the left of this last formed xylem-bundle; no cambium was present, and the phloem arose by the meristematic activity of the cells in this region. At a still lower level the protoxylem ( $x_1$ ) branches to the right and to the left, and in this way a pentarch root is produced.

The variation just described, taken in connexion with the somewhat similar case figured for *C. tortuosus* (Diagram 5, Fig. 3), appears to indicate the manner in which the simple transition from stem- to root-structure in the genus *Cereus* may have been brought about. A suppression of one xylem branch of each cotyledonary bundle, followed by a total obliteration

tion of the intercotyledonary arms, would result in the curious structure seen in most of the roots of the seedling *Cerei*.

*Cereus Jamacaru*, D.C. The transition-phenomena of these seedlings were practically identical with those of *C. tortuosus*; there were, however, one or two differences of minor importance. For instance, the seedling had no lateral bundles in the cotyledons, and the strand which supplied the cotyledonary bud was very small, dying out before reaching the seed-leaf-trace. In another seedling four lateral roots arose almost simultaneously, alternating with the phloem groups; in general only two appear, and these opposite the protoxylem.

*Cereus Spachianus*, Lem. Both in the transition-phenomena, and in other respects, the seedlings of this plant closely resemble those of *C. Jamacaru*.

*Cereus triangularis*, Mill. With the exception that the cotyledonary bud is but slightly developed, and has no vascular supply, the structure and transition-phenomena in these seedlings are similar to those of *C. tortuosus*.

#### PILOCEREUS.

The two species of *Pilocereus* which were examined showed a great similarity to *Cereus* in their external appearance; and, as in some species of that genus, small cotyledonary buds were present in the axils of the seed-leaves.

*Pilocereus exerens*, K. Schum. Each of the two cotyledons has a small endarch bundle throughout its length, and at its base the slightly differentiated tubercle-bundle appears. The seed-leaf-traces and the tubercle-bundles in Series 1 perform differently: in one cotyledon they form the 'double' bundle in exactly the same way as is illustrated for *Cereus peruvianus* (Diagram 6, Figs. 2, 3), in the other cotyledon the seed-leaf-trace shows no bifurcation of its phloem, but rotating slightly, forms one half of the 'double' bundle, the second half being produced by the tubercle-bundle. The two 'double' bundles once formed, the transition is practically completed, since, except for a slight centripetal displacement of the vascular elements, no further changes take place, and the root is of the *Cereus* type. In Series 2 both cotyledon-bundles bifurcate, and the tubercle-strands, in which no xylem is present, fuse laterally with one half of the seed-leaf-phloem; in other respects the transition takes place as in Series 1.

*Pilocereus albispinus*, Salm-Dyck.

Series 1. Each cotyledon contributes one endarch bundle to the hypocotyl, and also one tubercle-bundle which is composed of phloem only. The tubercle-phloem-strand bifurcates, and the two halves pass outwards until they lie one on either side of the cotyledonary bundle; the phloem of the latter then bifurcates, and the two halves separate slightly and fuse with the adjacent tubercle-phloem groups. Further rotation of

the bast takes place until it occupies the position characteristic of the *Cereus* root-structure, and with these movements the transition is completed. In this series and in all the seedlings of *Pilocereus* examined, only protoxylem was present at the stage observed.

Series 2. In this seedling, towards the base of the cotyledon, four small vascular strands were present, but at the cotyledonary node the number was reduced to one. This bundle soon bifurcated, the tubercle-bundle fused laterally with one half of it, and the remainder of the transition followed the course described for Series 1.

#### RHIPSALIS.



FIG. 9.  
*Rhipsalis*  
*dissimilis*.  
× 1½.

Seeds belonging to two species only of this genus germinated. The seedlings were very small and resembled those of the genus *Mamillaria* in the possession of a globular hypocotyl; they differed, however, in owning two minute pointed cotyledons. The cuticle was much more strongly developed in these seedlings than in those of any other genus, but in other respects the general characteristics of the cotyledons and hypocotyl are those of such a group as *Echinopsis*.

*Rhipsalis Warmingiana*, K. Schum. One small endarch bundle passes into the hypocotyl from each cotyledon, but during its inward passage bifurcation of the phloem takes place; at this stage, and indeed throughout the whole length of the hypocotyl, only protoxylem is present. Rotation of the phloem groups follows immediately on their bifurcation, and the movement continues round the stationary protoxylem, until they lie at right angles to their former position. A general centripetal displacement of all the vascular elements follows until the opposite groups of phloem lie close together, when fusion of them takes place. New xylem elements develop centripetally to the protoxylem until a central plate is formed; thus the diarch root-structure is arrived at.

*Rhipsalis dissimilis*, K. Schum. The seedlings of this species are very similar to those of *R. Warmingiana*, but the stem apex is rather more depressed, giving rise to a short cotyledonary tube. Further, bifurcation of the phloem takes place at the base of the cotyledons, and is not delayed until the hypocotyl has been entered, as in the former species.

#### ECHINOCEREUS.

The seedlings of the species of *Echinocereus* examined show a fairly close resemblance to those of *Cereus*, but there is a slight diminution both in the length of the cotyledons and of the hypocotyl, and the latter is rather more globular than in any of the species of *Cereus*. The epidermal cells of the cotyledons are elongated and almost pointed; but there is no differentiation of these cells as is the case in some of the *Cerei*.

Transition.

*Echinocereus Ehrenbergii*, Engelm. The cotyledons have at their broadest part five small vascular bundles, which by anastomosing have been reduced, by the time the base of the cotyledon is reached, to two in one cotyledon ( $c_1$ ) and one in the other ( $c_2$ , Diagram 7, Fig. 1).

During the passage into the hypocotyl the phloem of the bundle  $c$  bifurcates and the two halves rotate round the protoxylem; no metaxylem is present. The two bundles in  $c_1$  rotate slightly until their protoxylems are directed towards one another. Six epicotylar phloem groups ( $e$ ) are also shown in the hypocotyl at this level (Diagram 7, Fig. 2), but fusion of two of the adjacent bundles soon reduces their number to four. These four groups ultimately join with the cotyledonary phloem next to

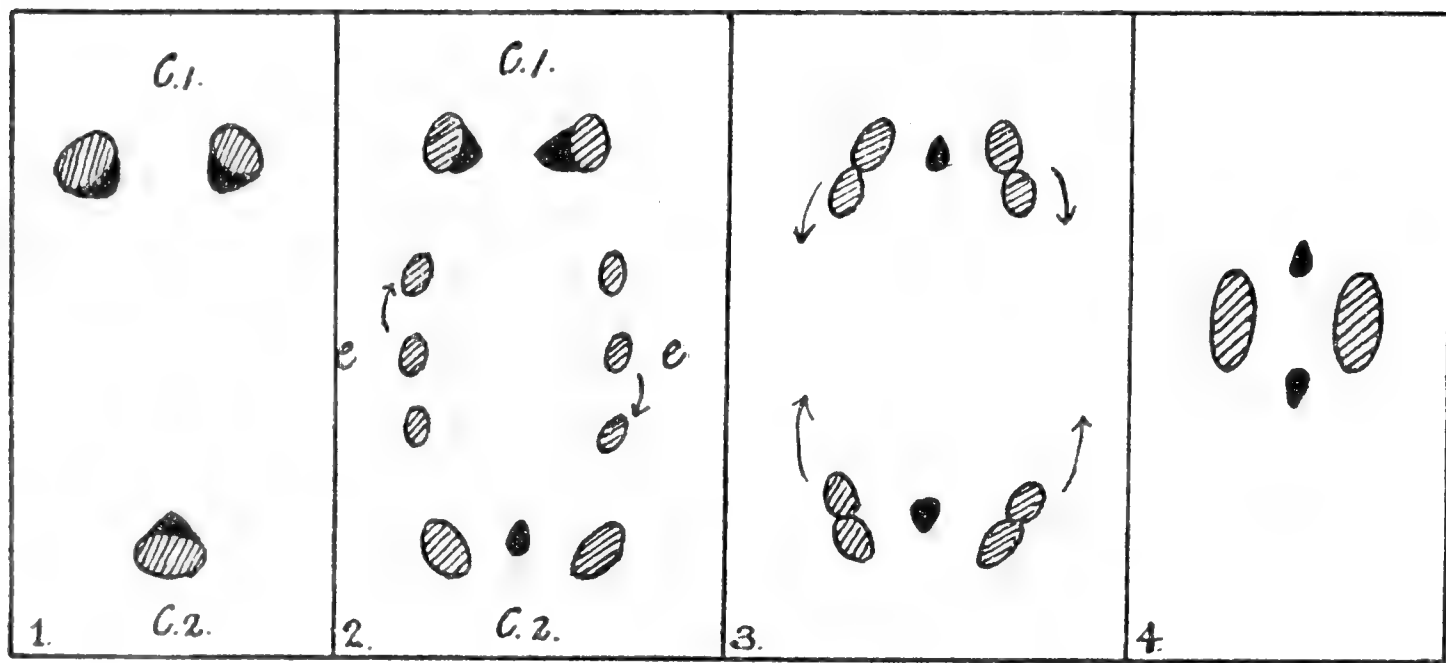


DIAGRAM 7. *Echinocereus Ehrenbergii*.

them, but before this is accomplished the two protoxylems of  $c_1$  have fused. A diarch root finally results by the union of the two adjacent groups of bast (Diagram 7, Figs. 3 and 4).

Series 2 showed two differences from the transition just described: there were fewer bundles in the cotyledons, and the two 'double' bundles of the hypocotyl were both produced as in  $c_1$  (Diagram 7) by the union of two strands, not by the bifurcation of one.

*Echinocereus cinerascens*, Lem. These seedlings had only three bundles in the cotyledons, which fused towards the base into a single strand, bifurcation of which took place during the passage into the hypocotyl. With the exception that no epicotylar phloem was developed, the transition was essentially like that in *E. Ehrenbergii*.

ECHINOPSIS.

The seedlings belonging to the *Echinopsis* group are distinctly smaller than those of the genus *Cereus*. They are characterized by an almost

globular hypocotyl, which terminates in a short, slender root (Fig. 10). The marked increase in succulence in the hypocotyl is correlated with



FIG. 10.  
*Echinopsis*  
*Lagermannii.*  
× 2.

a decrease in the size of the cotyledons, which in some species of the genus show extreme reduction. In the seedlings in which the cotyledons attain their maximum development, they consist of small pointed structures with an almost circular base of insertion; while, on the other hand, the almost complete suppression of the pointed apex in *Echinopsis multiplex* results in the diminution of the seed-leaves to such an extent that they are merely represented by two papillae. In every case, however, no matter how reduced the cotyledons may be, a difference in size between those of any one plant can always be observed.

#### THE TUBERCLE.

The tubercles with their tufts of spines, which are so characteristic a feature in so many of the Cactaceae, have received attention from several observers with a view to the elucidation of their morphology; and Darbishire<sup>1</sup> very briefly summarizes the principal views which have been put forward. The appearance of the cotyledonary buds at the extreme base of the seed-leaves has been already noted above for some species of *Cereus*, *Pilocereus*, and *Phyllocactus*, e.g. *C. tortuosus*, *C. Famacaru*, *Pilocereus exerens*, *P. albispinus*, and *Phyllocactus Hookeri*, and in the following species of *Echinopsis* precisely similar buds appear: *E. oxygona*, *E. Eyriesii*, and *E. Zuccarinii*. In other species, e.g. *E. multiplex*, *E. tubiflora*, and *E. Lagermannii*, these cotyledonary buds attain comparatively large dimensions, and where their development is most complete they closely resemble the tubercles which arise on the epicotyl. In some species of *Mamillaria*, to be described later, the cotyledonary bud is absolutely indistinguishable from the epicotyledonary tubercle, and can only be identified by its subsequent fusion with the seed-leaf. A well developed cotyledonary bud consists of a mass of tissue composed of large, parenchymatous cells, and throughout its length runs a single vascular bundle; at the apex of the bud is a cushion of tissue on which the ends of the spines are inserted, and a cork cambium separates this cushion from the underlying tissues (Fig. 11). When a comparison is made between the anatomy of a 'cotyledonary bud' and that of the tubercle of an adult plant, such as is described by Darbishire,<sup>2</sup> there is seen to be a very close resemblance in structural details between the two. From these considerations it is concluded that the 'cotyledonary buds' of the seedling are morphologically identical with the tubercles of the mature plant.

<sup>1</sup> Darbishire, O.: loc. cit.

<sup>2</sup> Loc. cit.



Owing to the greatly depressed stem apex, which often results in the appearance of a short apparent cotyledonary tube, the tubercles may seem in some cases to be situated on the cotyledon itself, instead of in its axil; but longitudinal sections through the seedlings indicate that the tubercles are really auxiliary structures and are not outgrowths from the cotyledon.

With regard to the morphological nature of the tubercle and spines it has been stated by Goebel<sup>1</sup> that in many Cactaceae, 'the spines are transformed leaves which arise upon very much reduced lateral shoots standing in the axils of the leaves.' In another connexion he remarks<sup>2</sup> that in many Cacti, 'the thorns are here usually arranged in tufts on very short shoots,'

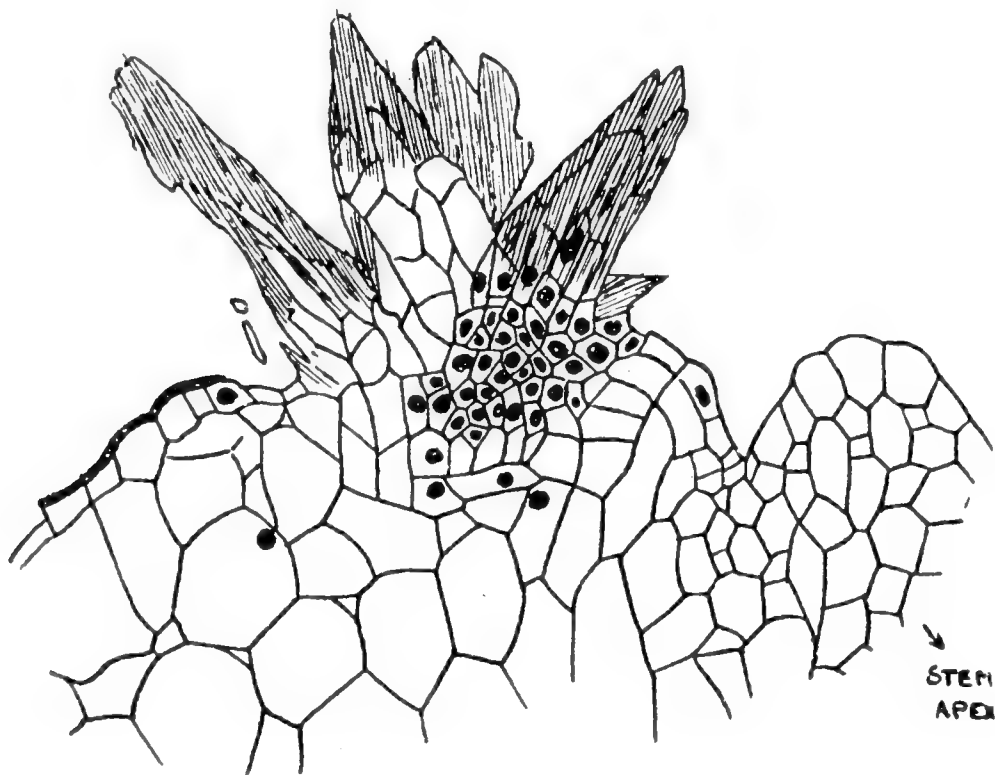


FIG. 11. *Echinopsis Lagermannii*. Group of thorns on the cotyledonary tubercle. L. S.  $\times 109$ .

and that 'the thorns are transformed leaves'. He further observes<sup>3</sup> that in many Cactaceae, especially in the Mamillarieae, 'the axillary shoot "grows up upon" its subtending leaf—that is to say, the common base of the two is elongated.' Darbishire,<sup>4</sup> however, 'can see in the mature tubercle only the highly developed leaf-base. The spines together represent the leaf-blade, the leaf-stalk being absent.'

The development of tubercles in the axils of the cotyledons appears to support Goebel's view that the tubercles represent short shoots, but it is desired to carry out further investigation along experimental lines before expressing any very definite opinion as to their morphological nature.

The tubercles in the *Echinopsis* group show a very well marked rise in importance when compared with the cotyledons; increase in size of the

<sup>1</sup> Goebel, K.: Organography of Plants, Oxford, 1905, Part I, p. 168.

<sup>2</sup> Loc. cit., Part II, p. 429.

<sup>3</sup> Loc. cit., Part II, p. 436.

<sup>4</sup> Loc. cit.



one is usually accompanied by decrease in size of the other. In *R. Eyriesii*, *E. oxygona*, and *E. Zuccarini* they closely resemble the 'cotyledonary buds' of the *Cereus* group, but in *E. Lagermannii* and *E. multiplex* the cotyledons are, comparatively speaking, small, and the tubercles are well developed, tufted with spines, and have a vascular bundle running to the base of the cushion.

#### VASCULAR RELATIONSHIPS BETWEEN THE COTYLEDONS AND THE TUBERCLES.

The behaviour of the cotyledon- and tubercle-bundles is extremely erratic, and varies not only within the species but even in the opposing pairs of bundles of a single seedling.

The simplest case was that found in *E. multiplex*, Series 1 (Diagram 8,

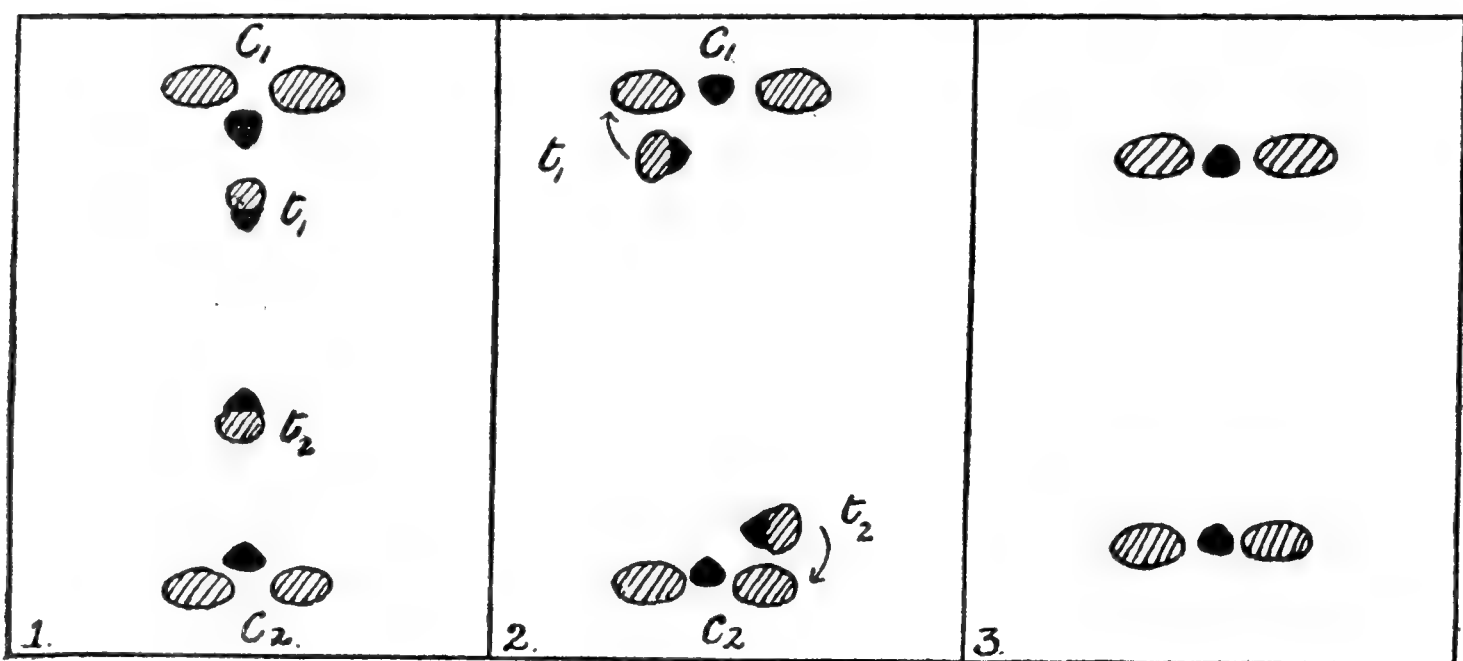


DIAGRAM 8. *Echinopsis multiplex*, Series 1. Behaviour of cotyledonary and tubercle bundles.

Fig. 1), in which the cotyledonary bundles ( $c_1, c_2$ ) both showed bifurcation of the phloem by the time the tubercle-bundles ( $t_1, t_2$ ) appeared. The strand  $t_1$  rotated through an angle of  $90^\circ$ , moving outwards as it did so to meet the incoming cotyledonary trace ( $c_1$ ), and  $t_1$  finally fused laterally with  $c_1$  (Diagram 8, Fig. 2); in a similar manner  $t_2$  fused with  $c_2$ . By this means two bundles, such as are shown in Diagram 8, Fig. 3, are produced. This is a parallel case to what occurs in *Cereus tortuosus*.

*Echinopsis multiplex*, Series 2, showed a similar sequence of events as far as the larger cotyledon was concerned, but in the smaller seed-leaf the two strands behaved differently. In this case the tubercle-trace rotated and moved outwards to meet the seed-leaf-trace; this latter also rotated slightly but did not bifurcate, and the two strands together formed one bundle by the fusion of their protoxylems. In this cotyledon the tubercle-

and the cotyledon-strands were of equal importance in effecting the formation of one of the root-poles. In a third series of *Echinopsis multiplex* yet another variation was seen. The two strands derived from the larger cotyledon and the corresponding tubercle rotated towards one another and the xylems fused ( $c_2, t_2$ , Diagram 9, Figs. 1 and 2); when the junction was almost completed the cotyledonary phloem ( $c_2$ ) showed a belated bifurcation, one half immediately joining with the tubercle phloem ( $t_2$ ) lying near it (Diagram 9, Fig. 3). In the smaller cotyledon ( $c_1$ ) the tubercle bundle ( $t_1$ ) soon lost its xylem, and its phloem bifurcated. The cotyledonary trace ( $c_1$ ) remained *in situ*, and the two phloem groups ( $t_1$ ) passed outwards on either

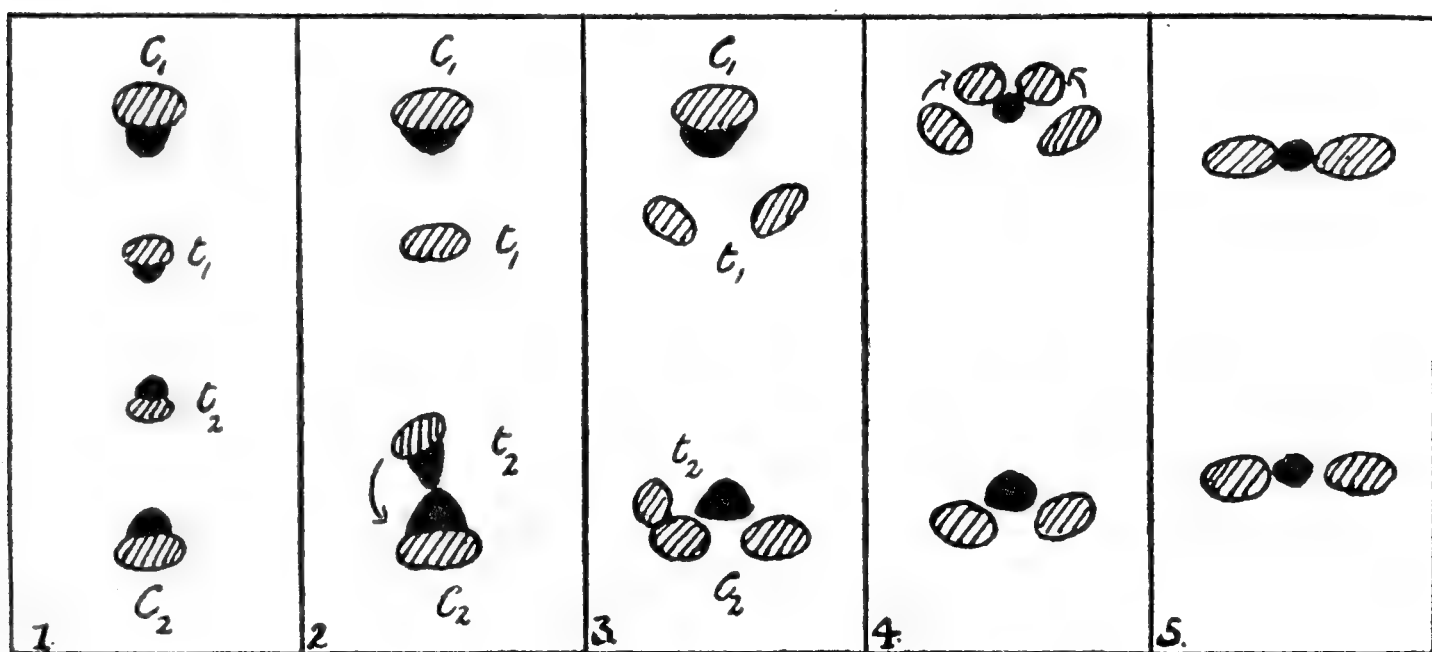


DIAGRAM 9. *Echinopsis multiplex*, Series 3.

side of it, and fused with the cotyledonary phloem ( $c_1$ ), which by this time had bifurcated (Diagram 9, Figs 1 to 5).

It is thus seen that a tubercle-trace may act in a manner usually associated with the vascular strand of a cotyledon.

*Echinopsis Lagermannii*. Two vascular strands are present in each cotyledon. These two strands rotate towards one another, their protoxylems fuse, but the phloems remain separated, one mass on either side of the wood; thus a bundle is produced exactly like that figured in Diagram 8, Fig. 1 ( $c_1$ ); it is derived, however, by the fusion of two bundles, and not, as in *Echinopsis multiplex*, by the bifurcation of one. The behaviour of the cotyledon- and tubercle-traces in all other respects resembles that described in *E. multiplex*, Series 1.

*Echinopsis tubiflora*, Series 3, is essentially similar to *E. multiplex*, Series 1.

In Series 2 the behaviour of the cotyledon- and tubercle-bundle of one seed-leaf is like that of Series 3; in the other seed-leaf two equal bundles replace the median cotyledon-bundle, and these two strands, together with the tubercle-bundle, act as in *Echinopsis Lagermannii*.

One cotyledon in Series 3 is also similar to *E. Lagermannii*, but in the second cotyledon there are three equal bundles present; two of these fuse and later form one half of the 'double' bundle, while the second half is produced by the fusion-product of the third cotyledonary bundle and the tubercle-trace.

A study of the various methods of formation of the two bundles always found towards the upper part of the hypocotyl in the species of *Echinopsis* examined, leads to the conclusion that the behaviour of the cotyledon-bundle depends almost entirely upon the stage of development of the vascular supply of the tubercle; where the latter is absent or is poorly differentiated, the former dominates the transition; on the other hand, where it is well developed it shares equally with the seed-leaf-trace, and may even play the more important part.

A further detail in connexion with *E. multiplex* assumes interest when compared with the Mamillarias to be described later. In this species the epicotylar strands contribute a fair share to the root-structure, while in the other species of *Echinopsis* examined, the bundles, even when fairly well developed, play no part in the formation of the root.

#### THE HYPOCOTYL.

The globular hypocotyl in *Echinopsis* is remarkable for the enormous development of its large parenchymatous cells; the vascular tissue appears but of slight dimensions when compared with the non-vascular tissue.

The curious cortical lobing of the hypocotylar base, which was mentioned above as occasionally occurring in some members of the *Cereus* group, is an almost constant feature of *Echinopsis* species. Towards the base of the hypocotyl the outer layers of the cortex grow downwards for a short distance, very closely enveloping the upper part of the root. The growth may take place in a complete circle around the axis, so that a transverse section, at a level towards the base of the region of downward growth, shows a remarkable zoned structure, the primary root having the appearance usually associated with an adventitious root embedded in the cortex of a rhizome. More usually, however, it occurs only at two or more places, so that at a lower level the cortex is lobed. This growth rarely attains an equal depth all round the axis, hence it often appears, in transverse section, as though an outer layer of cortex were being gradually peeled off.

The upper part of the root, as in most of the other genera, just below the region of exfoliation of the cortex, is closely invested with long root-hairs; below this, again, there is a more or less well marked superficial cork formation.

Transition.

A. Species in which the vascular symmetry of the root is attained by the cotyledonary bundles only.

*Echinopsis Eyriesii*, Pfeiff. and Otto. Each cotyledon supplies the hypocotyl with one endarch collateral bundle, approximately equal in size to a single mesophyll cell. Soon after entering the node the phloem bifurcates, and its two halves rotate until they lie almost in a straight line with the protoxylem; no metaxylem is present. The four strands thus produced show no further change beyond a slight centripetal displacement, and the root structure is of the typical *Cereus* type (Fig. 12).

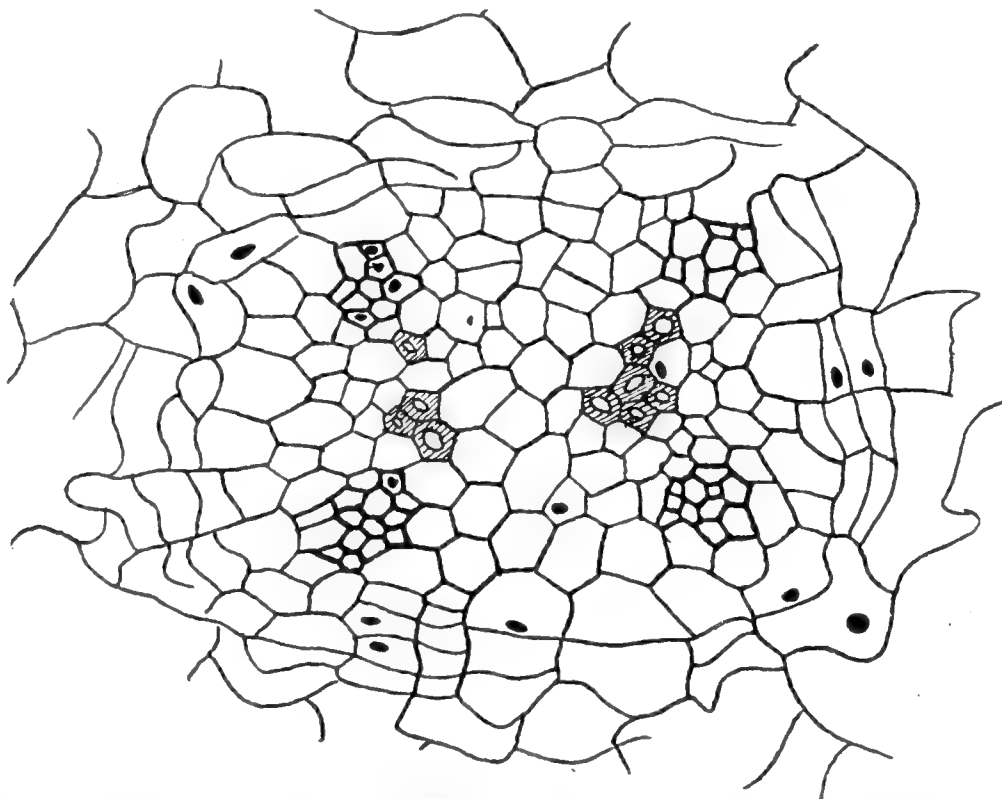


FIG. 12. *Echinopsis Eyriesii*. × 220.

*Echinopsis Zuccarinii*, Pfeiff. and Otto. These seedlings differ from those of *E. Eyriesii* in but one respect; in one series the root-structure gives place towards the root-tip to a completely diarch arrangement by the fusion of the opposing groups of phloem.

*Echinopsis oxygona*, Pfeiff. and Otto. In this species the bifurcation of the phloem in the cotyledonary bundles takes place before the node is reached; in other respects the transition-phenomena resemble those of *E. Eyriesii*. The *Cereus* type of root is not maintained, however, for just as differentiation is about to cease the opposing phloem groups fuse, giving rise to a diarch root.

B. Species in which the vascular symmetry of the root is attained by means of tubercle and cotyledonary bundles.

The upper region of the hypocotyl in all the seedlings examined showed two vascular strands, in which the phloem groups appeared on either side of a centrally placed group of protoxylem elements, which often

consisted of one or two tracheides only. The formation of these two strands from the tubercle and cotyledonary traces has already been described in the account of the tubercle, so that no further description is necessary, and only the subsequent transition features will be given.

*Echinopsis multiplex*, Pfeiff. and Otto. In the upper region of the hypocotyl there are present only the two 'double' bundles (Fig. 13), but very soon six epicotylar bundles appear; they are grouped in threes on either side and occupy the intercotyledonary plane.

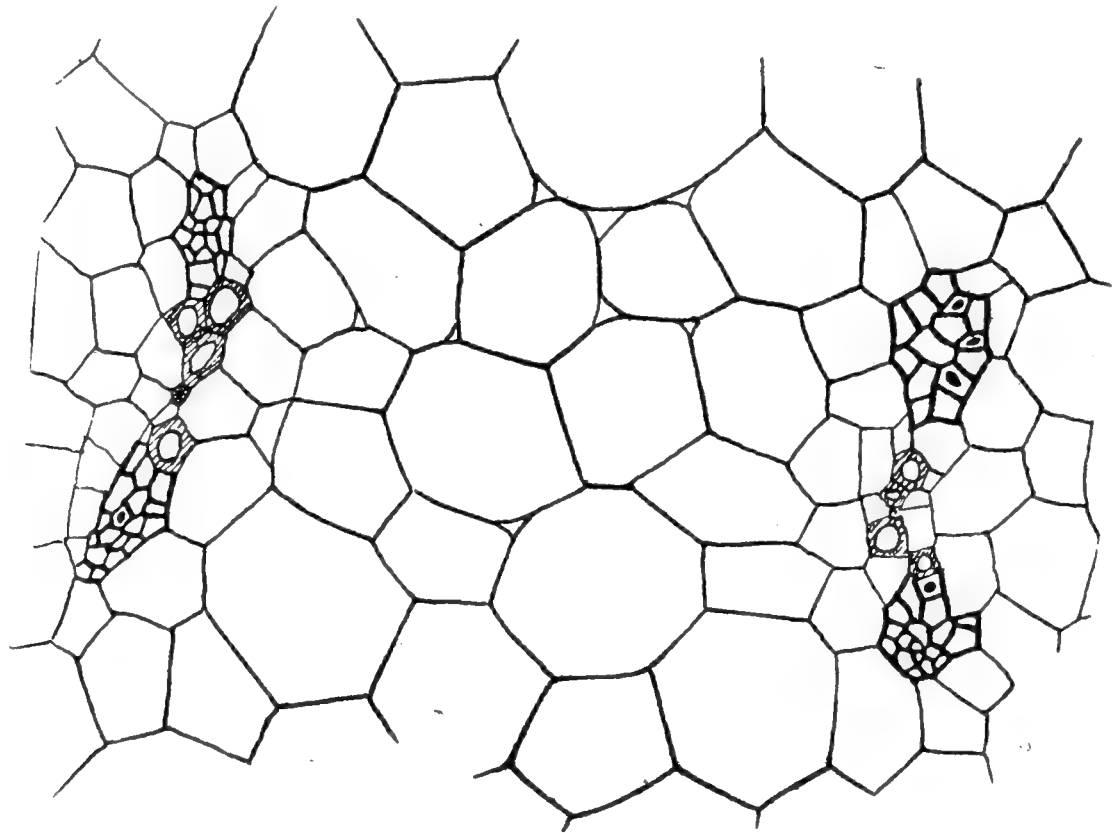


FIG. 13. *Echinopsis multiplex*. Upper part of the hypocotyl.  $\times 109$ .

The median bundle of each group of three gradually dies out, the remaining two move slowly outwards towards the cotyledonary bundles and fuse laterally with them. Towards the base of the hypocotyl 'barrel' tracheides are developed; at first they bridge the gap which exists between the protoxylem and its phloems, but they gradually extend inwards, until they occupy most of the central part of the axis. The four phloems do not fuse, thus the root shows the arrangement characteristic of the species of *Cereus*.

*Echinopsis Lagermannii*. The transition-phenomena in this species resemble very closely those just described for *E. multiplex*, with the exception that the phloems fuse in pairs, so that the root is diarch.

*Echinopsis tubiflora*, Zucc., is similar to *E. multiplex*.

#### ECHINOCACTUS.

The seedling *Echinocacti* show a close resemblance to those of *Echinopsis* in their external features, but their cotyledons are usually very unequal in size; in *E. Ottonis* they are reduced to microscopic papillae,



which fuse with the well developed tubercle at the cotyledonary node (Fig. 14). A small cotyledonary bud is developed in *E. denudatus* and *E. bicolor*, which in one seedling of the latter species possesses a vascular bundle; *E. hexaedrophorus* and *E. Wislezeni* show no sign of this structure.

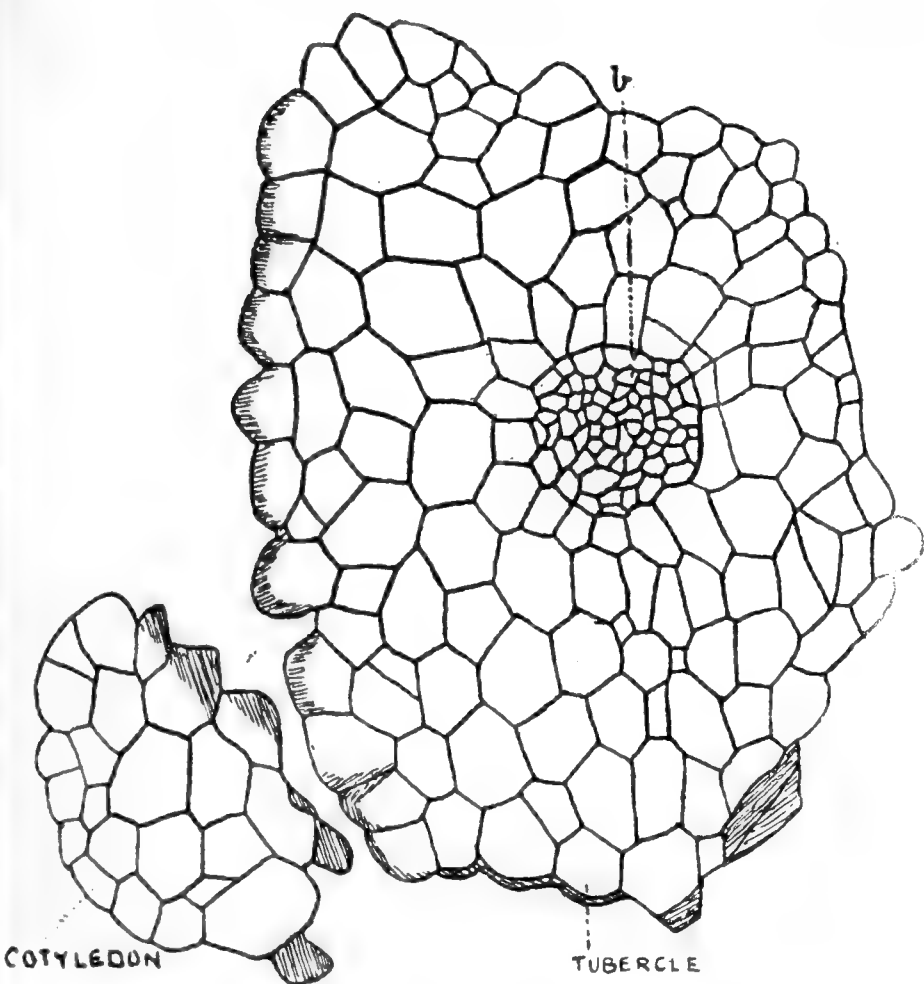


FIG. 14. *Echinocactus Ottonis*. Base of cotyledon and its tubercle. *b* = base of spine group.  $\times 109$ .

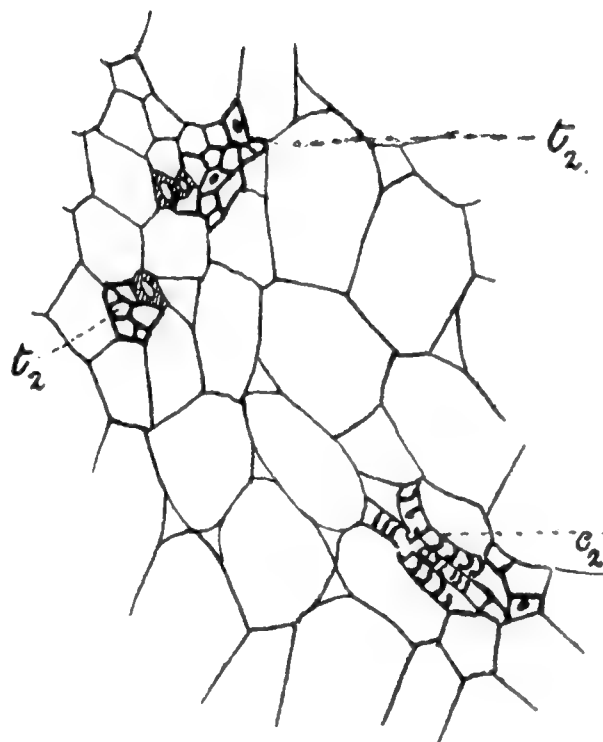


FIG. 15. *Echinocactus Ottonis*. Cotyledon- and tubercle-bundles.  $\times 109$ .

### Transition.

*Echinocactus hexaedrophorus*, Lem. Each cotyledon supplies the hypocotyl with one small endarch bundle, in which the phloem bifurcates during the passage through the cortex; protoxylem only is present in the bundles and the phloem groups move round this as round a pivot, until they lie one on either side of it. About the central region of the hypocotyl new xylem elements arise between the two protoxylem groups until the two become connected; this diarch plate, however, does not long persist, for new tracheides arise to the right and left of its central region until a cross-shaped xylem mass results. Towards the base of the hypocotyl the central elements of the cross disappear and leave four xylem groups alternating with the four phloem-bundles of a tetrarch root.

*Echinocactus Wislezeni*, Engelm. The bifurcation of the single bundle of the cotyledons in this species takes place in the cotyledon. The phloem, accompanied by a small quantity of metaxylem, rotates and separates from the protoxylem, which it thus leaves exposed in the exarch position. Four



well developed epicotylar bundles are present in the upper part of the hypocotyl; these move towards the adjacent halves of the cotyledonary 'double' bundles and fuse with them. Towards the base of the hypocotyl the four phloem groups have lost their accompanying metaxylem elements, and the root-structure is thus of the type characteristic of species of *Cereus*.

*Echinocactus denudatus*, Link and Otto. Each of the cotyledons of this seedling has a median bundle and two small lateral ones, which at the base of the seed-leaf fuse with the central strand. Bifurcation and rotation of the phloem take place as in *E. hexaedrophorus*, but, at a later stage, two of the phloem groups fuse ( $ph_4 + ph_1$ , Fig. 16, A).

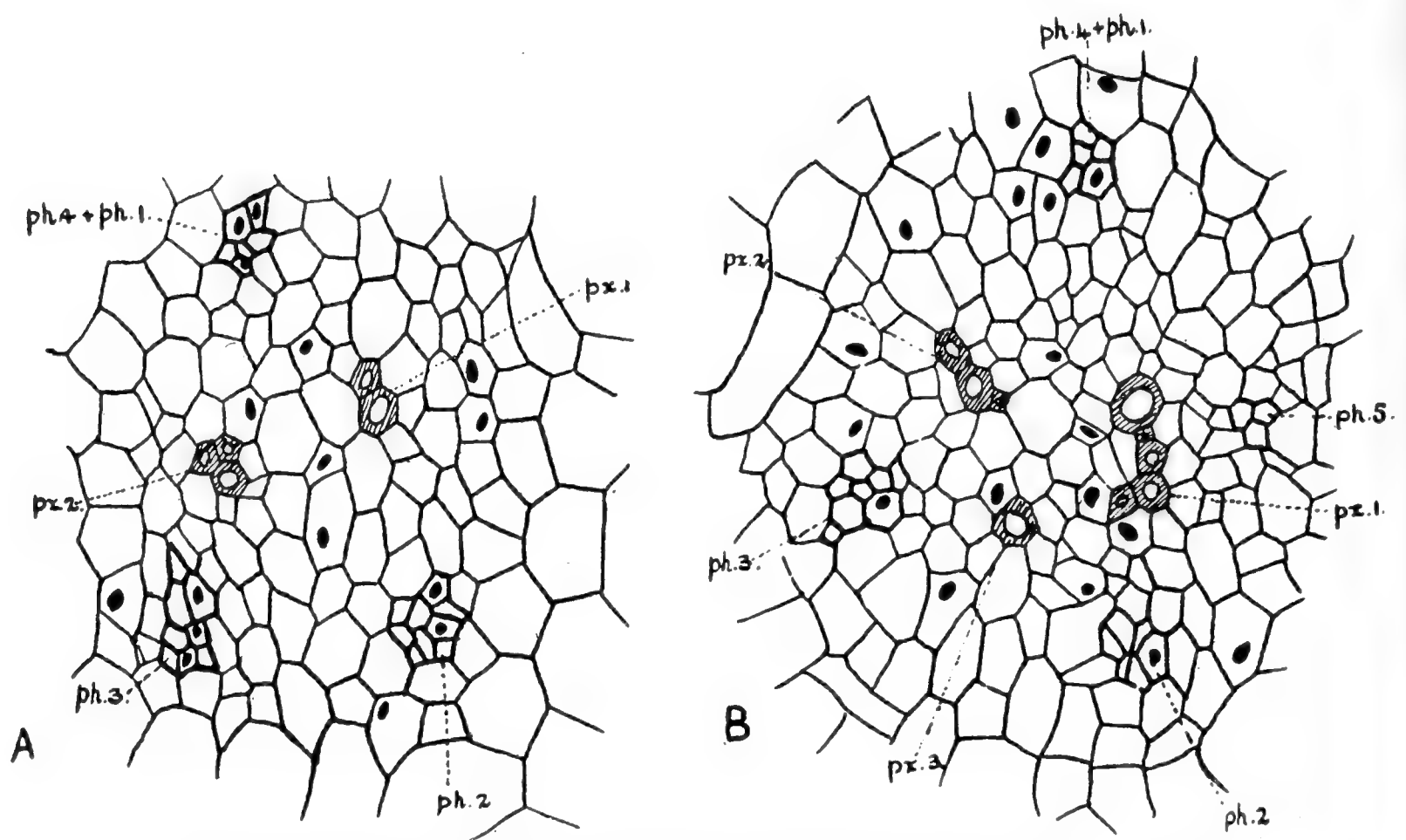


FIG. 16. *Echinocactus denudatus*. Formation of the tetrarch root.  $\times 220$ .

At a still lower level new xylem elements are differentiated on either side of  $px_1$ , producing a slightly elongated strand of tracheides. Opposite to the middle of this xylem arc a phloem group arises ( $ph_5$ , Fig. 16, B), and between  $ph_2$  and  $ph_3$  (Fig. 16, B) the appearance of a new xylem element shows the origin of a third xylem-bundle. The fourth bundle results from the breaking of the protoxylem group  $px_1$ , one branch passing between the phloem groups  $ph_4 + ph_1$  and  $ph_5$ , the other remaining *in situ*; by means of these changes a tetrarch root is produced.

*Echinocactus bicolor*, G. The top of the hypocotyl is occupied by two endarch cotyledonary bundles and six epicotyledonary strands; the latter arrange themselves in two groups of three, alternating with the seed-leaf-traces, and, at a slightly lower level, two of each group fuse. The phloem

of each cotyledonary bundle bifurcates and rotates round the xylem, which consists only of protoxylem tracheides; as soon as this is completed the epicotyledonary strands, which have been moving outwards, fuse with the cotyledonary bundles. This *Cereus* arrangement persists almost to the apex of the root, but finally the phloems fuse and a diarch root results.

*E. tricolor*, Series 2, shows a slight difference from the above in its possession of a cotyledonary bud-bundle, in addition to the six epicotyledonary strands. These bud-bundles ( $t_1$  and  $t_2$ , Diagram 10, Fig. 1) pass obliquely to one side and fuse with one of the epicotyledonary bundles, the remaining two strands ( $e$ ) also fuse (Diagram 10, Fig. 1). The four strands thus produced ( $t_1+e$ ,  $e$ ,  $t_2+e$ , and  $e$ , Diagram 10, Fig. 2) move outwards and join on to the cotyledonary strands, which have by this time bifurcated. The remaining details of the transition are essentially similar to those of the other seedlings of the genus.

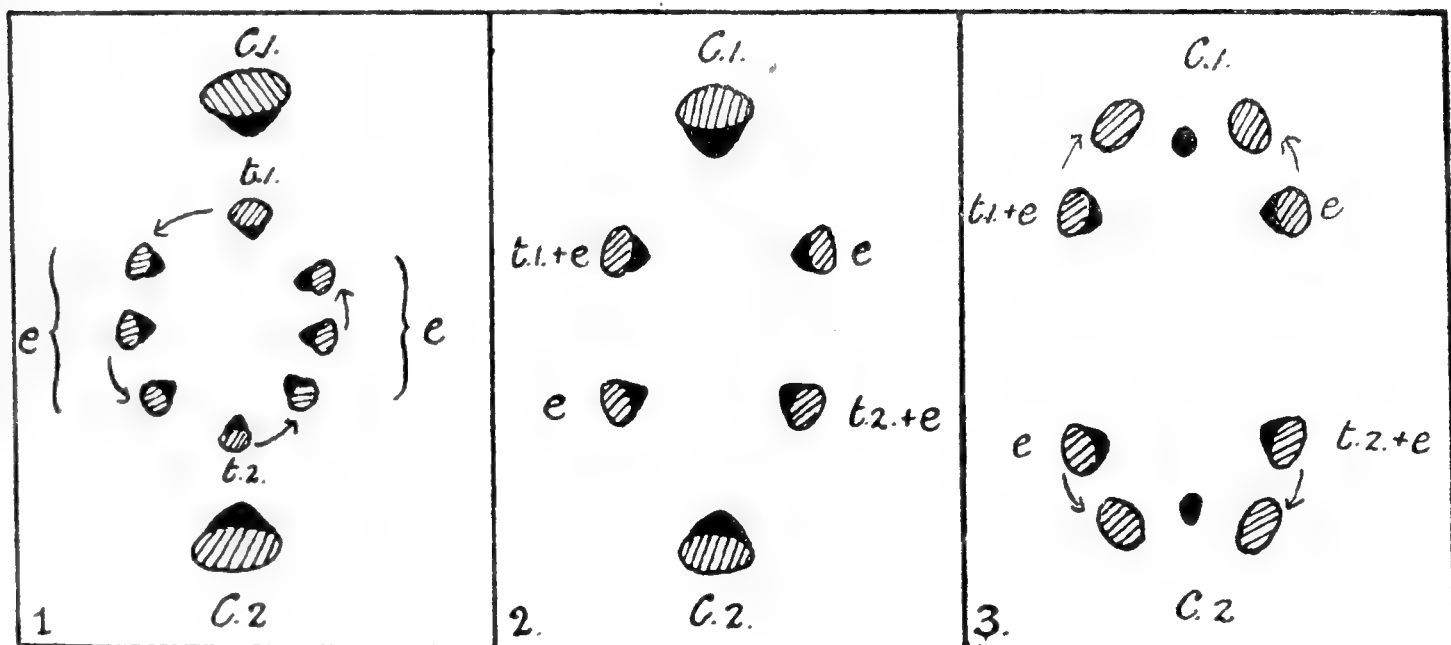


DIAGRAM 10. *Echinocactus bicolor*, Series 2.

*Echinocactus Ottonis*, Link and Otto. The seedlings of this species differ very considerably from those of the other members of the genus in their possession of well-marked cotyledonary tubercles, which far surpass in importance the seed-leaves themselves, for the latter are reduced to mere papillae. This increasing importance of the tubercle over the cotyledon is shown in the behaviour of the vascular strands. In all the seedlings examined, the tubercle-traces ( $t_1$ ,  $t_2$ , Diagram 11, Fig. 1) appeared some time before those which supplied the cotyledons, but this would naturally be expected from the greater comparative development of the tubercle.

*Echinocactus Ottonis*, Series 3, illustrates the simplest course of events. In its larger cotyledon the two traces ( $c_1$ ,  $t_1$ , Diagram 11, Fig. 2) rotate and fuse, thus producing one bundle, as in *Echinopsis multiplex*, Series 2; in the smaller seed-leaf the cotyledonary strand  $c_2$  remains *in situ* while the

tubercle-strand  $t_2$  bifurcates; its halves pass outwards and fuse with the seed-leaf-trace, forming the second bundle (Fig. 15, and Diagram II,

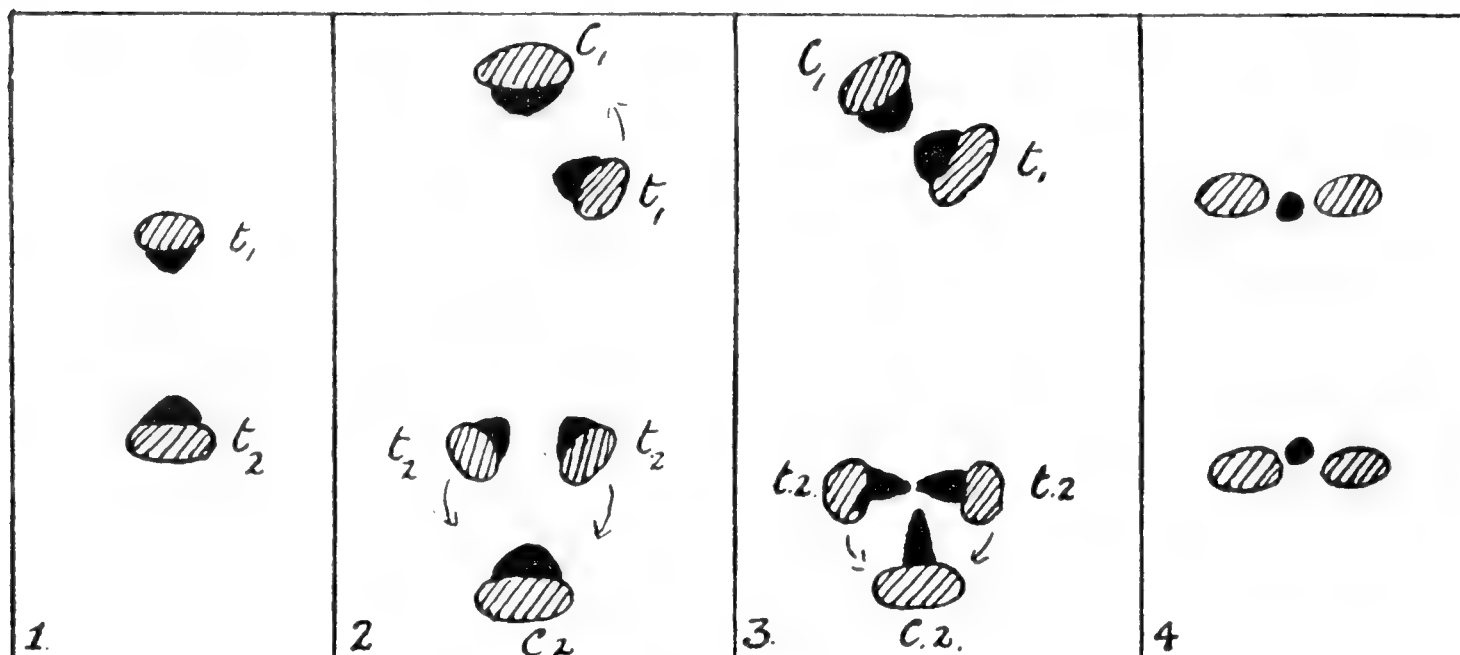


DIAGRAM II. *Echinocactus Ottonis*, Series 3.

Figs. 1-4). In this seedling we thus have a further example of a tubercle-trace acting in the manner usually associated with a cotyledonary bundle.

*Echinocactus Ottonis*, Series 1, shows an interesting feature in its

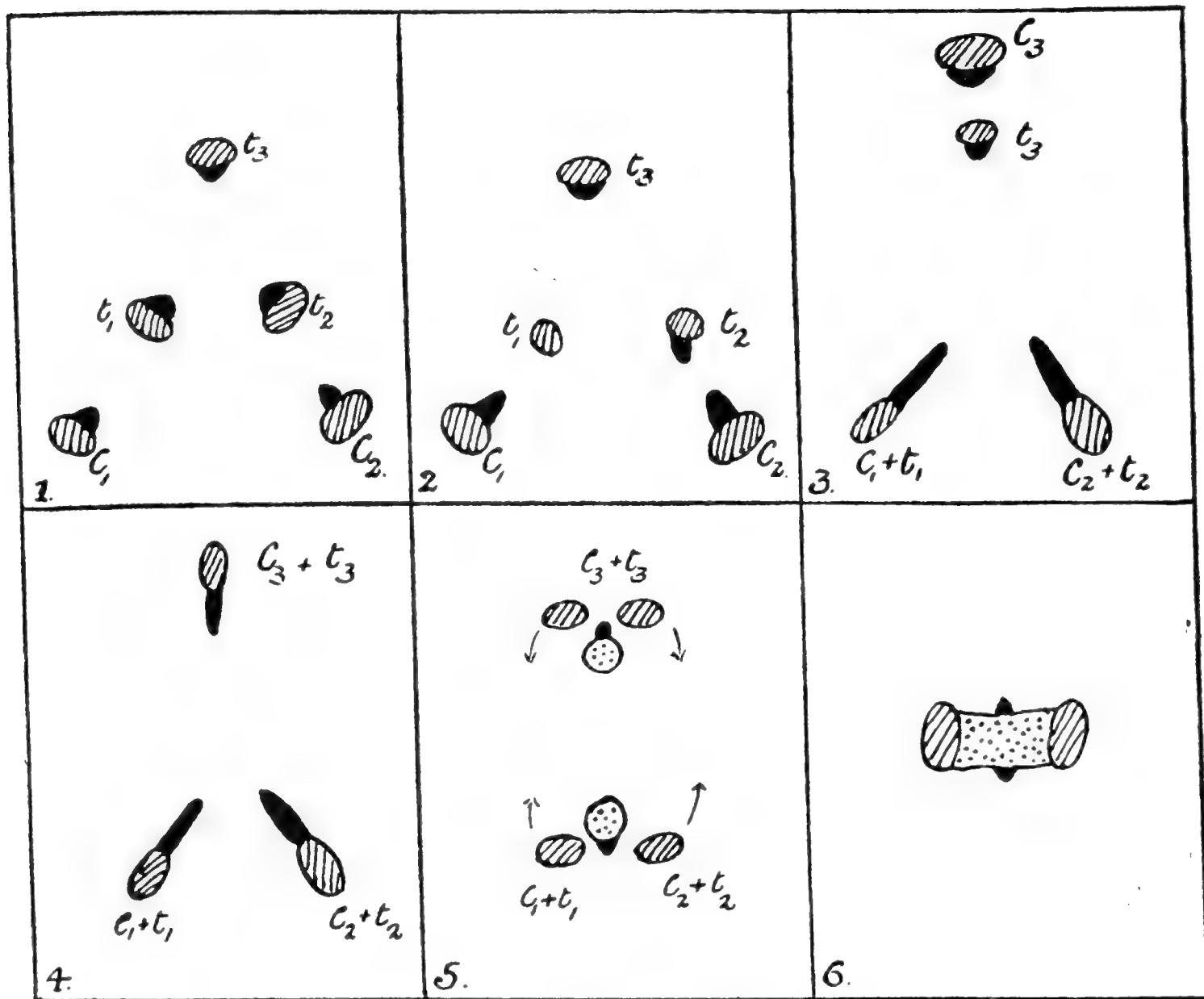


DIAGRAM 12. *Echinocactus Ottonis*, Series 1.

possession of three cotyledons subtending three tubercles. The behaviour of the vascular tissue points to the fact that two of the cotyledons ( $c_1, c_2$ , Diagram 12, Fig. 1) have been derived from the splitting of one larger one to its extreme base. The three pairs of vascular strands run into the hypocotyl so very obliquely that it is impossible to say definitely exactly what occurs, but fusion of the members of a pair takes place, resulting in the formation of three bundles, as shown in Diagram 12, Figs. 1-3.<sup>1</sup> The strand ( $c_3 + t_3$ ) which supplied the smaller cotyledon and its tubercle

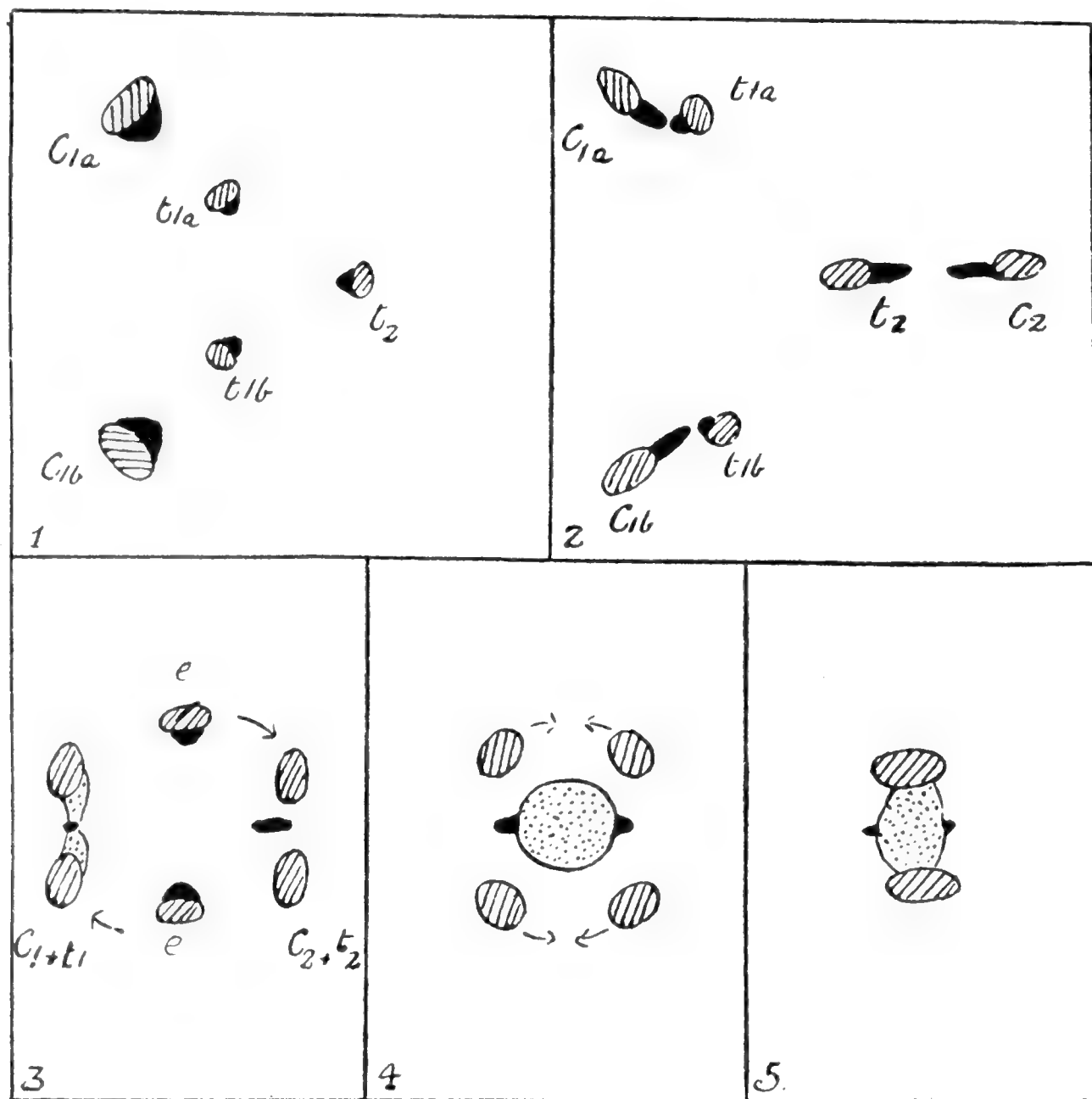


DIAGRAM 13. *Echinocactus Ottonis*, Series 2.

soon showed a bifurcation of the phloem (Diagram 12, Fig. 5), while the other two bundles rotated towards one another and together formed a V-shaped strand in which the protoxylem occupied the apex of the V. Large metaxylem tracheides were enclosed by the arms of the V; these, however, soon died out and others differentiated centripetally, as is shown in Diagram 12, Fig. 5.

<sup>1</sup> The elongation of the bundles shown in Diagram 12, Figs. 3 and 4, and in Diagram 13, Figs. 2 and 3, is intended to represent their appearance in almost longitudinal section. This is due to the rapidity of their centripetal displacement.

*Echinocactus Ottonis*, Series 2, shows a seedling in which the splitting of the larger cotyledon has not completely taken place, for while it possesses three seed-leaves, there are only two tubercles. Two of the cotyledons subtend one tubercle, and these two may be considered to represent one which has split nearly to its base. The tubercle ( $t_1$ ) belonging to the split cotyledon is, however, supplied with two vascular bundles instead of the usual one ( $t_{1a}$ ,  $t_{1b}$ , Diagram 13, Fig. 1). These two strands pass outwards and fuse with those of the cotyledons ( $c_{1a}$ ,  $c_{1b}$ , Diagram 13, Fig. 2); the two strands thus produced undergo rapid centripetal displacement, rotate slightly towards one another, fuse as regards their protoxylems, and finally together form one bundle (Diagram 13, Fig. 3). The second bundle is formed by the union of the remaining cotyledon- and tubercle-traces ( $c_2$ ,  $t_2$ , Diagram 13, Figs. 2-4).

#### *Transition.*

In Series 2 there were present in the upper part of the hypocotyl two 'double' bundles, produced in the manner described above, and two epicotyledonary bundles ( $e$ , Diagram 13, Fig. 3). The behaviour of these bundles in the transition is indicated in Diagram 13, Figs. 3-5. Extraordinarily large 'barrel' tracheides are developed in the central region of the axis.

In Series 1 and Series 3 the formation of the diarch root takes place in a similar manner by the fusion of the opposite pairs of phloem-strands, but in these seedlings there is no development of epicotylar bundles.

#### MAMILLARIA.

The Mamillarias have reached the ultimate stage in the development of a succulent habit in the seedling, which, in this group, consists of a tiny globular structure ending in a short, thread-like root (Fig. 17).

Cotyledons are practically absent, for even in the species in which they attain their maximum size they merely appear as two minute swellings at the apex of the spherical hypocotyl (Figs. 17 and 18). In spite of the extreme reduction of the cotyledons, a reduction to microscopic papillae in most of the species examined, a difference in size between the two can always be observed. In general the stem apex is very much depressed (Fig. 18).

Tubercles are well developed in those members of the genus in which the cotyledons attain their greatest size, e. g. *M. multiceps*, *M. rodantha*, *M. centricirrha*, *M. polyedra*, and *M. meiacantha*; in these species it is only possible to differentiate cotyledonary from epicotyledonary tubercles by the position of the former in the axils of the cotyledons. The tubercle

possesses a vascular strand which traverses its tissue to the base of the spine group, but the cotyledon is usually without a vascular supply. In some cases a bundle may reach the base of the seed-leaf papilla, but more usually it does not do so, but ends blindly in the hypocotyl; the name cotyledonary bundle will still be applied to these strands, however, for, from their position, they obviously represent the seed-leaf-traces of the other genera. The behaviour of the tubercle- and cotyledon-bundles will be described later, in the account of the transition-phenomena.

The still further reduction of the cotyledons shown by *M. pentacantha*, *M. hexacantha*, and *M. eriacantha* is accompanied by the partial suppression of the tubercles, which are here represented by cotyledonary buds, such as were described as occurring in some species of *Cereus*; these buds may



FIG. 17.  
*Mamillaria*  
*rhodantha*.  
× 3.

Fig. 18. *Mamillaria multiceps*. L.S. through upper part of seedling.  
*s* = group of spines, *t* = tubercle, *c* = cotyledon, *a* = stem apex, *v* = vascular strands. × 62.

or may not possess vascular tissue. *M. spinosissima* and *M. Donatii* have neither tubercles nor buds, and the cotyledonary papillae are almost entirely suppressed; while, finally, *M. pusilla* has reached the stage in which the seedling consists of hypocotyl and root only; cotyledons are entirely absent.

The structural details of the hypocotyl and the root so closely resemble those described in *Echinopsis* that any further account of them is quite unnecessary.

#### Transition.

The details of the transition-phenomena in the different species of *Mamillaria* examined differ considerably, so that it is quite impossible to give any general description; for this reason the account of the various species will be given separately.



A. *Species in which tubercle- and cotyledon-bundles play a part in root formation.*

*Mamillaria multiceps*, Salm-Dyck. The upper part of the hypocotyl contains four vascular bundles, two of which are supplied by the cotyledon and two by the epicotyledonary tubercles (Diagram 14, Fig. 1).

Very soon four smaller epicotylar bundles make their appearance, alternating with the other four (Diagram 14, Fig. 2), and the eight bundles almost immediately fuse into two groups ( $e+t_1$ ,  $e+t_2$ ), which take up

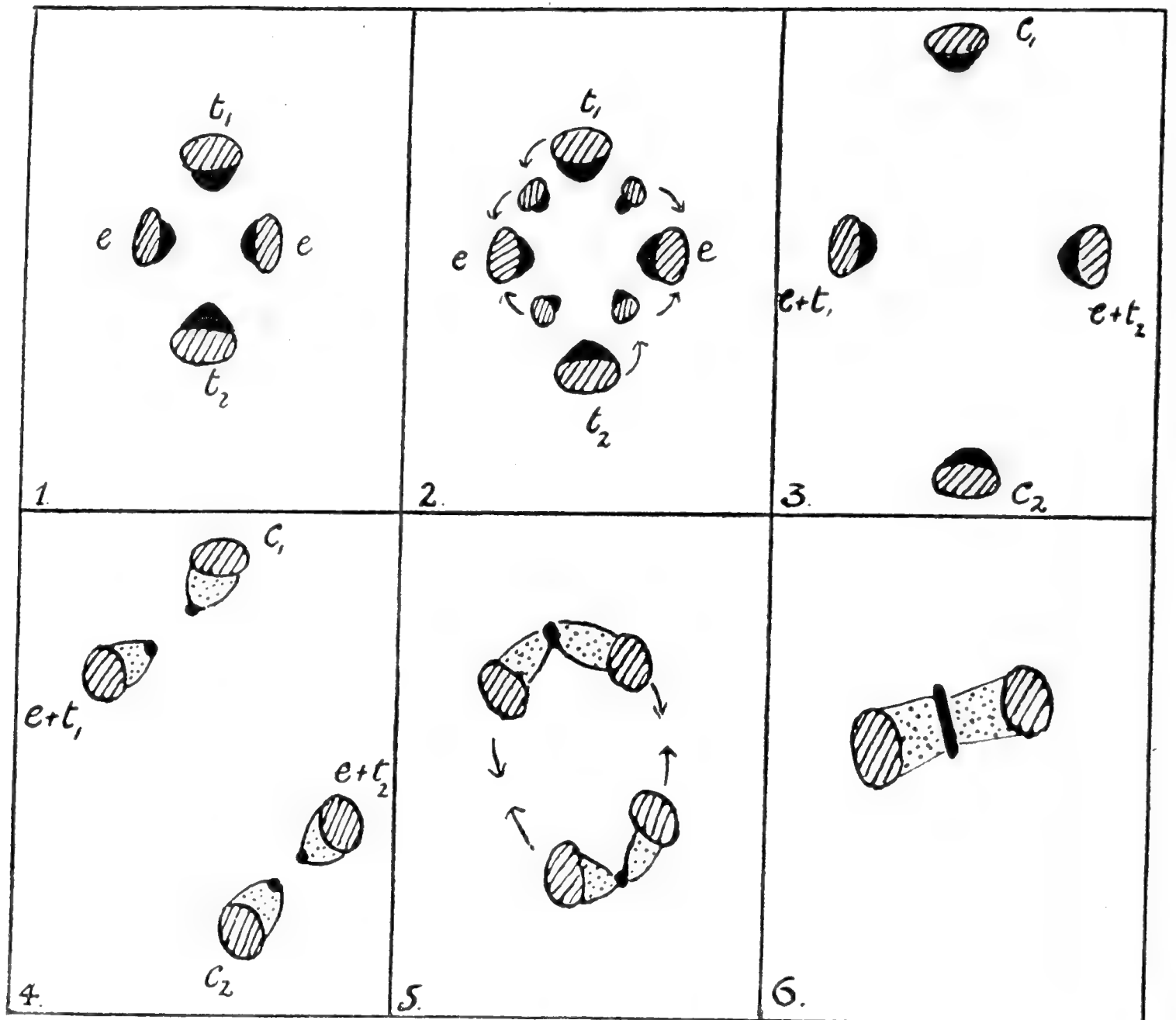


DIAGRAM 14. *Mamillaria multiceps*.

a position in the intercotyledonary plane alternating with the two cotyledon-bundles ( $c_1$ ,  $c_2$ ), which have by this time differentiated (Diagram 14, Fig. 3). The two strands ( $c_1$ ,  $c_2$ ) rotate slightly and pass inwards to meet the two rotating bundles  $e+t_1$  and  $e+t_2$  (Diagram 14, Fig. 4). The protoxylems of the adjacent bundles fuse, and by the continued movements of the phloems they are left in the exarch position (Diagram 14, Fig. 5). The opposite phloem groups finally fuse, resulting in the formation of a diarch root (Diagram 14, Figs. 5 and 6). In this species the metaxylem elements, which make their appearance towards the middle region of the

hypocotyl, are of the 'barrel' or spindle type, and are very sharply marked off from the protoxylem (Fig. 19).

*Mamillaria rhodantha*, Link and Otto. One of the seedlings examined showed a transition almost precisely similar to that described in *M. multiceps*, but the others differed.

In these last the cotyledonary bundles ( $c_1, c_2$ ) appeared at the node ;

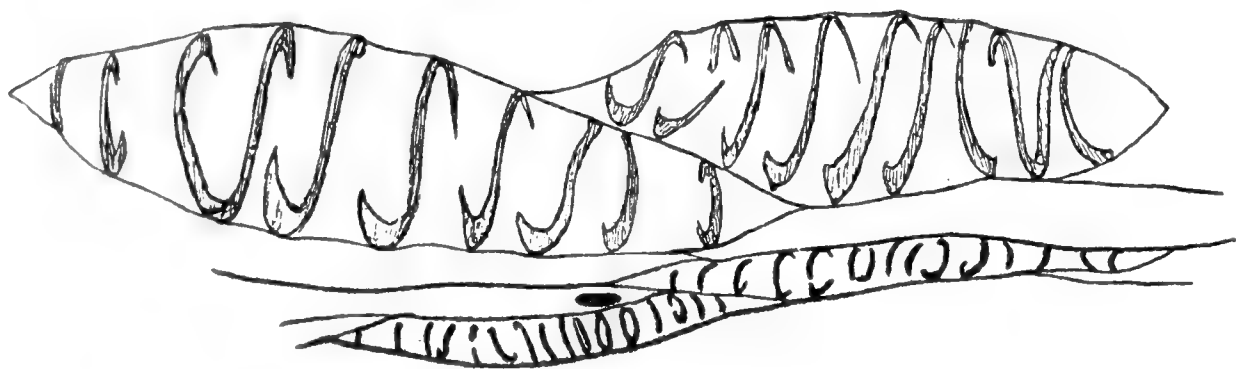


Fig. 19. *Mamillaria multiceps*. Protoxylem elements and 'barrel' tracheides.  $\times 420$ .

in addition to them were two tubercle-strands ( $t_1, t_2$ ) and two epicotyledonary traces ( $e$ ), and, very shortly, four smaller epicotyledonary strands were differentiated. Fusion of these bundles took place in the manner indicated in Diagram 15, Figs. 1 and 2. At this stage the cotyledonary phloem bifurcated, and rotated through an angle of  $90^\circ$ , thus leaving the

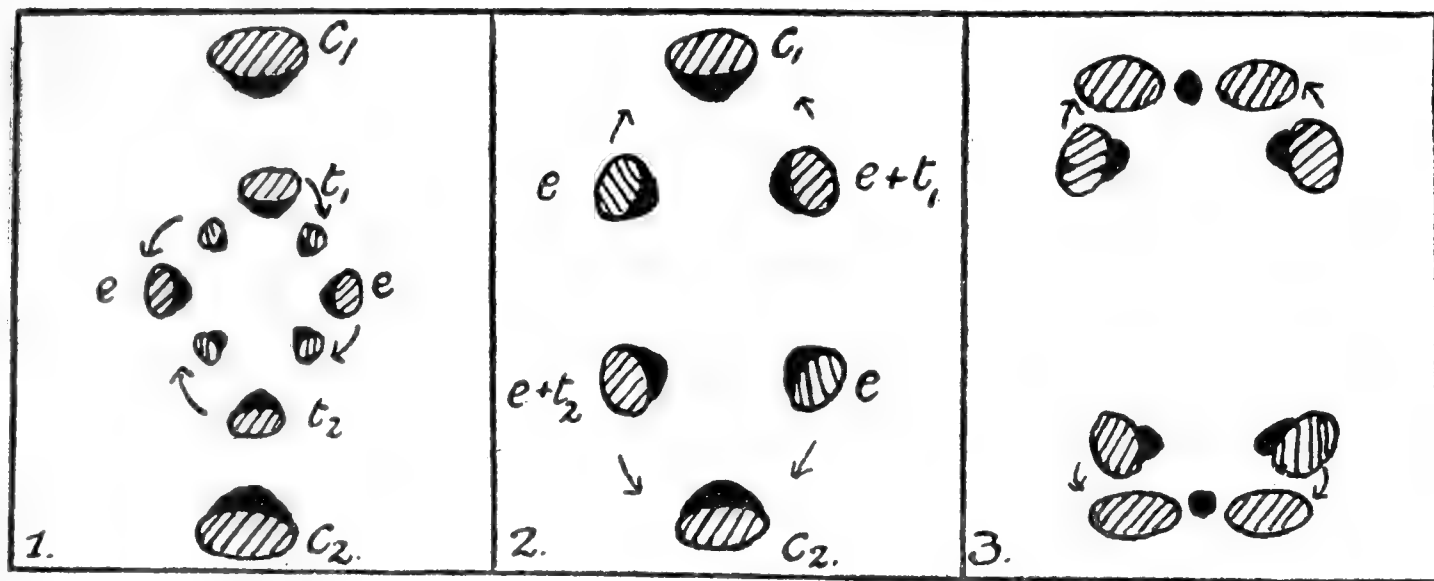


DIAGRAM 15. *Mamillaria rhodantha*.

protoxylem exposed. The remaining four bundles present in the hypocotyl moved outwards and fused laterally with the cotyledonary bundles (Diagram 15, Figs. 2 and 3). A few large metaxylem elements begin to develop at this stage between the protoxylem and the phloem, so that there are present two strands similar to those figured in Diagram 14, Fig. 5. The remaining details of the transition are similar to those of *M. multiceps*.

*Mamillaria meiacantha*, Engelm. These seedlings showed a transition which in all essential features resembled that of the last described individuals of *M. rhodantha*.

*Mamillaria centricirrha*, Lem. This species showed some slight

variation when compared with the seedlings of *M. rhodantha* described above. The upper region of the hypocotyl possessed, in addition to the tubercle- and cotyledon-traces, four epicotylar bundles; these four strands together with the tubercle-bundles ( $t_1$ ,  $t_2$ , Diagram 16, Fig. 1) by their movements finally occupy the intercotyledonary plane.

The adjacent epicotyledonary bundles soon fuse, and at about the same level cotyledonary bundle  $c_1$  bifurcates (Diagram 16, Fig. 2), the tubercle-strand ( $t_1$ ) and the epicotyledonary bundle ( $e$ ) pass outwards and fuse with the bifurcated bundle ( $c_1$ , Diagram 16, Fig. 2). The second cotyledonary bundle ( $c_2$ ) shows no sign of bifurcation, it simply rotates as a whole towards  $e$  and fuses laterally with it; this fusion bundle ( $e + c_2$ ) then fuses as regards its protoxylem with the tubercle-bundle ( $t_2$ ), in the

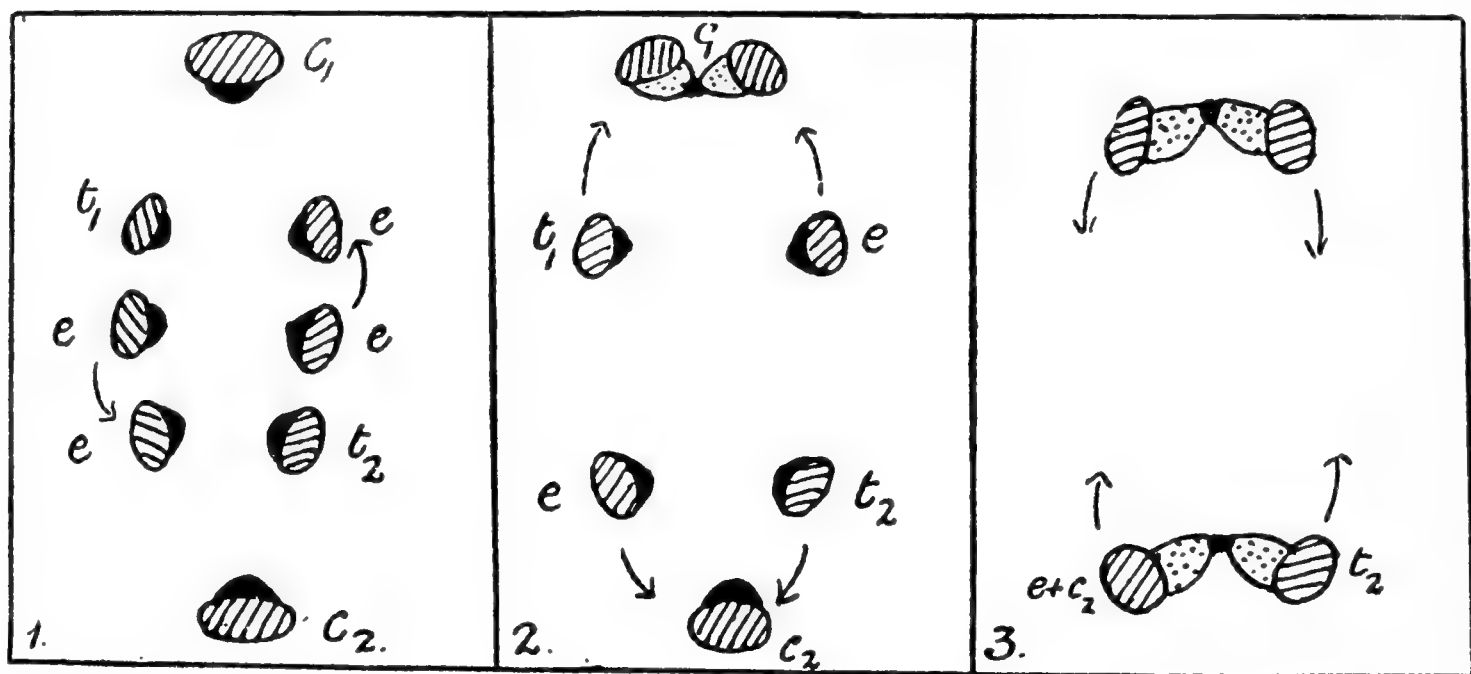


DIAGRAM 16. *Mamillaria centricirrha*.

manner shown in Diagram 16, Fig. 3. The remaining details of the transition resemble those described in *M. multiceps*.

*Mamillaria polyedra*, Mart. The two bundles supplied to the hypocotyl by the two cotyledons bifurcate early, and the transition takes place in the manner shown in Diagram 16, Fig. 2 ( $c_1$ ); unlike *M. centricirrha*, however, both cotyledonary bundles behave in a similar way. A slight variation was shown by one seedling of the species, in that the two halves of the seed-leaf-trace separated widely after bifurcation, and remained some distance apart throughout the greater part of the passage through the hypocotyl.

*Mamillaria pentacantha*, Pfeiff. In this species the tubercles were present in the form of cotyledonary buds, which in two of the seedlings examined were supplied with a vascular bundle, while in the third no such bundle was present; the transition-phenomena varied in the two cases.

In the seedlings belonging to the first category, viz. those with a tubercle-bundle, the upper part of the hypocotyl possessed four epicotyledonary, two cotyledonary, and two tubercle strands. By the gradual

movement of the tubercle and epicotyledonary strands until they occupied a position similar to that indicated in Diagram 16, Fig. 1, and by the subsequent fusion of the three bundles lying between the cotyledon-traces, the eight bundles originally present were reduced to four, which performed during the remainder of the transition in the manner indicated for *M. multiceps* in Diagram 14, Figs. 3-6.

The seedling in which no tubercle-bundles appeared showed a transition very closely resembling that described for *M. rhodantha*, but the four bundles occupying the intercotyledonary plane in the latter species and shown in Diagram 15, Fig. 3, were, in *M. pentacantha*, all epicotylar in origin.

*Mamillaria eriacantha*, Hort. As in *M. pentacantha*, a tubercle-bundle supplying the cotyledonary bud may or may not be present; but, unlike that

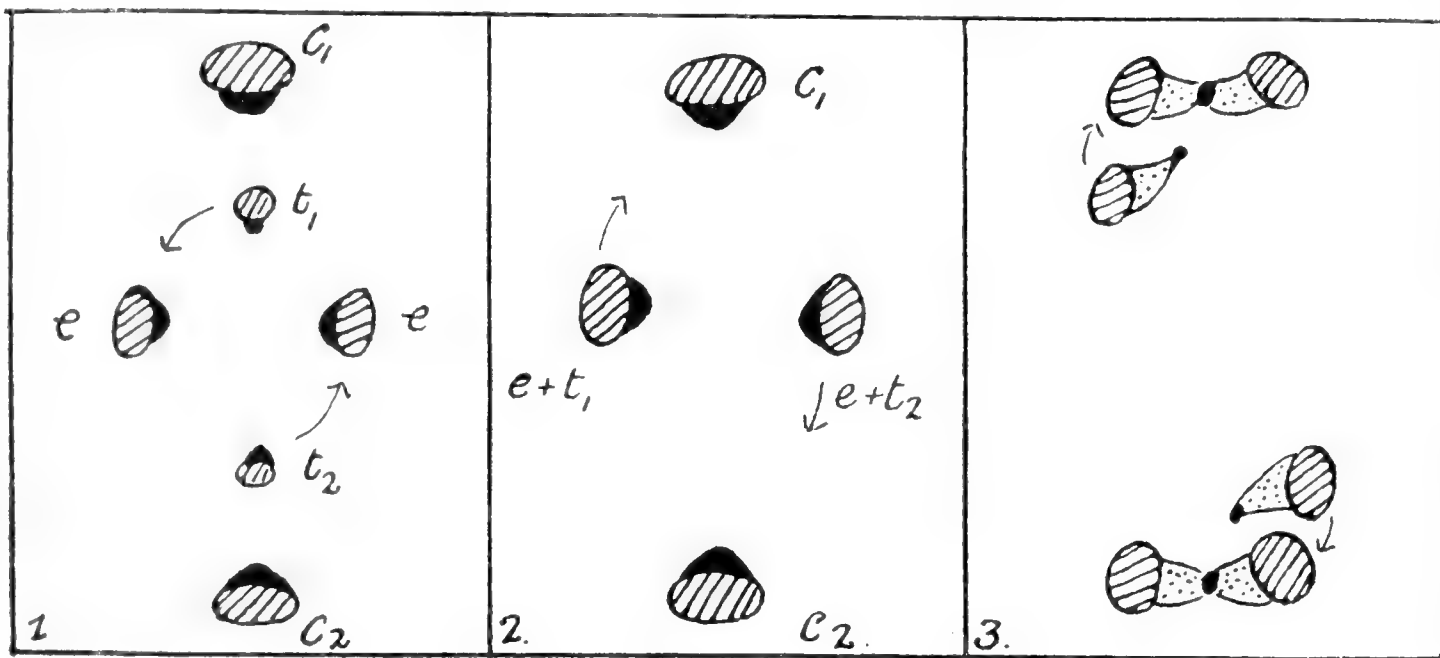


DIAGRAM 17. *Mamillaria eriacantha*.

species, the transition takes place in essentially the same manner in both cases. Two cotyledonary and two epicotyledonary bundles are present at the top of the hypocotyl; when the tubercle-bundles are developed they simply move outwards and fuse with the epicotyledonary strands (Diagram 17, Figs. 1 and 2). Almost concurrently with this fusion the cotyledonary bundles bifurcate, their phloems separate and rotate until they lie on either side of the protoxylem, which by this means is left exposed; during this change large metaxylem elements appear, in the position indicated in Diagram 17, Fig. 3. The epicotyledonary strands have by this time moved laterally towards the cotyledon-traces, fusion with them takes place, and the resulting bundles give rise to a diarch root in a precisely similar way to that indicated in Diagram 14, Figs. 5 and 6.

B. *Species in which only cotyledon-bundles play a part in root formation.*

(Strictly speaking some seedlings of *M. pentacantha* and *M. eriacantha* fall into this category and not into Group A, but for the sake of simplicity they have been described above.)

*Mamillaria hexacantha*, Salm-Dyck. The transition-features in these seedlings are practically identical with those of *M. eriacantha*, in which cotyledonary bud-bundles are absent.

*Mamillaria spinosissima*, Lem. The transition-phenomena of most of the seedlings of this species which were examined were practically identical with those of *M. eriacantha*, in which the tubercle-bundle was absent; one, however, showed a somewhat different method of procedure. In this seedling the upper hypocotylar region possessed six epicotyledon-traces lying in two groups of three, in alternation with the cotyledonary bundles. At a lower level each of the two groups fused into one strand, which, unlike what usually obtains, remained in position during the remainder of the transition. Concurrently with the fusion of the three bundles, the cotyledonary phloem bifurcated; the two halves thus produced rotated and at the same time moved gradually inwards; thus towards the middle region of the hypocotyl the epicotyledonary bundles each showed a phloem group derived from the cotyledon-bundles lying on either side of it; these phloem groups soon fuse laterally with epicotyledonary phloem; the epicotyledonary xylem dies out, but this is probably to be attributed to the age of the seedlings. During these changes the protoxylems of the cotyledon-strands, which by the movement of the phloems had been left exposed, undergo centripetal displacement; finally large metaxylem elements bridge the space between them and the diarch root is completed.

*Mamillaria Donatii*, Berge. The transition-phenomena in these seedlings very closely resemble those which are found in the seedlings of *M. pentacantha* which possess no cotyledonary tubercle-trace. The bundles are, however, very small, and the xylem is composed of very few elements.

*Mamillaria missouriensis*, Sweet. The seedlings of this species differ in one respect from all of the above described Mamillarias. The papillate cotyledons possess at first one median vascular strand, which almost immediately branches into three. In one of the two seedlings available for examination two of these strands (*b* and *c*, and *d* and *e*, Diagram 18, Fig. 1) fused in the upper part of the hypocotyl, so that there were present four cotyledonary and four epicotyledonary bundles.

The pairs of cotyledonary strands rotated towards each other as is shown in Diagram 18, Fig. 2, and at a lower level one epicotyledonary bundle (*e*) fused with each seed-leaf-trace (Diagram 18, Fig. 3), but this fusion does not take place simultaneously. After it has been accomplished the four phloem-strands separate; accompanied by the metaxylem tracheides, which from this stage gradually die out, they finally take up a position such as is shown in Diagram 18, Fig. 4. Towards the base of the hypocotyl each of the protoxylems branch, and as a result a tetrarch root is produced (Diagram 18, Figs. 5 and 6).



The other seedling of this species behaved as did the preceding one as regards the branching of the cotyledonary traces; the branches, however, soon fused again, leaving only one cotyledon-bundle for each papillate seed-leaf. This bundle bifurcated at a lower level and the divided bundle played

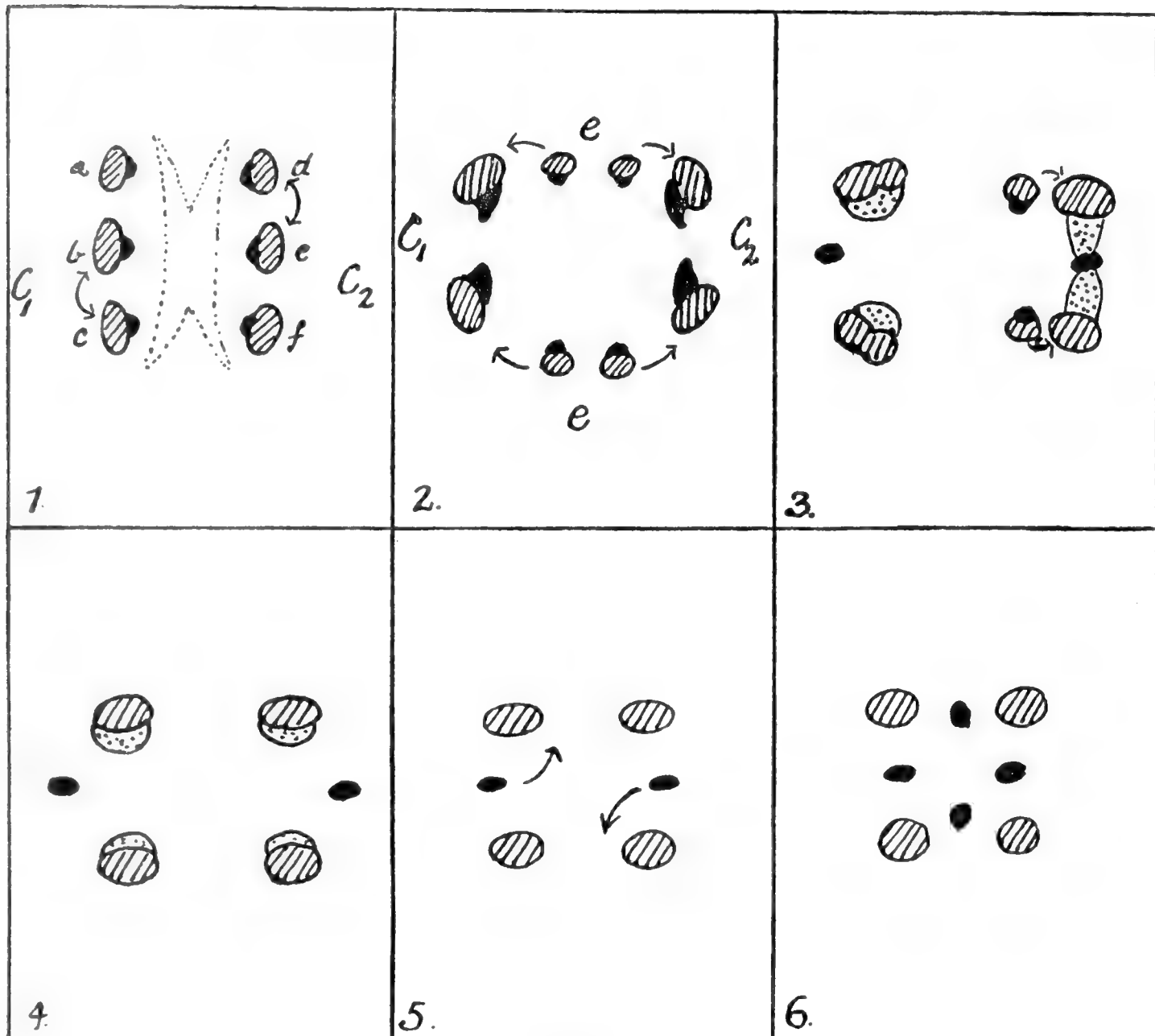


DIAGRAM 18. *Mamillaria missouriensis*.

the same part in the transition as did the paired bundle of the first described seedling. In all other respects the two seedlings were precisely similar.

The belated formation of a tetrarch root by the branching of the xylem arms recalls the similar feature described in *Cereus tortuosus*.

C. *Species in which cotyledons are absent, and epicotyledonary bundles alone produce the root structure.*

*Mamillaria pusilla*, Sweet. The hypocotyl of these seedlings possesses in its upper region four vascular strands. These bundles move gradually towards the centre, and at the same time rotate slightly towards one another in pairs; finally the protoxylems of a pair fuse and metaxylem elements begin to appear centripetally, until a solid mass of large 'barrel' tracheides results. During this development the phloem-strands have been gradually



moving laterally, until the opposite groups finally fuse, and a diarch root results. The transition is thus seen to be very similar to that illustrated in Diagram 14, Figs. 3–6, but the four groups are in *M. pusilla* all epicotylar in origin.

#### VALUE OF SEEDLING ANATOMY IN CLASSIFICATION.

The Cactaceous seedlings examined can be classified into two groups so far as their external morphology is concerned. The first of these groups includes those seedlings which bear a more or less close resemblance to those of an ordinary Dicotyledon, having a long hypocotyl and distinctly leafy cotyledons. Three genera are included in this division, namely, *Pereskia*, *Opuntia* and *Nopalea*; the seedlings of the first show no sign of a succulent habit, but in the last two genera evidence of it is given in the somewhat swollen hypocotyl and in the thick seed-leaves.

The second group is composed of all the other genera which have been examined, viz. *Phyllocactus*, *Cereus*, *Pilocereus*, *Rhipsalis*, *Echinocereus*, *Echinopsis*, *Echinocactus* and *Mamillaria*. The members of this group show a considerable diminution in length and a marked increase in succulence as compared with the seedlings of the first group; in them the hypocotyl is always short and is ovoid or globular in shape, while the cotyledons are small, pointed structures often microscopic in size.

Generally speaking, two types of transition are found in the order; the *Anemarrhena* type of Miss Sargent is characteristic of the seedlings of Group I, while Van Tieghem's Type 3 forms the ground-plan of the transition phenomena of the members of Group II; in this respect the seedling anatomy follows the morphology.

#### GROUP I.

In the seedlings of this group the transition in the upper part of the hypocotyl follows closely the *Anemarrhena* type, with the exception of *Opuntia maculacantha* and *O. Pseudo-tuna*. The type is maintained and a tetrarch root is established in the usual way in *Nopalea n. sp.*, *Opuntia Ficus-indica*, *O. Tuna*, *O. polyantha*, *O. imbricata*, *O. Bergeriana*, and *O. stricta* Series A and C, but a modification of it occurs in *O. stricta* Series B, *O. albicans*, *Pereskia n. sp.*, and *P. Pititache*. The seedling structure is, however, of very little use in delimiting the genera in the group, for the resemblance between two species of the same genus is sometimes much less close than between species of different genera; thus *Nopalea n. sp.* and *Opuntia Ficus-indica*, or *Opuntia albicans* and *Pereskia n. sp.*, would appear from their seedling anatomy to be much more closely related than *Opuntia Ficus-indica* and *O. albicans*.

## GROUP II.

The transition in the majority of the species examined in this group was of Van Tieghem's Type 3; many variations on it occurred, of which the following were the most important:—

(a) In *Phyllocactus*, *Cereus*, *Pilocereus*, *Echinocereus*, sp. of *Echinopsis*, and certain species of *Echinocactus*, the root is not of the typical diarch nature, but possesses four phloem- and two xylem-bundles; this is due to the fact that the opposing groups of bast remain apart in the hypocotyl instead of fusing.

(b) In many seedlings a cotyledonary tubercle-bundle is developed; it usually merely fuses laterally with a portion of the cotyledon-strand, but in *Echinocactus Ottonis*, *Echinopsis multiplex* Series 2, *Pilocereus exerens* Series 1, *Mamillaria multiceps*, *M. rhodantha* (one seedling), and *M. pentacantha*, it played an essential part in the root formation, forming half of each of the 'double' bundles.

(c) In *Echinocactus hexaedrophorus* and *Mamillaria missouriensis* a tetrarch root is formed in a manner which recalls the similar feature in *Opuntia maculacantha*.

In Group II, as in Group I, the transition-phenomena afford little assistance in the delimiting of genera within the group, for the details of the seedling anatomy are not always constant in a single species, and much less in a particular genus; this is well seen in *Echinopsis multiplex*, *Pilocereus exerens*, *Mamillaria rhodantha*, and *M. pentacantha*. Further, although in some cases, as for example in the genus *Cereus*, the seedling anatomy of the species is a fairly constant feature, yet individuals of other genera bear so close a resemblance to them that it is impossible by this means to separate one genus from the other; in other words, it is impossible to define a genus in terms of its seedling structure.

On the other hand, just as the seedlings of Group I are distinctly marked off by their external appearance from those of Group II,<sup>1</sup> so the transition-phenomena of the members of Group I are fairly constant and are sharply marked off from the type of transition characteristic of Group II. From this it would seem that the physiological factors which have ultimately resulted in the specialized seedlings of Group II have reacted on the seedling anatomy, producing a transition very different from that of the less reduced members of Group I.

## RELATION OF THE SEEDLING ANATOMY TO SOME PHYLOGENETIC PROBLEMS.

One of the most interesting features in the seedling anatomy of the Cactaceae is the occurrence in some genera, e. g. *Opuntia* and *Nopalea*, of the type of transition shown by Miss Sargent to be characteristic of

<sup>1</sup> Compare Figs. 1, 2, and 6 with Figs. 7, 8, 9, 16, and 17.

*Anemarrhena asphodeloides*. So far as has been ascertained this formation of a tetrarch root-stele from two cotyledonary traces has been previously described in only two other dicotyledonous seedlings, *Eranthis hiemalis*<sup>1</sup> and *Podophyllum peltatum*,<sup>2</sup> in both of which the tetrarch arrangement is fugitive and the root is really a diarch structure. In the Cactaceae, on the other hand, the resemblance to *Anemarrhena* is more complete, for the tetrarch structure persists to the root-tip; indeed, the only important difference between the two seems to lie in the fact that the two cotyledonary bundles are derived in the Cactaceae from two seed-leaves and in *Anemarrhena* from one.

The occurrence of the *Anemarrhena* type in a modified form in a member of the natural order Ranunculaceae which, on other grounds, has been considered to bear a somewhat close relation to the Monocotyledons, has been used as a reason for formulating an hypothesis as to the origin of the monocotyledonous condition, namely, a theory that 'the two cotyledons of Primitive Angiosperms have united to form the single member in Monocotyledons'.<sup>3</sup> The close resemblance between *Eranthis* and *Anemarrhena* led to the conclusion that there is probably 'a real genetic connection between *Eranthis* and *Anemarrhena*; that they are descended from a common ancestor with two distinct seed-leaves, each represented by a single trace in the hypocotyl'.<sup>4</sup> Now the cotyledons of *Eranthis* are united as regards their petioles, and this led to the statement that even if there were no historical connexion between them, 'the structure of *Eranthis* may nevertheless illustrate the double origin of the *Anemarrhena* cotyledon. For without the analogy of *Eranthis*, the assumption that each trace in the cotyledon of *Anemarrhena* represented a distinct seed-leaf was groundless. Not only was direct evidence of such a double origin absent, but there was nothing to show that the union of two cotyledons, if it did take place, would actually give rise to such a type of vascular symmetry.' The second Dicotyledon in which the *Anemarrhena* type was found was also a geophilous seedling in which the petiolar tube was well developed; hence the 'fusion' hypothesis appeared to receive additional support, for the known facts seemed to show that union of cotyledons might bring about such a vascular arrangement as was found in *Anemarrhena*. But at the same time it must be observed that the formation of a cotyledonary tube does not necessarily produce this *Anemarrhena* type of structure, for in two other Ranunculaceous seedlings, *Delphinium* sp. (probably *nudicaule*) and

<sup>1</sup> Sargent, E. : The Origin of the Seed-Leaf in Monocotyledons. *New Phyt.*, i, 1902, p. 112.

<sup>2</sup> Sargent, E. : Reconstruction of a Race of Primitive Angiosperms. *Ann. Bot.*, xxii, 1908, p. 170.

<sup>3</sup> *Loc. cit.*, p. 183.

<sup>4</sup> Sargent, E. : A Theory of the Origin of Monocotyledons, founded on the Structure of their Seedlings. *Ann. Bot.*, xvii, 1903.

*Anemone coronaria*,<sup>1</sup> a cotyledonary tube is present, but the transition is of the ordinary Ranunculaceous type in which there is a diarch root and no hint of tetrarchy. Thus it is seen that the development of a cotyledonary tube may or may not give rise, even in seedlings of the same family, to the *Anemarrhena*-type of vascular rearrangement; while this same type may occur, as has been shown above, in seedlings in which there is not the slightest sign of cotyledonary fusion.

It cannot be considered that the resemblance of *Opuntia*, for example, to *Anemarrhena* is the result of a close genetic relation between the two; nor can it be conceded that it is due to a response of two unrelated forms to similar conditions; hence we cannot but conclude that the resemblance is accidental. This being so, then it is quite possible that the similarity of *Eranthis* and *Podophyllum* to *Anemarrhena* is also accidental, more especially as the other two related seedlings, *Delphinium* and *Anemone*, do not diverge from the normal Ranalian type, though they might naturally be expected to do so, on analogy with *Eranthis*.

The theoretical importance of the anatomical resemblance of *Eranthis* to *Anemarrhena* has already been denied on other grounds by Tansley.<sup>2</sup> Miss Sargent, in her paper on the 'Reconstruction of a Race of Primitive Angiosperms',<sup>3</sup> has put forward many reasons for the probability of a dicotyledonous Proangiosperm; she concludes that the monocotyledonous condition arose by a fusion of the cotyledons in adaptation to a geophilous habit, the fusion taking place on account of the need for strict economy which must be observed by the seedling. But, as has been already pointed out, the evidence on which the 'fusion' hypothesis was based has been considerably weakened by the discovery of the *Anemarrhena* type in seedlings of such a specialized order as the Cactaceae; moreover, the economizing of time and material may have taken place in at least two other ways without necessitating any theory of fusion. It may have occurred:—

1. By the gradual suppression of one cotyledon of an ancestral pair, or,
2. By the assumption of different functions by the two cotyledons.

The first of these views, the 'suppression' hypothesis, was supported by Prof. Henslow,<sup>4</sup> and Miss Sargent<sup>5</sup> has summarized the most important evidence given by him in support of his views.

Charles and Francis Darwin<sup>6</sup> have shown that dicotyledonous plants

<sup>1</sup> Sargent, E.: loc. cit.

<sup>2</sup> Tansley, A. G.: Reduction in Descent. New Phyt., i, p. 132.

<sup>3</sup> Loc. cit.

<sup>4</sup> Henslow, G.: A Theoretical Origin of Endogens from Exogens by Adaptation to an Aquatic Habit. Linn. Soc. Journ., xxix, 1892.

<sup>5</sup> Sargent, E.: Reconstruction of a Race of Primitive Angiosperms. Ann. Bot., xxii, 1908, p. 175.

<sup>6</sup> Darwin, C. and F.: Power of Movement in Plants, 1880, p. 94.

may possess cotyledons in which one shows signs of reduction, or even of complete abortion, and they cite as instances of this *Citrus Aurantium*, in which the cotyledons differ in size and are not necessarily placed opposite to one another; *Pachira aquatica*, which shows similar features; species of *Abronia*, in which one of the cotyledons is quite rudimentary; and *Chaerophyllum* and *Corydalis*, where only one cotyledon is present. They infer that 'there is some close connection between the reduced size of one or both cotyledons and the formation by the enlargement of the hypocotyl or of the radicle of a so-called bulb . . . and that one or both cotyledons, from being superfluous, decreased in size'. A further illustration of this reduction in size of the cotyledons is afforded by the Cactaceae seedlings.

Additional evidence of this suppression is given by Goebel<sup>1</sup> for *Trapa*, in which not only is there a difference in the size of the cotyledons, but the larger one arises as a terminal structure upon the embryo, while the smaller is lateral to the stem-bud.

Finally, the experimental work of A. W. Hill<sup>2</sup> on the genus *Cyclamen* has shown that normally only one cotyledon is developed, which first serves as an absorbing organ, and, later, as an assimilating one; the rudiment of the second cotyledon is always present, and should anything happen to the first cotyledon this rudiment develops into a normal green leaf. Further, in some species the second cotyledon may closely resemble the first one, while in others it is more like a foliage leaf.

Thus there is no lack of evidence to show that the partial or complete suppression of one of the seed-leaves may occur in the Dicotyledons when the necessity for economy occurs; and it is possible that a similar suppression, owing to the adoption of a geophilous habit, may have taken place in the ancestors of Monocotyledons.

For these reasons it does not appear necessary to postulate a theory of fusion to account for the occurrence of the monocotyledonous condition.

The second theory relating to the evolution of the Monocotyledons has been put forward by A. W. Hill,<sup>3</sup> as the result of his observations on the germination of apparently monocotyledonous species of *Peperomia*, in which one cotyledon is hypogeal, and acts as a sucker, while the other has assumed the appearance and functions of a foliage leaf. He suggests that evolution along similar lines may have produced the normal seedling habit of such monocotyledonous orders as Araceae, and that the cotyledon and

<sup>1</sup> Goebel, K.: Organography of Plants, Part II, Oxford, 1905, p. 257.

<sup>2</sup> Hill, A. W.: The Seedlings of certain Pseudo-monocotyledons. Section K, Brit. Ass., York, 1906.

<sup>3</sup> Hill, A. W.: Morphology and Seedling Structure of the Geophilous species of *Peperomia*, together with some Views on the Origin of Monocotyledons. Ann. Bot., xx, 1906, p. 395.



so-called 'first leaf' which are directly opposite to each other in the seedlings of some species of *Arum* and *Arisaema* may be the equivalent of the two cotyledons of *Peperomia*. He has further shown<sup>1</sup> that in *Arisarum vulgare* the 'first leaf' can perform the function of a cotyledon when the latter has become aborted or been torn off.

Among the Monocotyledons the Araceae have been considered to be most nearly related to the Piperaceae. 'The affinities between two such simple orders as the Piperaceae and Araceae appear to be much more close and definite than between the anomalous Ranunculaceae and the highly specialized Liliaceae, and in the former case the modified pseudo-monocotyledonous *Peperomias* show definite homologies in their adult condition with the Monocotyledons.' It seems probable that in some of the Monocotyledons, at any rate, the monocotyledonous condition has arisen from the dicotyledonous one along similar lines to those followed by the *Peperomias* in their development of the geophilous habit.

In this connexion it may be noted that there has always been much difference of opinion as to the interpretation of the organs present in the grass embryo, and the view has been put forward that the scutellum and the germ-sheath represent highly differentiated parts of a single cotyledon;<sup>2</sup> in view of A. W. Hill's work it is possible that they represent two cotyledons which have each taken on a separate function. A similar explanation may serve to explain the complicated structure of those monocotyledonous seedlings in which the seed-leaf is differentiated into an haustorium, a middle portion, and a sheath, e. g. *Tradescantia*, *Cyperus*, &c. In other monocotyledonous families, e. g. Juncagineae, Butomeae, and Alismaceae, the cotyledon becomes green, and according to Goebel<sup>3</sup> does not differ in form and structure from the first foliage leaves in any essential feature, though its anatomical differentiation is somewhat simpler.

According to Miss Sargent,<sup>4</sup> however, the seedling anatomy of the Monocotyledons does not support A. W. Hill's view, for the first leaf is characterized by a midrib and usually has lateral bundles in addition to it, while the cotyledon usually has no midrib; it has instead a double bundle or two single and quite distinct bundles, while in the cases in which a midrib is present it usually shows its double character during the transition.

The presence or absence of a midrib seems then to be the chief difference between the cotyledon and the first leaf, and the question as to the possibility of the first leaf being a second cotyledon really rests upon the importance which can be attached to the 'double' bundle.

<sup>1</sup> Hill, A. W.: The Origin of Monocotyledons. Ann. Bot., xxii, 1908.

<sup>2</sup> Rendell, A. B.: The Classification of Flowering Plants, i, p. 235.

<sup>3</sup> Goebel, K.: loc. cit., p. 408.

<sup>4</sup> Loc. cit., p. 178.



On this point the seedling structure of the Cactaceae can throw some light. In general the upper part of the hypocotyl of these seedlings is occupied by two 'double' bundles, which may have been formed in one of the following ways:—

1. By the bifurcation of the cotyledonary bundle, e. g. *Echinopsis multiplex*.
2. By the fusion of separate vascular strands.

The strands which fuse may be:—

- (a) The two separate bundles present in a cotyledon, e. g. *Echinopsis Lagermannii*, *Cereus peruvianus*, and *Echinocereus Ehrenbergii*.
- (b) The cotyledon-bundle and its tubercle-bundle, e. g. *Echinocactus Ottonis*, Series 3.
- (c) The fused cotyledon- and tubercle-bundles to form one half of the double bundle, the other half being similarly produced, e. g. *Echinocactus Ottonis*, Series 1, *Mamillaria multiceps*.
- (d) The fused cotyledon- and tubercle-bundles to form one half of the double bundle, the other half being formed by the fusion of the other two cotyledonary strands, e. g. *Echinopsis tubiflora*.
- (e) The epicotyledonary strands only, e. g. *Mamillaria pusilla*.

Further, in *Mamillaria missouriensis* the 'double' bundle may be produced either by bifurcation of a single strand or by the fusion of the separate ones; while in *Mamillaria centricirrha* one 'double' bundle is derived by the splitting of the cotyledon-trace, and the other by the interaction of a tubercle- and a cotyledon-strand.

A consideration of these various methods of formation of the 'double' bundle in the Cactaceae points to the fact that it is unsafe to formulate any theory on the homology of such variable structures;<sup>1</sup> for they would appear to be nothing more than an arrangement of the vascular elements in such a way that the change from stem to root structure may take place as conveniently as possible; they would certainly not appear to have any definite morphological value. If then the 'double' bundle, which appears to be a fairly constant feature of the cotyledon of Monocotyledons, is not necessarily the homologue of two separate bundles, that is, if the 'double' bundle has no definite morphological significance, it is quite possible to account for the distinction between the vascular symmetry of the cotyledon and of the 'first leaf' in the Monocotyledons. Thus, supposing that the ancestor of the group was a seedling with two equivalent cotyledons, adaptation to a geophilous habit led to these cotyledons assuming different functions; one serving as an organ for the absorption of the endosperm, the other retaining its assimilating function. Since the need for economizing

<sup>1</sup> Thomas, E. N.: A Theory of the Double Leaf-trace founded on Seedling Structure. *New Phyt.*, vi, p. 88.

time was urgent, one seed-leaf delayed its appearance more and more until finally the symmetry of the root-stele of the seedling had to be attained without its aid, and the second cotyledon became practically the first leaf. We may consider that, since the cotyledons originally were equivalent in function, their vascular structure would be similar, each of them would contribute equally to the root-structure, and each would furnish a strand opening out somewhere in its course as a 'double' bundle. In the 'sucking' cotyledon it would obviously be an advantage to delay the closing up of the 'double' bundle and to separate its parts, producing the appearance of two distinct bundles, especially where much endosperm had to be absorbed. In these cases the structure of the first cotyledon and the second would be different, but the second might still, although its appearance was delayed, contribute to the root formation. Finally, when the second seed-leaf did not develop until very late, the first would necessarily carry out the transition unaided; its two strands, formed as indicated above, would now each act as a 'double' bundle, and the difference between the two originally equivalent cotyledons would be still further increased. In this connexion it is important to remember that 'seedlings are thrown on their own resources at so early a period in their life history that the struggle for existence, repeated through many generations, often transforms their whole structure. At the time of germination this structure is still so little differentiated as to be extraordinarily plastic.'<sup>1</sup>

It is, therefore, quite conceivable that the difference in structure between the cotyledon and the so-called 'first leaf' in Monocotyledons can be explained as due to the physiological needs of the young plant, and if so, A. W. Hill's theory is still left without any serious objection against it.

In conclusion, of the three hypotheses which have been put forward in explanation of the evolution of Monocotyledons from a dicotyledonous ancestry:—

1. The *fusion* hypothesis is seriously weakened by the discovery of the facts described above.
2. The *suppression* hypothesis is but slightly affected.
3. The *first leaf* hypothesis is supported, for the chief objection which has been urged against it has been shown to be no longer valid.

In regard to the transition-phenomena of the seedlings of the Cactaceae, much variation is to be found throughout the group; it does not always appear to be uniform even in the members of a single genus, as is seen in *Opuntia* and *Mamillaria*, although it may be fairly constant, as for example in *Cereus*. Ganong<sup>2</sup> has already pointed out that in all probability the adaptations to physiological needs in the adults have worked back into the

<sup>1</sup> Sargent, E.: The Origin of the Seed-Leaf in Monocotyledons. *New Phyt.*, i, p. 108.

<sup>2</sup> Ganong, W. F.: *loc. cit.*

embryo, for the form of the seedlings corresponds very closely with that of the adults. A study of the seedling anatomy shows that not only the form but also the structure has been so influenced; this is seen, for example, in the appearance of tubercles and of 'barrel' tracheides in seedlings of those plants in which similar features are characteristic of the adults.

Miss Sargent has shown that much variation in the transition-phenomena occurs in the Liliaceae, and T. G. Hill<sup>1</sup> has found that 'the details of the transition in the Piperaceae are anything but rigid', while there is probably 'much variation in the Centrospermae'.

Since, then, there is such variation in small groups of plants, and since seedling structure can be influenced by physiological factors working on the adults, to me it does not appear justifiable to use such characters as indicators of phylogenetic connexions.

#### SUMMARY.

##### *Cotyledons.*

1. There is a gradual increase in succulence combined with a marked decrease in size in the seedlings from *Pereskia*, which is normally dicotyledonous in form, through *Opuntia*, *Nopalea*, *Phyllocactus*, *Cereus*, *Pilocereus*, *Echinocereus*, *Rhipsalis*, *Echinopsis*, *Echinocactus* to *Mamillaria*, in which the seed-leaves are either microscopic or absent.

2. In general the cotyledons are two in number; they are always unequal in size, the difference being most marked in *Nopalea*. Seed-leaves are absent in *Mamillaria pusilla*.

Three cotyledons were found in *Opuntia stricta*, Series C. In *Echinocactus Ottonis*, Series 1, one of the two cotyledons was split to its base, giving the seedling the appearance of possessing three distinct seed-leaves, and three cotyledonary tubercles were also present.

In *Echinocactus Ottonis*, Series 3, one of the two cotyledons was bifurcated almost to its base and the split cotyledon subtended one tubercle which was laterally elongated.

3. Cotyledonary buds have been observed in *Cereus triangularis*, *Echinopsis Eyriesii*, *E. Zuccarinii*, *E. Oxygona*, *Echinocactus bicolor*, *E. denudatus* and *Mamillaria hexacantha*.

There is an increase in the size of the buds, and a vascular bundle to supply them in *Phyllocactus Hookeri*, *Cereus tortuosus*, *C. peruvianus*, *C. Jamacaru*, *C. Spachianus*, *Pilocereus exerens*, *P. albispinus*, *Echinocactus bicolor* (one seedling), *Mamillaria pentacantha* and *M. eriacantha*.

Tubercles with spines of the form found in the mature plant occur in *Echinopsis multiplex*, *E. Lagermannii*, *E. tubiflora*, *Echinocactus Ottonis*,

<sup>1</sup> Hill, T. G.: On the Seedling Structure of certain Piperales. *Ann. Bot.*, xx, 1906, p. 174.

*Mamillaria multiceps*, *M. rhodantha*, *M. centricirrha*, *M. meiacantha* and *M. polyëdra*.

4. The cotyledonary bud-bundles behave in various ways. They may or may not be essential to the formation of the root-structure.

In the following species they are essential in the transition, forming one half of a 'double' bundle: *Echinopsis multiplex*, Series 2, *Echinocactus Ottonis*, *Mamillaria multiceps*, *M. rhodantha* (some seedlings), and *Pilocereus exerens*, Series 1.

In the following species they are not essential in the root formation. They may:—

(a) Fuse laterally with one half of the double bundle, e.g. *Phyllocactus Hookeri*, *Cereus tortuosus*, *C. peruvianus*, *Pilocereus*, *Echinopsis multiplex*, Series 1, *E. Lagermannii*, *E. tubiflora*, and *Mamillaria polyëdra*, Series 1.

(b) Fuse with an epicotyledon-strand, and the fusion product unite laterally with the cotyledon-trace, *Echinocactus bicolor*, Series 2, *Mamillaria rhodantha* (some seedlings), and *M. polyëdra*, Series 2.

(c) Bifurcate and rotate, each half bundle fusing with the cotyledon-bundle, e.g. *Echinocactus Ottonis*. In *Pilocereus albispinus*, Series 1, and in one cotyledon of *Echinopsis multiplex*, Series 3, the tubercle-bundle which bifurcated consisted of phloem only.

#### Transition-Phenomena.

5. The transition-phenomena are of the *Anemarrhena* type in *Opuntia Ficus-indica*, *O. imbricata*, *O. Tuna*, *O. polyantha*, *O. Bergeriana*, *O. stricta* and *Nopalea n. sp.* This type is slightly modified in *O. Tuna*, Series C, and in one seedling of *O. polyantha*, and is considerably modified in *Echinocactus hexaedrophorus* and *E. denudatus*.

The following species show a branching into three of the cotyledonary xylem, but the lateral arms die out again at a later stage, and the two intercotyledonary root-poles arise independently of the cotyledonary xylem:—

*Pereskia n. sp.*, *Opuntia stricta*, *O. albicans*. In *P. Pititache* the two intercotyledonary root-poles do not arise, so the root-structure is of the *Cereus* type. In *O. maculacantha* and *O. Pseudo-tuna* the tetrarch root arises in a similar way to *O. stricta*, but there is no previous suggestion of the *Anemarrhena* type.

In all the remaining Cactaceae seedlings examined the two 'double' bundles which are found in the hypocotyl follow a somewhat similar course, and the transition is of Van Tieghem's Type 3. The bundles rotate until the phloems lie on either side of the xylem in which the protoxylem has become exarch; the phloems then either fuse in pairs to form a diarch root, or else remain isolated, when the *Cereus* root type results.

6. The epicotyledonary vascular tissue plays no essential part in the transition-phenomena in any of the species examined except *Mamillaria pusilla*, in which seedling no cotyledons are present; it is, however, differentiated very early, especially in the *Mamillarias*, and supplements the existing vascular supply.

7. There are differences in the method of transition between seedlings of the same species, in the case of *Opuntia stricta*, *Echinopsis multiplex*, *Mamillaria rhodantha* and *M. pentacantha*.

8. The two 'double' bundles of the hypocotyl show a very great variation in their method of formation. They may arise:—

(a) By the bifurcation of the cotyledonary bundle, e.g. *Echinopsis multiplex*, *Opuntia sp.*, &c.

(b) By the fusion of separate vascular strands. The strands which fuse may be:—

(1) The two separate bundles present in the cotyledon, e.g. *Echinopsis Lagermannii*, *Cereus peruvianus* and *Echinocereus Ehrenbergii*.

(2) The cotyledon-bundle and its tubercle-bundle, e.g. *Echinocactus Ottonis*, Series 3, *Mamillaria multiceps* and *M. centricirrha*.

(3) The fusion product of a cotyledon- and a tubercle-bundle to form one half of the 'double' bundle, the other half being produced by another similar fusion, e.g. *Echinocactus Ottonis*, Series 1.

(4) The fusion product of a cotyledon- and a tubercle-bundle to form one half of the 'double' bundle, the other half being produced by the fusion of two cotyledon-strands, e.g. *Echinopsis tubiflora*.

(5) The epicotyledonary bundles, e.g. *Mamillaria pusilla*.

9. 'Barrel' tracheides are present in the hypocotyl and root of *Opuntia stricta*, *O. maculacantha*, *Cereus tortuosus*, *C. peruvianus*, *Echinopsis multiplex*, *E. tubiflora*, *E. Lagermannii*, *Echinocactus Ottonis*, and all the species of *Mamillaria* examined.

#### ROOT.

10. A tetrarch root is characteristic of *Pereskia n. sp.*, the *Opuntias*, *Nopalea n. sp.*, *Echinocactus hexaedrophorus*, *E. denudatus* and *Mamillaria missouriensis*.

A 'Cereus' type of arrangement, with four phloem and two xylem bundles, is found in the roots of *Pereskia Pititache*, *Phyllocactus Hookeri*, *Cereus tortuosus*, *C. peruvianus*, *C. Jamacaru*, *C. Spachianus*, *C. triangularis*, *Pilocereus exerens*, *P. albispinus*, *Echinopsis Eyriesii*, *E. multiplex*, *E. tubiflora* and *Echinocactus Wislezeni*.



A normal diarch root is produced in *Rhipsalis Warmingiana*, *R. dissimilis*, *Echinocereus Ehrenbergii*, *E. cinerascens*, *Echinopsis oxygona*, *E. Lagermannii*, *E. Zuccarinii*, *Echinocactus bicolor*, *E. Ottonis*, and the species of *Mamillaria* with the exception of *M. missouriensis*.

#### THEORETICAL.

11. The adaptations shown by adult plants in response to their environment, which have been impressed on the *form* of the young seedling, have had a corresponding influence on their internal structure.

12. It is not justifiable to use the seedling structure as an indicator of phylogenetic connexions.





# The Reproduction and early Development of *Laminaria digitata* and *Laminaria saccharina*.

BY

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With Plates XIV and XV.

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## INTRODUCTION.

THE possibility of obtaining the early stages of the life-history of some of the Laminariaceae, and of following their growth in artificial culture solutions, was suggested to me by some experiments undertaken by Dr. E. J. Allen and Mr. E. W. Nelson, in the winter of 1907-8, to determine the conditions of growth of the Diatomaceae. In some of these experiments young specimens of *Laminaria digitata* were noticed growing in culture solutions which had been inoculated with Plankton.

The present work was undertaken in the winter of 1908-9. I have to thank the Council of the Marine Biological Association for placing at my disposal a table at their Plymouth Laboratory. I have especially to thank Dr. Allen and Mr. Nelson for their kind assistance and advice.

No account of the reproductive processes in the Laminariaceae has been published. A good description of the structure of the reproductive areas is given in Oltmanns' 'Morphologie und Biologie der Algen' and

in other works, but in all publications the reproductive areas are described as consisting of sporangia embedded among a mass of paraphyses. I have been forced to the conclusion that the organs so described are in reality gametangia.

Briefly summarized, my results point to the conclusion that a number of planogametes are liberated from the gametangia, isogamous conjugation occurs, the resulting zygospore divides and gives rise to a chain of cells which may represent the '2 x' generation, and this in turn gives rise to the *Laminaria* plant, which represents the gametophyte, or 'x' generation.

#### SUMMARY OF THE MORE IMPORTANT CHARACTERS OF THE PLANTS.

*Laminaria digitata* grows on rocks and stones, from the level of the lowest spring tides to about the fifteen fathom line, and appears to be widely distributed in the temperate waters of the Northern Hemisphere. It is found all round the coasts of the British Isles and along the European seaboard from Norway to Spain. In the North Atlantic, it is found around the southern coasts of Greenland and along the American coast as far south as Massachusetts (Harvey). In the North Pacific it has been reported from Kamtschatka (Harvey) and Alaska (Setchell and Gardner), but is not found as far south as Japan.

*Laminaria saccharina* grows in similar situations, from a little above the low tide mark to the five or ten fathom line. Its geographical distribution is much the same as that of *Laminaria digitata*, with the exception that it is not found quite so far south as the latter.

The plant in both cases consists of a thallus, which externally shows differentiation into a flattened lamina, a rounded or oval stipe, and a root provided with a number of stout hapteres, by means of which the plant gains attachment to rocks. Internally, the thallus shows partial differentiation into cortical parenchymatous cells and a central or medullary region, consisting of cells somewhat resembling a tightly packed fungal mycelium. Mucilage canals are present in the cortex (Oltmanns). Growth takes place from an intercalary meristem, situated at the junction of the lamina with the stipe; accordingly, when growth is proceeding, continuous additions are made to the base of the lamina and apex of the stipe. The length of the lamina remains fairly constant, owing to the constant erosion of the apex by wave action, whilst the stipe tends to increase in length with age. This continuous physiological growth ceases in the autumn, at the beginning of what may be termed the reproductive period. After this there is a rapid growth of the intercalary meristem with the formation of a new lamina, which has the old lamina still attached to its extremity (Setchell). Finally the old lamina degenerates and becomes detached.

In *L. digitata*, the lamina is broad and subdivided into a number of lobes by longitudinal incisions, its surface is smooth and uniform; in

*L. saccharina*, the lamina consists of one long tapering lobe, the central portion of which is somewhat corrugated.

Both species contain a mucilaginous substance in their tissues ; this is slightly soluble in distilled water, and readily soluble in alkalies : from an alkaline solution it is reprecipitated by acids as a gelatinous substance. The colouring matter, to which the plants owe their olive-brown tint, is contained in the chromoplasts of the cells of the parenchymatous layers. Besides chlorophyll they contain a pigment, known as phaeophyll : this can be shown to consist of two substances, phycophaein and phycoxanthin, the former soluble in distilled water, the latter in alcohol (West). Neither phycophaein nor phycoxanthin gives distinct absorption bands in the spectrum. Both are readily decomposed by the action of light, and in the process of decomposition become first of a pale green tint, and finally colourless.

The reproductive areas of *L. digitata* occur as cloudy patches of irregular shape and size, situated on the older portions of the lamina, that is, towards its distal extremity. The areas vary in colour from a light brown to a brownish black, and are slightly raised above the surrounding surface ; in most cases each corresponds in size and position with a similar area on the other side of the lamina. The colour appears to depend on the ripeness of the reproductive organs, which darken as they become more mature. In *L. saccharina* similar patches are found, but they are more extensive and usually continuous. They form a very dark area which extends along the greater part of the lamina, but is chiefly confined to the central corrugated portion. In the neighbourhood of Plymouth, plants of *L. digitata* bearing mature reproductive areas are common during the months of October and November, and those of *L. saccharina* a few weeks later.

#### METHODS.

##### *Collection of specimens.*

The most satisfactory results were obtained with plants collected in situations free from any possibility of contamination by river water, sewage, &c. In the neighbourhood of Plymouth, the best plants were obtained from Wembury Bay, where they grow in great profusion, and are relatively free from growths of other Algae, which are so often found on the thallus of *Laminaria*. Culture experiments with plants obtained from within Plymouth Sound resulted in such plentiful growth of Bacteria, that the water rapidly became thick and cloudy, and portions of the thallus in the culture solution soon decomposed.

##### *Preservation of living specimens.*

Under normal conditions most of the larger Algae refuse to grow in the Laboratory tanks. Mature specimens of any of the Laminariaceae or Fucaceae soon die and begin to degenerate. On the other hand, most

of the smaller Algae grow with comparative ease; species of the Ectocarpaceae in particular grow so readily that they soon tend to cover any undisturbed spot where the light is sufficiently powerful for their needs. The water which circulates in the Laboratory is drawn from Plymouth Sound, allowed to clear in a settling tank, and then pumped through vulcanite pipes into the tanks. The same supply of water may be used again and again for several weeks.

The most obvious physical difference between the artificial and natural environment is the absence of wave motion in the former. Acting on this suggestion, the action of the waves was imitated as far as possible by keeping the plants slowly and continuously in motion in a tank with free circulation of water, and by avoiding overcrowding. The plants were tied by their roots to a weighted glass rod which was connected with the 'stirring' apparatus of the Laboratory. This consists of a siphon which automatically fills and discharges about once a minute, and so raises and lets fall a heavy float. The range of motion is about two feet, and the power is distributed throughout the Laboratory by a system of wires and pulleys, so that it can be used where required. By this means the plants were kept in a healthy condition for some months, and cultures were successfully made from the reproductive areas, though the whole thallus in time tended to become covered by a free growth of various species of *Ectocarpus*. This method of keeping the plants alive was first adopted in November, and in the following July young *Laminaria* plants, from one-quarter to half an inch in length, were found growing attached to the sides of various tanks, some of them at a considerable distance from the tank in which the experiments were conducted. Young *Laminaria* plants had never before been observed growing naturally in the Aquarium.

#### *Examination of the reproductive areas.*

Sections are best cut by the freezing method. If any process involving treatment with alcohol be used, the resulting dehydration produces a shrinking of the mucilaginous contents of the cells which results in considerable distortion. No fixing agent was found which would prevent this change. A good fixative for use before cutting sections by the freezing method is a saturated solution of picric acid in sea-water; this should be allowed to act for at least twenty-four hours. Almost equally good results are obtained by the use of a 2 per cent. solution of 40 per cent. formalin in sea-water.

A satisfactory staining method for sections is the following:—

1. Stain for one minute with Löffler's methylene blue solution, undiluted.
2. Decolorize by successive washings with acid alcohol, until only the extreme edges of the section retain the stain.
3. Counterstain with very dilute safranin.

The methylene blue is apparently only retained by portions of the cells whose walls have partially degenerated. Safranin acts as a body stain with an especial attraction for the mucilaginous contents of the cells.

The structure of the gametangia and paraphyses can best be determined by making scrapings of the mature reproductive areas with a sharp scalpel; the scraping is teased out on a slide in sea-water slightly tinged with methylene blue, and can then be conveniently examined under a  $\frac{1}{12}$  inch objective. By this method, after a little practice, it is possible to obtain both the gametangia and paraphyses unharmed and completely separated from neighbouring cells.

*Culture methods.*

Small pieces of the mature reproductive areas were cut from plants that appeared clean, and free from growths of other Algae. These pieces were washed for some time in a stream of sea-water, and gently brushed over with a soft brush; they were then washed in several changes of sea-water that had been heated to 70° C. and allowed to cool. The object of this treatment was to remove as far as possible the growth of diatoms, Algae, &c. which is always found infecting the thallus.

The culture medium used in these experiments was recommended to me by Mr. Nelson, who kindly kept me supplied with prepared water, and gave me these directions:—

‘The following solutions, which are slight modifications of those recommended by Dr. P. Miquel for the culture of Algae, are prepared.

Solution A.	NaNO <sub>3</sub>	.	.	.	.	.	2	gms.
	KNO <sub>3</sub>	.	.	.	.	.	2	„
	NH <sub>4</sub> NO <sub>3</sub>	.	.	.	.	.	1	„
	Distilled water	.	.	.	.	.	100	„
Solution B.	Na <sub>2</sub> HPO <sub>4</sub>	.	.	.	.	.	4	gms.
	CaCl <sub>2</sub>	.	.	.	.	.	4	„
	FeCl <sub>3</sub> cryst. puriss.	.	.	.	.	.	2	„
	HCl concentrated	.	.	.	.	.	2	„
	Distilled water	.	.	.	.	.	80	„

‘When making up solution B, dissolve the sodium phosphate in 40 c.c. of the water, and then add first the hydrochloric acid, and then the ferric chloride dissolved in 20 c.c., and lastly the calcium chloride dissolved in 20 c.c. Shake well and filter.

‘To each litre of sea-water (obtained from well beyond the Plymouth breakwater) 2 c.c. of solution A and 1 c.c. of solution B are added. The water is then sterilized by slowly heating it in a flask to 70° C. When cold the clear liquid is decanted off from the precipitate which will have formed on the addition of solution B, and is stored in sterilized bottles.’



The cultures were made in glass jars of about 500 c.c. capacity. These were first thoroughly washed with boiling water, dried, and then filled with the culture solution, after which they were inoculated with pieces of the reproductive areas, of about one square inch in size. The jars were covered with a sheet of glass, and placed in a window with a north light. The room temperature varied between 13° C. and 17° C.

In the case of *Laminaria saccharina*, considerable difficulty was caused by the plentiful growth of yeasts and Bacteria, which was possibly favoured by the presence of mannite in the thallus. Accordingly it was found advisable to pipette off some of the flagellated gametes, and to make subcultures. By this means cultures that appeared free from all bacterial growth were eventually obtained.

In the case of *Laminaria digitata*, it was found possible to rear the young plants in the original jars without making subcultures, but the results were complicated by the free growth of various species of *Ectocarpus* and diatoms, which apparently were always introduced with portions of the reproductive areas. By continuous washing and brushing in sterile water, in the manner described, it was found possible to reduce the amount of this growth, but it was never entirely eliminated. Where a free growth of *Ectocarpus* occurred, it was found that most of the young *Ectocarpus* plants of the second generation floated on the surface film of the water, and, provided the jar had not been shaken, they could be skimmed off and so removed.

On several occasions I observed the 'swarming' conjugation of the gametes of various species of *Ectocarpus*.

When the young *Laminaria* plants had grown about one-quarter of an inch long, it was usually found advisable to empty out the culture solution and smash the jars, having first marked them out in small squares with a diamond. The small pieces of glass with the attached plants were then placed in glass dishes filled with the same solution. By this means overcrowding was avoided, and fresh growths of *Ectocarpus* that developed in the later stages of impure cultures could be conveniently removed without disturbing the young *Laminaria* plants. Experiments conducted in Petri dishes were unsuccessful, although some plants were grown from the gametes in small flasks holding 50 c.c. in which the depth of the solution was about two inches.

At first the various stages of growth were observed in the cultures by pipetting out drops from the region of the focus of the light (away from the source of light in a round jar), where the liquid appeared faintly cloudy from the presence of the gametes: in the later stages it was necessary to make scrapings from the sides of the jars. Pipettes that had been sterilized with boiling water were always used.

THE REPRODUCTIVE PROCESS.

In both *Laminaria digitata* and *Laminaria saccharina* the processes of reproduction appear to be very similar. The reproductive areas (Pl. XIV, Fig. 1) consist of modifications of the limiting layer of cells, which become elongated and develop into gametangia and paraphyses.

The gametangia (Fig. 2) are about .05 mm. in length, somewhat ovoid in shape, and broader at the distal extremity than at the proximal end. Under low powers of magnification they appear full of a yellow granular substance with the exception of the region nearest their attachment to the cortical cells, where they show a clear colourless layer.

The paraphyses (Fig. 3) are somewhat flask-shaped, the neck forming the attachment to the underlying cortical cells: they average .065 mm. to .07 mm. in length, and enclose a narrow cavity in which twelve to sixteen chromoplasts are usually present. In number the paraphyses greatly exceed the gametangia, but the numerical ratio between them varies widely in different specimens.

By examining scrapings of the reproductive areas, various stages in the development of the gametangia can be observed. At first they appear to contain a yellowish granular material, interspersed with a number of small highly refractive globules, which are soluble in alkalis, but insoluble in alcohol or ether. Later, the granular material appears to become aggregated into a number of small round bodies of yellowish colour, about .0025 mm. in diameter, which slowly increase in size until at maturity their diameter is .003 mm. (Fig. 2).

From a consideration of the after life-history of the plants, which is described later in this paper, I have come to the conclusion that these bodies are the gametes.

During this process of maturation of the gametes, the small highly refractive bodies, present in the immature gametangia, tend to run together, and form large well-defined spheres.

In scrapings containing mature gametangia, many were seen to have ruptured at the unattached end with consequent liberation of the contents. The actual process of rupture was seen in several cases after keeping uninjured gametangia under observation for considerable periods (Fig. 4). In sections stained with methylene blue and decolorized with acid alcohol, it was found that only the distal extremities of the gametangia retained the stain, and the portion stained was larger in mature than undeveloped specimens; it would thus seem probable that some degenerative change occurs in the cell-wall before rupture, and that only this degenerated area retains the stain.

The next stages in development were followed by examination of the cultures.

In from two to three days after inoculation with pieces of the mature reproductive areas, the solution became slightly clouded; this cloudiness was more noticeable in the focus of the light and near the surface of the liquid. On examination, a drop of water showed a number of flagellated gametes, about .003 mm. in diameter (Fig. 5, *b*), spherical in shape, and consisting of granular protoplasm which usually showed a minute dark spot in the region opposite the attachment of the flagella. Each gamete has two flagella, the longer measuring about .015 mm., and the shorter about .004 mm.; the longer flagellum shows an active wriggling motion, whilst the shorter is more sluggish, and is usually held straight and motionless. Both flagella are inserted close together. The movement of the gametes is not very rapid, and appears to consist partly in revolution round a centre situated a little outside the centre of the body of the gamete, and partly in progression in the direction of the longer flagellum.

When observing the actual process of rupture of the gametangia, it was seen that the gametes were liberated as spherical non-flagellated bodies, containing a faint trace of a yellowish colouring matter (Fig. 4). At first these bodies are found embedded in the mucilaginous substance secreted by the cells of the lamina, and whilst in this position appear to lose their colouring matter and then protrude the flagella. Numbers of these non-flagellated bodies can be found by carefully removing the mucilage from the surface of the reproductive areas, after they have been standing in the nutrient solution for a few days. After the protrusion of the flagella the gametes soon escape from the surrounding mucilage and swim towards the light.

On examining the free swimming gametes obtained in culture experiments, forms were found in which two gametes were partially fused; some were in contact at the points of origin of the flagella, and others showed more complete fusion, but in these the flagella had disappeared (Fig. 5, *c* and *d*).

Observation of the actual process of conjugation presents considerable difficulties. Owing to the minute size of the gametes, it is necessary to use a  $\frac{1}{12}$  inch objective, or a lens of similar resolving power, if satisfactory results are to be obtained. If the gametes are in a hanging drop or in a sufficiently thick film of water to approximate to natural conditions, it is found that they soon move out of focus, or out of the field of view, so that it is difficult to keep any one gamete under observation for a prolonged period.

Before conjugation, two gametes approach one another, and the longer flagella often become entangled. As they draw nearer, the long flagellum of each gamete partially embraces the body of the other, and while in this position both gametes appear to slowly revolve round a common centre. Contact between the bodies of the gametes first takes place at the point of

origin of the flagella, and in this stage the similar flagella of each gamete point in opposite directions. Later stages in conjugation were observed in which partial fusion of the gametes had occurred, with retraction of the flagella, and from this stage the formation of the spherical non-flagellated zygospore was traced.

The whole process of conjugation, from the first meeting of two gametes to the formation of the zygospore, takes some hours. I was never able to keep any two particular gametes under the field of view for a sufficiently long period to watch the complete process in the case of the same two gametes, but I observed so many stages of fusion on so many different occasions that no possibility of doubt of the occurrence of true isogamous conjugation is left in my mind.

After conjugation, the resulting zygospore was found in the culture as a colourless, non-flagellated, body, situated on the bottom and sides of the vessel, mostly in the focus of the light (Fig. 5, *e*). About twelve days after inoculation, a clear tube with a rounded end grows out, the rounded end expands, becomes spherical, and soon equals the zygospore in size. The protoplasmic contents of the parent cell then pass down the tube, eventually leaving an empty case behind. This in time degenerates and becomes separated (Fig. 5, *f-j*).

This body now forms chromoplasts, and increases in size. During growth the first formed chromoplasts subdivide, and an outer and inner cell-wall are formed (Fig. 5, *i, l*). When the cell has reached a diameter of about .025 mm., division occurs, and a chain of cells is formed, each showing an outer and an inner cell-wall. In some cases division results in an aggregated mass of cells, but typically a chain is formed (Fig. 6).

This stage will in future be alluded to as the 'sporophyte' (see p. 188).

In from six to eight weeks after inoculation, many of the cells of the sporophyte develop a bulging protuberance. The outer cell-wall then ruptures at the apex of the protuberance, and the contents of the spore, enclosed in the inner cell-wall, partially emerge. This cell then starts active division, and forms the characteristic thallus of the young *Laminaria* plant. After a few divisions, the young thallus shows differentiation into a flat lamina, consisting of roughly cubical cells containing chromoplasts and a number of colourless unicellular rhizoids, which are each attached to one of the basal cells of the lamina. The rhizoids are at first enclosed within the ruptured outer cell-wall, which appears somewhat flask-shaped, and has colourless transparent walls and a circular opening at the neck. In time this outer coat degenerates, and the rhizoids become attached to surrounding objects (Fig. 6).

During this development of the rhizoids, the cells of the lamina are in a state of active division. They form parallel rows, one cell thick, in a direction at right angles to the long axis of the lamina. The number of

cells in each row depends on its position, the rows at the apex and base containing fewest cells. In later stages, as the apex becomes relatively more pointed, this definite arrangement is lost. Each cell shows a central area coloured brown by the presence of the chromoplasts, and a clear peripheral region. The central portion stains an intense mahogany brown with iodine.

This division of the protoplasm into two regions is found under natural conditions and does not appear to be due to any plasmolytic action.

The cell-walls are extremely delicate and somewhat difficult to resolve unless oblique illumination be used.

There is a single nucleus in each of these cells, situated near the centre: this can be demonstrated by staining with dilute nuclear stains after fixing, and decolorizing the chromoplasts with alcohol.

#### DEVELOPMENT OF THE YOUNG PLANT.

The lamina now increases in size owing to active cell-division. The cells near the base divide into two, in a direction at right angles to the plane of the lamina, which thus becomes two cells thick. These then again divide, and the outer cells go to form the limiting layer, whilst the inner form the cortical layer. The apex and periphery of the lamina remain one cell thick. The narrow basal portion of the lamina, to which the rhizoids are attached, increases in thickness and forms an oval stalk which constitutes the rudimentary stipe (Figs. 8 and 9). In this, the layers of cells of the cortical tissue are thicker than in the lamina. There will obviously be two layers of cortical cells which meet in the centre; each layer is derived from one of the two cells formed by the first division of the primary cells of the lamina. Usually in sections a small space is noticeable between these two layers, but this may be due to distortion produced by treatment with alcohol when cutting serial sections. Owing to the minute size of the plants it was found impossible to cut sections by the freezing method in this stage of growth.

The next stage appears to consist in an upgrowth of tissue from an active growing point, originating in the cells to which the rhizoids are attached. In this way chains of elongated cells are formed which grow up between the two layers of the cortical tissue. At the same time the innermost cortical cells divide, and certain of them start active growth, forming chains of much elongated cells, somewhat resembling fungal hyphae in appearance. These cells ramify among those growing vertically upwards from the base, and the whole mass thus formed is embedded in a structureless hyaline matrix. This material seems to be secreted by the cells themselves; it stains strongly with safranin, and also with haematoxylin, and is probably of a mucilaginous nature (Pl. XV, Figs. 13 and 14).



During this formation of the medullary tissue, the stipe becomes more rounded and increases in length and thickness. Then a disk-shaped expansion develops at the base, it is slightly convex in the direction of the apex of the plant, and partially covers over the rhizoids (Fig. 10). The next stage consists in the outgrowth from the disk of thick circular processes with rounded ends, which extend along the surface to which the young plants are attached (Fig. 11). These processes form the hapteres, by means of which the plant gains permanent attachment to the rocks. At first they exceed the stipe in thickness. They consist of the limiting layer, cortical cells, and a well-developed medullary layer. The cells of the latter arise from the growing point at the base of the stipe in the same way as the cells of the medulla in other parts of the plant (Fig. 15). Thus strands of medullary tissue can be traced, which radiate from the growing point through the tissues of the disk to each haptere.

The gradual upgrowth of the medullary tissue in the young plant can be seen to correspond to a darker area in the lamina visible to the naked eye on holding the plants up against the light. In some cases, the unicellular layer at the apex and periphery of the lamina degenerated and became separated, without apparently much affecting the after-growth of the plant.

If the young plants be removed from the attachment which they have obtained by means of their primary rhizoids, they do not readily again take root. In this detached condition the stipe shows marked negative heliotropism. If the plants be placed in a dish standing on white paper, where most of the light comes from below, the stipe soon turns upwards away from the light, whilst if the dish be placed on black paper, the stipe turns downwards and the rhizoids may again find attachment to the glass.

In impure cultures, filaments of *Ectocarpus* were nearly invariably found entangled among the rhizoids, but they can be distinguished by the fact that the rhizoids of *Laminaria* possess no chromoplasts.

It is impossible at present to say how far the development in artificial culture solutions resembles that in nature, but it is probable that all processes are somewhat accelerated. Assuming that the young plants found growing in the Aquarium tanks in July arose from those kept in the tanks in the preceding November, the rate of growth in the artificial solution would appear to be nearly three times as rapid as that in the tank water. In one case a piece of *Laminaria saccharina* was found on which some spores were developing, and the young plants thus formed resembled in every respect those grown in cultures.

All that has been said with regard to reproduction and development applies both to *Laminaria digitata* and *Laminaria saccharina* with the following small exceptions:—In the youngest stages the lamina of *L. saccharina* consists usually of a single row of cells, while that of *L. digitata* is



shorter, and several cells broad in the same stage of development. In later stages, when there is a partial differentiation into lamina and stipe, the lamina of *L. saccharina* is somewhat ovate, while that of *L. digitata* is more elliptical.

As regards the productivity of the plants, in one experiment a piece of the reproductive area of *L. saccharina*, one square inch in area, was placed in 500 c.c. of the culture solution; this was left for four days, then thoroughly shaken, and a subculture made with 1 c.c. of the solution. The resulting plants were much overcrowded, and probably only a small proportion developed. A rough estimate gave 4,000 as the number present after ten weeks. In the same proportion it is obvious 2,000,000 plants would have developed from each square inch of the reproductive area.

Attempts were made to acclimatize plants grown in the modified Miquel's solution to the tank water by successively adding larger and larger quantities of the latter. This, however, resulted in such a plentiful growth of various species of the Ectocarpaceae, and green Algae, that the *Laminaria* plants became smothered and did not long survive. Plants transferred direct from the culture solution to the tanks have, up to the present, lived for about nine months and average  $1\frac{1}{2}$  to  $2\frac{1}{2}$  inches in length, but their growth in the tank water is extremely slow, and they show a tendency to become eroded at the edges of the lamina.

#### SUMMARY OF RESULTS.

There is direct evidence of the existence of conjugation between the bodies which have hitherto been generally called zoospores, hence it is justifiable to infer that they are in reality gametes, and are of the 'x' type. Consequently the zygospore formed by the fusion of the gametes must be of the '2 x' type.

The mass of cells produced by division of the zygospore may also be of the '2 x' type, but on the other hand the reduction division may occur in the early divisions of the zygospore. This point can only be settled by direct observations. Unfortunately, owing to the difficulty of finding a fixing agent which would permit of the treatment of the cells with a solvent for the colouring matter, without producing distortion, no cytological work was done.

Perhaps the fact that the young *Laminaria* plant arises as a direct outgrowth from the structure formed by division of the zygospore faintly suggests that the reduction occurs at an early stage in the formation of the mass of cells.

For convenience of description I have alluded to this structure as the 'sporophyte', though this is scarcely justified on morphological grounds, and I have no cytological observations to support it.

Briefly summarized, my results are as follows:—

1. The *Laminaria* plant is the gametophyte.
2. The reproductive areas consist of gametangia and paraphyses.
3. Flagellated gametes escape from the gametangia, and isogamous conjugation occurs.
4. The resulting zygospore divides and gives rise to a chain or mass of cells. These may be of the '2 x' type, or the reduction may occur in the early divisions of the zygospore.
5. The cells of this structure rupture, and their contents grow out and form the gametophyte.
6. The young gametophyte consists of a flat lamina, one cell thick, and is attached at its base to surrounding objects by a number of unicellular rhizoids.
7. The cells of the lamina divide, and eventually form the limiting and cortical layers and part of the medullary tissue.
8. The stipe is formed by a modification of the base of the lamina.
9. Part of the medullary tissue is formed by an upgrowth of cells from the base of the rudimentary stipe.
10. A disk-shaped expansion is formed at the base of the stipe, and from this the hapteres originate.

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DESCRIPTION OF PLATES XIV AND XV.

Illustrating Mr. Drew's Paper on *Laminaria.*

Fig. 1.  $\times 350$ . Section of reproductive areas showing gametangia embedded among paraphyses.

Fig. 2 *a, b, c.*  $\times 1,000$ . Gametangia separated by making scrapings of the reproductive areas. Stained with very dilute methylene blue. Stages in the development of the gametes are shown and the process of aggregation of the oil globules. The cell-wall at the apex has commenced to degenerate and retains the blue stain.

Fig. 3.  $\times 1,000$ . A paraphysis separated by making scrapings of the reproductive areas. Stained with very dilute methylene blue.

Fig. 4.  $\times 1,000$ . A gametangium which has ruptured at the apex and is liberating the gametes and oil globules.

Fig. 5.  $\times 1,000$ . *a.* A gamete as liberated from the gametangia. *b.* A gamete which has lost its colouring matter and developed flagella. *c* and *d.* Stages in the fusion of the gametes. *e.* A zygospore resulting from the fusion of the gametes. *f* to *l.* Stages in the formation and growth of a cell of the sporophyte generation.

Fig. 6.  $\times 350$ . *a* and *b.* The sporophyte generation of *Laminaria digitata*, showing some cells in a state of division and the origin of the young plants of the gametophyte generation. *c.* A young *Laminaria saccharina* plant, the rhizoids still enclosed in the outer cell-wall.

Fig. 7.  $\times 350$ . A young *Laminaria digitata* plant separated from the outer cell-wall, showing the lamina and rhizoids.

Fig. 8.  $\times 20$ . Young *Laminaria digitata* plant, showing the formation of the stipe.

Fig. 9.  $\times 20$ . Young *Laminaria saccharina* plant, of the same age as that in Fig. 8.

Fig. 10.  $\times 20$ . Young *Laminaria digitata* plant, showing the formation of the disk at the base of the stipe.

Fig. 11.  $\times 20$ . Young *Laminaria digitata* plant, showing origin of the hapteres from the disk.

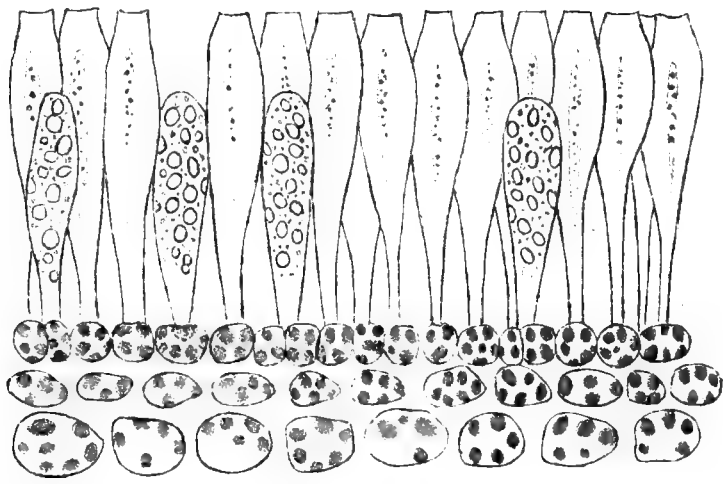
Fig. 12.  $\times 100$ . Longitudinal section of a very young *Laminaria digitata* plant.

Fig. 13.  $\times 100$ . Longitudinal section of a young *Laminaria digitata* plant through the stipe, showing the first stages in the formation of the disk.

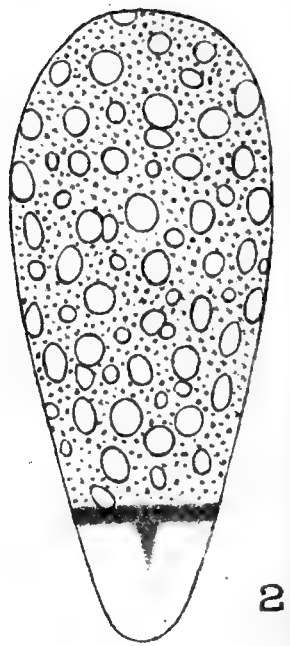
Fig. 14.  $\times 50$ . Transverse section through the basal portion of a slightly older *Laminaria digitata* plant, showing the medullary tissue.

Fig. 15.  $\times 50$ . Longitudinal section through the stipe, disk, and one haptere of a *Laminaria digitata* plant.

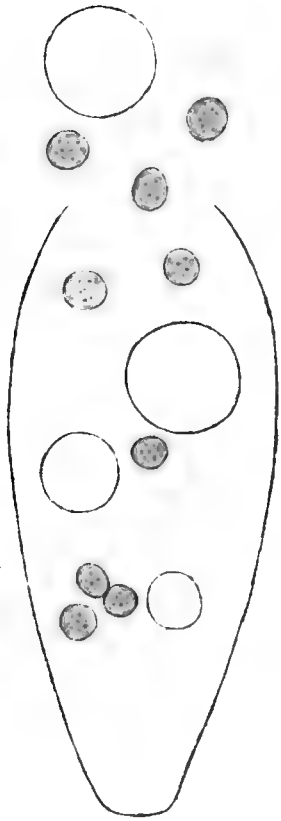




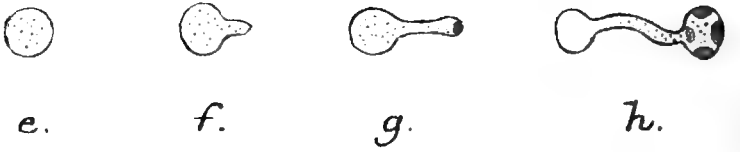
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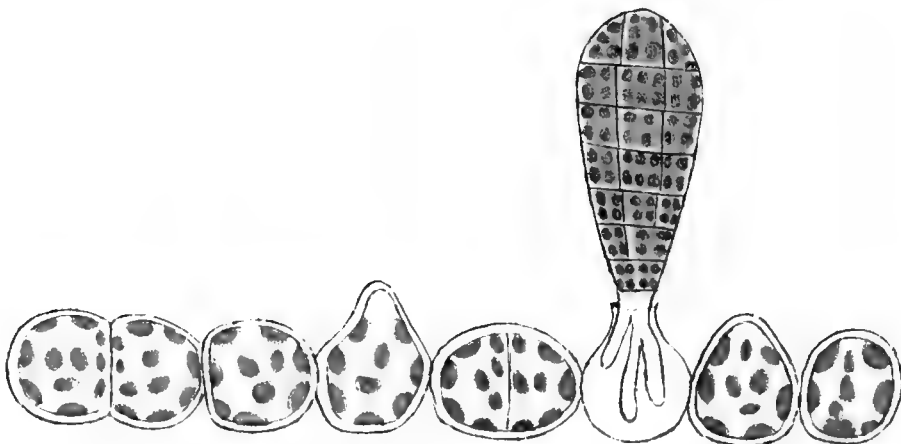
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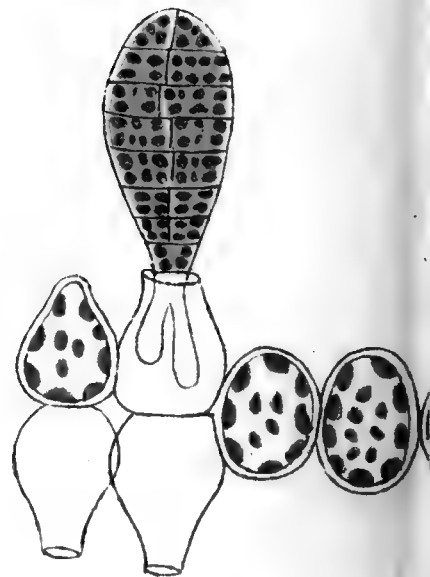
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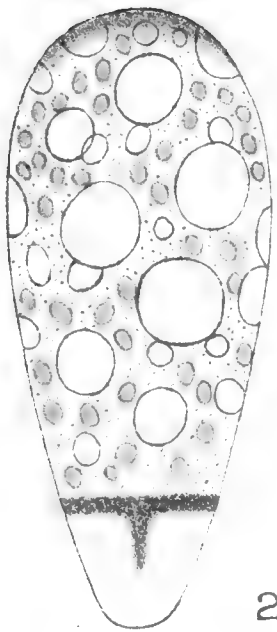
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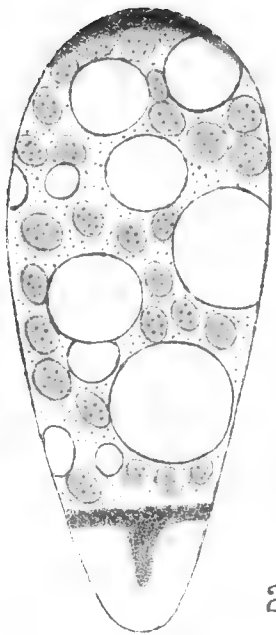
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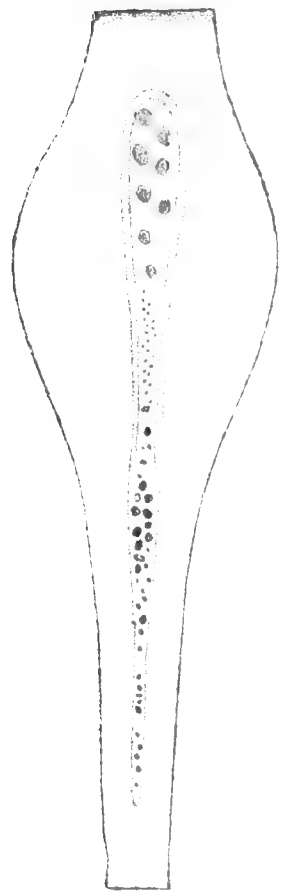
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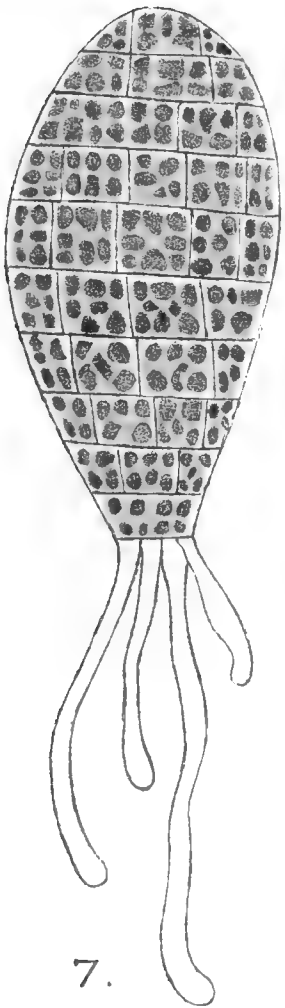
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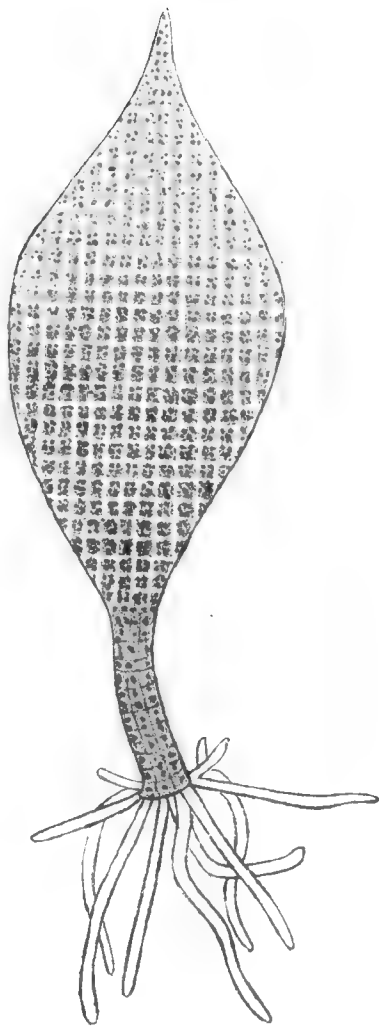
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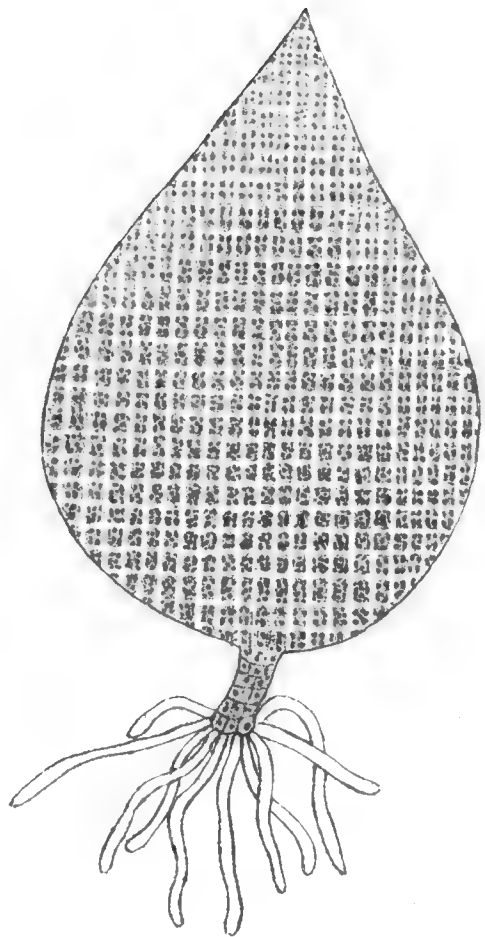
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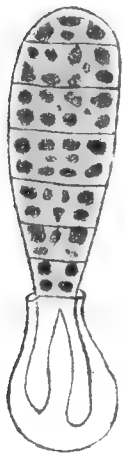
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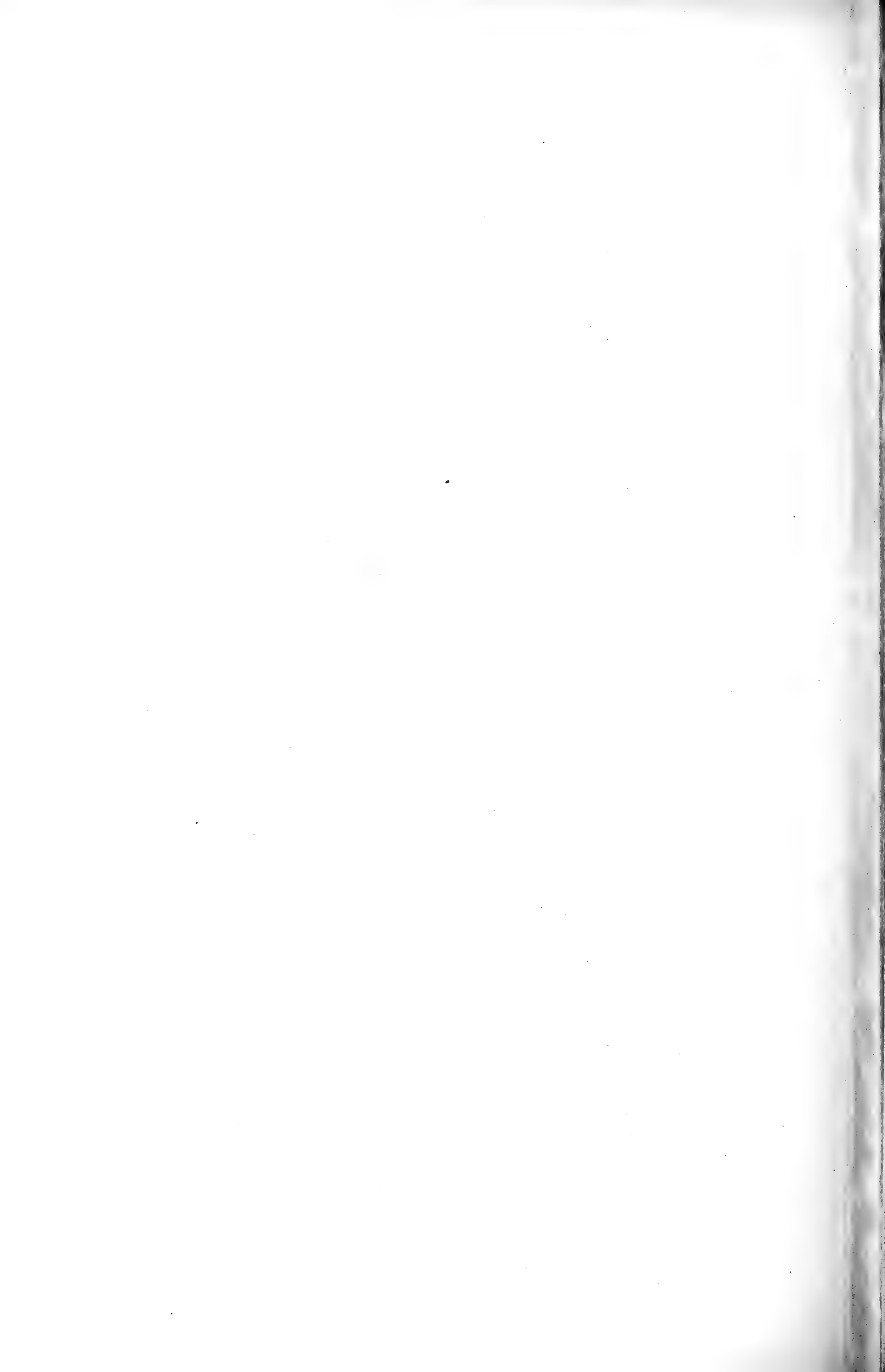


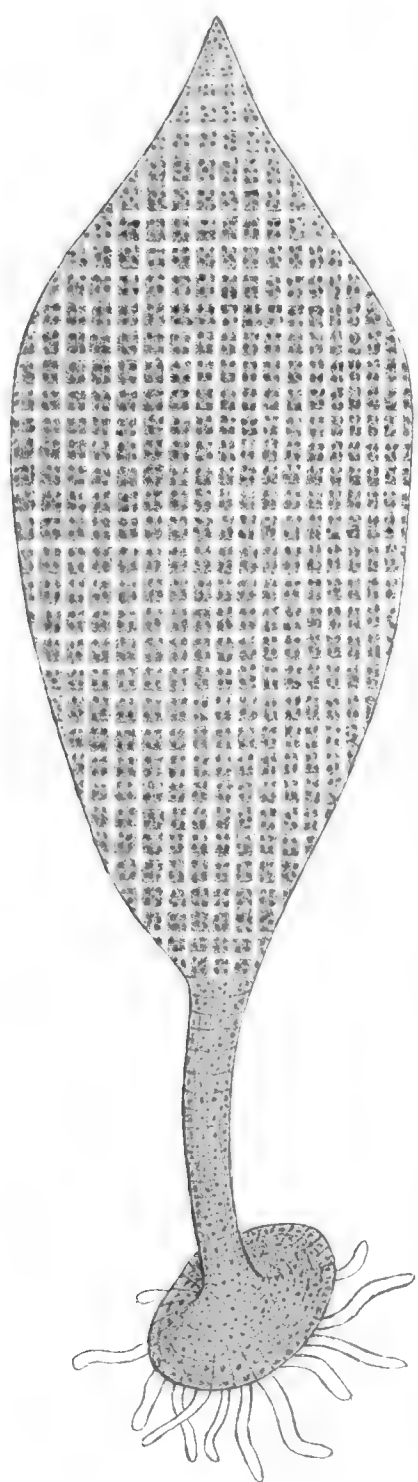
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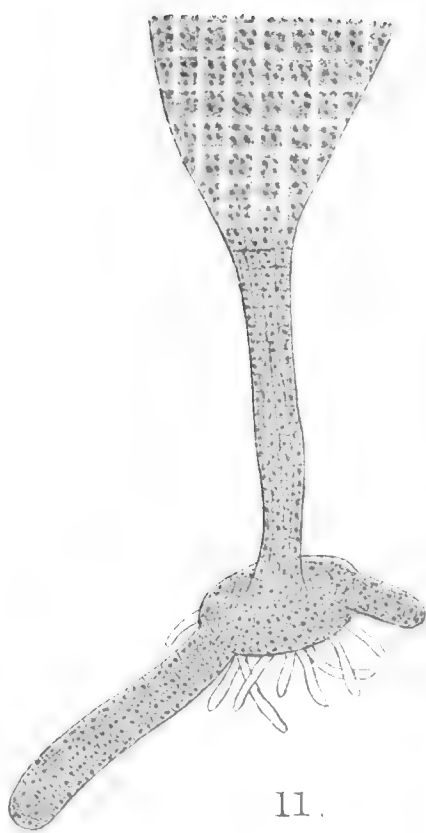
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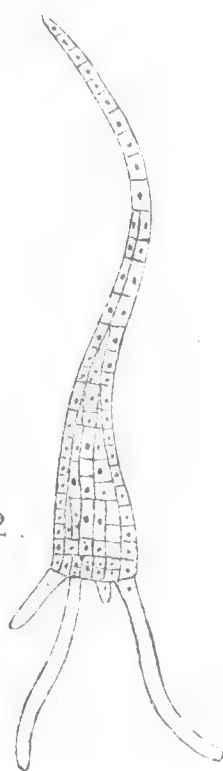




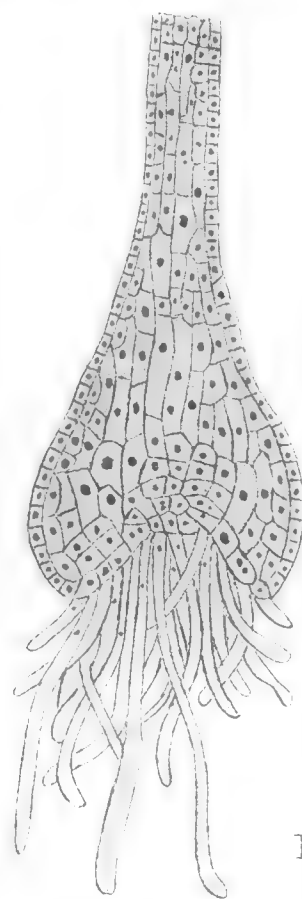
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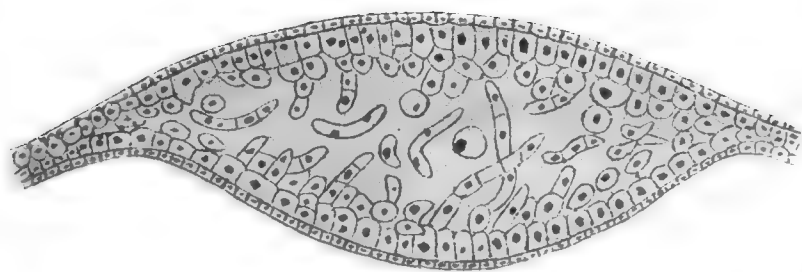
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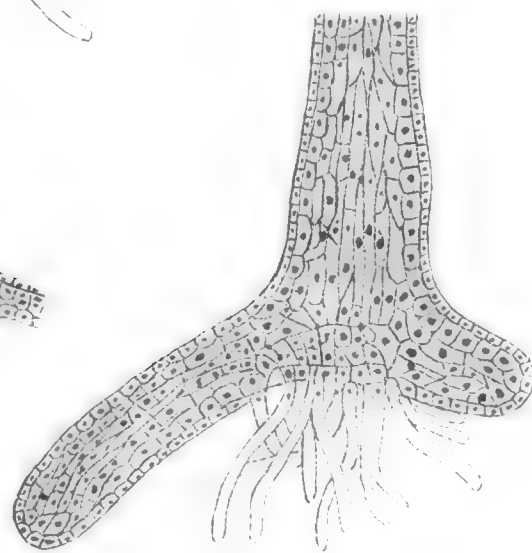
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G.H.D. del.

DREW — LAMINARIA.

Huth lith. et. imp.



# On the Cytological Features exhibited by certain Varietal and Hybrid Ferns.

BY

J. BRET LAND FARMER, F.R.S.,

AND

L. DIGBY.

With Plates XVI-XVIII.

THE last few years have witnessed the publication of the results of a number of investigations on the cytology of hybrid plants, and far-reaching inferences as to the significance of the chromosomes have been drawn from them. But the views of different investigators are somewhat widely divergent. Some have concluded that the frequent sterility of the hybrid is the result of an incompatibility between the parental chromosomes which enter into the nuclear constitution of the hybrid, whilst others, notably Tischler,<sup>1</sup> seem to attribute the failure of the reproductive processes to a disturbance of the normal relations of nucleus and cytoplasm.

This latter conclusion is in harmony with the views advanced by R. Hertwig, according to whom the individual chromosomes are of comparatively little significance, whilst the matter of supreme importance consists in the maintenance of certain definite relations between the nucleus and the cytoplasm which he embodied in his well-known formula K/P.

The recent work of Popoff,<sup>2</sup> as well as the very valuable results obtained in earlier years by Klebs,<sup>3</sup> also points to the need of keeping in mind the mutual relations of nucleus and cytoplasm which have been too much ignored by some writers. We have seen no reason for departing from the position already taken up by one of us in 1905<sup>4</sup> to the effect that whilst the cytoplasm chiefly supplies the raw material for development, and

<sup>1</sup> Tischler, G., Zellstudien an sterilen Bastardpflanzen. Arch. f. Zellforschung, Bd. i.

<sup>2</sup> Popoff, M., Experimentelle Zellstudien. Arch. f. Zellforsch., Bd. i.

<sup>3</sup> Klebs, G., Die Bedingungen der Fortpflanzung, 1896, and other papers by the same author.

<sup>4</sup> Farmer, J. B., and Moore, J. E. S., Quart. Journ. Micr. Sci., xlviii, 1905, p. 553.

to this extent limits the possible directions of the course of metabolism which ultimately finds expression in structure and form, it is the nucleus which determines and controls the line which is actually followed.

We think the highly important and interesting experiments of C. Herbst<sup>1</sup> strengthen this view. Herbst found that by starting the development of an ovum on parthenogenetic lines he was able to influence the character of the offspring in the maternal direction—and he has more recently<sup>2</sup> succeeded in correlating this with the cytological facts—and to show, though indirectly, how large a share the nucleus takes in determining the nature of the individual to which such an ovum will give rise.

It is evident that questions such as these can only be answered by extending the scope of investigation. Whilst the method of direct experiment will ultimately bring us nearest to the goal, the less direct method of observation of those disturbances which often follow on a less artificial deviation from natural conditions cannot fail to be profitable. Hybrids have already yielded results of importance, and recalling the opportunity which presented itself some years ago of investigating a hybrid fern, *Polypodium Schneideri*,<sup>3</sup> we determined to inquire into the cytological features attendant on the development of the spores. We hoped that some definite information might thus be obtained which would throw light on certain controversial points connected with meiosis, and especially the prophase stages of the heterotype mitosis. Although the fern has not fulfilled our expectations in this respect, owing mainly to the small size and the large number of the chromosomes, it has nevertheless yielded other results which appear to us to be of sufficient interest to be worth recording.

*Polypodium Schneideri* was raised by Mr. Schneider in the nurseries of Messrs. Veitch and Sons, Chelsea. It was stated to be a cross between *P. aureum* and *P. vulgare* var. *elegantissimum*, the latter a beautiful laciniate variety of the common polypody which was found in the wild state. In its general external and anatomical features the hybrid bears out the statements made as to its parentage, and the existence of leaves on which pinnae that have 'reverted' to the simple form of the type in each parent lend further support to the claims made on its behalf. These 'reverted' pinnae sometimes possess the character of a coarse polypody leaflet, but occasionally they also have the glaucous colour and bloom of the other parent. There is at the present time growing at the Chelsea Physic Garden (where our material has all been cultivated) a plant showing a fine instance of such a 'reversion' in some of its lower pinnae.

Sporangia are produced fairly freely on both the ordinary and the 'reverted' fronds, but in no case have we ever found the spores to be

<sup>1</sup> C. Herbst, Vererbungsstudien, IV. Arch. f. Entwickl.-mechanik, Bd. xxii.

<sup>2</sup> C. Herbst, Vererbungsstudien, VI. Arch. f. Entwickl.-mechanik, Bd. xxvii.

<sup>3</sup> Farmer, J. B., On the structure of a Hybrid Fern. Ann. Bot., xi.

capable of germination. The causes for this will become evident later on. Our material was fixed with special precautions, and we found Fleming's and Hermann's solutions, in various strengths, to give the best results. The stains we chiefly employed were Heidenhain's haematoxylin, Flemming's triple, and the Polychrome methylene blue and orange tannin. The latter gave very sharp differentiation, and may be recommended as a valuable addition to the stains in more general use.

In order to elucidate the peculiarities of the hybrid it is obviously necessary to study the meiotic divisions in the parent forms, in order to ascertain whether the influence of either parent preponderates in the cellular structure of the hybrid. It at once becomes evident that there is a great difference between the numbers of the chromosomes in the two species. The haploid nuclei of *P. aureum* contain about 34 (Pl. XVI, Fig. 4), those of *P. vulgare* var. *elegantissimum* at least 90 (Pl. XVII, Fig. 19), and our impression is that this number may be too low. *P. vulgare* (type) resembles its *elegantissimum* variety in respect of these numbers, that is, there are about 90 haploid chromosomes (Pl. XVI, Fig. 10) or perhaps somewhat fewer. The exact numbers are of relatively little moment; the comparative differences form the main point of interest. The hybrid plant possesses distinctly more chromosomes than either *P. vulgare elegantissimum* or the type form, but they do not, at least usually, amount to the sum of those of the two parents. They are variable, and commonly range from 95 to 105, but they do not, in the great majority of cases, amount to 124, as they should do if they represented the sum of those contributed by the parents. These facts are of interest in view of the relative numbers that have been given for other hybrids, and we shall discuss their significance later on. We will now proceed to the more detailed description of the features as exhibited by the plants themselves.

#### POLYPODIUM AUREUM.

The sporangia of this fern are produced in great abundance. Their development follows the ordinary course, a flattened tapetal cell being cut off from the tetrahedral archesporium; the former then divides tangentially into two layers, the outer of which retains the tabular shape whilst the inner layer becomes glandular in appearance. The sporangium grows faster in the peripheral region than in its central sporogenous portion, and by the time the spore mother-cells are formed and begin to separate from each other they lie as a hollow cluster in the centre of the sporangial cavity. The tapetal cells begin to break down as the nuclei pass into the stage of synapsis, the glairy mass to which they give rise forms a plasmodial nucleated substance bathing the spore mother-cells, and the nuclei of these nutritive cells can still be recognized at much later stages.



The details of the meiotic phase were carefully studied in the hope that they might serve to throw light on the processes that obtain during and after synapsis, but they proved too difficult for satisfactory analysis. Cases were often seen which might have been interpreted as due to a lateral approximation of two parallel filaments (Pl. XVI, Fig. 2), but it was impossible to feel sure that such an explanation was not illusory. We have, however, seen examples of nuclei which at this stage very closely resembled the excellent figure of Janssens' illustrating the corresponding stage in the spermatocyte of *Alytes*.<sup>1</sup> But as we observed numerous other instances in which more than two filaments were apparently involved in the thickened thread, the evidence for approximation at this stage was less cogent than it would otherwise have been. It must also be remembered that during synapsis there is in any event a bunching together of a tangled threadwork, and that it would consequently be singular rather than otherwise if parallelism between some of the threads did not occur. This should be no less true for those filaments which extend from the central tangle to the nuclear periphery than for the rest. Indeed, on merely mechanical grounds, such parallelism might be expected to be found in these threads quite apart from the question of any significance arising such as has been attributed to them on theoretical grounds. We have not succeeded in convincing ourselves that the apparent reduction in the threadwork of the nucleus which certainly does occur at synapsis is to be explained otherwise than as the result of contraction and concomitant thickening of the leptotene filament.

During the very earliest stages of synapsis the cytoplasm in the vicinity of the nucleus begins to exhibit that structural modification termed kinoplasm by Strasburger. It is probable that the extrusion of nuclear substance (Pl. XVI, Fig. 1), in the form of granules or droplets of chromatin, is concerned with its appearance; the kinoplasm surrounds the nucleus as a fine feltwork, and recalls the arrangement described and figured by Wilson Smith<sup>2</sup> for the pollen mother-cells of *Osmunda*. The spindle in *P. aureum* is very regular, and shortly after synapsis rapidly assumes a bipolar character, in sharp contrast to what happens in the other parent. We examined several other species of the genus, e. g. *P. guatamalense*, *Dryopteris*, &c., and found them to resemble *P. aureum* in this respect.

When the spindle is formed (Pl. XVI, Fig. 3), the granules in the cytoplasm, just referred to, are fairly numerous. They are often to be found in the neighbourhood of the spindle poles, but do not seem to exert any appreciable influence on the distribution of the fibres, although some-

<sup>1</sup> Janssens and Willems, La spermatogenèse dans l'*Alytes obstetricans*. La Cellule, t. xxv, Pl. I, Fig. 3.

<sup>2</sup> Wilson Smith, The achromatic spindle in the spore mother-cells of *Osmunda regalis*. Bot. Gaz., xxx.

times a few threads may diverge towards one of them here and there. In this respect they are to be contrasted with what occurs in many other plants, e. g. *Lilium*, *Equisetum*, &c.

As the later stages of the division in this species were not traceable in sufficient detail to enable us to add materially to our knowledge of the process, we shall pass on to the consideration of the other plants.

#### POLYPODIUM VULGARE (Type form).

It was desirable to examine this plant, inasmuch as its variety *elegantissimum* is one of the parents (or putative parents) of the hybrid. We have already remarked on the large number (about 90) of the haploid chromosomes in this species. In so far as the development of these bodies from the resting nucleus is concerned the process is similar to what obtains in *P. aureum*, but as the larger size of the nucleus (cf. Pl. XVI, Figs. 4 and 10) is more than compensated by the number of the chromosomes themselves, the details of chromosome-formation were not satisfactorily followed. But the character of the spindle is very different in the two plants, and it also exhibits unexpected abnormalities which recur in the varietal form, as well as in the hybrid.

The granules in the cytoplasm, already described for *P. aureum*, are also formed here, and some at any rate are derived from the chromatic constituents of the nucleus. They are seen to be budded off from the nuclear contents into the cytoplasm just as has been described by one of us for *Galtonia*.<sup>1</sup>

As the nucleus of the spore mother-cell enters on the stage of synapsis, the kinoplasmic nuclear sheath is easily demonstrable, but instead of the bipolar type of spindle a *quadripolar* (or multipolar) structure (Pl. XVI, Figs. 7, 8) is differentiated.<sup>2</sup> The arms (nearly always four in number) are very prominent objects in the cytoplasm, and they persist as well-marked cones which extend from the nucleus to the periphery of the cell. It is not till diakinesis is passing over and the nuclear wall breaks down that the quadripolar arrangement merges into the bipolar one by the fusion and concentration of the poles in pairs (Pl. XVI, Fig. 9).

There are certain features connected with the nuclear contents that are important in relation to the first differentiation of the quadripolar spindle. As the synaptic tangle passes into the more open thread stage which always immediately succeeds it, the chromatic linin is seen to be first less evenly distributed within the nucleus than is more often the case in plants. On the contrary, it is, though rather obscurely, massed at four spots about equidistant from each other just within the nuclear wall. As soon as this becomes

<sup>1</sup> See V. Derschau, Max, Beiträge zur pflanzlichen Mitose: Centren, Blepharoplasten. Pringsheim's Jahrb. f. wiss. Bot., xlv. Digby, L., Observations on 'Chromatin Bodies', &c. Annals of Botany, xxiii.

<sup>2</sup> Cf. Lawson, A. A., Studies in spindle formation. Bot. Gaz., xxxvi.

evident, and not before, the kinoplasmic sheath already described is observed to *protrude* from the surface of the nucleus at these points. In this way the quadripolar structure is initiated. The inference that it arises as the result of a specific influence attributable more or less directly to the chromatic linin seems irresistible. The lines of kinoplasm assume curvatures which unless one is careful may easily lead to misinterpretation. In surface view the appearance is sometimes such as to suggest the existence of a so-called 'Hermann's Spindle'; owing to the fact that in the regions between the cones the fibres form curves which start from the surface of the nucleus (cf. Pl. XVI, Fig. 7, to the left).

Instances also occur where individual threads of the spindle are attracted towards, or perhaps originate from, isolated granules in the cytoplasm; but in this fern, whatever the individual differences may be, a quadripolar (or multipolar) spindle is the rule. We have also found it to occur in the premeiotic archesporial divisions, though not so obviously as at meiosis (Pl. XVI, Fig. 6).

The cones consist each of a dense sheaf of fibres, and these are very 'rigid' in character, as is shown by the fact that a cone may often be seen to cause a bulging out of the cytoplasm (Fig. 7) at the periphery when, through the action of reagents employed in fixing, the cell protoplasm has contracted away from the wall. This 'rigidity' has been observed before and in other plants, and it has been interpreted as the result of a 'growth' of the fibres. We do not share this view of the matter, at least if the word growth is to bear its ordinary meaning; but, as we have already indicated, the whole process seems to us to depend primarily upon repulsive forces developed within the cell. We think the facts support the view that electrical disturbances, and perhaps also phenomena of induction, play the most important part in the formation of these structures,<sup>1</sup> and whilst they are normally maintained by the continuance of those conditions that were in the first place responsible for their formation, it by no means follows that the cones, when once organized, will lose their identity immediately on the cessation of the operation of the causes which first produced them unless the new conditions are such as to actively render their further continuance impossible.<sup>2</sup> We have used the term 'kinoplasm' as designating a particular differentiation of the cytoplasm, but we make no assertion as to whether the stuff which this differentiation renders visible really represents a specific substance or not. It is of course easily conceivable that such a substance might exist, but would only become visible, would only become organized, when appropriate conditions were supplied, just as the figure

<sup>1</sup> Lillie, R. S., *Am. Journ. Physiol.*, viii; also *Biol. Bull.*, iv. See also Gallardo, A., *L'interprétation bipolaire de la division karyocinétique*. *An. del Mus. Nacional de Buenos Aires*, t. xiii, 1906.

<sup>2</sup> Hartog, M., *The dual force of the dividing cell*. *Proc. Roy. Soc.*, B. 76.

assumed by iron filings in a magnetic field would differentiate such filings, if present, in a mixture of iron and other dust, provided that the mixture were brought into a suitable magnetic field.

We do not think a question such as this can be really answered by an appeal to stains. The latter act differently according to the physical, as well as the chemical, state of a substance, and it may be that the well-marked staining reactions of 'kinoplasm' ought to be regarded as an expression of physical differentiation (which may be transient) rather than as a proof of chemical difference. On the other hand, it is possible that a chemical change may also be involved in the operation of those very physical conditions that are at the same time responsible for the differentiation itself. The aggregation of the nuclein-charged linin beneath the points of origin of the spindle cones indicates the probability that there exists a causal connexion between the two phenomena, and a suggestion that the immediate cause is electrical in nature appears to be supported by the absence of any direct connexion between the chromatic aggregations and the fibres of the spindle cones, whether at this or at an earlier stage, such as any hypothesis of 'growth' would imply.<sup>1</sup>

As the course of the mitosis advances, the state of aggregation of the chromatic linin disappears, and finally the chromosomes, when fully formed, become uniformly distributed just within the nuclear wall at diakinesis. But the quadripolar character of the spindle persists through these changes. We have already given reasons for not regarding this fact as anomalous; perhaps it may be taken as an expression of a state of 'lag', such as has been regarded as probable by Hartog.<sup>2</sup> But it cannot be a merely fortuitous circumstance that as the brief stage of diakinesis ends with the dissolution of the nuclear wall (itself a coagulum or a precipitation, and probably to be regarded as due to the interaction or agglomeration of substances present in the colloidal masses of which the cytoplasm and the nucleus respectively are composed, and only lasting so long as certain definite physical conditions persist), the character of the spindle should concomitantly change as well. The cytoplasm, gaining access to the interior of the nucleus, instantly brings about the differentiation of the portion of the fibres which are attached to the chromosomes. At the same time the four cones rapidly coalesce into two opposite bundles,<sup>3</sup> thus forming at this late stage the bipolar spindle (Pl. XVI, Figs. 8, 9), which is, in the majority of plants, commonly reached at a much earlier stage. And just as the chromatin at the earlier stage appeared to determine the formation of the four or more poles, so now the chromosomes again appear to be the active agents in effecting the resolution of the quadripolar into the bipolar arrangement.

<sup>1</sup> We are of course aware that attempted explanations based on the assumption of diffusion currents have been advanced, but these appear to us to rest on no real foundation.

<sup>2</sup> Hartog, M., loc. cit.

<sup>3</sup> Cf. Lawson, A. A., loc. cit.

When the chromosomes are fully developed it can readily be seen that they vary in size (Figs. 8, 10). This difference is obvious both at diakinesis, at the equatorial plate stage, and also during the anaphase of the heterotype mitosis. Another feature of this division consists in the somewhat irregular congregation of the chromosomes at the equatorial plate, and the same thing applies to the manner of their separation to their respective poles. It thus often happens that several chromosomes lag behind the rest, though they ultimately all reach their destination. We call attention to this circumstance here because the same irregularities, in an accentuated form, occur in both the varietal and in the hybrid plants.

We have once in this fern met with a simulation of amitosis in the first division of the spore mother-cell (Fig. 11). This is of interest, inasmuch as it is fairly common in both the sport and the hybrid, where we shall describe it at some length.

The remaining features of this and the homotype division call for no special remark here, beyond the mention of the fact that the spores, when mature, are commonly fertile.

#### POLYPODIUM VULGARE, var. ELEGANTISSIMUM.

This fern produces, as we have already stated, not only the beautifully dissected fronds proper to the variety, but also other leaves which lack, wholly or in part, the lacinate character. They may be said to have 'reverted' more or less completely to the ancestral type. The 'reversion' may only affect a few pinnae or it may involve the entire frond.

The sporangia are produced on both the varietal and on the 'reverted' pinnae, and germination tests revealed the unexpected fact that those borne on the 'reverted' pinnae were more sterile than those obtained from the lacinate ones. The fertility in either case is, however, a low one, and a large proportion of the spores are seen at a glance to be obviously depauperate and valueless. The development of the sporangia usually runs through a normal course until the mother-cells of the spores are formed, but degeneration commonly sets in during or after synapsis.

During the early stages of prophase the spindle is developed just as in the type form (Figs. 16, 17). The kinoplasmic web becomes repelled at four or more points on the nuclear periphery, and a quadripolar (or multipolar) arrangement is the result. The poles subsequently fuse, in the manner already described, into a bipolar structure as the nuclear membrane disappears. We may remark that we have observed, in another species of polypody, *P. tripartitum*, a case in which the spindle, though often bipolar, is sometimes quadripolar.

When diakinesis passes over, and the chromosomes are moved towards the equatorial plane, they present a far greater degree of irregularity (Fig. 18) than is the case with the type. By the time that the majority



have reached the equator some are already to be found at the poles, whilst still others are scattered over the spindle fibres. The impression is conveyed that the *irregular* chromosomes, which in this fern are not numerous although almost always present, fail in some way to respond to the controlling influence by which the majority are governed. Such a condition indicates a difference in physical and perhaps chemical nature which may well be correlated with the variability, and probably also with the sterility, of the plants now under consideration.

When the chromosomes arrive at the poles they still exhibit that same 'indiscriminate' and confused arrangement which characterized their previous behaviour. They also vary much in size and, so far as we have been able to arrive at a conclusion, they seem to be inconstant in number also. It may be that individuals here and there fail to form bivalents, but when the numbers are so great it is difficult to be sure as to this, and we only feel justified in recording our impression that such is the case.

The degeneration of the cells is associated with a degeneration of the cytoplasm which becomes more and more marked as the spore mother-cells pass through the meiotic phase. This degeneration has been described by Tischler<sup>1</sup> and others for a number of hybrids in flowering plants, and it is of special interest to encounter it, along with some other characteristics of hybrids, in a form which, though a 'sport', is clearly not of hybrid origin.

A second feature not seldom to be met with during the nuclear divisions of the spore mother-cells of this plant (and, as will appear later, it also occurs in the hybrid as well) consists in a curious form of division nearly resembling amitosis. We have already mentioned that we found a single case in *P. vulgare*, type. It is to be seen in the spore mother-cells produced on both the lacinate and the 'reverted' pinnae, and it may occur in both of the two meiotic divisions. The stage in question usually sets in about the time when diakinesis should pass over to the metaphase, though it may occur at a still earlier period; the nuclear membrane remains intact, and the ordinary spindle is absent or disappears, the nucleus becomes pulled out into a form roughly resembling that of an hour-glass, while the chromosomic contents are numerous, irregular, and small in size. Then the nucleus assumes the condition of a double sac, the swollen portions being connected together by a tube which becomes narrower as the process advances. Fibres then become differentiated in the cytoplasm between the two sacs, and even a cell-plate is formed across this interzonal spindle. When, as is often the case, the sac-like protuberances are not situated symmetrically within the cell, the cell-plate shows corresponding irregularities. Thus it may be well formed on one side of the connecting tube (Fig. 21), but almost absent from the other. Sooner or later the tubular

<sup>1</sup> Tischler, loc. cit. See also Juel, H. O., Beitr. zur Kenntnis d. Tetradenteilung. Pringsh. Jahrb. f. wiss. Bot., Bd. 35.



connexion is pulled apart, and the two nuclei thus become completely free from each other, and the cell-plate is formed across the intervening cytoplasm. The final division of the spore mother-cell may also take place in the same manner (Fig. 21). The whole process is strikingly like that described for hybrid sorts of *Syringa* (*S. chinensis*) by Juel,<sup>1</sup> whose account was confirmed by Tischler, and, as will be seen, it also reappears in the hybrid *P. Schneideri*. But its occurrence is evidently not dependent on hybridity, but probably on a disturbance of the normal intracellular processes such as may indeed be brought about by hybridization, but which may also be connected with more proximate nutritional disturbance, whether produced by unfavourable environment<sup>2</sup> or by less obvious causes such as are associated with the formation of a 'sport', as in the present instance. For there can hardly be any question of hybrid origin here; the fern was found growing wild, and, besides, there is no other species which seems likely to have contributed to produce its peculiarities, and moreover the type species has long been known to be subject to sporting.

#### POLYPODIUM SCHNEIDERI.

This fern is of large and somewhat robust habit. In this respect it recalls the *P. aureum* parent, although, except in some of the 'reverted' leaves, there is otherwise no very close resemblance between the two plants. In the lacinate foliage it is like a very vigorous form of *P. vulg. elegantissimum*. Perhaps it is even still more like the variety known as *cambrica*. As we have already stated, the 'reverted' parts of the fronds may recall the appearance of either a gigantic polypody leaf, though in the wavy edge, and especially in the glaucous tinge, it approaches more nearly to the *aureum* type.

Sporangia are freely produced both on the normal and on the 'reverted' leaves. The cells of the sporangia resemble the *elegantissimum* parent rather than the *aureum* type in their large size, a feature in which, as will be remembered, the two parents are dissimilar. Fertile spores have never been obtained, either by ourselves or, so far as we have been able to ascertain, by others; the sporangial contents often die away at an early stage of development, and when this occurs the entire sporangium may degenerate, so that even the basal cell situated at the surface of the placenta is involved in the process (Pl. XVIII, Figs. 23 and 24).

In other instances development advances as far as the differentiation of the spore mother-cells (Pl. XVII, Fig. 22), and then these may begin to undergo regressive changes. Even the comparatively few spore mother-cells that pass through meiosis afford abundant proof that they also

<sup>1</sup> Juel, H. O., loc. cit.

<sup>2</sup> Mr. R. Beer informs us that he has observed this form of nuclear division in pure specimens of *Oenothera biennis* when these flowered *late in autumn*, but not in individuals blooming at a more favourable time of the year.

are for the most part really abnormal, as is shown by the deficiency of cytoplasm which they contain, as well as by the deviations from the usual course of cellular changes which they exhibit. Relatively few of these cells succeed in forming spores, and when they do so the spores themselves are commonly depauperate, and always barren.

We pass now to the description of the cytological features of the development of the sporangia and spores.

In the case of the sporangia which are destined to degenerate early, the nuclei of the cells are seen to change their character and to assume a gelatinous or mucus-like appearance. The cytoplasm diminishes and the cells die away. As we have already said, these lethal changes may involve the basal cell at the periphery of the placental epithelium on which the sporangium is borne. This cell represents the proximal portion of the primordial cell from which, distally, the sporangium is produced.

When the development of the sporangium is not arrested so early, the tapetum is cut off from the archesporium in the usual manner, but it seems never to assume that strikingly nutritive and glandular character so generally associated with this tissue (see Pl. XVII, Fig. 22). On the contrary, the cells rapidly become relatively poor in contents. It often happens that the nucleus—or nuclei—may be of large size, but the cytoplasm is small in amount and poor in appearance. The tapetum as a whole breaks down at an early stage, and by the time that the spore mother-cells are passing into synapsis the tapetal contents form a scanty plasmodial nucleated mass within the sporangial cavity. It is evident, we think, that the failure of so many sporangia to advance beyond the synaptic stage is to be directly connected with this weakening of the nutritive function on the part of the tapetum. It often happens that, despite the poverty of the tissue in question, the archesporial cells, up to the isolation of the spore mother-cells and the passage of their nuclei into synapsis, do not show marked signs of deviation from the normal course of development. Possibly their supply of nutrition may be less limited, or the demands on it less exigent, before the characteristic growth of the cell which marks the onset of the meiotic phase with its attendant nuclear changes.

The spore mother-cells which enter on meiosis exhibit a dense synaptic contraction (Pl. XVIII, Fig. 25) in which it becomes impossible to make out the detailed structure, but, as usual, this condition passes over into the open spireme in which the thread is very clear. There appear to be anastomoses between different parts of the thread as a whole, but a little later on the separate chromosomes can be identified. They are, from the time of their first appearance, somewhat abnormal. They give the impression of being badly fixed, but we believe this character is not really due to any such cause but to their actual nature. They are somewhat ragged in outline, and there are great differences in size, as well as of form, between them.

These peculiarities persist throughout their existence, and are very strongly marked in the later stages of diakinesis. As regards numbers they exceed those of the corresponding nuclei of either parent, though careful counting has convinced us that there is no very great constancy in the numbers actually present in the nuclei of this plant. We have already alluded to this circumstance in connexion with the other species, but it is far more obvious here. The chromosomes, so far as we could estimate them, vary from about 95 to 125, though about 95 to 105 is the more common range. The striking difference in individual size (see Figs. 30, 31, 32) is a point of some interest, inasmuch as it might be taken to indicate that the larger ones represented bivalents whilst the smaller ones constituted the univalent remainders which had failed to pair. This hypothesis would fit the fact that the chromosomes contributed by the two parents respectively are numerically different (about 34 in *P. aureum* and 90 or more in *P. vulg. elegant.*). But in any case such unions to form bivalents must at best be irregular, and we entirely failed to find anything which would justify us in asserting that there were 34 bivalents or indeed any other constant number of larger chromosomes which could be really identified as such. Certainly there is no such regularity in this instance as exists, for example, in the hybrid *Drosera* according to Rosenberg. We would, however, point out that this seems to us to be in no way surprising. Indeed it is difficult, on the assumption that the chromosomes ordinarily contributed by the male and female parent respectively are to be regarded as homologous, and therefore destined to pair at meiosis, to see how any such homology can exist at all *when the numbers are different* in the two parents. For it is, in the first place, extremely improbable that any single one of those of the *more* numerous lot can be equivalent to any single one of the *less* numerous group, and it is obviously impossible that there can be any close resemblance between the whole of the lot comprised in the smaller number and an *equal number* (less than the whole) of the larger set furnished by the other parent. In many of these cases there are, as in the *Drosera* plants under consideration, twice as many chromosomes contributed by one parent as by the other. It seems not unlikely that the double number of the one has arisen by a division of the individual chromosomes in the ancestor of *D. longifolia* which have thenceforth remained distinct. If this has occurred by a transverse fission, then we might expect one chromosome derived from the (10) parent (*D. rotundifolia*) to unite at meiosis with *two* chromosomes from the (20) parent (*D. longifolia*), so that there would be practically ten bivalents produced. If, however, it be argued that the twenty chromosomes of *D. longifolia* have arisen not by transverse, but by longitudinal fission, and that they have, in the absence of hypothetical 'regulative' processes, persisted unchanged, and so have come to be characteristic of the species in question, we are thereby driven to the

admission that the whole case for individuality and differentiation amongst the chromosomes rests on a far more insecure and speculative foundation than, on other grounds, would be generally admitted. We do not urge these considerations in any sense as suggesting that we doubt the statements that have been made respecting the process in *Drosera*. Our only motive for alluding to this case is because it is, from the relatively few number of the chromosomes, a much more favourable case than is our plant for ascertaining the facts, and at the same time to indicate that, in our opinion, the special importance which has been attached by some writers to the course of events in *Drosera* can nevertheless hardly be maintained as being of general application. Furthermore, that there are also, as it seems to us, obstacles which from a theoretical standpoint make the interpretation which has been put on the pairing of the chromosomes in this and in similar cases difficult of acceptance.

The important results which have recently been published by Gates,<sup>1</sup> as the result of his investigations on hybrid *Oenotheras*, prove that there are other factors connected with the possible modes of chromosome distribution of the nature of which we are as yet practically ignorant. Gates finds that the hybrid *Oenothera lata* × *O. gigas* has 21 chromosomes in its premeiotic cells. At meiosis this premeiotic number reappears, owing to the failure to pair on the part of the chromosomes, and finally each of the resulting nuclei receives as nearly as possible the half of this number, i. e. either 10 or 11 respectively. There are irregularities also, but these are of no consequence for our present purpose. The feature around which our interest centres is that despite the fact that the two parents contribute different numbers of chromosomes, an equal, or almost an equal, number are distributed to each of the daughter-nuclei in the heterotype mitosis. That is to say, the distribution is not the result of 'a pairing and separation of homologous chromosomes of maternal and paternal origin, but the segregation tends to be into two numerically equal groups'.<sup>2</sup> These results are in harmony with those we have obtained in *Polypodium Schneideri*, for the chromosomes, whether they are bivalent or not, are about equally distributed between the two daughter-nuclei at the heterotype mitosis, in spite of the fact that the share, numerically speaking, which is contributed by the two parents respectively is so markedly dissimilar. We are far from desiring to suggest that these results affect, at any rate necessarily, the views which are entertained by many respecting the allelomorphic distribution of chromosomes in a normal plant. It may well be that the peculiarities that characterize the behaviour of the chromosomes in these hybrids are directly correlated with their sterility. But however this

<sup>1</sup> R. Ruggles Gates, The behaviour of the chromosomes in *Oenothera lata* × *O. gigas*. Bot. Gaz., xlviii.

<sup>2</sup> Ibid., p. 195.

may be, the facts cannot be without importance as we gradually arrive at a position which may enable us to formulate a coherent theory of the mechanism which is responsible for the distribution of the chromosomes to the daughter-nuclei at mitosis.

Before considering the various aberrant features which the chromosomes of *P. Schneideri* exhibit during the heterotype mitosis, we may proceed to describe the mode of spindle-formation in these cells. As in the common polypody, the spindle is usually quadripolar instead of bipolar. It is, however, never so well developed as in the parent species (cf. Figs. 26, 27, with 16, 17), and this fact is to be correlated with the relatively poor amount of cytoplasm which, as we have already said, is present in the cells. Probably the abnormal character of the nuclear metabolism may also act as a contributory cause. The first indications of spindle-formation are to be seen during the later stages of the thick spireme, just when what we have elsewhere termed the 'second contraction' is setting in. The fact that sometimes its inception may apparently be deferred to even later stages is perhaps to be attributed to the difficulty of recognizing the earliest stages in cells that under any circumstances do not form the achromatic structures as distinctly as in the majority of allied forms.

The kinoplasmic differentiation around the nucleus can be made out, though often with difficulty, at earlier stages. Then there occurs a similar aggregation of the chromatic linin such as we have described above for the other ferns. The chromatic linin forms a bunched mass (Figs. 26, 27), often very prominent and striking, just beneath the nuclear wall, and at several spots. The kinoplasm on the outer side of the nucleus, at places exactly corresponding to these aggregations, is repelled, or at any rate diverges there, from the surface of the nucleus. It differentiates into spindle fibres which, though feebly developed, ultimately traverse the cytoplasm to the periphery of the cell, and end in denser masses of protoplasm just beneath the cell-wall. Sometimes there are clusters of granules at these spots, but the cytoplasm also contains other granules distributed in its mass. The appearance of the fibres in surface view is often very remarkable, as is shown in Pl. XVIII, Fig. 29. It may happen, however, that the spindle is not quadripolar but bipolar at these stages, though this is not of frequent occurrence. The nuclear wall remains intact till diakinesis, and at this stage the quadripolar arrangement of the chromosomes is often very striking. At other times the chromosomes are apparently grouped in three clumps (Fig. 32), two being smaller than a prominent central group.

As the wall of the nucleus breaks down, the spindle immediately becomes bipolar, but the three or four chromosome clusters may persist. Such cases lead to very irregular figures, as the two smaller sets move at once towards the poles, whilst the more central group or groups take some time to point off to their respective destinations.



A peculiar feature, already observed in *P. vulg.* var. *elegantissimum*, and once also in the typical *P. vulgare*, is to be seen in the nuclei which go through division without the disappearance of the nuclear wall (Pl. XVIII, Figs. 33, 34). Such examples, as already pointed out, at first sight suggest amitosis, but the fact that the chromosomes are quite distinguishable serves to indicate the need for caution in drawing this conclusion respecting them. As a matter of fact, they are more properly to be described as imperfect mitoses—imperfect inasmuch as the nuclear wall does not become destroyed. The whole nucleus is pulled out like a sac, and this then is distended at its two ends. The ends gradually receive a moiety each of the chromosomes, which are sometimes much smaller and shorter than in the normal mitoses, or, if the process has set in earlier, they may be in the long thread stage. The two end-sacs are for a time connected by a tubular bridge which gradually becomes more and more attenuated and finally is empty of chromosomes, these having passed to the distended ends. In the cytoplasm which lies between the latter the interzonal spindle fibres are developed. These are often somewhat irregular, but they are invariably present, and when the tubular bridge is centrally and symmetrically placed in the cell, it is seen to be equally surrounded by the fibres. When, however, as is often the case, the tubular connexion is excentrically placed, it may happen that the interzonal fibres are absent or sparsely developed on the outer side. The whole process seems to us to afford a confirmation to the view that the fibrous system of lines is due to an indirect influence of the nuclear contents on the cytoplasm and to be dependent on the induction of a state of stress as the result of the chemical changes going on in connexion with the nuclein transformation that is taking place. At any rate there is here no question of any kind of open communication between the nuclear contents and the cytoplasm such as accompany an ordinary mitosis. A cell-plate is formed across the interzonal fibres, and sometimes this seems as if it were about to nip the tubular connexion into two parts.

The homotype mitosis (Fig. 35) calls for no special comment beyond the statement that this curious form of division may also occur in connexion with it; indeed it seems to do so generally when it has occurred in the preceding (heterotype) mitosis.

During the reconstruction of the normal daughter-nuclei the cytoplasm is seen to contain numerous granules which are coloured by dyes that are capable of staining chromatin. These are very well known to all who are familiar with the details of karyokinesis, and there is some doubt as to their origin. We have been able to trace at any rate some of them in these plants quite definitely to a nuclear source. They are given off (Fig. 35) by the vesiculating chromosomes as buds which pass out from the nucleus into the cytoplasm (cf. Pl. XVII, Fig. 12), in somewhat the same way as occurs at the commencement of the division (Pl. XVI, Fig. 1). Whether



this elimination of chromatic substance is to be regarded as always possessed of the same significance seems to us to be doubtful, but the facts before us are not as yet sufficient to enable us to usefully discuss the question at the present time. It may well be that we are here dealing with matters destined to throw light on the bodies known as chromidia, but overmuch speculation in proportion to the amount of positive knowledge has already grown up around these matters, and it seems premature to add to it on this occasion.

#### GENERAL CONCLUSIONS.

The main points of general interest which appear to us to emerge from the study of the ferns as detailed in the foregoing account relate to the formation of the spindle and to the behaviour of the chromosomes in the sports and hybrid respectively.

The mode of differentiation of the kinoplasmic weft around the nucleus supports the view long ago advanced by one of us to the effect that this is to be regarded rather as a specialized and temporary arrangement in the cytoplasm than as indicating a permanent substance in the cell. Strasburger, to whom we are indebted for the clear recognition of the differentiation in question, also now holds that no absolute distinction between kinoplasm and the cytoplasm can be maintained, although so long as it does exist the protoplasm, thus distinguishable, is the seat of metabolic change of a nature often very definite in character.

The spindle fibres are clearly produced at the expense of such cytoplasm in the cases studied here, and indeed more clearly so than is frequently the case in other plants. We think that the particular mode of the formation of the spindle points strongly in the direction of its originating as the result of electrical disturbance within the colloids of which the cytoplasm is made up. We have shown how it arises just above the aggregations of chromatin-containing linin, and we believe that a real causal relation is here indicated. It will be remembered that one of the characters of albumin is its capacity of absorbing, or otherwise associating with itself, dissociation products which may carry electrical charges of different sign.<sup>1</sup> The apparent magnitude of the observed results is to be attributed, at least in part, to the smallness of the field (the cell) in which the forces are operating.

The view that the spindle owes its existence as well as its character to electrical conditions has been repeatedly advocated. In recent years Hartog<sup>2</sup> has endeavoured, by working models of an ingenious form, to show that the poles of the spindle may be compared with unlike poles of a magnet, and that the curved system of lines assumed by the achromatic fibrils are to

<sup>1</sup> See Lillie, R., On the differences in the electrical connexion of certain free cells and nuclei. *Am. Journ. of Physiol.*, viii.

<sup>2</sup> Hartog, M., The dual force of the dividing cell. *Proc. Roy. Soc., B.* 76.

be regarded as approximately representing the position of the lines of force in a magnetic field between such unlike poles. Gallardo,<sup>1</sup> on the contrary, has argued that the difficulties that must underlie the assumption of unlike poles under the conditions in which they could exist in the cell are wellnigh insuperable. It is difficult to see how they could be kept asunder, much less to understand how they could actually move still further apart.<sup>2</sup> Gallardo's contention is that the apices of the spindle represent not unlike but like poles, and he regards them as due to an inductive effect, dependent on the charge carried by the contents of the nucleus. At the same time he remarks that there are cases in which the achromatic fibres are entirely differentiated in the cytoplasm under conditions in which the nucleus is excluded from all share in the process. Such an example is afforded when, by the agency of drugs, the spindle fibres are caused to appear in cells from which the nucleus has been removed. In this case Gallardo thinks that the poles are really unlike.<sup>3</sup>

It seems improbable that there can exist so fundamental a difference in this respect, and we are ourselves of opinion that the solution of the problem will be found to lie in the proof that the poles are always similar to each other and of opposite sign to that of the acid nucleus. The matter is complicated by the colloidal nature of the material in which the process is going on, and the state of lag, so well insisted on by Hartog, may well account for much that is otherwise anomalous. We recognize that the so-called 'Hermann's Spindle', as well as those other instances in which the centrosomes diverge from each other, although the connecting fibres form a convergent system of curves, are difficult cases. But the remarkable manner in which the sheaves of fibres in these ferns diverge from the proximity of the chromatin-charged linin, and are so repelled by each other that they press out equidistantly at the periphery of the cytoplasm, seems to us to provide an almost overwhelming support for the hypothesis that the linin with its contained chromatin, by virtue of the chemical changes involved in its metabolism, has brought about an electrical condition of opposite sign similar in each of the spindle-cones formed from the substance of which the kinoplasm is made up. This hypothesis also is in harmony with the fact that the disappearance of the nuclear membrane is closely associated with the spreading of the chromosomes beneath it just before their retrogressive movement to the equator, whilst the spindle poles have shifted away from the nuclear surface. The change of size of the nucleus,

<sup>1</sup> Gallardo, A., *L'interprétation bipolaire de la division karyocinétique*. An. Mus. Nac. de Buenos Aires, t. xiii.

<sup>2</sup> The subsidiary hypotheses which have been advanced to explain the spindle mechanism on the assumption of unlike poles do not appear to us to be convincing.

<sup>3</sup> In a paper just published in the *Archiv f. Entwicklungsmechanik* (vol. xxviii) Gallardo has given up this view, and asserts that the sign is always similar in the cytoplasm, i. e. electropositive.

so obvious during the progress of mitosis, a change which has often been interpreted as indicating alteration in osmotic relations, or, less precisely, has been ascribed to growth changes, seems to us to find a less strained explanation when regarded as due to change of electrical state than in any other way. It has been shown that the size of the nucleus may alter during the prophase of division, and that this may be correlated with the changes in the chromatin. Further, that the 'growth' is not regular, but may oscillate in the plus and minus directions. And it cannot escape notice that the periods of maximal size coincide with the spreading out of the chromatin beneath the nuclear wall, that is, with conditions that tend to lower the surface tension of the membrane, or even to bring about its disappearance altogether, by resolving the coagulated state to which it almost certainly owes its existence. The time has admittedly not yet arrived when it will be possible to give an explanation of these cellular changes that will prove completely satisfactory from a physical point of view. We have still much to learn concerning the modifications imposed by the physical and other properties of the colloids<sup>1</sup> in which the chemical disturbances that are bound up with the changes of form and with the genesis of structures such as those we have been considering are taking place. For example, as Hartog pointed out, the lines of force in a magnetic field,<sup>2</sup> although they may be mapped out by the arrangement of those cell constituents which, as he says, are more 'permeable', may be themselves abolished or otherwise modified, without a simultaneous effect becoming at once manifest in the 'chains' formed of material particles which are relatively immobile, and tend to retain any particular arrangement (in this case approximately that of lines of force) in which they have been cast until, by the action of other forces, they are compelled to assume new positions.

If we now turn our attention to the behaviour of the nucleus, we find the infertility of the varietal form as well as of the sport to be intimately related to degenerative changes in the nucleus. We have seen how degeneration may set in at an early stage in the history of the development of the sporangium, and that the lethal processes are first visible in the nucleus. The cytoplasm also is very characteristically altered, and gives the impression of being starved. The same is true of the separated spore mother-cells, except that in them the starvation in the cytoplasm is more definite, doubtless owing to the poor nutrition consequent on the failure of the tapetum to develop properly. It is at any rate quite clear that the normal relations between the cytoplasm and the nucleus have become

<sup>1</sup> e. g. the adsorption of ions, dissociation or association products, on the colloidal surfaces.

<sup>2</sup> It may be as well to point out that there can be no question of the existence of *magnetic* forces in the cell. The problem is one of electrostatics, and Hartog showed he was fully aware of this; cf. his paper on 'The dual force of the dividing cell' in *Science Progress*, 1907.

abnormal, and it may be that the disturbance of these relations, the upsetting of the *Kernplasma-relation*, lies near the root of the matter.

But it is of course evident that this does not really *explain* anything, for what we want to know is just what it is that disturbs these normal relations. It is certainly not merely hybridism. The hybrid is indeed more sterile than the sport, but the phenomena are of the same order in the two cases. We find the same early degeneration in both, the same 'tubular' nuclei simulating amitosis, the same irregularity in the distribution of the chromosomes on the heterotype spindle. We feel that it is of little use at present to endeavour to give explanations which can only rest on a very insecure base. But it is at any rate clear that the sterile 'sports' demand far more attention than has hitherto been paid them, and we confidently anticipate that they will furnish important clues to the understanding of the causes of sterility in hybrids. Many of the remarkable deviations that are so characteristic of the cells, and especially of the nuclei, in malignant neoplasms, are also to be encountered in the aberrant cells of these sports and sterile hybrids. Whether the underlying causes are similar is still to seek, but we think that there is an interesting field of inquiry in this direction, and one well worthy of investigation. As far as the immediate future is concerned, what is required is a greatly extended knowledge of the plain facts.

We do not propose to discuss the questions that arise in connexion with the mode of formation of the heterotype chromosomes further than we have already done, but reserve this to be dealt with in a future communication now on the way towards completion.

#### SUMMARY.

1. The evidence from the study of the cytology of *Polypodium Schneideri* is consistent with the view that it is of hybrid origin, although it is not very conclusive if taken by itself.

2. The nuclei of *P. aureum* are about two-thirds the diameter of those of *P. vulgare* and its var. *elegantissimum*. The number of chromosomes in *P. aureum* is about 34-36, in *P. vulgare* about 90, and there are about the same number in the variety *elegantissimum*. In *P. Schneideri*, the hybrid, there are variable numbers, the range lying between 95 and 125.

3. The sporangia are apt to produce a large proportion of abortive spores in var. *elegantissimum*, and in the hybrid no fertile spores have been found. This sterility is to be associated with the degenerative changes in the cytoplasm, and these become especially acute at meiosis. The nuclear apparatus also appears to be, at least in part, responsible for the failure to form fertile spores.

4. The achromatic spindle in *P. aureum* and some other species is

bipolar from its early appearance, in *P. vulgare* and its var. *elegantissimum* it is quadripolar in the great majority of cases, in the hybrid it is either bipolar or quadripolar, more commonly the latter.

5. The spindle is formed from a differentiation of the cytoplasm (kinoplasm), and it is influenced in its distribution within the cell by the aggregation of the chromatic linin within the nucleus. This is especially seen in the cases of quadripolar spindle formation. Electrical conditions are believed to be concerned in the spindle formation.

6. Chromatic droplets are ejected from the nucleus into the cytoplasm during the early stages of the heterotype mitosis, and also during telophase at other mitoses. It is left an open question as to whether this phenomenon always implies the same processes.

7. Nuclear divisions resembling amitosis occur frequently in the hybrid, but they also occur, though far more rarely, in *P. vulgare* (type) and in its variety *elegantissimum*. These divisions are examples of imperfect mitoses, and are brought about by the failure of the nuclear wall to disappear at the end of diakinesis.

8. Much irregularity as to pairing of the chromosomes to form the bivalents exists in the hybrid plant, and also, though less obviously so, in *P. vulgare* var. *elegantissimum*.

9. The processes that lead to depauperation of the reproductive cells, and ultimately to sterility, in hybrids are encountered in certain 'sports, which also exhibit sterility in a marked degree'.

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## EXPLANATION OF PLATES XVI–XVIII.

Illustrating Professor Farmer and Miss Digby's paper on the Cytological features exhibited by certain varietal and hybrid Ferns.

All the figures, with the exception of Figs. 22, 23, and 24, were drawn with the camera lucida under a 2 mm. apochr. hom. imm. Zeiss, N.A. 1.40 with comp. oc. 18. × 2250.

Fig. 22 was drawn with the camera lucida under a 3 mm. apochr. hom. imm. Zeiss, N.A. 1.40 with comp. oc. 6. × 498.

Figs. 1–5. *Polypodium aureum*.

Figs. 6–13. *Polypodium vulgare*.

Figs. 14–18. *Polypodium vulgare* var. *elegantissimum*.

Figs. 19–21. *Polypodium vulgare* var. *elegantissimum* ('reverted').

Figs. 22–34. *Polypodium Schneideri*.

### PLATE XVI.

Fig. 1. *Polypodium aureum*. Early heterotype prophase in the spore mother-cell nucleus. 'Chromatic droplets' are being given off from the nucleus; some lie free in the cytoplasm, whilst others are still attached to the nuclear contents by fine connexions. × 2250.



Fig. 2. Three adjacent spore mother-cells whose nuclei show successive stages in the withdrawal of the network from the nuclear periphery; the first indication of the preparation for 'synapsis'.  $\times 2250$ .

Fig. 3. The chromosomes are coming out of second contraction. Note the bipolar spindle, and the massing of the chromosomes at the two parts of the nuclear periphery which will be invaded by the fibres.  $\times 2250$ .

Fig. 4. Polar view of a heterotype equatorial plate.  $\times 2250$ .

Fig. 5. Heterotype equatorial plate.  $\times 2250$ .

Fig. 6. *Polypodium vulgare*. Archesporial nucleus in prophase showing the premeiotic spindle origin. One of the spindle cones is distinctly bilobed. Note the granules towards which many of the fibres converge.  $\times 2250$ .

Fig. 7. Fully formed chromosomes. The four spindle cones are in the same plane.  $\times 2250$ .

Fig. 8. The fibres have invaded the nucleus, and the chromosomes are becoming arranged on the spindle. Two of the spindle cones have come together, the other two are still wide apart.  $\times 2250$ .

Fig. 9. Slightly later stage in which the quadripolar spindle has become decidedly bipolar.  $\times 2250$ .

Fig. 10. Polar view of a heterotype equatorial plate.  $\times 2250$ .

Fig. 11. Aberrant mitosis, the result of an incomplete first meiotic division. The narrow bridge joins the two sac-like nuclear extremities. The interzonal spindle fibres are clearly to be seen.  $\times 2250$ .

#### PLATE XVII.

Fig. 12. *Polypodium vulgare* (continued). Telophase of the first meiotic division showing the extrusion of 'chromatic droplets', some of which are still joined to the nuclear chromatin by conspicuous threads.  $\times 2250$ .

Fig. 13. Nucleus of tetrad after the second meiotic division. Extranuclear 'chromatin droplets' are in direct connexion with the nuclear threadwork.  $\times 2250$ .

Fig. 14. *Polypodium vulgare* var. *elegantissimum* ('reverted'). Nucleus of tetrad showing 'chromatin droplets' still attached.  $\times 2250$ .

Fig. 15. Nucleus going into synapsis. A stage slightly later than Fig. 2.  $\times 2250$ .

Fig. 16. Nucleus having passed through the 'hollow spireme' stage is passing into second contraction. There is a marked massing of the spireme at the areas where the spindle radiations are in contact with the nucleus.  $\times 2250$ .

Fig. 17. A later stage showing the increased massing of the chromatin.  $\times 2250$ .

Fig. 18. Heterotype diaster. The chromosomes pass to the poles in an irregular manner.  $\times 2250$ .

Fig. 19. *Polypodium vulgare* var. *elegantissimum* (from varietal leaf). Polar view of a heterotype equatorial plate.  $\times 2250$ .

Fig. 20. Aberrant form of the first meiotic division, giving off droplets of chromatin. The cell-plate is incomplete.  $\times 2250$ .

Fig. 21. Aberrant nuclei of the second meiotic division. These nuclei will break apart as the tetrads mature.  $\times 2250$ .

Fig. 22. *Polypodium Schneideri*. General view of a sporangium with its spore mother-cells. Note the scanty tapetum.  $\times 498$ .

#### PLATE XVIII.

Figs. 23 and 24. *Polypodium Schneideri* (continued). An abortive sporangium dying back to the placenta (two drawings of the same sporangium in consecutive planes).  $\times 498$ .

Fig. 25. Nucleus passing into synapsis. A stage between Figs. 2 and 15.  $\times 2250$ .

Fig. 26. Nucleus coming out of the 'hollow spireme' and beginning to prepare for the second contraction. There is a slight concentration of the spireme towards the spindle fibres.  $\times 2250$ .

Fig. 27. Chromosomes coming out of second contraction. The spindle fibres are feebly developed as compared to those of *P. vulgare* and to the variety *elegantissimum* at the corresponding stage.  $\times 2250$ .



Fig. 28. Slightly later stage. Again the spindle cone is relatively inconspicuous.  $\times 2250$ .

Fig. 29. Superficial view of a quadripolar heterotype spindle. Many of the fibres are in connexion with granules.  $\times 2250$ .

Fig. 30. Formation of an irregular heterotype spindle.  $\times 2250$ .

Fig. 31. Quadripolar heterotype spindle. Only three limbs are seen, the fourth lies in the next section.  $\times 2250$ .

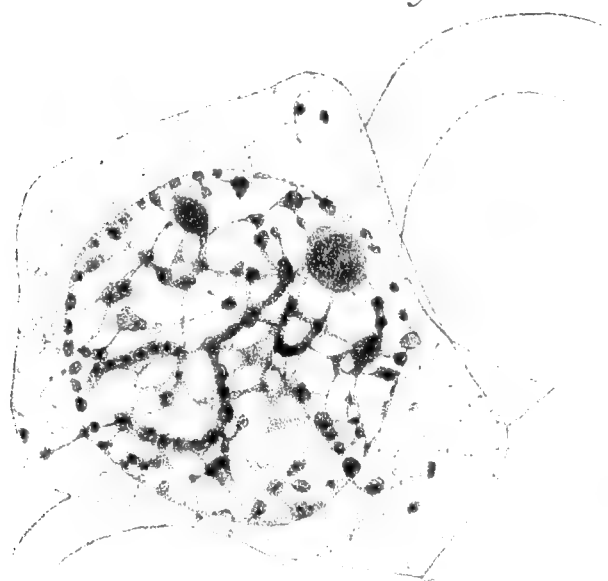
Fig. 32. Typical heterotype spindle of this plant, showing the large collection of chromosomes at the equator, and the smaller groups at either pole. The spindle fibres are few.  $\times 2250$ .

Fig. 33. Aberrant heterotype nuclear division.  $\times 2250$ .

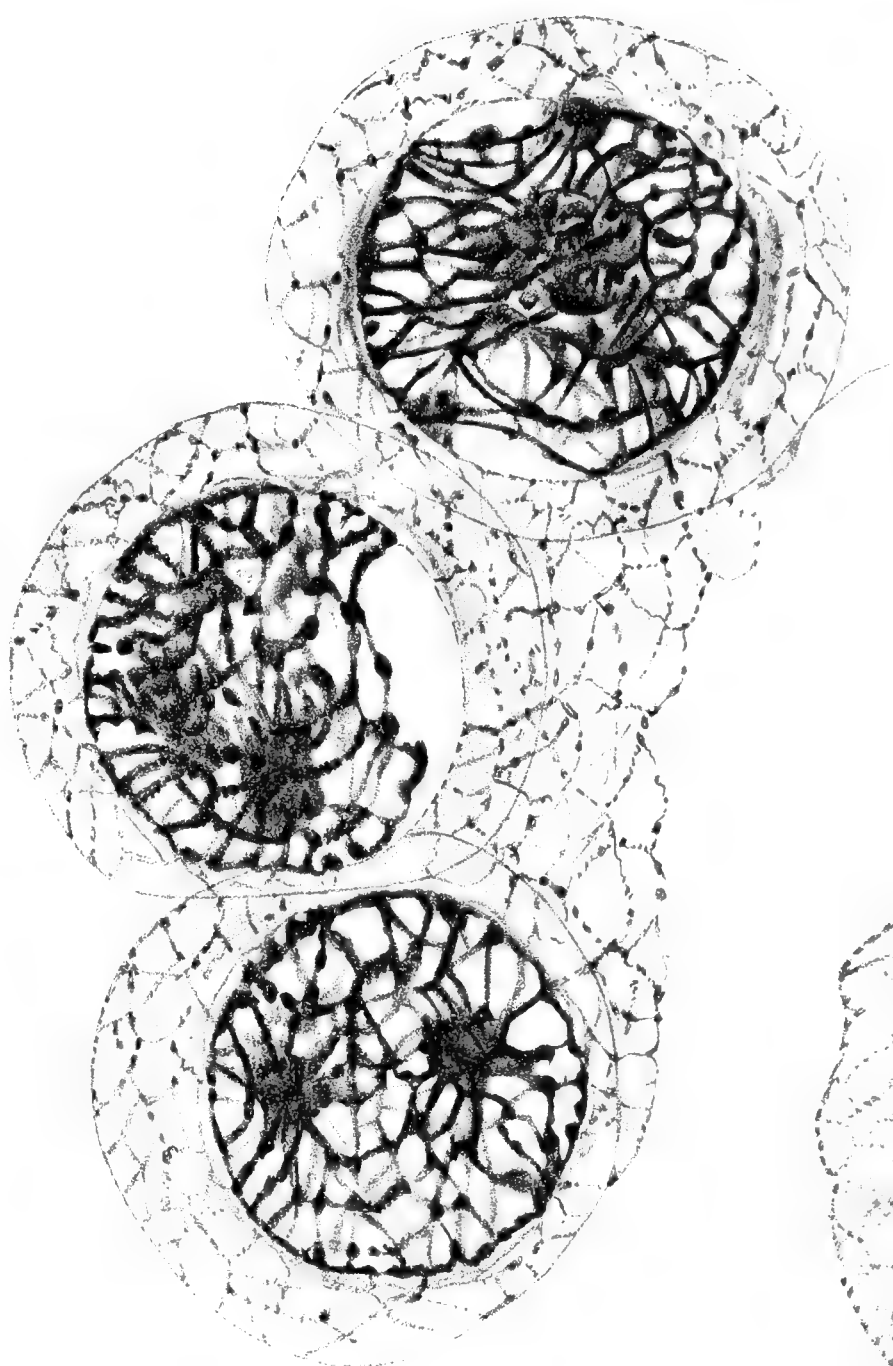
Fig. 34. Aberrant heterotype nuclear division, but somewhat later stages; the chromosomes are swelling and losing their sharpness. The axis joining the two nuclei is not in the focal plane, hence the apparent breadth of the tubular connexion.  $\times 2250$ .

Fig. 35. Second meiotic division of a fairly normal character, which is of rare occurrence in *P. Schneideri*. Note the 'droplets' and the belated chromosomes.  $\times 2250$ .

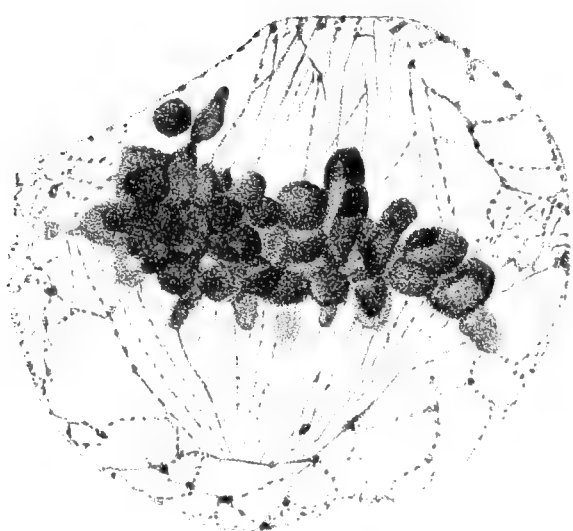




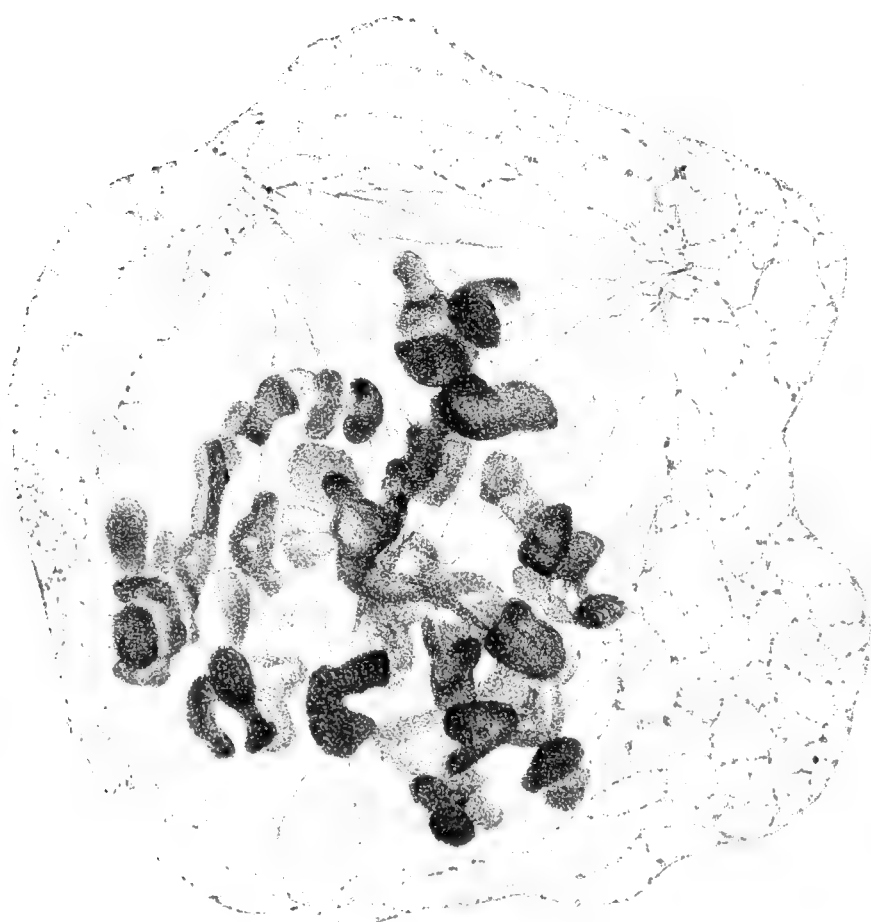
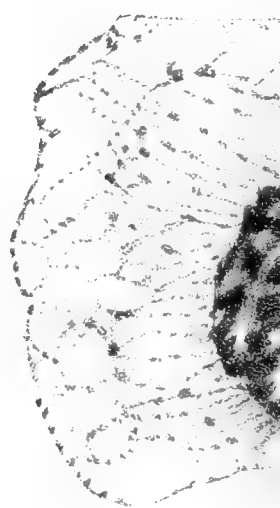
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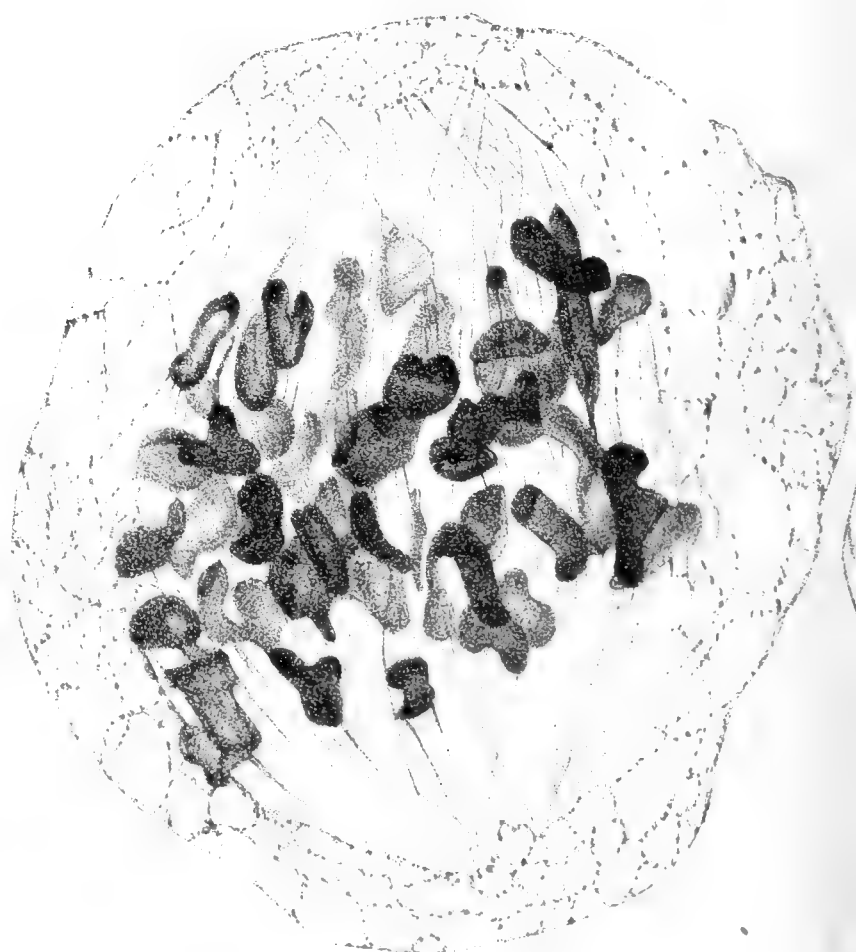
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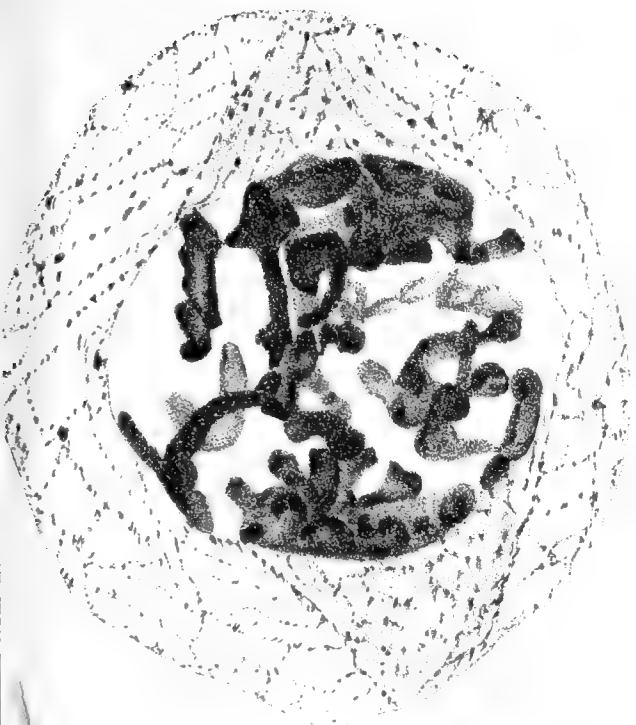
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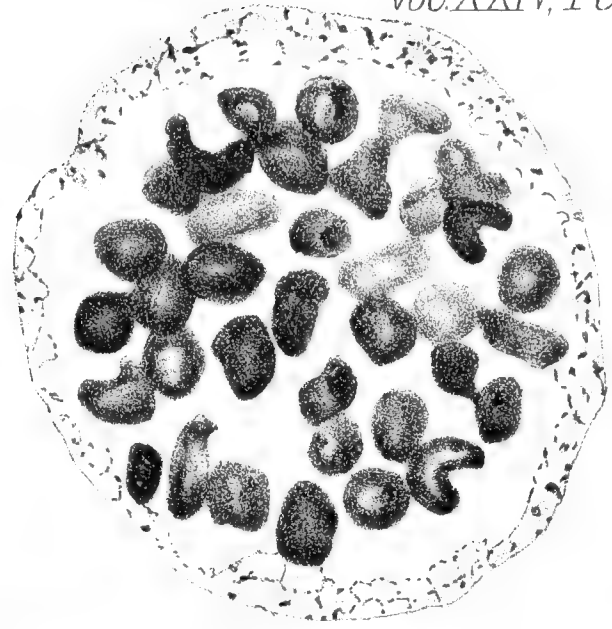
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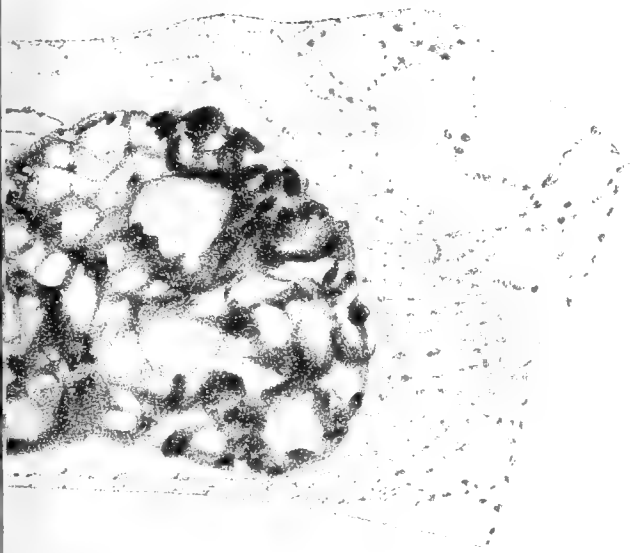
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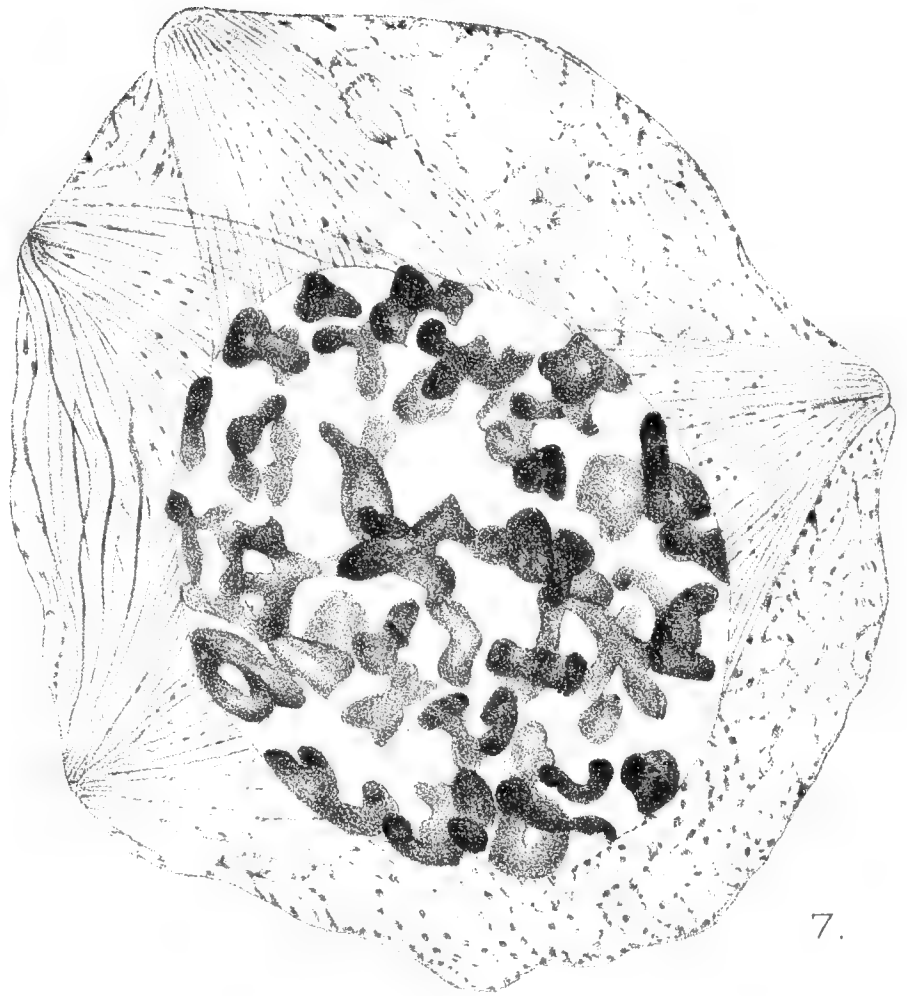
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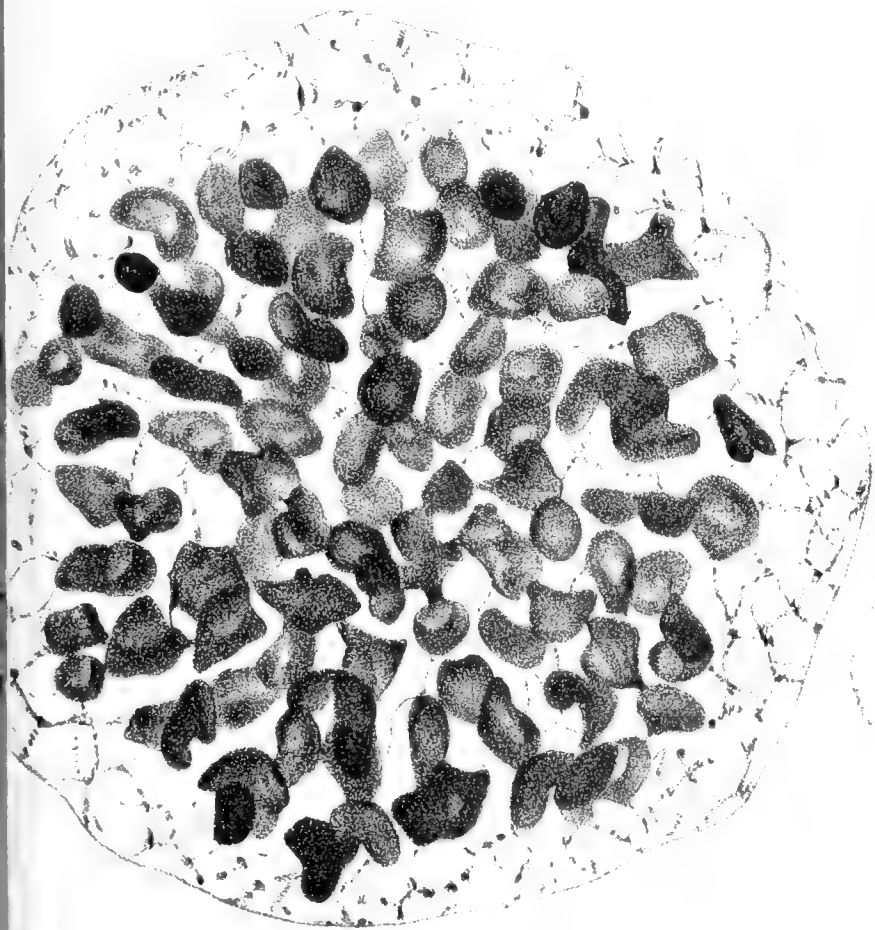
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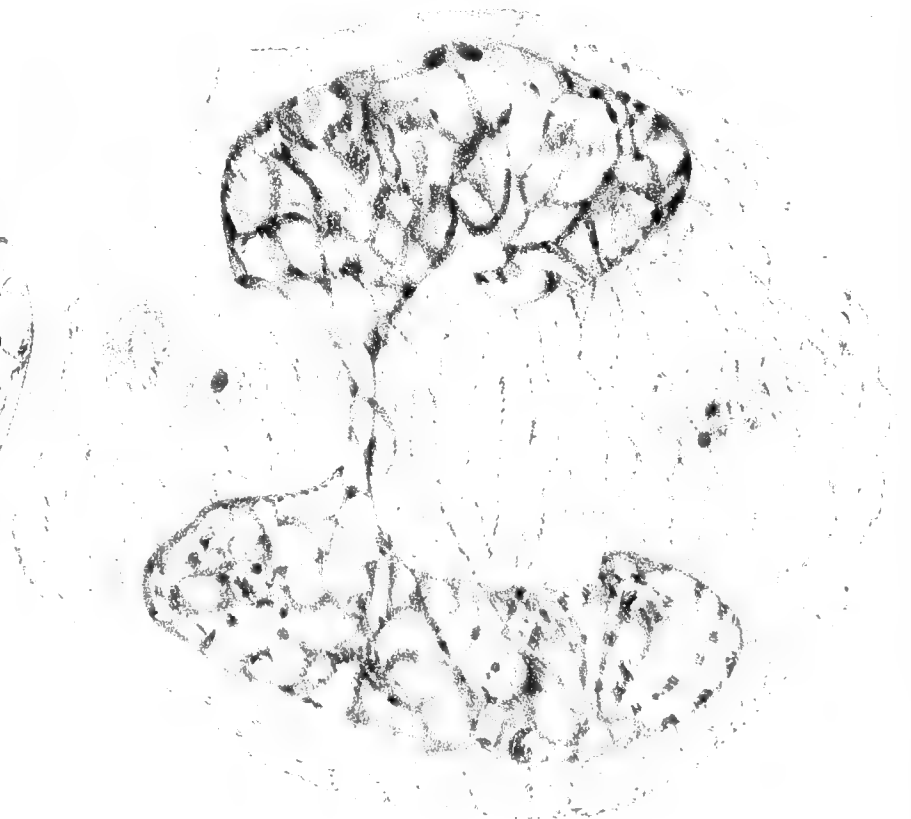
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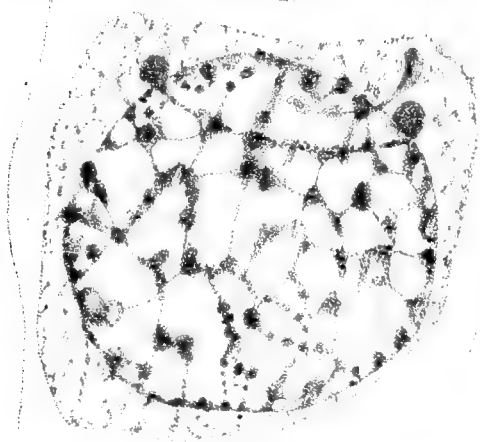


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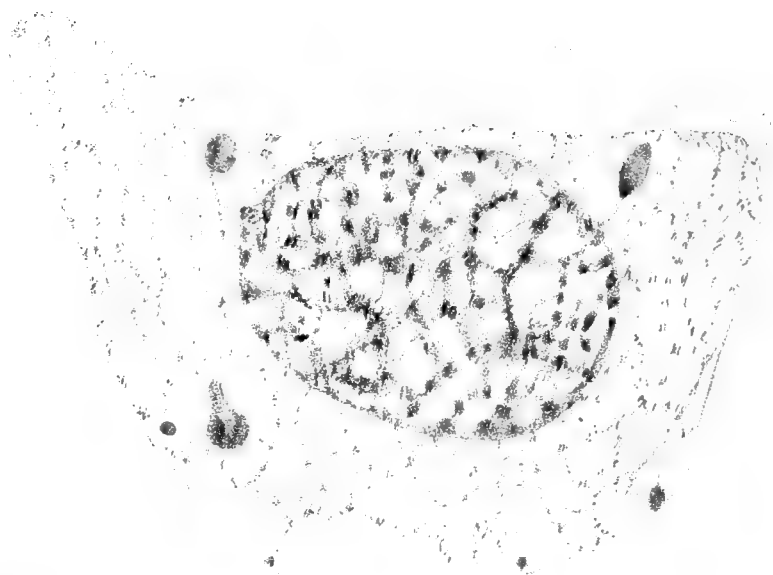




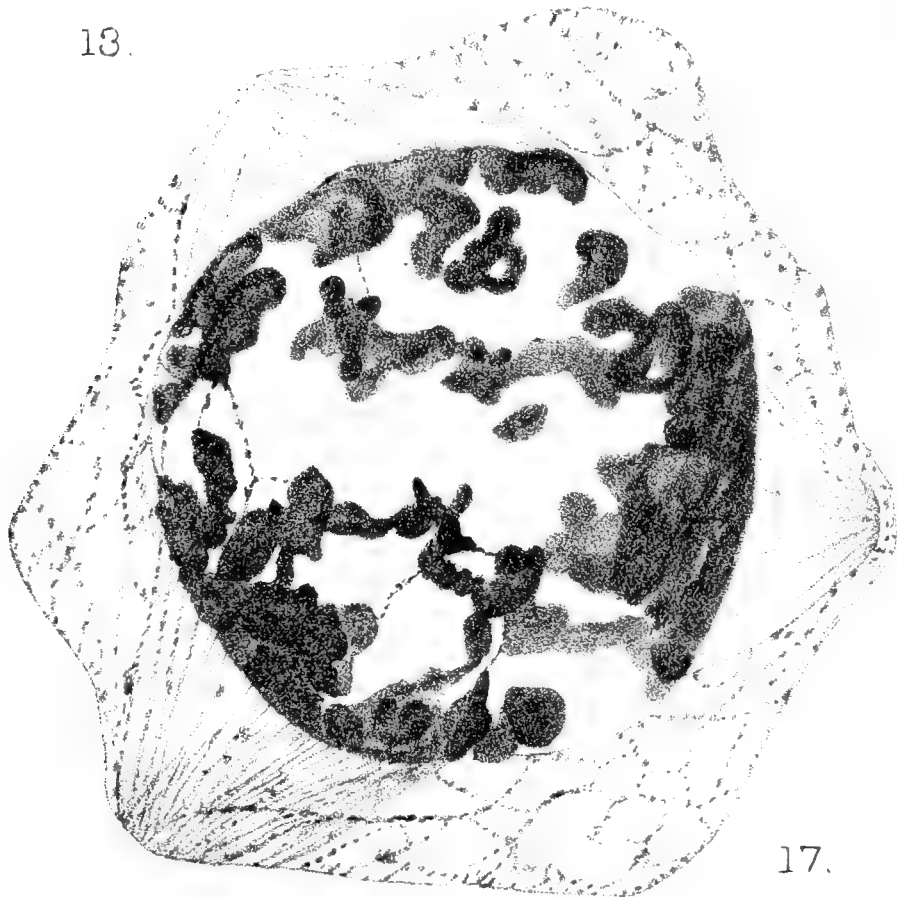




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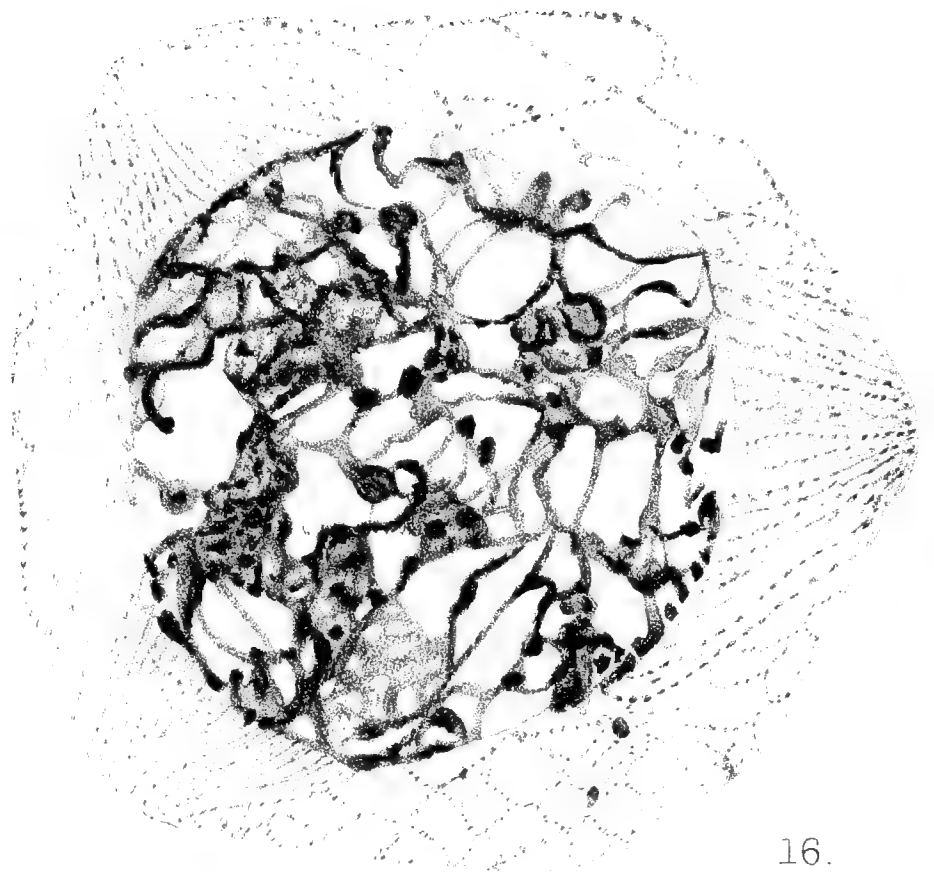
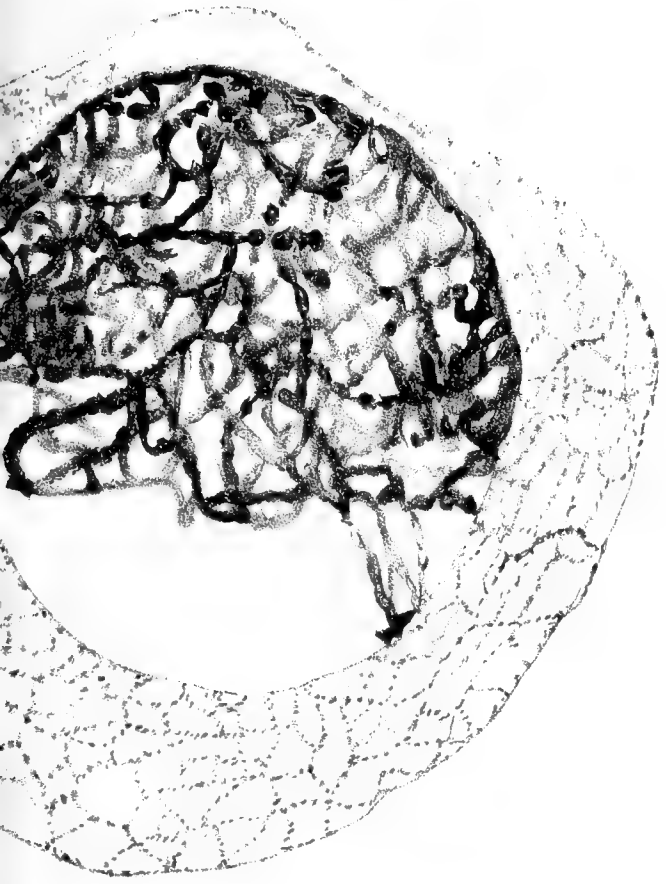


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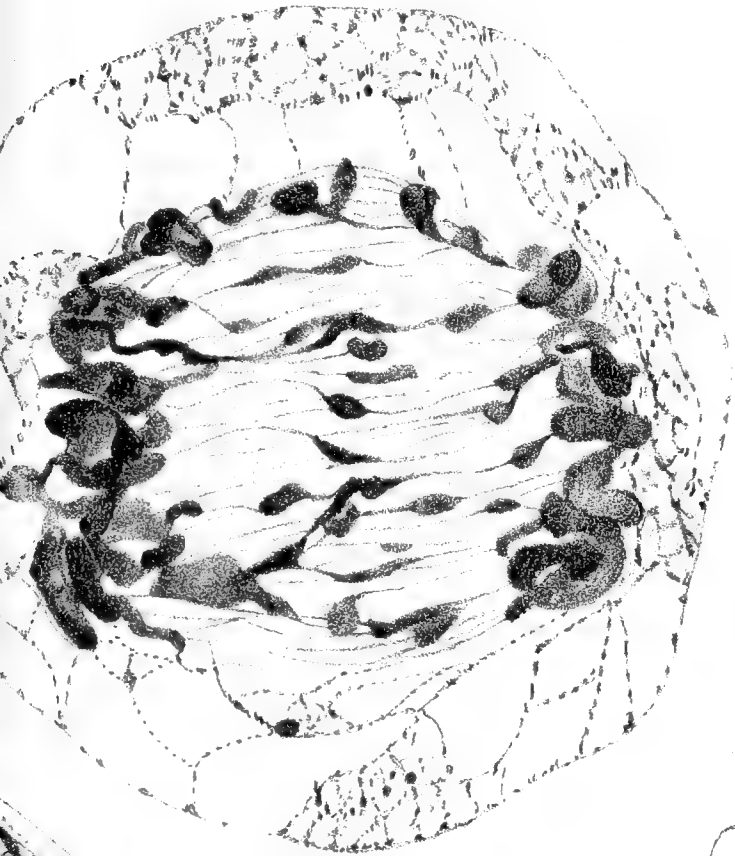


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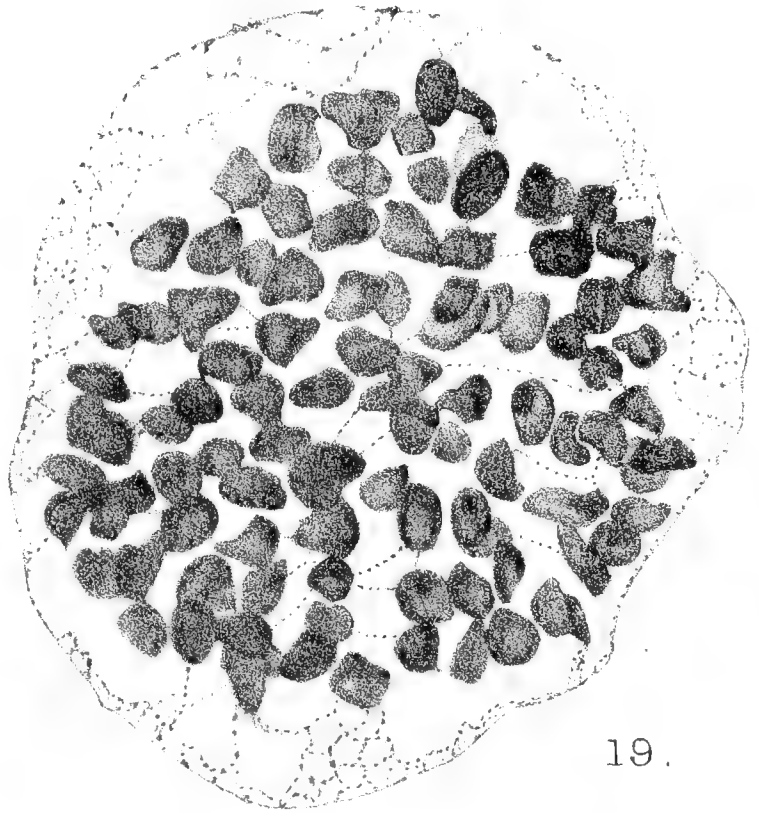




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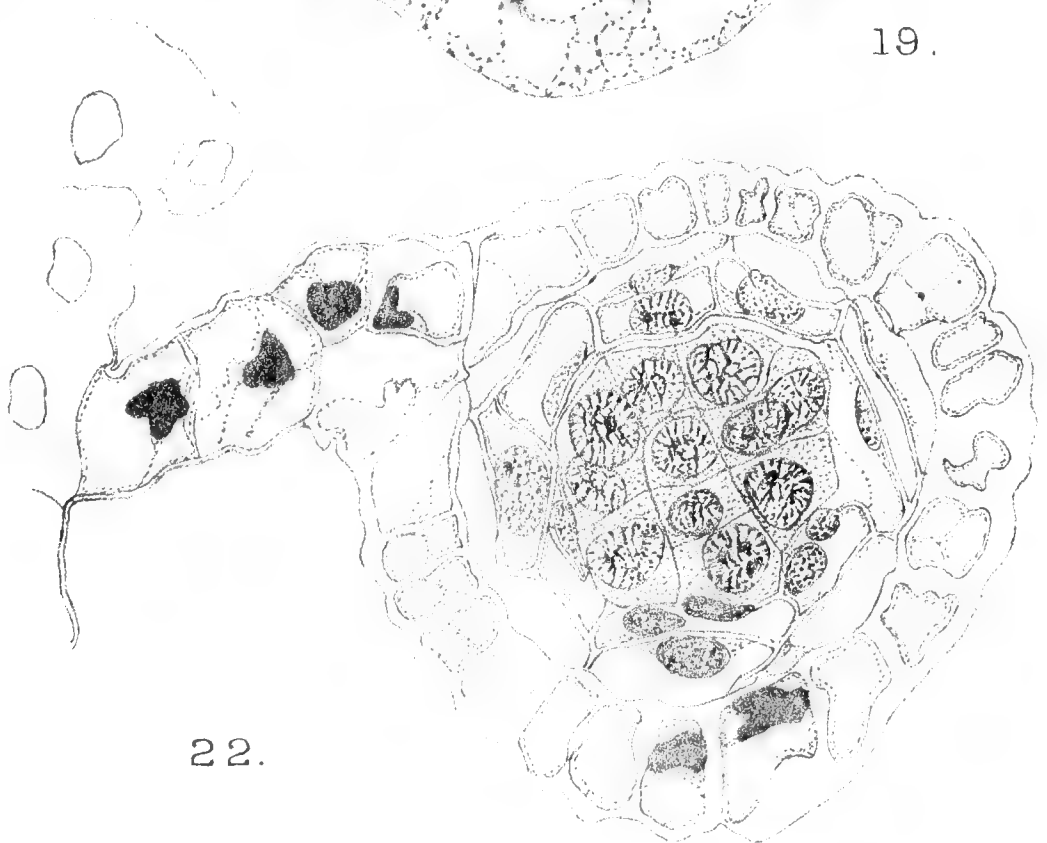
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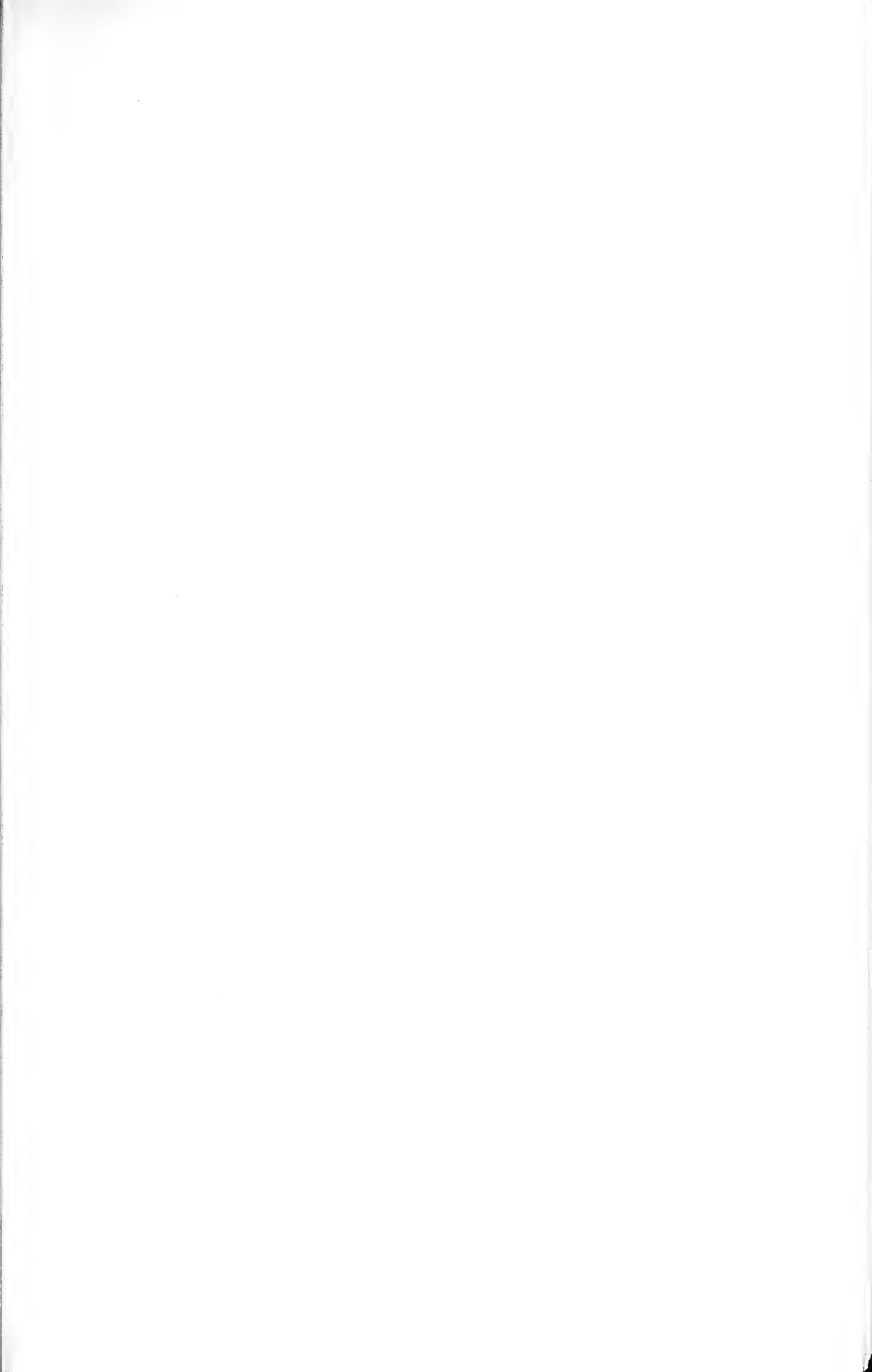


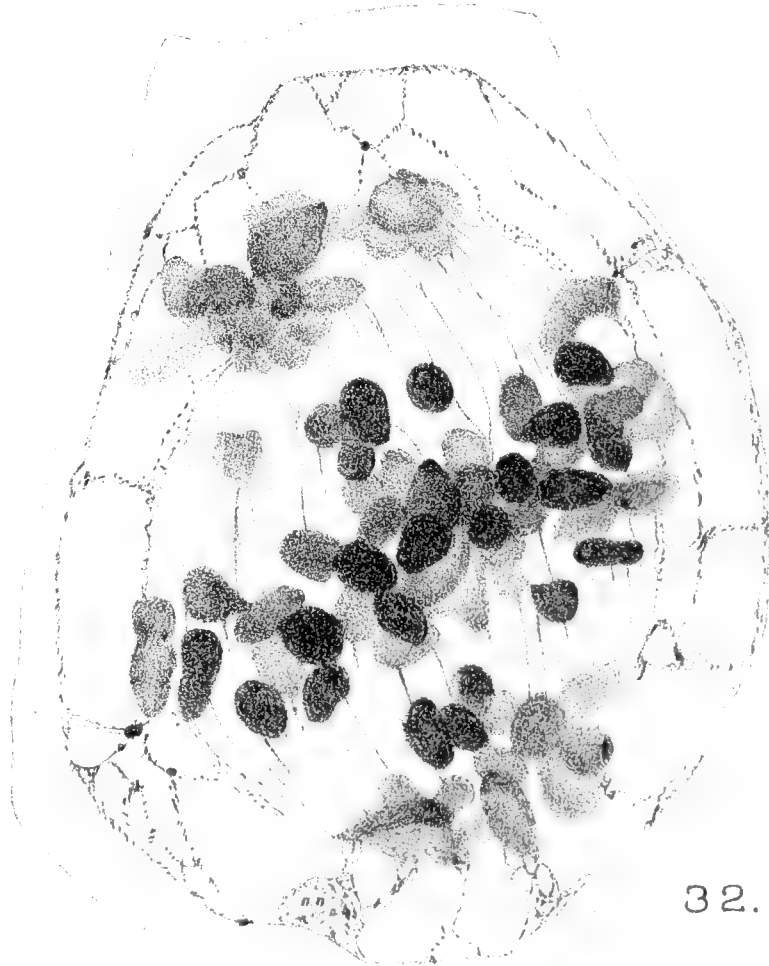
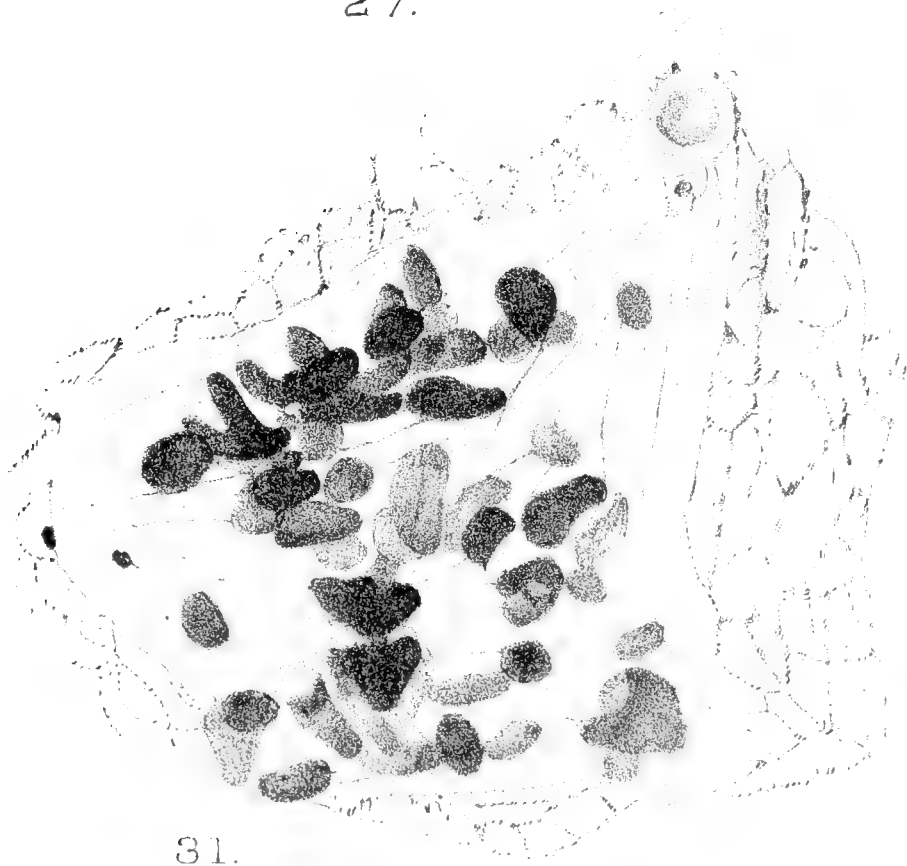
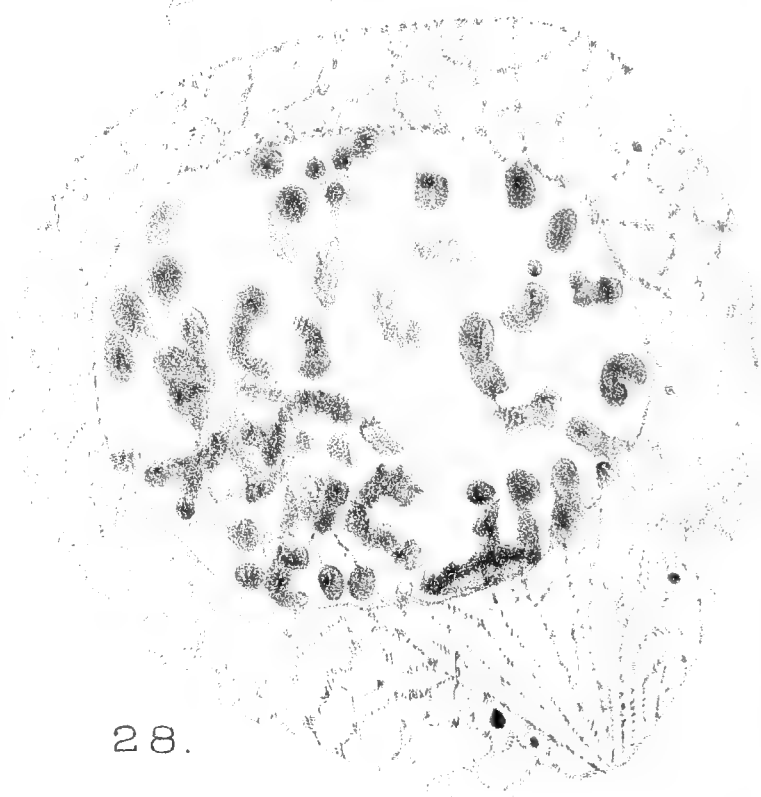
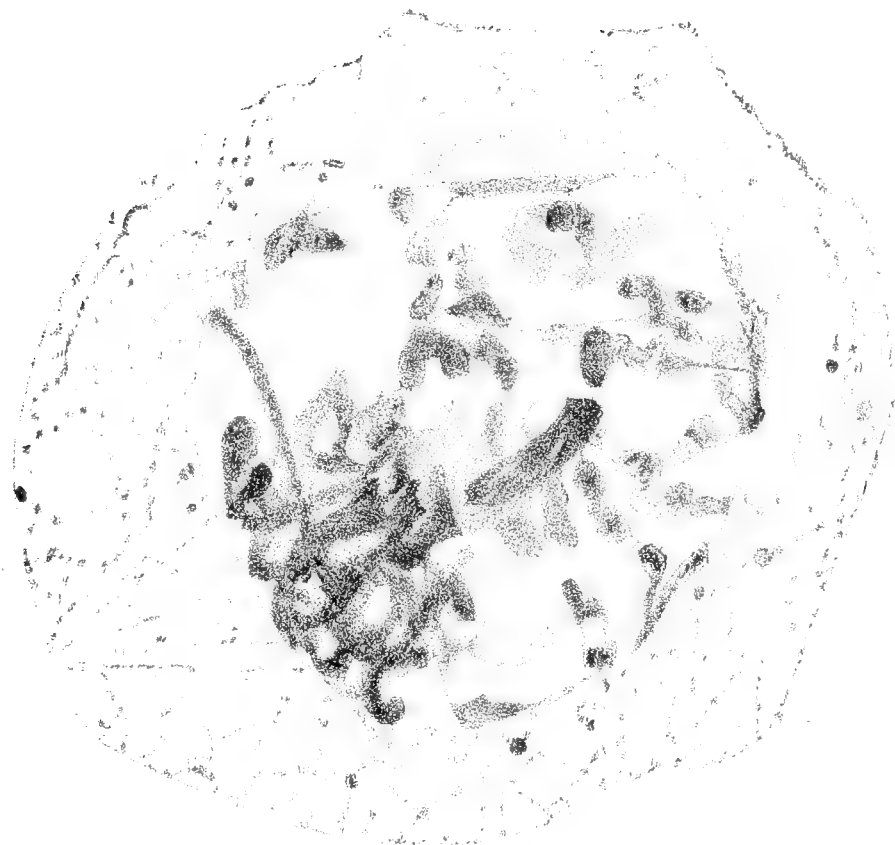
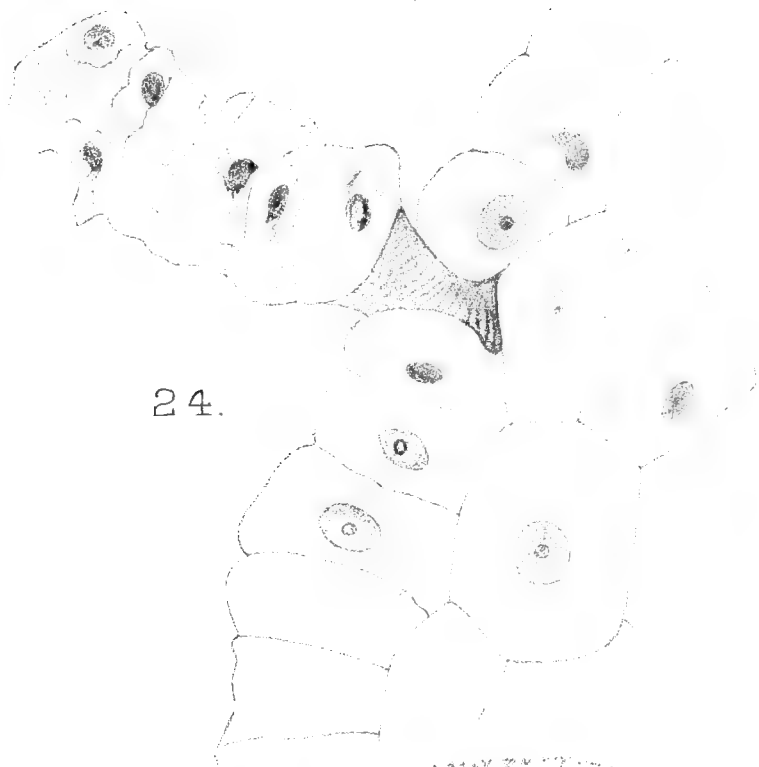
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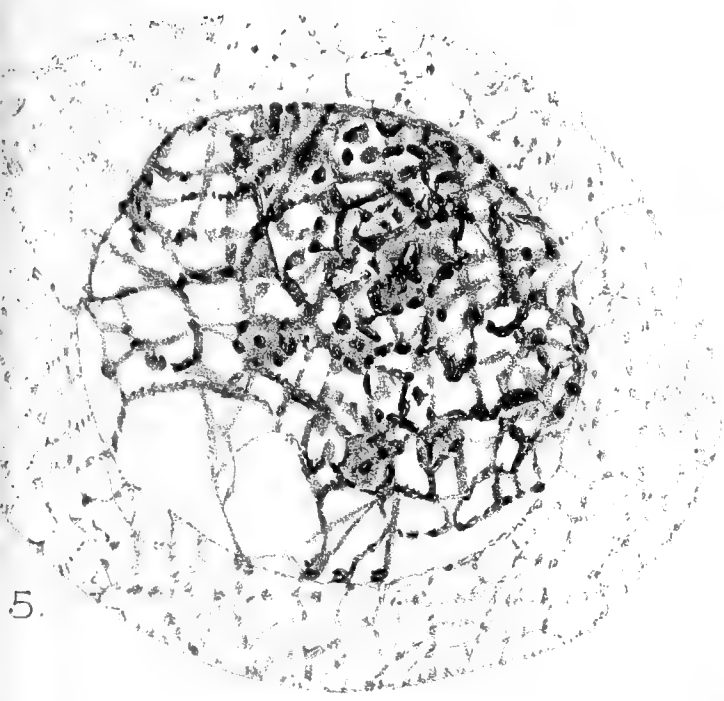
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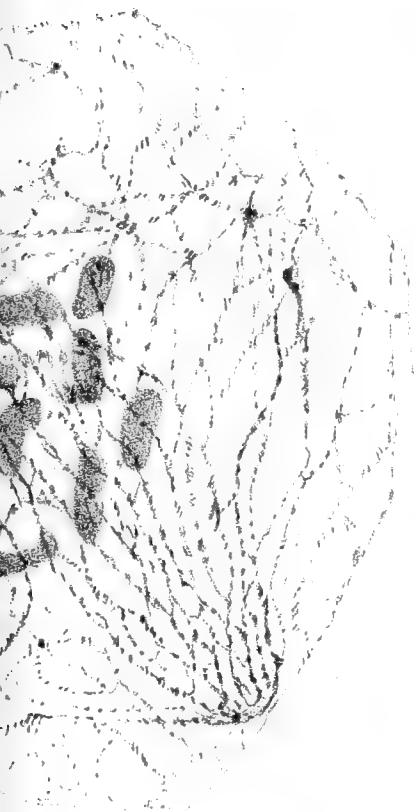




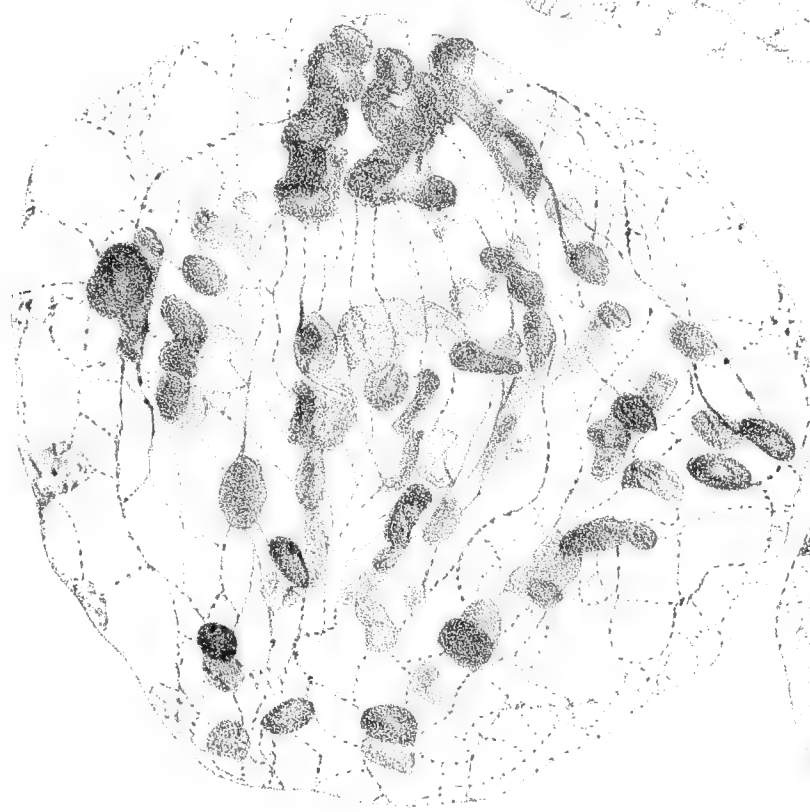
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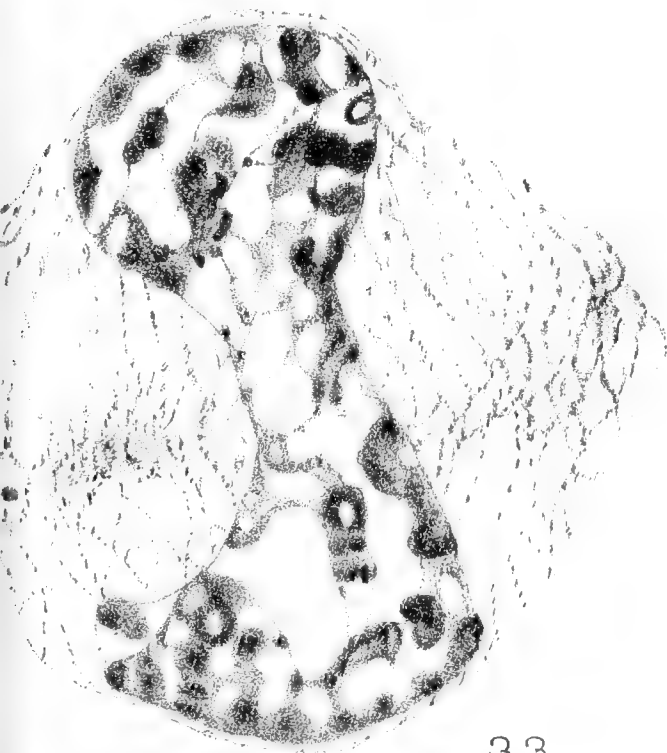
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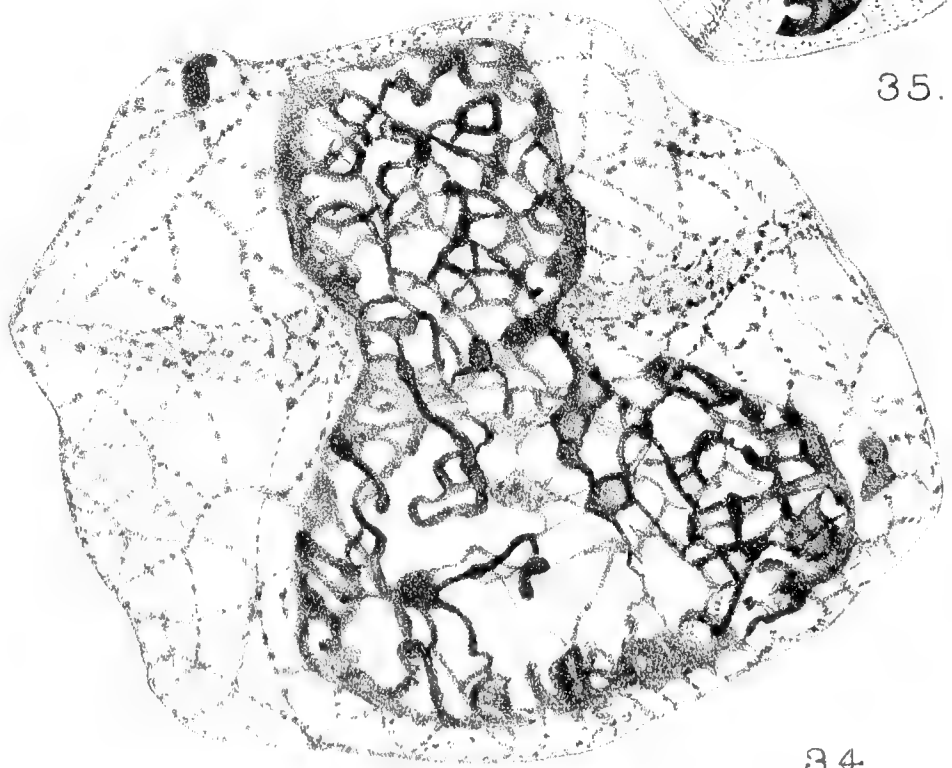
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# The Proteases of Plants (VII).

BY

S. H. VINES, F.R.S.

*Sherardian Professor of Botany in the University of Oxford.*

IN a previous paper upon this subject (1 ; 1905) I gave an account of experiments with malt, which led me to the conclusion that it contains two proteases, the one a peptase, peptonizing fibrin ; the other an ereptase, peptolyzing albumoses and peptones. I found further that the peptase was most active in an acid medium, whether of natural acidity, or when HCl had been added to the extent of 0.1% : the addition of HCl to 0.2%, or of Na<sub>2</sub>CO<sub>3</sub> to 1%, arrested digestion. The ereptase digested Witte-peptone most actively in an acid medium, up to HCl 0.2%, and somewhat less rapidly in an alkaline medium containing up to 4% Na<sub>2</sub>CO<sub>3</sub>. The reaction-range of the peptase was thus determined to extend from 0.2% HCl to 1% Na<sub>2</sub>CO<sub>3</sub>, whilst that of the ereptase was left undetermined, though the limit of alkalinity had been nearly reached at 4% Na<sub>2</sub>CO<sub>3</sub>.

It occurred to me subsequently that since malt has such well-marked proteolytic activity, it was highly probable that the preparations of diastase which are used for medicinal purposes would possess them also. The following is an account of some experiments made in pursuance of this idea.

## MALT-DIASTASE.

The first tentative experiment was made with an old specimen of diastase that had been in the laboratory for many years : the result was such as to justify further investigation.

2 grms. were mixed with 100 c.c. distilled water : the liquid was neutral, and gave no tryptophane-reaction. 40 c.c. of it were put into each of two bottles : to the one was added 0.2 gm. fibrin, to the other 0.2 gm. of Witte-peptone, with some HCN as an antiseptic. After 24 hours' digestion in the incubator at 40° C., the fibrin remained unaltered ; and the contents of the other bottle gave a faint tryptophane-reaction. 24 hours later, the fibrin still remained unaltered, but the tryptophane-reaction had become marked.

These results clearly indicate the presence of ereptase, and the probable absence of peptase.

I then obtained from Merck of Darmstadt some of his 'Maltine' and of his 'Absolute Diastase'.

#### *Experiments with Maltine.*

One gm. treated with 100 c.c. distilled water formed a somewhat viscid, neutral, liquid, which was filtered: the filtrate gave a slight turbidity on boiling and on adding  $\text{HNO}_3$ , also a distinct xanthoproteic reaction, faint biuret-reaction, but no trace of tryptophane-reaction. 40 c.c. of the filtered liquid were put into each of two bottles, to one of which 0.2 gm. fibrin, to the other 0.2 gm. Witte-peptone, were added, with a little HCN. After being in the incubator for nearly 48 hours, the contents of the bottle to which Witte-peptone had been added gave a faint tryptophane-reaction, indicating the presence of a small quantity of ereptase: the fibrin in the other bottle underwent no apparent change in 70 hours, and the liquid then gave a trace of biuret, but no tryptophane-reaction, showing that practically no fibrin-digestion had taken place.

I thought it worth while to repeat the experiment, using a stronger extract of maltine.

10 grms. were extracted for some hours with 100 c.c. water and then filtered: the filtrate was a clear, light-brown, distinctly acid liquid; on boiling a portion of it, a considerable precipitate of protein formed and was filtered off, the filtrate giving a distinct biuret but no tryptophane reaction. 40 c.c. of the filtered extract were put into each of two bottles, to one of which 0.2 gm. of fibrin was added, to the other nothing, in order that it might serve as an experiment in autolysis. Within 24 hours the fibrin had nearly disappeared: in 42 hours it had disappeared completely; the liquid in the bottle now gave marked tryptophane-reaction. The liquid in the other bottle gave a similar tryptophane-reaction at this time.

To test further the ereptic action of maltine, 0.2 gm. of Witte-peptone was digested with 40 c.c. of a 4% extract: within 24 hours the liquid gave a marked tryptophane-reaction.

Hence it appears that maltine contains proteases capable of digesting fibrin and Witte-peptone as well as its own proteins.

Subsequent experiments were made exclusively with absolute diastase, as it was found to digest much more actively than maltine.

#### *Experiments with Absolute Diastase.*

As the general method of these experiments was similar to that of those made with maltine, it will not be necessary to give full details in every case. It will suffice to say that distilled water was always used in making the extracts; that the antiseptic was HCN; that the temperature of the incubator was about 40° C.; and that several control-experiments were made with boiled extract, always with negative result.

EXPERIMENT 1. A 1% extract: it gave no precipitate on boiling nor on adding  $\text{HNO}_3$ : distinct xanthoproteic reaction and distinct biuret, but no tryptophane-reaction.

40 c.c. digested 0.2 gm. of fibrin in about 70 hours, the liquid then giving distinct biuret and marked tryptophane reactions.

40 c.c., to which 0.2 gm. Witte-peptone was added, gave marked tryptophane-reaction in 21 hours.

EXPERIMENT 2. A 5% extract. Some observations were made with the object of obtaining information as to the nature of the proteins present. A portion of the extract gave a dense precipitate on boiling: the filtrate still gave evidence of the presence of protein: it was saturated with ammonium sulphate and boiled, a dense precipitate being formed: the filtrate from this precipitate gave no further precipitate on boiling and no biuret-reaction. Hence it appears that absolute diastase contains an appreciable quantity of coagulable proteins as well as albumoses.

40 c.c. of this extract digested 0.1 gm. fibrin within 22 hours, the liquid giving strong tryptophane-reaction. An autolysis experiment, carried on simultaneously, gave evidence, by a marked tryptophane-reaction, of the digestion of the native proteins.

Having thus ascertained that watery extracts of absolute diastase can digest the native proteins, fibrin, and Witte-peptone, the investigation of the effect of variations in the reaction of the liquid upon the digestive processes was undertaken.

EXPERIMENT 3. A 5% extract used: it was distinctly acid, and gave a biuret-reaction, but no tryptophane.

In each of 4 bottles were put 35 c.c. of the filtered extract: to No. 1 nothing was added; to No. 2, 0.2 gm. of fibrin; to No. 3, 0.2 gm. of fibrin and  $\text{HCl}$  to 0.12%; to No. 4, 0.2 gm. of fibrin and  $\text{Na}_2\text{CO}_3$  to about 0.6%.

After 24 hours' digestion in the incubator the results were—No. 1, the liquid gave a slight biuret-reaction; No. 2, the fibrin had been partly digested, and the biuret-reaction was strong; No. 3, much the same as No. 2; No. 4, fibrin unaltered, biuret-reaction slight.

24 hours later, the fibrin had almost entirely disappeared in Nos. 2 and 3, and the biuret-reaction was strong; the fibrin in No. 4 had been slightly attacked, and the liquid gave a distinct biuret-reaction, as did also that in No. 1. The tryptophane-test was then applied to all the liquids: the reaction was marked in Nos. 1 and 2; strong in No. 3; distinct in No. 4.

These results show that an acid reaction of the liquid promotes both the peptonizing and the peptolyzing action of the proteases, whereas alkalinity retards both. They are confirmed, as regards peptolysis, by the following experiment with Witte-peptone:—

EXPERIMENT 4. 30 c.c. of a 5% filtered watery extract were put into each of 4 bottles: to No. 1 nothing was added; to No. 2, 0.2 gm. Witte-peptone; to

No. 3, 0.2 grm. Witte-peptone and HCl to 0.12 % ; to No. 4, 0.2 grm. Witte-peptone and  $\text{Na}_2\text{CO}_3$  to about 0.6 %.

After 24 hours' digestion in the incubator, the tryptophane-reactions were found to be—in No. 1, faint ; No. 2, marked ; No. 3, very strong ; No. 4, faint. 48 hours later, the only changes were that the reaction had become strong in No. 2 and distinct in No. 4.

The following are the results of an experiment without added protein, that is, of an autolysis-experiment :—

EXPERIMENT 5. A 5 % watery solution was prepared: the filtered liquid was slightly acid, and gave a good biuret-reaction, but no tryptophane-reaction.

40 c.c. were put into each of 3 bottles: to No. 1 nothing was added; to No. 2, HCl to 0.15 % ; to No. 3,  $\text{Na}_2\text{CO}_3$  to 0.625 %.

After 20 hours in the incubator, the tryptophane-reactions were—in No. 1, faint ; in No. 2, marked ; in No. 3, none: after 45 hours they were—in Nos. 1 and 2, strong ; in No. 3, none: at this time No. 3 still gave a distinct biuret-reaction, whilst Nos. 1 and 2 did not.

The results of all these experiments agree in proving that acidity promotes, whilst alkalinity retards or arrests, the digestive action of the proteases.

So far it has been assumed, on the strength of the previous experiments with malt, that there are two proteases, a peptase and an ereptase in the diastase-preparation. It remains now to adduce some evidence to justify this assumption.

With this object in view, attempts were made to isolate the proteases by means of alcohol ; three grms. of diastase were extracted, in different experiments, with 100 c.c. of 40, 50, and 60 % alcohol ; the alcoholic extracts were evaporated to half their bulk, to get rid of most of the alcohol, and then made up with distilled water to their original volume. Digestion-experiments showed that in no case had the extract any action on fibrin, but that it acted in some cases on Witte-peptone, as shown by the tryptophane-reactions.

40 % alcohol extract gave marked reaction in 24 hours, strong in 48 hours.

50 %     "     "     "     faint     "     "     and in 48 hours.

60 %     "     "     "     no reaction in 48 hours.

Hence it appears that the peptolytic enzyme (ereptase) can be extracted, without the peptonizing enzyme (peptase), by means of 40 % alcohol.

Having determined this point, I proceeded now to ascertain what effect extraction with alcohol had had upon the diastase. The residues after extraction with alcohol of 40, 50, and 60 % respectively, were extracted with distilled water, and the action of these watery extracts upon fibrin and

Witte-peptone was tested, with the following results:—(1) in no case did the watery extract made after previous extraction with alcohol have any digestive action on fibrin in 48 hours: (2) in all three cases Witte-peptone was digested more or less; thus the watery extract of the residue previously extracted with 40% alcohol gave distinct tryptophane-reaction after 48 hours in the incubator: those of the residues previously treated with 50% and with 60% alcohol both gave a strong reaction in the same time. Thus the more ereptase is extracted by alcohol, the less there is in the residue for subsequent extraction by water. The effect of treatment with alcohol upon the peptonizing activity of diastase is remarkable: it appears to be entirely destroyed. It should be added that the duration of the treatment with alcohol was 4 hours in the case of the 40% extract, rather longer in that of the 50% extract, and 24 hours in the case of the 60% extract.

The preparation of extracts which acted on Witte-peptone but not on fibrin is strong evidence of the presence of ereptase as an independent enzyme. The further question remains—is the fibrin-digesting enzyme peptase? This question can only be answered conclusively by preparing from diastase an extract which digests fibrin but is without action upon Witte-peptone. I have not yet succeeded in doing this, and cannot therefore give a definite answer at present, although, on analogy, it is probable that the enzyme in question is peptase.

#### TAKA-DIASTASE.

This substance is prepared commercially by Messrs. Parke, Davis and Co., to whom I am indebted for the material used in these experiments; and it is employed medicinally on account of its powerful amyloclastic action. They describe it as 'a pure ferment obtained by the cultivation of a fungus of the species *Eurotium Oryzae* upon wheat-bran'.

The mould, now usually called *Aspergillus Oryzae*, Cohn, is of special interest on account of its employment on a large scale in Japan for the preparation of the alcoholic drink known as *saké*, and of the sauce known as 'soy'. For the former purpose it is cultivated upon steamed rice with the object of converting the starch of the grain into sugar; from the mixture of rice and mould, known as *koji*, a wort is prepared which is fermented with yeast. For the latter purpose the mould is cultivated upon a mixture of boiled Soja-beans (*Glycine Soja*, Sieb. et Zucc.) and roasted wheat, forming a *koji*. When the conversion of starch into sugar has proceeded far enough, a wort is prepared in which various micro-organisms induce both alcoholic and lactic acid fermentations (see Lafar, 3, and Saito, 4).

My surmise that Taka-diastrase includes proteases as well as diastase has proved to be well founded; in fact it may be said to act as vigorously upon proteins as it does upon starch. This conclusion was communicated to Messrs. Parke, Davis and Co. a year ago.



EXPERIMENT 1. A 1.5% solution in water forms an opalescent liquid which is distinctly acid, gives no precipitate on boiling or on adding  $\text{HNO}_3$ , and no biuret nor tryptophane reaction; but its xanthoproteic reaction is distinct. Only a very small quantity, therefore, of protein is present.

30 c.c. of the solution were put into each of two bottles: to the one was added 0.2 gm. fibrin, to the other 0.2 gm. Witte-peptone; to each a few drops of HCN.

After 24 hours in the incubator at  $37^\circ\text{C}$ ., the fibrin had disappeared, and the liquid gave a marked tryptophane-reaction: the liquid in the other bottle gave a very strong tryptophane-reaction; the liquid in both bottles was now strongly acid.

These results indicate great proteolytic activity, both peptonizing and peptolyzing. The next experiment was made to ascertain in what way digestion was affected by the reaction of the medium.

EXPERIMENT 2. 40 c.c. of 1.5% solution were put into each of 5 bottles: to each bottle were added 0.2 gm. of fibrin and 0.2 gm. of Witte-peptone, and some HCN. To No. 1 nothing further was added; to No. 2, HCl to 0.1%; to No. 3, HCl to 0.2%; to No. 4, citric acid to 0.5%; to No. 5,  $\text{Na}_2\text{CO}_3$  to 0.5%.

After 19 hours in the incubator, the fibrin had disappeared in all the bottles; the tryptophane-reactions were—in Nos. 1 and 5, marked; in Nos. 2, 3, 4, strong.

Hence it appears that the amounts of acid and of alkali added had not materially affected fibrin-digestion, whereas the added acid had distinctly promoted the digestion of Witte-peptone.

In view of the activity of the proteases, a more dilute solution was used in the next experiment (1%), and the process of digestion was closely watched.

EXPERIMENT 3. 40 c.c. of a 1% solution were put into each of 2 bottles: to each were added 0.2 gm. of fibrin and 0.2 gm. of Witte-peptone, and some HCN; to the one was added HCl to 0.2%, to the other  $\text{Na}_2\text{CO}_3$  to 1%.

The fibrin disappeared in the acid bottle in 3 hours, in the alkaline bottle in  $4\frac{1}{2}$  hours; both liquids then gave marked tryptophane-reaction.

EXPERIMENT 4. This experiment was similar to the foregoing, except that a 0.5% solution was used.

The fibrin disappeared in  $2\frac{1}{2}$  hours in the acid bottle, and in  $3\frac{1}{4}$  hours in the alkaline bottle: the tryptophane-reaction was distinct in the former, faint in the latter: 19 hours later, the tryptophane-reaction was strong in the acid bottle, distinct in the alkaline.

These experiments show that both fibrin-digestion and peptolysis are more rapid in an acid than in an alkaline medium; and further, that peptolysis proceeds more slowly than fibrin-digestion.

I may quote yet one more experiment illustrating the relation between peptolysis and the reaction of the liquid.

EXPERIMENT 5. Witte-peptone was dissolved to the extent of 0.5% in a 0.5% solution of the diastase: the slightly acid liquid, which filtered rather slowly, gave a trace of tryptophane-reaction, and, of course, a good biuret-reaction. 40 c.c. were put into each of 3 bottles: to No. 1 no acid or alkali was added; to No. 2, HCl to 0.2%; to No. 3,  $\text{Na}_2\text{CO}_3$  to 1.25%.

After 4 hours in the incubator, the tryptophane-reaction in Nos. 1 and 3 was as at the beginning; in No. 2 it had become marked. After 24 hours' digestion the reactions were—strong in No. 1; very strong in No. 2; in No. 3 but little stronger than originally. The biuret-reaction was scarcely perceptible in Nos. 1 and 2, but was undiminished in No. 3. Similar results were obtained after 48 and 72 hours' digestion. It was noticed that the acid reaction of No. 1 increased perceptibly during the experiment.

It is thus shown conclusively that Taka-diastase contains active proteases which are especially adapted to an acid medium. The reaction-ranges for protein-digestion were not determined, but they appear to be wide: with a 0.5% solution of the substance, fibrin-digestion took place readily between 0.4% HCl and 1%  $\text{Na}_2\text{CO}_3$ ; and peptolysis within the same limits, though it was diminished by 1%  $\text{Na}_2\text{CO}_3$  and arrested (Expt. 5) by 1.25%  $\text{Na}_2\text{CO}_3$ .

Some experiments were made with crude extract of the fungus, kindly placed at my disposal by Messrs. Parke, Davis and Co.; the results did not differ materially from those of the experiments here described, which were with the substance as prepared for sale, except that the digestive action was more rapid.

The question as to whether the digestive action of Taka-diastase is due to one or to two proteases had next to be considered: here again the alcohol method was employed, with the result that it was found possible to obtain, by means of extraction with alcohol, a liquid which digested Witte-peptone but had little or no action on fibrin.

EXPERIMENT 6. 1 gm. of crude Taka-diastase was treated for a couple of hours with 100 c.c. of 50% alcohol, and then filtered; filtration was slow. The filtrate was evaporated to half its bulk at about 37° C., to get rid of some of the alcohol, and then diluted with distilled water to 105 c.c. 35 c.c. of the liquid were put into each of 3 bottles: to No. 1, 0.2 gm. of fibrin was added; to No. 2, Witte-peptone to 0.5%; to No. 3, Witte-peptone to 0.5% and HCl to about 0.06%. The contents of the bottles gave a trace of tryptophane-reaction.

After 24 hours in the incubator, the fibrin in No. 1 remained unaltered; the contents of No. 2 gave marked tryptophane-reaction, and those of No. 3 a distinct reaction. 24 hours later, the fibrin still remained unaltered in No. 1, and the tryptophane-reaction had become strong in No. 2 and marked in No. 3. 24 hours later, the fibrin in No. 1 was found to have been partly digested, and the liquid gave

distinct tryptophane-reaction; the reaction given by Nos. 2 and 3 had increased in intensity.

In the meantime the residue of the diastase on the filter had been again extracted with 80 c.c. of 25% alcohol. 20 c.c. of this filtrate were diluted with an equal volume of water and were put into a bottle with 0.2 gm. Witte-peptone; the remaining 60 c.c. were evaporated (at 37° C.) to 40 c.c., and then water was added to 80 c.c., half of it being put into each of 2 bottles, to one of which (No. 1) 0.1 gm. fibrin was added, to the other (No. 2) 0.2 gm. of Witte-peptone.

After 24 hours in the incubator, the diluted alcoholic extract gave a distinct tryptophane-reaction; the fibrin in No. 1 was mostly digested; and the contents of No. 2 gave a marked tryptophane-reaction. 24 hours later, the tryptophane-reaction of the diluted alcoholic extract was still distinct; the fibrin had entirely disappeared in No. 1, and the liquid gave faint tryptophane-reaction; No. 2 gave a distinct reaction.

The residue on the filter, which had been already twice extracted with alcohol, was further extracted with 80 c.c. of water: it did not entirely dissolve. 40 c.c. of the watery extract were put into each of 2 bottles: to No. 1 was added 0.1 gm. fibrin; to No. 2, 0.2 gm. Witte-peptone. After 24 hours in the incubator, the fibrin in No. 1 had almost disappeared; the contents of No. 2 gave a distinct tryptophane-reaction. 24 hours later, the fibrin had completely disappeared in No. 1, and the liquid gave a faint tryptophane-reaction; the tryptophane-reaction of No. 2 had become rather stronger.

This experiment shows that it is possible, by means of rapid extraction with 50% alcohol, to obtain a liquid which has well-marked peptolytic action but digests fibrin very slowly: that it is possible, in fact, to obtain a solution containing ereptase almost alone. This result was confirmed by other experiments in which the conditions were somewhat different. In one case the diastase was treated with 50% alcohol for 48 hours before filtration: the resulting liquid, prepared by evaporation as in the experiment just described, digested Witte-peptone, as shown by a faint tryptophane-reaction in 24 hours which increased daily in intensity at natural acidity, whereas it failed to digest fibrin at all, even though the experiment was prolonged for six days. A similar result was obtained when stronger alcohol (63%) was used for extraction. In both these cases the action upon Witte-peptone was much retarded, in consequence of the treatment of the substance with alcohol.

Since it was thus possible to prepare an alcoholic extract of ereptase, and since, as the last-mentioned experiment shows, the residue of Taka-diastase (unlike Malt-diastase) yielded extracts, made either with more dilute alcohol or with water, which rapidly digested fibrin and had but little action upon Witte-peptone, there was ground for the assumption that the whole of the ereptase might be extracted from a small quantity of Taka-diastase, leaving behind a residue which, on extraction with water, would yield a solution which would digest fibrin but would not act upon

Witte-peptone: a solution, in fact, containing only peptase. With this object in view, the following experiment was made:—

EXPERIMENT 7. 1 gm. of Taka-diastrase was mixed with 100 c.c. of 50% alcohol and immediately filtered: the residue on the filter was then treated with 60 c.c. 50% alcohol, and when that had filtered off, with 50 c.c. more of the same alcohol. The residue was then treated with 80 c.c. distilled water. 40 c.c. of the watery filtrate were put into each of 2 bottles: to the one was added 0.2 gm. fibrin, to the other 0.2 gm. Witte-peptone: the liquid containing Witte-peptone gave no tryptophane-reaction.

After 24 hours in the incubator, the fibrin had disappeared in the one bottle, and the contents of the other gave no tryptophane-reaction; 24 hours later, the contents of the latter bottle gave a trace of tryptophane-reaction, which did not increase during 48 hours.

I succeeded, therefore, in the attempt to remove the ereptase, and to obtain from the residue a solution which contained only peptase. This result, together with that of Experiment 6, proves that Taka-diastrase contains two distinct proteases, a peptase and an ereptase.

#### SUMMARY.

The foregoing experiments lead to the following conclusions:—

1. Malt-diastrase (whether maltine or absolute) and Taka-diastrase both contain proteases which digest fibrin and produce tryptophane from albumoses and peptones whether produced by digestion or added as Witte-peptone.

2. It is possible to extract from both of these substances, by means of alcohol of various strengths, a protease which digests Witte-peptone but has no action on fibrin: this protease is therefore ereptase.

3. On washing out all the ereptase from Taka-diastrase, the residue yields, on extraction with water, a solution which digests fibrin but has no action on Witte-peptone: this solution therefore contains peptase. Such a solution has not yet been obtained from Malt-diastrase.

4. Since both Taka-diastrase and Malt-diastrase digest fibrin more rapidly in acid than in alkaline medium; and since the enzymes are excreted, in the case of Taka-diastrase by the *Aspergillus Oryzae* into the organic matter upon which it is cultivated, in the case of Malt by the Barley-embryo into the endosperm; it would seem natural to refer the peptase to the group of proteases which, in my last paper on this subject (2; 1909), I termed *Ectopeptase*.

But in the course of some quite recent and still incomplete experiments I have found that solutions of the peptase of Taka-diastrase, freed from ereptase (and doubtless from other substances as well) by the alcohol method (see Experiment 7, above), digest fibrin more actively when the

liquid is neutral or alkaline (e.g. 0.6 %  $\text{Na}_2\text{CO}_3$ ) than when it is acid (e.g. 0.06 % HCl): that is to say, the purified peptase behaves in such a way as to suggest that it is rather an *endopeptase* than an *ectopeptase*. The question as to its nature must therefore be left open for the present.

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## Morphological Notes.

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### XII. GERMINATION OF THE DOUBLE COCO-NUT.

With Plates XIX and XX, and a Figure in the Text.

THE palm (*Lodoicea sechellarum*, Labill.) is remarkable in the vegetable kingdom for producing the largest seed of any known plant. Its native country was long unknown. But the seeds which were thrown up on the shores of the Indian Ocean, most frequently on the Maldivé Islands, though occasionally on Ceylon and S. India, and as far as Zanzibar in one direction and Sumatra in the other, were held in great repute by the Eastern peoples for their supposed medicinal virtues. Though there is every reason to suppose that this repute was very ancient, it did not reach Europe till the sixteenth century. The general belief was that they were the produce of some marine plant, and this was accepted by even so capable a botanist as Rumphius as late as 1680. He says: 'non est fructus terrestris qui casu in mare procidit . . . sed fructus est in ipso crescens mari' (xii. 8). Although the seeds were capable of a wide oceanic dispersal which must have continued over a long period of time, I am aware of no case of their having spontaneously established themselves in any new territory. This is in striking contrast with the ordinary coconut, which, probably originally a native of S. America, is now widely distributed throughout the tropics.

It was not till the middle of the eighteenth century that the mystery was cleared up. I take the account given by Sonnerat (*Voyage à la Nouvelle-Guinée*, pp. 3, 4): 'Parmi les Isles de cet archipel [Séchéelles], il y en a une que M. de la Bourdonnais désigna sous le nom de l'*Isle des Palmes*, lorsqu'il en fit la découverte en 1743 ou 1744. Cette Isle, examinée de plus près en 1767, a été nommée l'*Isle Praslin*, nom que l'usage, qui prévaut en tout, a changé depuis en cela d'*Isle des Palmiers*. C'est sur cette Isle qu'on trouva le palmier qui produit ce fruit si recherché, qu'on n'avoit connu jusqu'alors que sous les noms de coco de Mer. . . .' Sonnerat visited the island in 1771, and in his 'Voyage' published in 1776 he gives five plates which represent the



superficial characters of the palm with tolerable accuracy. He adds in a note that the description was communicated to the Académie des Sciences in 1773. Since Sonnerat's time *Lodoicea* has been known to also grow in the neighbouring islands, Curieuse and Round.

Gmelin in the thirteenth edition of the *Systema* (p. 569), 1791, named it *Cocos maldivica*. But Giseke (*Prael.* 1792, p. 88) correctly divined its affinities, naming it *Borassus Sonnerati*. Labillardière created for it the genus *Lodoicea*. But this, though maintained, is only distinguished technically from *Borassus* by the numerous stamens and large fruit.

It is not a little remarkable that our detailed knowledge of the morphology of a plant with so singular a history and such striking characteristics should still be very imperfect. But that this is the case is evident from the description given by Bentham and Hooker in the *Genera Plantarum*, iii. 939. I had hoped to do something to remedy this, and had collected some material for the purpose. The necessary leisure to accomplish the task has, however, always been wanting, and I must content myself with some detached notes which may be useful to some one more fortunate.

As long as the Coco-de-mer was only known from sea-borne specimens it was of course assumed that the Double coco-nut, as it was called, was the entire fruit. As soon as the palm producing it was discovered, it was at once obvious that this was not the case. The Coco-de-mer is in fact the stone of a gigantic drupe with a fibrous mesocarp. The complete fruit is rarely to be seen in Museums; but Kew possesses one, as well as a plaster model which the late General Gordon had made in the Seychelles and presented to it. The fruit is poorly figured by Sonnerat, but the best representations are in the fine series of pictures (Nos. 474-7 and 479) in the North Gallery at Kew, which Miss North visited the Seychelles in 1883 for the purpose of painting. According to Sir William Hooker, it is 'often a foot and a half in length, weighing twenty or twenty-five pounds'.

Sonnerat figures the drupe as ellipsoidal. This, if it ever occurs, except in the youngest stage, must be exceptional. The *Genera Plantarum*, no doubt correctly, describes it as 'oblique obovoideus'. Miss North, quoted by Sir Henry Yule (*Hobson-Jobson*, p. 178), says: 'the outer husk is shaped like a mango.' It is clearly therefore usually unsymmetrical; one side is somewhat flattened, and the other rounded. This arises from the fact that in the maturing ovary one carpel only usually develops.

One point which seems to require further investigation is the number of primary component carpels, or at any rate of cells in the ovary. The *Genera Plantarum* says it is '3- rarius 2-4- locale'. The whole symmetry of the flower is ternary, with three stigmas in the female. This would imply three component carpels, and therefore a three-celled ovary. It is possible, though the point requires further investigation, that the discrepancy has been produced by the misinterpretation of sections containing

the bilobed seed. Sir William Hooker figures in the *Botanical Magazine* (tab. 2737, fig. 1) a transverse section of an ovary which clearly points to a normal tricarpellary structure.

The stone, or nut as it is sometimes called, is, as is well known, deeply bilobed. As shown in Plate XIX the outline of the putamen in a longitudinal section is roughly that of an exaggerated dumb-bell. The cavities of the two lobes communicate in the middle. The upper parts of the lobes are separated by an open sinus, but the lower are more or less united, and if a cross section be made at this point, the nut would show, as in one of Sonnerat's figures, a bilocular structure.

Another point which has not been ascertained is the number of ovules in each ovarian cell. Analogy and such evidence as is available suggest there being only one. The 'nut' therefore, if developed from one carpel, would be only one-seeded. W. B. Hemsley, in the *Catalogue to the North Gallery* (p. 74), refers to 'the two-lobed nut, which usually contains only one seed'. But I am not aware of any proof of its ever containing more. It seems probable that the bilobed form of the nut has suggested that it might consist of two coalescing carpels, but there is no evidence for this.

*Juglans* affords a familiar instance of ingrowths from the pericarp into the seed cavity. The purpose of such spurious dissepiments, especially when they intrude on the developing seed and modify its form, is difficult to account for. The separation of the cavities at the base of the seed of *Lodoicea* is apparently due to such an ingrowth. But this can only be ascertained by following the development. The free lobes themselves are only lateral inflations in order to provide space for the enormous endosperm. They are much more distended on the dorsal than on the ventral surface, which is somewhat flattened; this produces the corresponding difference in the two surfaces of the fruit which has already been mentioned. In this case, which seems the most usual, the fruit contains only one nut and one seed. The *Genera Plantarum* describes the fruit as '1- v. imperfecte 2-3-locularis'. The latter condition can be only due to the more or less complete development of one or both of the other carpels.

The endosperm is voluminous. According to the *Genera Plantarum* it is hollow, 'late cavo.' My recollection of a specimen examined at Kew, though unfortunately I made no note at the time, is that it was solid. The account given by Sir William Hooker seems to confirm this. He says: 'The cavity is filled by the *almond*, which is very hard, white, and corneous, so that it may be rasped with a file, but is with difficulty cut with a knife.' I can only conjecture that this must have been described from an old and desiccated nut. A fresh one which afforded Dr. Walter Gardiner material for a study of the histology of the endosperm must have been immature, for sections were easily cut with a razor, and the consistence was not much harder than that of a turnip.

Apparently in an earlier stage of development the endosperm is unconsolidated and gelatinous. Sir William Hooker says: 'Before the fruit has attained its perfect maturity, the interior . . . contains a substance like a white jelly, firm, transparent and sweet to the taste. A single Coconut holds, perhaps, three pints of this substance; but if kept a few days, it turns sour, thick and unpalatable, giving out a very disagreeable smell.' Miss North gives a more graphic description: 'The outer shell was green and heart-shaped; only the inner shell was double, and full of white jelly, enough to fill the largest soup tureen.'<sup>1</sup> And elsewhere, as quoted by Sir Henry Yule: 'I ate some of the jelly from inside . . . of the purest white and not bad.'<sup>2</sup> The late General Gordon, who, as is well known, was deeply interested in the palm, on somewhat mystical grounds, informed me in a note: 'The nut when ripe is black and falls from the tree; the gelatinous jelly is then hard like ivory.' It would be extremely interesting to trace the histological changes which accompany that of texture.

Widely different statements have been made as to the time which the fruit takes to reach maturity. According to Sir William Hooker: 'Twelve months elapse, from the time of the appearance of the *germen*, before the fruits are fully ripe; and they have been known to hang three years on the tree before falling on the ground.' Mr. C. Button, who was at the time Conservator of Crown Lands and Forests, informed me in a letter that the period was much longer: 'It remains seven years before arriving to its perfect maturity and falls to the ground. This experience has been several times made by me personally; but the proprietors of Coco-de-mer trees generally break the fruit at about four years of age for commercial purposes, as the shell at that time is sufficiently hard.' General Gordon also informed me that the 'fruit attains maturity in seven years'. It looks as if the discrepancy had arisen from a confusion between the time at which the fruits are gathered and that at which they are really mature.

Frequent attempts have been made to cultivate the Coco-de-mer in European botanic gardens, but with little success. For some years a plant, which I think was imported, existed in the Liverpool Botanic Garden. And a young plant was raised and perhaps still exists in the Jardin des Plantes. In 1889 I began a prolonged attempt to add it to the rich collection of palms at Kew. I was energetically assisted by Mr. C. Button, who sent us repeated consignments of mature nuts. Many failed to germinate at all: others did so, but only imperfectly; others again sprouted satisfactorily, but only to end their existence by disaster almost suicidal.

According to the *Genera Plantarum*, *Lodoicea* has the 'embryo basilaris, sinum spectans'. But unless I am mistaken the sinus is the apex of the nut, and the embryo is therefore apical. In any case the sinus being open affords the embryo a free path for emergence.

<sup>1</sup> Recollections of a happy life, ii. 289.

<sup>2</sup> Hobson-Jobson, 178.

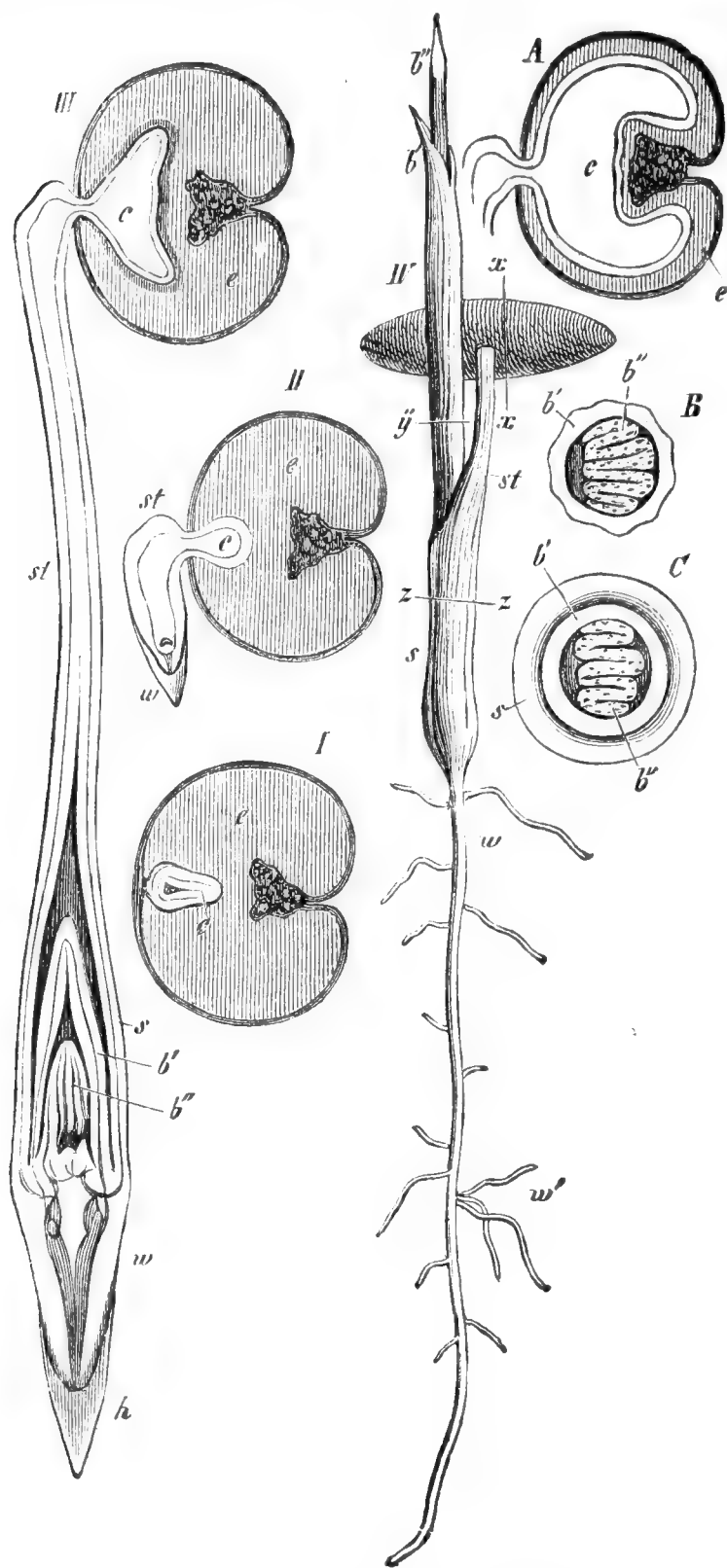
Everything connected with *Lodoicea* is on a gigantic scale, and the germination is no exception. According to Sir William Hooker: 'From the period of its falling from the tree, a year elapses before the nut begins to germinate.' Mr. Button, however, put the period at 'four or five months before germinating and sometimes less'. The germination morphologically is of an ordinary monocotyledonous type. The apex of the cotyledon remains immersed in the endosperm and develops into a vast suctorial organ, while its petiole, which is about an inch in diameter, emerges from the nut carrying with it the plumule and 'radicle'. The petiole, which acts as a sort of umbilical cord, according to Mr. Button, 'enters the ground to the depth of about one or two feet, then continues underground nearly parallel to the surface for a distance of four, five, six feet, sometimes more.' A note of General Gordon's is that it 'comes to sprout out of the ground twelve feet from nut'. Mr. Button subsequently informed me that it 'runs in the ground, sometimes to a distance of several yards before coming to the surface'.

The difficulties of successful germination under artificial conditions will now be apparent. At Kew the nuts were buried in coco-nut fibre in a hotbed. Germination, once commenced, proceeded rapidly. Unfortunately in at least one instance it proved abortive. Before the proceeding could be detected the growing apex managed to insinuate itself in some crevice, with the result that it was irretrievably injured. In a subsequent attempt the petiole did not grow to so great a length, and it was possible to guide its course and finally establish the young plant in a pot. This grew pretty rapidly, and in 1892 was exhibited in the Victoria Regia House at Kew, still drawing nutriment from the parent seed, a process which may apparently continue for some years.

The cultural failures were at any rate available for anatomical purposes. One of them supplied the material for the accompanying plates. These represent a structure which, as far as size is concerned, is amongst the most remarkable, if not the most so, in the vegetable kingdom. They are somewhat smaller than life-size. Plate XIX represents a longitudinal section through a germinating nut. The actual specimen (which may be seen with its companion in Museum No. II at Kew) is two and a half inches wider than in the figure, and the other dimensions are of course diminished in proportion. It shows a section of the foot-like suctorial apex of the cotyledon. This has developed at the expense of the endosperm, which it has completely broken down and absorbed. Nothing remains of it but mere exhausted flakes which adhere to the internal walls of the nut. The petiole of the cotyledon will be noticed passing outwards through the sinus. Plate XX represents the external surface of the other half of the suctorial structure. The position is reversed as compared with Plate XIX, and the attachment of the petiole is below. It again is reduced and the



actual specimen is an inch and a half wider. It is impossible not to be struck with the general resemblance of the whole preparation to that of



Germination of *Phoenix dactylifera*. I, transverse section of the dormant seed; III—IV, different stages of germination (IV the natural size); A transverse section of the seed at *xx* in IV; B transverse section at *xy*, C at *zz*; *e* the horny endosperm, *s* the sheath of the cotyledon, *st* its stalk, *c* its apex developed into an organ of absorption which gradually consumes the endosperm and at length occupies its place, *w* the primary root, *w'* secondary roots, *b'* *bb''* the leaves which succeed the cotyledon, *b''* becomes the first foliage-leaf: in B and C its folded lamina is seen cut across.

brought about by an extension of the cotyledon, which very gradually encroaches upon the endosperm. The surface of this advancing portion of the cotyledon is covered with a well-marked epithelium, which, according to Sachs, probably emits some special substance which provokes the absorption

of a human brain with the hemispheres drawn apart. The general form, the bilateral symmetry, the lobing and convolutions might deceive the anthropologist. It would be interesting to trace the gradual stages by which such a structure has been reached. There must have been a wild proliferation of botryoidal growths pressing on and compressed by their prey as it succumbed to absorption. One can only find a parallel to the process in the ravages produced by a tumour in an animal economy. It is obvious that at an earlier stage it must have completely filled the cavity of the nut previously occupied by the endosperm which it has replaced. As it has been in turn drained by the growing plant it has shrunk, and this has probably produced the lobulation of the surface. Except in scale the whole process in no way differs from that which takes place in *Phoenix* as admirably described by Sachs in 1862. This is at once apparent on comparing the figure reproduced from his *Lectures on the Physiology of Plants* (p. 344). As I have not the opportunity of consulting the original paper I borrow the following account from Brown and Morris:—

‘The absorption of the reserve-cellulose during germination is

of the cellulose, but he does not appear to have made any direct experiments in support of this view. . . . The epithelial cells increase in number, and are gradually pushed forward as the reserve-cellulose of the endosperm is absorbed.'<sup>1</sup>

Green in 1887, and Brown and Morris in 1890, were unsuccessful in extracting the enzyme of the Date, which the latter suggested might be used up as fast as formed.<sup>2</sup> It was, however, accomplished by Newcombe in 1899. Brown and Morris, however, extracted a cyto-hydrolytic enzyme from the germinating seeds of the Grasses. But this had no action on the reserve-cellulose, the seeds of *Phoenix*, or of plants of other natural orders.

That at any rate a similar cytase does the work in *Lodoicea* cannot be doubted. Its precise nature and that of the final and intermediate products of its action deserve investigation. We shall probably have to wait till some one with the necessary equipment can visit the Seychelles, where the scale on which the enzymes operate in *Lodoicea* should afford unique opportunities for their study.

What other constituents besides cellulose the endosperm contains has not been ascertained. In barley, according to the classical research of Brown and Morris, the amylo-hydrolytic enzyme 'is no doubt principally due to a secretion by the columnar epithelium of the growing embryo'.

'There is a steady accumulation of starch-liquefying diastase in the endosperm, and this in the course of time permeates its whole tissue, the ready passage of the enzyme through the contents of the grain having been enormously facilitated by the previous destruction of the cell-walls by the cyto-hydrolytic enzyme.'<sup>3</sup>

It is probable that in most cases in the absorption of an endosperm the process takes place in two stages: the thickened cell-walls are attacked by one enzyme, and the cell-contents are thus exposed to the action of others. But an enzyme might possibly get access to the cell-contents before the destruction of the cell-wall. In 1883 Gardiner was able to study its structure in a nut which I was able to place at his disposal, as it had failed to germinate. He found that the thickened cell-walls showed numerous deep pits which were closed by a membrane. But he describes them as 'affording one of the clearest examples of the perforation both of the wall and pit membrane'<sup>4</sup> by protoplasmic threads. It may be reasonably suspected that these would afford channels for the propagation of enzyme action.

<sup>1</sup> Researches on the Germination of some of the Gramineae. J. Chem. Soc. 57, pp. 497-8.

<sup>2</sup> l. c., p. 499.

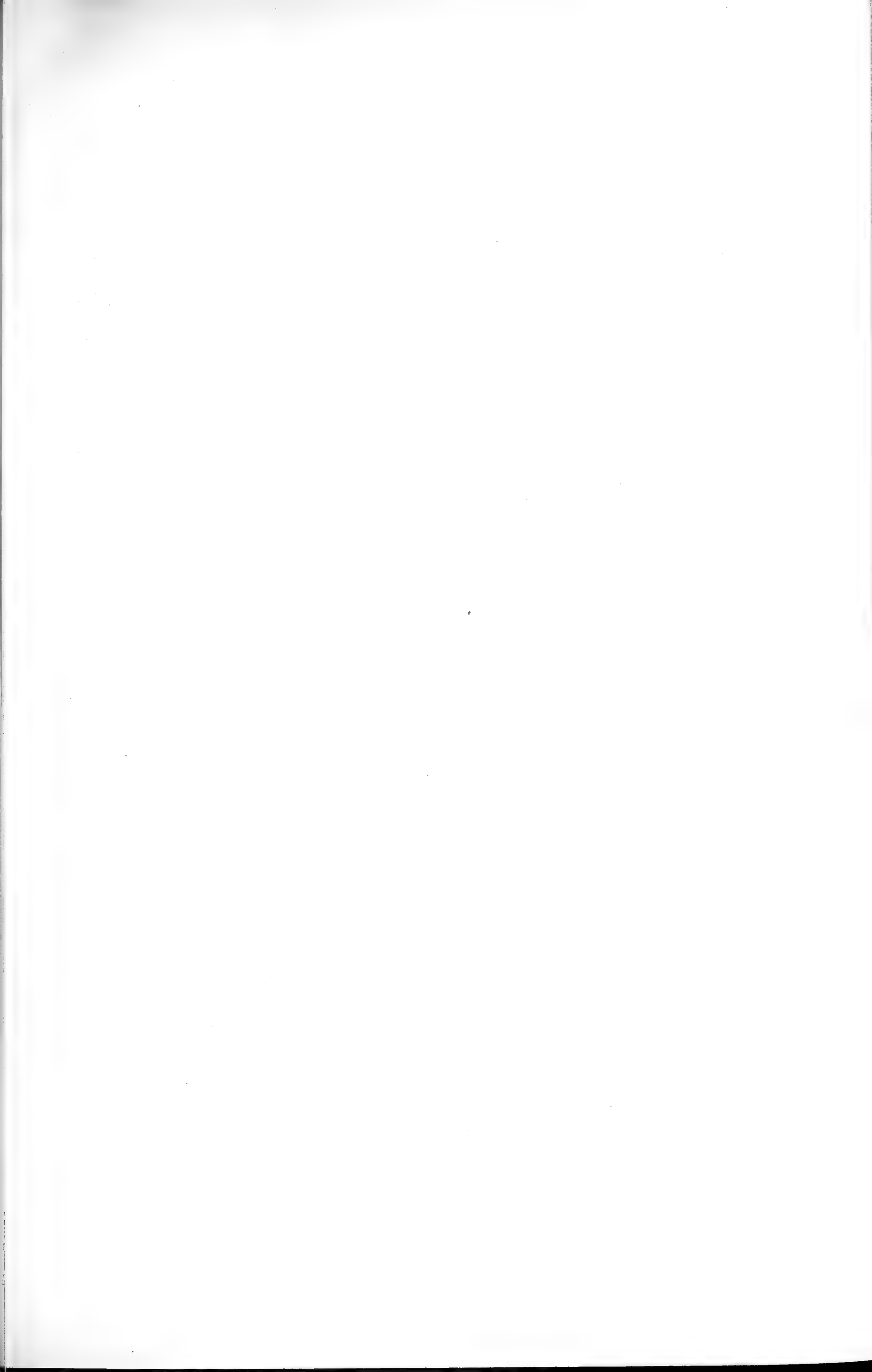
<sup>3</sup> l. c., p. 507.

<sup>4</sup> Phil. Trans., R. S., 1883, p. 848.

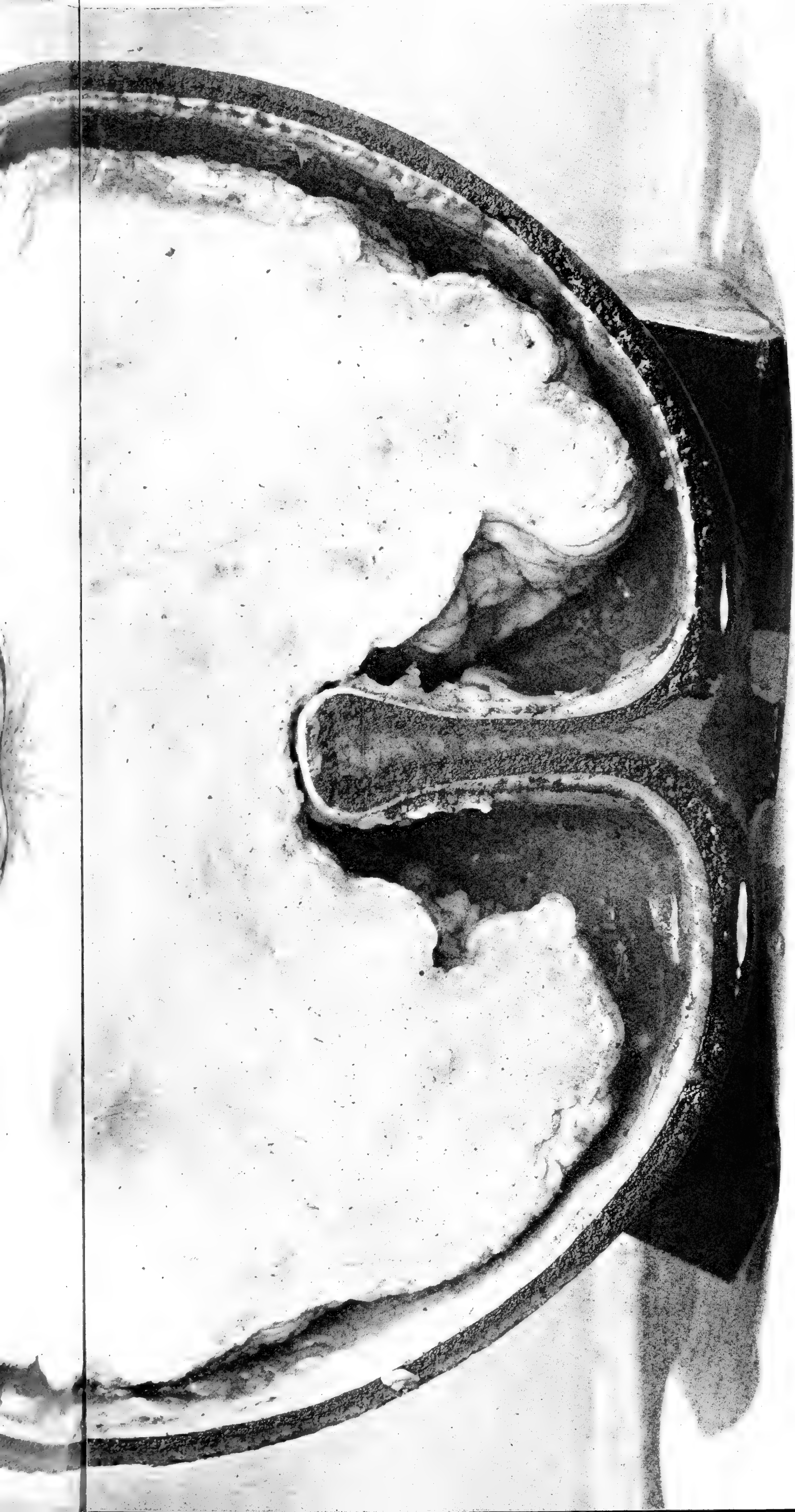
PS. While this was passing through the press, Prof. Farmer, who had seen a proof, wrote to me: 'It may interest you to know that in 1890,



when I was at Peradeniya, there was a young plant [of *Lodoicea*] growing in the Gardens which Trimen [the Director] told me had been planted about five years previously. The "nut" was still in the ground and connected by the sucker with the plant. The latter bore, I think, about five leaves; the later formed ones were very large, somewhat resembling those of *Corypha umbraculifera*.'

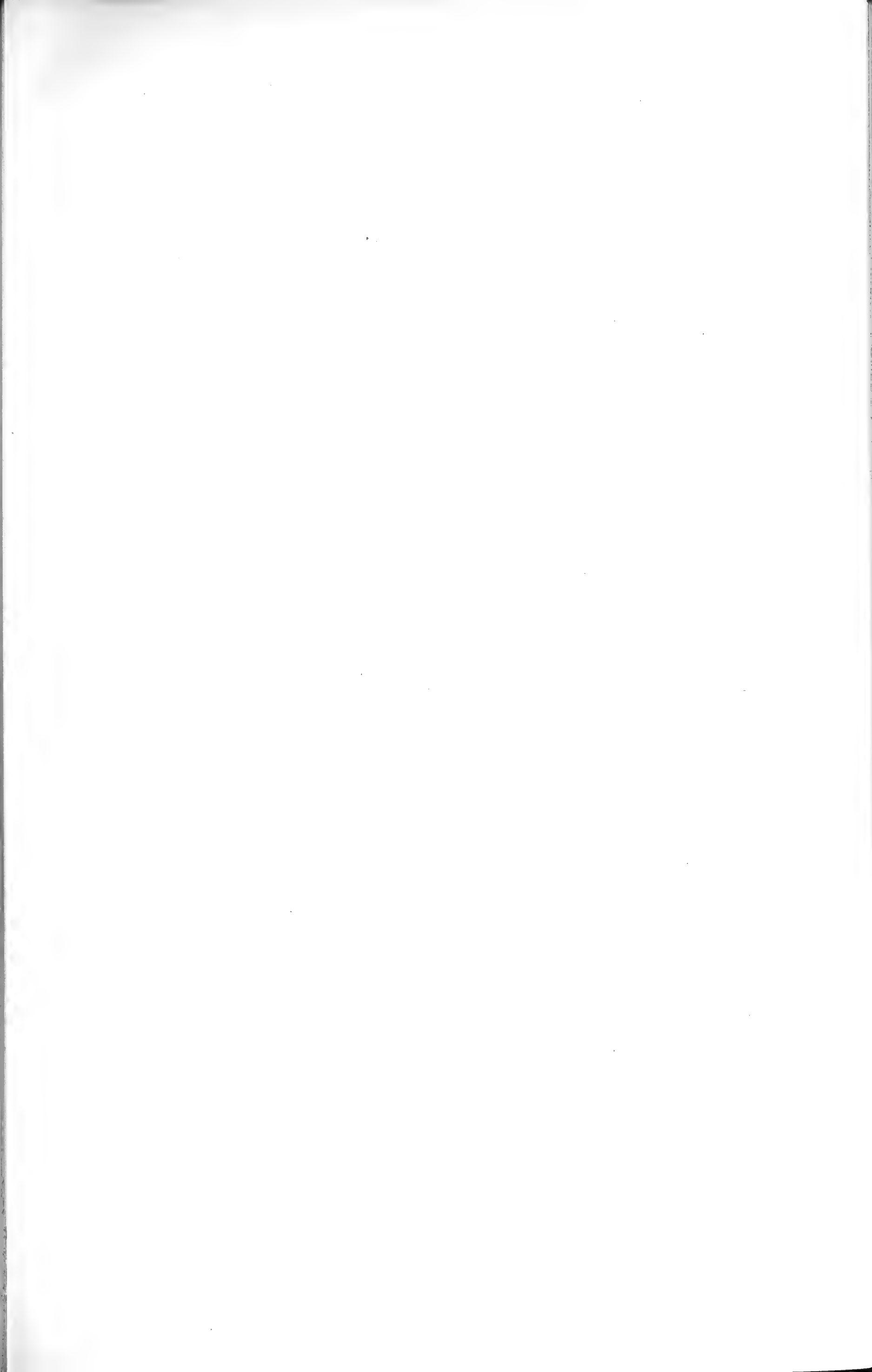






THISLTON-DYER-- LODOICEA.











ER—LODOICEA





## NOTES.

**STUDIES ON THE STRUCTURE AND AFFINITIES OF CRETACEOUS PLANTS.—(ABSTRACT).** (From the Proceedings of the Royal Society, 1909.)—The authors comment on the importance of the work done on the flora of the Palaeozoic period, and the botanical interest that would attach to similar petrifications of plants from all ages of the Mesozoic period. They have had the good fortune to find excellently preserved material from the Cretaceous of Northern Japan.

In the present paper they describe eighteen plants from this material, which is extraordinarily rich. As hitherto there has been very little known from anatomical material of plants of this age, the present paper is by no means final, but is in the nature of a pioneer chart of the ground.

The petrification of the cells of the plants is often extremely good, though the fragments are not so complete as could be desired. The plant structures include stems, roots, leaves, cones, fern sporangia, and even an Angiospermic flower, the first petrification of a flower to be described. The débris lie together in the nodules in much the same way that the débris lie in the Coal-balls of the Palaeozoic, though they are mixed with fragments of shells. The latter are largely Ammonites and serve to determine the age of the petrifications.

The flora, as a whole, represents an interesting mixed flora such as has not hitherto come to light among petrifications.

Roughly speaking, the flora seems to have consisted of about one-third Angiosperms, slightly more than one-third Gymnosperms, and the rest of ferns and lower plants. The anatomy of the early Angiosperms being such a desideratum in botany, their presence in the petrifications renders them doubly interesting, and particularly when they are found in so evenly balanced a mixed flora.

All the specimens described in this paper were cut in Tokio in the botanical department by the authors.

The plants described are as follows:—

*Petrosphaeria japonica*, gen. et spec. nov. A fungus which has numerous microsclerotia, in the periderm of one of the Angiosperms, *Saururopsis*.

*Schizaeopteris Tansleyi*, gen. et spec. nov. The sorus and sporangia of a Schizaeaceous fern.

*Fasciostelopteris mesozoica*, gen. et spec. nov. The stem and petiole of a fern with a dictyostelic anatomy. Probably allied to the Dicksoniaceae.

*Fern rootlets*, in excellent state of preservation, showing the diarch stele of the leptosporangiate ferns.

*Niponophyllum cordaitiforme*, gen. et spec. nov. The leaf of what appears to be some plant of Cycadean affinity, the anatomy bearing considerable resemblance to that of *Cordaites*.

*Yezonia vulgaris*, gen. et spec. nov. A Gymnosperm, of which stems, unthickened twigs, and leafy axes are all very plentiful. It is the commonest plant in the material, and at the same time the most unique. In the anatomy of both main axis and foliage it is not like any known type.

*Yezostrobus Oliveri*, gen. et spec. nov. The fructification of a Gymnosperm, the cone bearing simple scales with seeds, one on each, which are like those of Cycads in some respects, but have a nucellus standing up entirely free from the integument with a well marked epidermis between.

Though continuity is lacking between these two fossils, there seems considerable ground for suspecting them of belonging to the same plant from anatomical points of likeness.

*Araucarioxylon tankoense*, spec. nov. Secondary wood, showing remarkably clear pittings in the transverse sections.

*Cedroxylon Matsumurae*, spec. nov. Well preserved secondary wood.

*Cedroxylon Yendoi*, spec. nov. Secondary wood, with traumatic resin canals.

*Cunninghamiostrobus yubariensis*, gen. et spec. nov. A cone, as its name implies, belonging to the family of the Cunninghamias, with its external appearance partly preserved, and the cone scales and axis fairly well petrified. The seeds have apparently been scattered.

*Cryptomeriopsis antiqua*, gen. et spec. nov. Stem with leaves attached, the foliage very like that of a *Cryptomeria*.

*Saururopsis niponensis*, gen. et spec. nov. The stem and attached roots of an Angiosperm, probably to be included in the Saururaceae.

*Jugloxylon Hamaoanum*, gen. et spec. nov. The secondary wood of an Angiosperm.

*Populocaulis yezoensis*, gen. et spec. nov. The stems of an Angiosperm, with cortical tissue.

*Fagoxylon hokkaidense*, gen. et spec. nov. The secondary wood of an Angiosperm.

*Sabiocaulis Sakurii*, gen. et spec. nov. Minute stems, and older twigs of an Angiosperm, with cortex, and well preserved and characteristic anatomy.

*Cretovarium japonicum*, gen. et spec. nov. The flower of an Angiosperm, of which there are several specimens.

Of this list of plants, the commonest, i. e. those which have yielded the greatest number of specimens in the course of the work, are *Yezonia*, *Sabiocaulis*, and *Cretovarium*. It is noteworthy that these are among the most unusual and the most interesting of the plants.

The authors acknowledge much assistance in the work from the Royal Society Government Grant Committee, which made it possible for one of them (M. C. S.) to attempt the work; and from the various departments of the Imperial Government of Japan in the course of collecting and preparing the material.

MARIE C. STOPES.

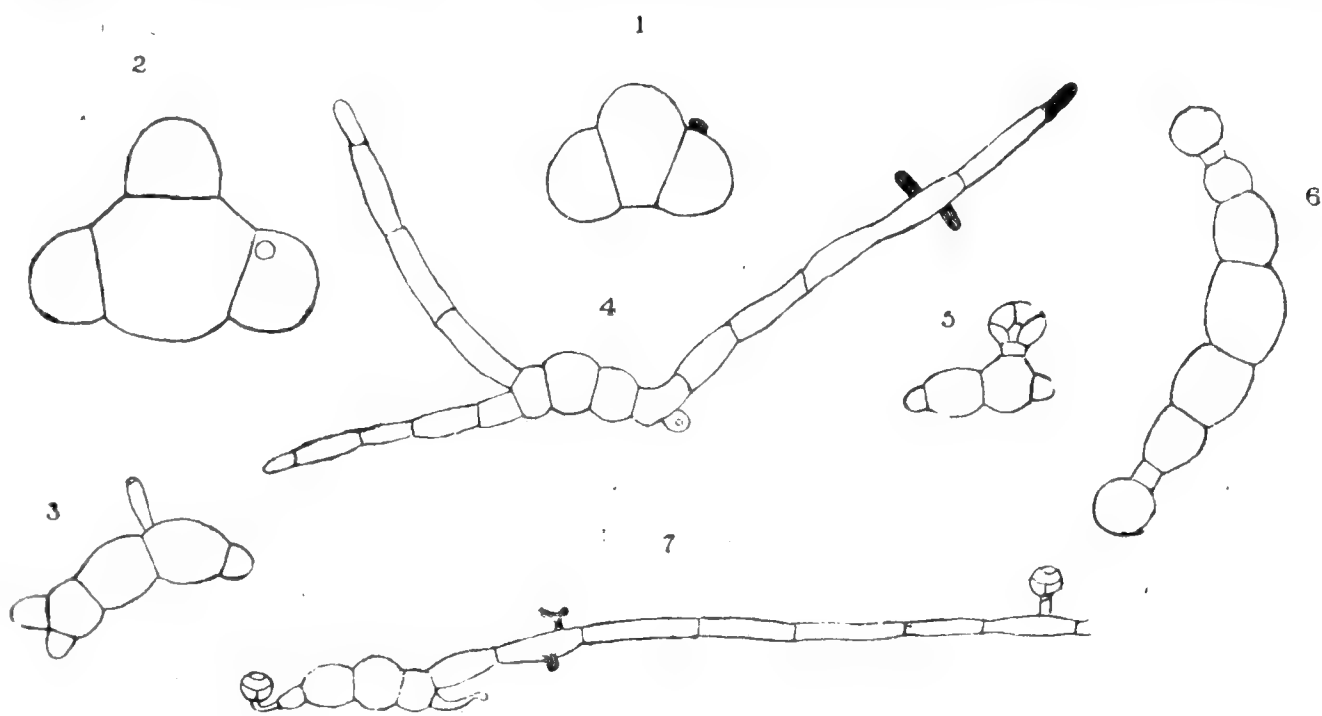
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**PRELIMINARY NOTE ON AOSPORY AND APOGAMY IN TRICHOMANES KAULFUSSII, HK. ET GREW.**—Bower<sup>1</sup> has already written on apospory and the production of gemmae in *Trichomanes Kaulfussii*, HK. et Grew. His investigations, however, especially with regard to the development of gemmae, are by no means complete, as he has only described the primary stages of this development. Thus he has observed that 'the filamentous growths originate from single marginal or superficial cells of the frond, and bear lateral rhizoids'. I can confirm this observation of Bower's, and may add that, as a rule, the apex of a pinna in which a midrib ends forms the origin of a prothallium, from which by continued cell-division an end-cell is cut off. On the other hand, if a marginal cell grows to a prothallium it twists away from the leaf surface and turns towards the apex.

It is interesting to note that the nucleus changes sympathetically and takes on the same curved form as the cell itself. The prothallium as it grows assumes a filamentous



form which ramifies in different directions and bears, at its extremities or laterally, sterigmata singly or in tufts. At the end of each of these branches is balanced a spindle-shaped gemma as described by Bower. The gemma is bullet-shaped or elliptical in form, and is divided by a cell-wall which runs perpendicular to the long axis of the sterigma. One or both daughter-cells of the gemma divide in the same direction, and from this a many-celled spindle-shaped growth results. The junction of the gemma and the sterigma may always be observed as a brownish scar on the mother-cell of the gemma. Very often the cell-division of the gemma takes place from one only of the two primary daughter-cells, out of which a three-celled stage develops (Fig. 1).

In this case the middle cell divides first, and cuts off a cell perpendicular to the long axis of the gemma (Fig. 2). Often, however, a gemma divides in such a way that after a four-cell stage, an end-cell of the gemma divides twice in succession and in different directions (Fig. 3). By further divisions of these end-cells in the direction indicated many-celled prothalloid growths arise which bear lateral rhizoids and thus betray the nature of a prothallium (Fig. 4). By continued cell-division of these

<sup>1</sup> On Apospory and production of Gemmae in *Trichomanes Kaulfussii*. *Annals of Botany*, viii, 1894.



ramifications of gemmae arise many-celled prothallia which are characterized as such by the presence of rhizoids and sexual organs. Bower did not observe these advanced stages, for he states 'after the first steps the further growth is exceedingly slow' (p. 467). I have observed very often that after the four-cell stage of the gemmae a development of the antheridia takes place by the cutting off of a neck-cell from the middle papilla whilst the antheridium develops from the other part (Fig. 5).

Furthermore I have observed that the antheridia are formed at both extremities of the spindle-shaped gemma in the same way (Fig. 6).

Lastly, I have seen cases where antheridia and prothallia with antheridia grew on the same gemma. Such cases are illustrated in Fig. 7.

At the left extremity of a four-celled gemma is seen a typical antheridium, and at the other extremity a cell which has divided twice and from which has developed a many-celled prothallium and one aborted cell destined to form a rhizoid.

This prothallium bears a typical antheridium and several rhizoids which are unmistakable signs of its gametophyte nature.

I would emphasize this fact because in the case of the genus *Trichomanes* the existence of sexual organs in the gemmae has been denied.

Bower has expressly stated this with regard to *Trichomanes Kaulfussii*, HK., saying: 'The filaments show characters similar to those of *T. alatum*, and though no sexual organs have been found upon them in *T. Kaulfussii*' (p. 467).

As far as the transition from the sporophyte to the gametophyte is concerned I can only support Farmer and Digby<sup>1</sup> and state 'that the transition from the sporophyte to the gametophyte in this Fern is attended by no reduction or alteration in the number of the chromosomes . . .' (p. 164).

I have done my utmost to obtain the exact number of chromosomes in sporophyte and gametophyte.

To this end I have counted the chromosomes in the cells of the leaves on the surface as well as at the point, and can fix the approximate number of chromosomes at about eighty.

The counting was carried out at different stages of mitosis, and in every case almost the same number was arrived at.

The chromosomes in prothallium cells were also counted, and the same number (about eighty) was found. During this counting the same precautions were used as before. No difference between the chromosomes of one and of the other generation could be discovered.

Moreover, the number and the appearance of the nucleoli in both generations were the same, so that this distinctive mark was also wanting. On the basis of these observations I can only confirm the statement of Farmer and Digby 'that so far as our present knowledge goes, apospory is always found to imply the absence of the meiotic phase from the life-cycle of the organism', and extend it by the result of my own research.

In accordance with this it is impossible to draw a sharp line of distinction between sporophyte and gametophyte.

BELGRADE.

PETER GEORGEVITCH.

<sup>1</sup> Studies in Apospory and Apogamy in Ferns. *Annals of Botany*, vol. xxi, no. lxxii, 1907.

**PRELIMINARY NOTE ON THE SPERMATOGENESIS OF MNIUM HORNUM.**—In a recent paper Arens<sup>1</sup> has investigated the spermatogenesis of *Polytrichum juniperinum* and *Mnium hornum* and finds that in both cases the nuclear divisions are of the normal type. In the latter plant eight (later corrected to six<sup>2</sup>) chromosomes of equal size appear at each mitosis. Drs. van Leeuwen-Reijnvaan<sup>3 4</sup> have also described the spermatogenesis in several Mosses, and in all the plants examined they state that a reduction in the number of chromosomes takes place at the final division of the spermatogenetic cells. In *Mnium* sp.<sup>4</sup> eight chromosomes are present, four being long and four short, and, at the last division, two long and two short chromosomes pass to each daughter-cell.

In the present investigation no such reduction has been discovered. The early divisions in the antheridium resemble the premeiotic mitoses already described in the developing archesporium,<sup>5</sup> with the difference that here six chromosomes are present. During the early stages of the penultimate and of the final division a body is cut off by constriction from the nucleolus, similar to that described in the first meiotic division;<sup>5</sup> this body has not been observed to pass outside the nuclear membrane as long as the latter is present. No centrosomes are found during the later stages in these or in the earlier division figures. The resting nucleus, before the final division, is of large size in relation to the cell, but possesses a small nucleolus. A continuous spireme is not present, the chromatic material first appearing in a number of small masses, and from these the chromosomes are gradually formed. Stages showing the six free chromosomes are frequently found; the latter are usually curved and intertwined, but are of approximately equal size. The metaphase is normal and is quickly passed over, the axis of the spindle usually coinciding with the long axis of the cell; there is no diagonal division. In polar views of the anaphase six chromosomes can be distinguished, and it is quite clear that no reduction has taken place. The telophase is normal, and is characterized by the presence of large vacuoles in the cytoplasm. The spermatocytes each possess a distinct wall, but the protoplasm soon contracts away from this, forming a rounded mass; a large vacuole is usually present. The nucleus is large and contains a small deeply staining nucleolus.

The development of the spermatozoid from the spermatocyte is a very complex process, and full details of this and of the preceding divisions will be given in a further communication.

M. WILSON.

ROYAL COLLEGE OF SCIENCE,  
S. KENSINGTON.

Dec. 1909.

<sup>1</sup> Zur Spermatogenese der Laubmoose. Inaugural-Dissertation, Bonn, 1907.

<sup>2</sup> Zur Spermatogenese der Laubmoose. Ref., Bot. Centralblatt, Bd. cvii, 1908, p. 611.

<sup>3</sup> Über eine zweifache Reduktion . . . bei einigen *Polytrichum*-Arten. Recueil des Travaux botaniques Néerlandais, iv, 1907.

<sup>4</sup> Über die Spermatogenese der Moose. Ber. d. deutsch. Bot. Gesell., Bd. xxvi a, 1908, p. 301.

<sup>5</sup> Wilson, M.: Spore Formation and Nuclear Division in *Mnium hornum*. Annals of Botany, xxiii, 1909, p. 141.

**A NEW PARASITIC DISEASE OF THE JUNCACEAE.—PRELIMINARY NOTICE.**—The roots of various species of *Juncus* are subject to the attack of a Myceto-zoan parasite, which I propose to call '*Sorosphaera Junci*' as being allied to *S. Veronicae*. The terminal stages in the life-histories of both these Fungi are strikingly similar, the wedge-shaped spores being collected into spherical balls, the sorospheres, although in the case of *S. Junci* many of these balls are of elliptical shape, and often merely loosely aggregated masses of spores fill the root-cells. The stages of nuclear division in both parasites are also similar. In old infected roots the cortical cells are filled with sorospheres and spores, and in those of recent infection the cells contain the nucleated amoebae of the parasite, and in some the nuclei may be seen collecting in masses previous to spore-formation. The infection of the root takes place by the entry of an amoeba into a root-hair and thence into the cortex of the root. The roots show no hypertrophy. This Fungus is in no way related to the *Entorrhiza*, which has been described by Weber in Bot. Zeit., 1884, as being the cause of tubercle-formation in the roots of *Juncus bufonius*. The latter is doubtless one of the Ustilagineae; its young spores are binucleate, and it infects the root by means of conidia which push their way down the root-hairs. I hope shortly to publish in detail an investigation into the life-history of *Sorosphaera Junci*.

E. J. SCHWARTZ.

**ON MESOXYLON, A NEW GENUS OF CORDAITALES.—PRELIMINARY NOTE.**—The relation of *Poroxylon* to *Cordaites* has been recognized ever since the discovery of the former genus by Renault, in 1879. The main anatomical distinction lies in the presence, in the stem of *Poroxylon*, of well-marked strands of centripetal wood, forming part of the leaf-trace bundles at the margin of the pith; the stem of *Cordaites*, as Renault states, is 'absolutely deprived of centripetal wood',<sup>1</sup> the leaf-traces only acquiring it on entering the leaf. Another character of importance is the usually discoid pith of *Cordaites*, while that of *Poroxylon* is continuous, and further distinctions are to be found in the denser wood of *Cordaites* and in the structure of the phloem.

Within the last five years several stems have come to light in the calcareous nodules of the Lower Coal-Measures of Lancashire, combining the characters of *Poroxylon* and *Cordaites*. The object of the present Note is to briefly place these observations on record, and to establish a new genus for the fossils in question. The generic name *Mesoxylon* has been chosen, to express the intermediate position of the genus.

One of the species now placed in *Mesoxylon* has already been shortly described by one of us under the provisional name *Poroxylon Sutcliffii*,<sup>2</sup> while others have been referred to under *Cordaites*.<sup>3</sup> It is now proposed to unite these forms, with others since discovered, making five species in all, in the genus *Mesoxylon*. There are already indications that further species may shortly have to be added.

<sup>1</sup> Bassin Houiller et Permien d'Autun et d'Épinac. Flore Fossile. II<sup>m</sup>e Partie, 1896, p. 332.

<sup>2</sup> Scott, Studies in Fossil Botany, 2nd edition, 1909, p. 511, Fig. 184.

<sup>3</sup> Loc. cit., p. 526.

The new genus, the species of which may be said to combine the anatomical habit of a *Cordaites* with the centripetal xylem of a *Poroxylon*, may be characterized as follows:—

MESOXYLON, gen. nov.

Pith relatively large, discoid.<sup>1</sup>

Wood dense, with narrow, usually uniseriate medullary rays, and relatively small tracheides.

Leaf-traces double where they leave the pith, the two strands uniting at a lower level, but undergoing further subdivision in the pericycle and cortex, before entering the leaf.

Centripetal xylem present in the stem, where it forms part of the leaf-traces at the margin of the pith and throughout their course outwards into the leaves.

Outer cortex strengthened by a system of sclerenchymatous bands of the Dictyoxylon or Sparganum type.

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.

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.

A brief diagnosis of the species follows:—

1. *Mesoxylon Sutcliffii*<sup>2</sup>; *Poroxylon Sutcliffii*, Scott, Studies in Fossil Botany, 2nd edition, 1909, p. 511; Fig. 184 (transverse section of stem).

Leaf-bases crowded, completely covering the surface of the stem.

Pith large, discoid, with a persistent outer zone.

Twin-bundles of the leaf-trace, at the margin of the pith, remaining separate through several internodes before fusing; subdividing in the cortex to form about eight bundles in all.

Petiole of leaf flat, containing about sixteen bundles.

Centripetal xylem distinct, persisting below the point of fusion of the two leaf-trace bundles.

Tracheides of the leaf-traces spiral or scalariform; those of the intermediate secondary wood pitted, except sometimes at the extreme inner margin.

Medullary rays uniseriate, 3–6 cells in height.

An axillary bud present in the axil of every leaf.

Dictyoxylon zone of cortex somewhat narrow.

Roof-nodules; Shore, Littleborough.

The very numerous sections of this species appear to represent about nine distinct specimens. The diameter is pretty uniformly about 3 cm., including the leaf-bases, the pith alone having an average diameter of 1.4 cm. This is the only species in which anything is known of the leaf.

2. *Mesoxylon poroxyloides*, sp. nov.

Leaf-bases somewhat less crowded than in the preceding species.

Pith of moderate dimensions, discoid, with a persistent outer zone.

<sup>1</sup> This point is not yet demonstrated in the case of *Mesoxylon platypodium*; see below.

<sup>2</sup> The specific name, in honour of the owner of the colliery at Shore (reopened on account of its richness in fossil remains), was originally suggested by Mr. J. Lomax, who sent out the sections under the name *Cordaites Sutcliffii*. All the species described are derived from the Shore workings.

Twin-bundles of the leaf-trace soon uniting after reaching the pith, subdividing in the cortex to form eight bundles.

Centripetal xylem distinct, persisting after fusion of the two leaf-trace strands.

Tracheides of the inner part of the intermediate secondary wood, as well as those of the leaf-traces, spiral or scalariform.

Medullary rays usually uniseriate, 1–8 cells in height.

No axillary buds or branches observed.

Dictyoxylon zone of cortex very broad, fibres not much thickened.

Seam-nodule, Shore, Littleborough.

Only one specimen has been investigated, though others are probably referable to the same species. The mean diameter of the stem is about 2.5 cm., that of the pith about 8 mm. The great distinctness of the centripetal xylem, the early union of the leaf-trace strands at the margin of the pith, and the moderate size of the latter accentuate the resemblance to *Poroxylon*.

3. *Mesoxylon multirame*, sp. nov.

Leaf-bases moderately crowded, as in *M. poroxyloides*.

Pith large, discoid, with a persistent outer zone.

Twin-bundles of leaf-trace approximated on reaching the pith, but remaining separate for several internodes; subdividing in the cortex as in other species.

Centripetal xylem dying out rather rapidly after the leaf-trace reaches the pith.

Tracheides and medullary rays essentially as in *M. poroxyloides*.

An axillary branch present in most of the leaf-axils. Base of branch leafless, with a flattened stele, recalling a phylloclade.

Dictyoxylon zone of cortex broad, fibres strongly thickened.

Seam-nodule, Shore, Littleborough.

One specimen only has been investigated. Among the preparations, a series of fifteen transverse sections was available, in which the course of the leaf-traces and axillary steles could be traced with great precision.

The phyllotaxis, however, remains a difficulty. The orthostichies are sufficiently numerous to correspond to a  $\frac{5}{13}$  arrangement, if not to a higher fraction, but the actual divergence between two successive leaf-traces seems to be considerably less than on any usual phyllotaxis.

The naked axillary branches are remarkable, and very different from the little buds of *M. Sutcliffii*—evidently the two organs had quite distinct functions.

The stem observed is about 2.5 cm. in diameter, the pith measuring just half this—12.5 mm.

4. *M. Lomaxii*, sp. nov.

Leaf-bases scattered.

Pith large and discoid, with a persistent outer zone.

Twin-bundles of leaf-trace converging, and fusing immediately on reaching the pith.

Centripetal xylem very distinct and persistent.

Xylem-strands at the margin of the pith sheathed by radiating parenchyma.

Scalariform tracheides almost limited to leaf-traces, only appearing at the extreme inner edge of the intermediate secondary wood.



Medullary rays uniseriate, 1-12 cells in height.

Dictyoxylon zone of cortex narrow; periderm very broad.

Roof-nodule, Shore, Littleborough.

This is a very distinct form, characterized by the very early fusion of the two leaf-trace strands, and the definite sheath about the primary xylem.

Three specimens have been observed (possibly fragments of one), all being about 5 cm. in diameter, with a pith about 2 cm. across.

The species is named after Mr. James Lomax.

5. *Mesoxylon platypodium*, sp. nov.

Leaf-bases very broad, scattered.

Pith large, with a persistent outer zone; interior not preserved.

Twin-bundles of each leaf-trace very far apart at margin of pith; where they leave the wood each of these bundles already subdivided, as regards its primary xylem. Leaf-trace in cortex consisting of two distinct rows of four bundles each.

Two distinct axillary steles to the same leaf.

Centripetal xylem very well developed and persistent at margin of pith.

Scalariform tracheides apparently limited to leaf-traces or their neighbourhood; those of secondary xylem sometimes pitted throughout.

Medullary rays 1-12 cells in height.

Sparganum zone of cortex narrow.

Roof-nodule, Shore, Littleborough.

This is the most isolated of the five species, and is characterized by the extreme separation (2 mm.) of the twin-bundles of the trace, and by their secondary division while still in the woody zone.

The two axillary steles corresponding to a single leaf constitute a very striking feature. It is interesting to note, in this connexion, that the axillary stele in *M. Sutcliffii* is sometimes double, though the two steles appear always to reunite before entering the bud. The specific name, *platypodium*, refers to the great breadth of the leaf-base.

The systematic position of the genus *Mesoxylon* will be discussed in our full paper which is in course of preparation. In the meantime it may be pointed out that these stems appear to completely bridge the gap, so far as anatomy is concerned, between the Poroxyleae and the Cordaiteae, and thus form valuable links in the chain of forms connecting the Pteridosperms with the typical Gymnosperms.

All the specimens were discovered by Mr. James Lomax and his son, in the Shore material, and the sections cut by them.

D. H. SCOTT.

A. J. MASLEN.





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## Remarks on the Oecology of Coniferae.

BY

PERCY GROOM, M.A., D.Sc., F.L.S.

*Assistant Professor of Botany, Imperial College of Science and Technology, London.*

**I**N this paper I propose to discuss three problems concerning the Coniferae, namely :—

1. The cause of their xerophytic foliage and tracheidal wood.
2. The cause of their survival in competition with dicotylous trees.
3. The cause of the suppression of many forms in past ages.

As the physiology of the Coniferae is known only in regard to north-temperate forms, the discussion will solely concern these.

The evergreen nature of the Gymnospermae has long been admitted to be primitive in the class, and in the sub-group Coniferae. The evergreen leaves of Coniferae display xeromorphy in their relatively and usually absolutely small surface, and exhibit xerophytic structure in their epidermal structure and often in other respects (hypoderma). Even the deciduous larch shows these ancestral features stamped on the form, and, to a slight extent, on the epidermal structure of the leaves.

The xeromorphy and xerophytic structure of evergreen coniferous leaves were, for a time, regarded as providing protection against the cold of winter, and against injury by excessive loads of snow.

If this explanation were regarded as adequate, we should be compelled to assume that Coniferae living in warm temperate or sub-tropical climes had migrated from cold temperate regions.

Another explanation was offered by A. F. W. Schimper ('90), according to whom the evergreen nature of the Coniferae must be combined with a slow rate of transpiration, because during the physiologically dry season—the cold season in cold temperate regions—absorption of water is largely arrested.

That this explanation, which accounts for the *survival* of Coniferae in north-temperate regions where there is a physiologically dry season, may not be a complete rationale of the origin and persistence of the coniferous mechanism is suggested by :—

1. The wide range of distribution as regards climate and habit of Coniferae.



2. The occurrence of Coniferae in evergreen tropical humid regions.<sup>1</sup>

3. The demand on the part of certain cold-temperate Coniferae for a relatively humid habitat.<sup>1</sup>

I propose, therefore, to discuss several points in connexion with the coniferous mechanism in relation to water.

(a) THE CONIFEROUS MECHANISM DOES NOT NECESSARILY INVOLVE A SLOW RATE OF TRANSPIRATION PER UNIT SURFACE OF LEAF, NOR XEROPHYTIC STRUCTURE IN THE LEAF.

Though the xerophytic structure of the evergreen Conifer leaf is associated with a slow rate of transpiration, the deciduous larch-leaf transpires rapidly. This is shown by results obtained by Von Höhnel ('80).

The subjoined statistics record the number of grammes of water transpired per gramme air-dry weight of leaf-substance during the period April 1 to September 31, 1879. (Von Höhnel's results have been converted into approximate numbers.)

<i>Sorbus torminalis</i>	...	...	1,750	} Deciduous
<i>Larix decidua</i>	...	...	1,150	
<i>Tilia grandifolia</i>	...	...	1,030	
<i>Fagus sylvatica</i>	...	...	860	
<i>Betula alba</i>	...	...	845	
Elm	...	...	755	
<i>Quercus Robur</i>	...	...	660	
<i>Acer platanoides</i>	...	...	520	} Evergreen
<i>Picea excelsa</i>	...	...	210	
<i>Pinus sylvestris</i>	...	...	105	
<i>Pinus Laricio</i>	...	...	100	
<i>Abies pectinata</i>	...	...	75	

But these results do not necessarily imply all they seem to, as misconception will arise if Von Höhnel's method of calculation and mode of experiment are not taken into account.

This will be clear, so far as the method of calculation is concerned, from the following example. If we suppose that there are two flat leaves, say a sun-leaf and a shade-leaf of the same species and of equal surface, and that the former leaf weighs twice as much as the latter; then, if the sun-leaf under the influence of direct sunlight is transpiring twice as much water in a given time as the shade-leaf in weak diffused light, the rate of transpiration will nevertheless seem to be the same in the two leaves if we estimate according to Von Höhnel's method, that is, per gramme dry

<sup>1</sup> Compare Schimper, 1898. English Version, pp. 564-5.

weight of leaf. The effect of the misconception arising becomes obvious when we examine the results obtained by Von Höhnel in connexion with different specimens of one species. Von Höhnel conducted his observations by means of potted plants, the pots and soil being protected from direct evaporation. He periodically weighed and watered the plants (and pots). One set of plants he kept in a well lighted position, and the other set in a constantly shaded position. Examining his statistics, the latter plants *seem* to be transpiring more rapidly than the plants exposed to sunlight. Von Höhnel sought to explain this as partly due to the fact that the shaded leaves were not exposed to rain or dew, that the structure of the shade-leaves is such as to promote rapid transpiration, and finally that the soil was moister in the shade. The first and third alleged causes were probably operative, and may well account for the truly greater transpiration per unit leaf-surface of some of the plants in the shade; but the second assigned reason is not justifiable. The explanation of the apparently greater transpiration of the shaded leaves lies chiefly in their lighter weight: for I find that when judging either by absolute amount of water transpired by the whole plant or by the weight of transpiration per square centimetre of leaf-surface, the leaves exposed to the light in most cases transpired more rapidly. This is proved by an inspection of columns 3 and 4 (giving Von Höhnel's results) and columns 5 to 8 (giving my calculations) of Table I.

It is, therefore, possible that the estimate given above of *Sorbus torminalis* is too high, for I notice that it refers to a solitary shaded specimen.

In Table I the estimates of the leaf-surface of the dicotylous plants are Von Höhnel's; those of the Conifers are mine, and result from numerous measurements of the surfaces of leaves combined with Von Höhnel's statistics as regards the numbers and lengths of the leaves. The following were my results:—

DIMENSIONS OF CONIFEROUS LEAVES.

	<i>In Sunlight.</i>		<i>In Shade.</i>	
	<i>Mean length.</i>	<i>Mean perimeter or width of cross section.</i>	<i>Mean Length.</i>	<i>Mean perimeter or width of cross section.</i>
<i>Pinus sylvestris</i>	47.3 mm.	3.48 mm.	54.75 mm.	3.32 mm.
<i>Picea excelsa</i> .	17.18 ,,	2.88 ,,	16.9 ,,	2.47 ,,
<i>Abies pectinata</i>	12 ,,	2 ,,	—	—
<i>Larix decidua</i> .	27.2 ,,	0.713 ,,	23.1 ,,	0.862 ,,

TABLE I.

I.			II.	III. IV.		V.	VI.	VII. VIII.	
			<i>Aggregate leaf surface of the tree in sq. cm.</i>	<i>Absolute amount of water transpired June 1 to Dec. 1, 1878, in grammes.</i>		<i>Number of grammes of water transpired June 1 to Sept. 1, 1878, per sq. cm.</i>		<i>Minimum number of grammes of water transpired in one of the months June to Aug. 31 per sq. cm.</i>	
				<i>Sun.</i>	<i>Shade.</i>	<i>Sun.</i>	<i>Shade.</i>	<i>Sun.</i>	<i>Shade.</i>
1	<i>Quercus Cerris</i>	Shade	1,764		2,065		0.83		0.113
14	" "	Sun	3,316	1,975		0.49		0.140	
18	" "	"	3,356	2,352		0.58		0.135	
3	<i>Carpinus Betulus</i>	Shade	842		504		0.42		0.113
21	" "	"	1,829		1,337		0.45		
55	" "	Sun	1,624	3,848		1.9			
59	" "	"	1,385	2,568		1.43		0.43	
28	<i>Fagus sylvatica</i>	Shade	1,776		1,721		0.65		0.156
29	" "	"	728		1,160		1.1		
53	" "	"	705		971		0.87		
62	" "	"	365		510		0.9		
30	" "	Sun	1,803	2,461		1.02			
54	" "	"	798	1,986		2.2			
65	" "	"	1,033	858		0.75		0.156	
70	<i>Betula alba</i>	Shade	707		1,868		1.92		0.558
71	" "	"	462		1,740		2.66		
50	" "	Sun	987	2,523		2.2			
51	" "	"	1,418	4,154		1.55		0.545	
16	<i>Fraxinus excelsior</i>	Shade	1,503		1,918		0.75		0.183
17	" "	"	1,940		2,987		1.14		
15	" "	Sun	3,067	4,857		1.45		0.34	
56	" "	"	1,385	4,270		2.5			
13	<i>Acer Pseudoplatanus</i>	Sun	2,858	1,657		0.51			0.107
56	" "	"	1,085	2,721		2.1		0.45	
9	<i>Acer platanoides</i>	Shade	2,677		1,636		0.39		0.096
10	" "	"	1,466		1,368		0.599		
8	" "	Sun	3,412	3,524		0.87			
11	" "	"	4,435	2,698		0.46		0.13	
24	<i>Tilia grandifolia</i>	Shade	1,015		1,474		1.05		0.3
25	" "	Sun	1,138	3,094		2.3		0.627	
36	<i>Picea excelsa</i>	Sun	(14,256)	2,315		0.13 (mean)			
37	" "	"		2,362					
39	<i>Pinus sylvestris</i>	Sun	(5,323)	1,074		0.17			
47	<i>Abies pectinata</i>	Shade			1,986				
49	" "	"			2,022				
69	" "	Sun	(12,394)	2,840		0.18			

Thus Von Höhnel's method of calculation causes the transpiration of evergreen Conifers to seem disproportionately slow, because each leaf has relatively great weight and small surface. Yet when judged by trans-

piration per square centimetre the evergreen Conifers are seen to transpire very slowly, whereas the larch transpires at a rate equalling that of rapidly transpiring deciduous dicotyledons. This is demonstrated by statistics concerning Von Höhnel's second series of experiments conducted during 1879-80 as shown below.

In these experiments, as in the earlier ones by Von Höhnel, great differences showed themselves in the rate of transpiration even of the same species and in the same situation. These particular differences, I think, must have been due to differences in the amount of water supplied, as the grave defect of the pot-method of measuring transpiration is that the rate of transpiration is so largely determined by the amount of water supplied to the roots. Moreover parsimonious watering leads to a shedding of the leaves and a further decrease in the apparent rate of transpiration. Hence, to show indubitably the rapid expenditure of water by the larch-needles, I compared their rate of transpiration with the maximum rate attained by dicotylous trees.

The subjoined statistics are derived from my calculations based upon Von Höhnel's results, and they record the approximate maximum transpiration in grammes per square centimetre during the year 1879-80.

<i>Fagus sylvatica</i>	...	...	7.5	} Exposed to the light.
<i>Larix decidua</i>	...	...	5.4-3.4	
<i>Quercus Cerris</i>	...	...	3.2	
<i>Betula alba</i>	...	...	3.13	
<i>Carpinus Betulus</i>	...	...	2.5	
<i>(Larix decidua</i> in half shade 1.4.)				

In reference to these results, I note the maximum shown by the beech was attained in the smallest specimen, so that it is probable that the high result was due to high rate of watering. Two numbers are given in connexion with the larch exposed to light, because the air-dry weight of larch-needles as measured by Von Höhnel and myself did not agree. Von Höhnel found that 100 air-dry 'lighted' larch-needles weighed 0.165 grm.; whereas mine weighed only 0.1034, while 100 shaded leaves weighed 0.0958 grm. Hence Von Höhnel's larch-needles were either larger, denser, or less dry; and I have calculated the transpiration according to the first possibility (viz. 3.4), as well as according to the surface of the larch-needles measured and weighed by myself (viz. 5.4).

The existence of the larch, with its rapidly transpiring leaves, is in itself fatal to Miss Stopes's hypothesis ('07) that Conifers necessarily have a limited rate of transpiration and 'xerophytic' foliage because the wood is incapable of 'allowing a rapid flow of water'.

(b) THE TRANSPIRATION CURRENT UP THE CONIFEROUS STEM  
MAY BE RAPID.

In view of the rapid transpiration of the larch, and the consequently rapid flow of water along the tracheides of the leaf, and in view of the large number of leaves borne indirectly or directly on the slender twigs, it seemed to me probable that the transpiration current in the larch would be quite as rapid as in dicotylous trees; and this anticipation was strengthened by the fact that the layer of sap-wood of the larch is unusually narrow.

I therefore made experiments as to the rate of ascent of water by means of the ordinary eosin method, in shoots of *Larix decidua*, *Pseudolarix Kaempferi*, *Abies pectinata*, *Pinus sylvestris*, and *Fagus sylvatica*.

The experiments were conducted in such a manner as to give maximum results. They were performed in July, 1909, on hot or warm, mainly sunny days, on which a breeze or wind was blowing: the shoots were exposed out of doors to the breeze, and in most cases to direct intense sunlight, or in a minority of cases to strong diffuse light. So far as possible the shoots were selected with a considerable head of foliage, either on a single axis, or on a branched axis which surmounted a considerable basal leafless portion of the stem; thus the current of the solution at the base was not distributed over several stems but travelled up a single axis. The shoots were amputated from the tree in such a manner that the region of severance was under water at the time of severance. Yet care was taken to avoid extreme results due to 'negative pressure', as before being tested with the eosin solution the detached shoots were kept (in some cases in a confined space in darkness) with their cut ends dipping in water for periods varying from 35 minutes up to more than 24 hours. The duration of these preparatory periods designed to do away with 'negative pressure' is recorded in the second vertical column of the succeeding tables.

I was unprepared for the rapidity of ascent of the dye, so that in a number of cases, when measuring the height to which the eosin had ascended in the shoot, I made the first cut, and sometimes some of the subsequent cuts, too low down the stem. The result was that in these cases the transpiring leaves on the upper part of the shoot were still exercising their suctional power and drawing up the eosin solution. Consequently, the time during which the eosin was ascending the stem was considerably greater than that intervening between the moments of placing the shoot in the eosin and removing it from the eosin. At the time of performing the experiments, I unaccountably failed to appreciate the extent to which this would modify the results as regards rate of flow; and I omitted to record the actual moment at which the highest point reached by the eosin was located, or whether, in making the first cut, I had made it above or below the highest



point reached by the eosin. As, however, my aim is not to give statistics showing the usual rate of ascent of sap, but it is merely to demonstrate that water can and does flow rapidly through coniferous wood under the influence of transpiration, these deficiencies in observation do not matter. In the subjoined tables and calculations I have assumed that the leaves were acting and drawing up the eosin for the maximum time in each case, and have therefore added a 'time-allowance' to the time actually intervening between the moment of dipping the stem in the eosin and removing it. The consequence is that in a number of cases the calculated rate of ascent of the eosin solution is certainly less than the true rate. But where this calculated rate of ascent seems startlingly great, I have been able to prove what was the lowest possible minimum by referring to my notebook and noting the moment at which observations on the next succeeding shoot had commenced. By this method it is proved, for instance, that in the cases of the larch specimens 3, 9, 10, 11, and 14, the lowest possible rates at which the eosin ascended were respectively 132, 153, 138, 131, and 126 centimetres per hour. The statistics given below, then, must not be regarded as doing more than prove that *coniferous wood can and does conduct water at a high speed, and that in the larch the rate may equal that attained under identical external circumstances by a very rapidly transpiring dicotylous tree*, such as the beech. In the larch, rates of 204, 240, and 233 cm. per hour were attained, while in the fewer beech shoots experimented upon, the highest speed calculated was 232.

The calculated rates of ascent in each of the different species varied considerably; for instance, in the case of the larch, between 240 and less than 24 cm. per hour. It is probable that the low rates in the larch may in some cases have been due to the admission of air into the cut end of the stem either at the moment of severance or during transport. In some cases the variation in rate could be correlated with change in the surroundings or differences in the amount of foliage. For instance, in the first set of observations the rates in the cooler, less breezy, less sunny afternoon were less in both the larch and beech than in the morning.

The final table of this series shows the comparative rates of ascent of the eosin in different species under identical external factors, as the measurements made upon the different shoots within 5–15 minutes of one another are recorded on the same horizontal lines of the table.

After the larch and beech, the Scots pine showed the highest speed of flow, namely, 120 cm. per hour, and a minimum of 30 cm. per hour. It thus surpassed in the rate of current the deciduous *Pseudolarix*, whose maximum rate was 78 and minimum 28.5. In *Abies pectinata* the maximum rate was 60, and the minimum 35 or 32.5. These results show that even an evergreen Conifer can have a relatively rapid flow of water through its wood; but they must not be regarded as demonstrating the



relative speeds in the various species, as only three or four shoots of several of the species were experimented upon.

In Ewart's experiments ('05), the maximum rates measured in centimetres per hour for different woody plants were: elder 131, apple 121, elm 205, pear 248, yew 23. Ewart worked with shoots longer (4-8 ft. long) than those I used.

In the subjoined tables the respective vertical columns, commencing at the left hand, represent:—

1. The number by which the shoot was designated.
2. The number of minutes during which the cut shoot remained dipping in water prior to eosin being supplied to it.
3. The number of minutes during which the stem remained dipping in eosin *plus* the number of minutes during which the eosin may have continued to ascend subsequent to the stem being removed from the eosin solution.
4. The height in centimetres to which the eosin had ascended.
5. The calculated rates of ascent of the eosin in centimetres per hour.
6. Any remarks on the shoots or the results obtained.

### LARCH.

(Experiments made in July.)

Specimen Number.	Minutes of Immersion.	Duration in minutes of period of passage of eosin together with allowance time.	Distance covered by eosin in cm.	Rate of ascent of eosin in cm. per hour.	
1	35	25.5 + 1	30.5	72.5	<p style="text-align: right;"><i>Set I</i></p> <p>The dye had entered the foliated part of the axis and the measurement is thus a maximum, but the record of the hour of the next experiment proves that the lowest possible minimum rate was 132.</p> <p>The dye had reached the terminal bud of the shoot, so that this measurement 110 does not record the full rate of ascent.</p> <p>This shoot had six long branches and plenty of foliage: possibly the slow ascent was due to entrance of air at the cut end of the stem.</p> <p style="text-align: right;">Morning: warm, breezy, sunny; shoots exposed to direct sunlight.</p> <p style="text-align: right;">Afternoon: cooler, less breeze, not sunny.</p>
2	70	10 + 1	12	65	
3	90	13.25 + 1	42	177	
4	95	20.25 + 1	39 +	110 +	
5	120	3 + 1	2.5	38.5	
6	150	5 + 1	7.5	75	
7	180	6 + 1	9	77	

## LARCH (continued).

Specimen Number.	Minutes of Immersion.	Duration in minutes of period of passage of eosin together with allowance time.	Distance covered by eosin in cm.	Rate of ascent of eosin in cm. per hour.	
19	One whole day	7 + 1	11	82.5	<p>(In this and the other shoots of this set, before experimenting with eosin, a piece, <math>1\frac{1}{2}</math>-<math>2\frac{1}{2}</math> cm. in length, was cut off the lower end of the stem under water.) This shoot was unbranched, 40 cm. long, well foliated at the end, and had three isolated tufts of leaves on the otherwise unfoliated basal part. The dye had gone past these three tufts, but not among the other foliage.</p> <p>This shoot gave off from the lower unfoliated part of the stem a long shoot, so that two measurements are given, the first recording the ascent of the eosin up the main stem, and the second up the basal part of the main stem and the long branch. In connexion with the second the time was reckoned as being taken at the <i>conclusion</i> of the observations on the ascent, so that the measurement is a minimum one.</p> <p>This slow rate of ascent is possibly due to entrance of air into stem.</p>
20	One whole day	11.75 + 1	21	99	
		16.5	31	113	
21	One whole day	13 + 1	13	56	<p><i>Set III</i></p> <p>Morning: sunny, breezy; shoots exposed to direct sunlight.</p>
8	50	9.5 + 1.5	26	142	
9	70	5 + 2.5	25.5	204	
10	75	6.5 + 1	30	240	<p><i>Set II</i></p> <p>The stem ended in a long leafless dead part (up which no eosin travelled), but gave off a long branch foliated at its terminal part; otherwise there were no branches.</p> <p>This shoot bore three long foliated branches and ended in a dead part of the relatively main stem: the measurement is up the main stem and thence up the topmost foliated branch. Full allowance is made for the ascent of eosin after removing from the solution, but details of the time of observations in the succeeding experiment prove that the minimum possible is not less than 153 for the rate of ascent of the eosin per hour.</p> <p>This shoot was branched and ended in a dead part of the main stem: the measurement concerns the ascent up the main stem and highest foliated branch. The time of the next observation absolutely proves that in this case the lowest possible minimum rate of ascent was 138 cm. per hour.</p> <p>The shoots were exposed to direct sunlight on a warm breezy July day in the morning.</p>

LARCH (*continued*).

Specimen Number.	Minutes of Immersion.	Duration in minutes of period of passage of eosin together with allowance time.	Distance covered by eosin in cm.	Rate of ascent of eosin in cm. per hour.	
11	80	3 + 1.5	17.5	233	<p>This shoot was unbranched, with the foliage confined to the more terminal parts; the dye had entered the foliated part. The time when the next observation was made proves that the lowest possible minimum rate of ascent was 131 cm. per hour.</p> <p>This measurement records the rate of movement up the leafless part of the main stem and of the lowest long branch among whose foliage the eosin entered. The time of the next observation proves that the lowest possible minimum rate of ascent was 126 cm. per hour.</p> <p>The eosin had ascended less than 3 cm., solely up the leafless basal part of this branched shoot.</p> <p><i>Set II (continued)</i></p> <p>The shoots were exposed to direct sunlight on a warm breezy July day in the morning.</p>
12	140	5.5 + 1	3.5	32	
13	145	6 + 1	5.5	47	
14	155	4 + 2	17.5	175	
15	160	6.5 + 1	3-	24-	
16	110	5 + 1	13.5	135	<p><i>Set IV</i></p> <p>This branched shoot was only feebly foliated; hence the low rate of ascent.</p> <p>There was an uncertainty as to the exact time of commencing the experiment, as it may have been initiated one minute earlier than recorded in my notes. This shoot had been blown over, so that it did not dip in the eosin during the whole of the time.</p> <p>Plants exposed to the sun in an afternoon that was moderately bright, and with a considerable breeze blowing.</p>
17	120	11.5 + 1.5	17.5	81	
18	155	6 (or 7)	7.5	75 (or 64)	

## SILVER FIR.

1	170	16.25 + 2	18.25	60	<p>A branched well foliated shoot. The eosin had ascended the main leafless stem past one foliated branch.</p> <p>Exposed to full sunlight on warm, sunny, breezy, July midday.</p>
2	125	11 + 1	7.5	37.5	Shoot about 45 cm. long.
3	154	12 (or 11)	6.5	32.5 (or 35)	Shoot well foliated, 50 cm. long. But the apparatus was blown over in the course of the experiment, so that the shoot was not dipping in the eosin throughout the whole time.

## SCOTS PINE.

Specimen Number.	Minutes of Immersion.	Duration in minutes of period of passage of eosin together with allowance time.	Distance covered by eosin in cm.	Rate of ascent of eosin in cm. per hour.	
2	A whole day	16.5 + 3.5	36 +	108 +	<p>The dye had reached the terminal bud, so that the distance recorded is probably not the full one that might have been attained, hence the rate of passage exceeds 108 cm. per hour. The time of the next observation conclusively proves that the minimum rate could not have been less than 93 cm. per minute.</p> <p>The dye had gone solely up the basal leafless part of the shoot, which was 30 cm. long.</p> <p>The dye had gone up to the terminal bud, hence the full possible distance is not recorded, and hence the rate of ascent may have exceeded 120. The dye also went up a branch, as denoted in the second statistics.</p>
3	A whole day	10 + 1	10	54.5	
4	A whole day	12.5 + 2.5	30 +	120 +	
		12.5 + 3.5	32	120	
1	115	10 + 1	6	35	<p>The second measurements deal with the journey of the eosin up the main stem and a branch; while the first concern the journey of the eosin up the main stem only.</p>
		10 + 2	6	30	

Set III

Shoots exposed to full sunlight on a bright breezy morning in July.

Set IV  
Afternoon: breezy, clear, and warm.

## PSEUDOLARIX.

1	100	5 + 1.5	3.1	28.5
2	120	6 + 1.5	6.5	52.5
3	175	9 + 1	13	78

## BEECH.

1	35	23 + 1	17 +	42.5 +	<p>The dye had reached the terminal bud, hence the distance and rate of travelling of the eosin are not up to the full possible.</p>
2	70	6 + 1	26 +	223 +	
3	90	7.5 + 1	19	134	<p>The eosin had reached the terminal bud, hence the distance and rate of travelling of the eosin are not up to the possible full.</p>
4	95	15.5 + 1	44.5	162	
5	160	5 + 1	11	110	

Set I

Warm, sunny, breezy morning.

Cooler, though warm, less breezy, not sunny.

BEECH (continued).

Specimen Number.	Minutes of Immersion.	Duration in minutes of period of passage of eosin together with allowance time.	Distance covered by eosin in cm.	Rate of ascent of eosin in cm. per hour.	
6	45	8.5 + 1	22.5	142	This shoot was forked and bore foliage only towards the ends of the two arms. The first measurement is given first; probably a larger time allowance should be made for the second.
		8.5 + 2	27.5	157	
7	175	4 + 3.5	29	232	This main stem gave off a number of dwarf foliated shoots, and the eosin was carried past six of these. The time allowance is so ample that the rate per hour is probably considerably below the real speed.
8	90	6 + 1	14	120	Shoot was about 52 cm. long.
9	140	4.5 + 1	8.5	93	The shoot was branched and the eosin had gone up past one foliated little branch, but scarcely entered this.

Set II

Warm, sunny, breezy July morning.

Set IV

Warm, moderately bright, rather breezy.

RATE OF ASCENT OF EOSIN IN CENTIMETRES PER HOUR.

(On same horizontal line the experiments synchronize in time to within fifteen minutes.)

Silver Fir.	Scots Pine.	Larch.	Beech.	
		72.5 65 177 (110+) 38.5 75 77	(42.5+) 223+ 134 162  110	Set I (July). Morning: hot, sunny; fresh breeze blowing: shoots exposed in the open to direct sunlight. Afternoon: cooler (though warm), a gentle breeze blowing, not so sunny: plants exposed to full light.
		142 204 240 240 32 47 175 (24-)	142 (157)       232	Set II (July). Warm sunny morning with a breeze. Shoots exposed to direct sunlight.
	(108+) 54.5 (120+) 120	82.5 99 113 56		Set III (July). Morning: sunny, with a breeze.
37.5 32.5 or 35	35 30	135 81 75 or 64	120 93	Afternoon: not so sunny as in the morning, yet warm: stronger breeze than in the morning.

But apart from the positive results demonstrating the rapid rate of the transpiration current in the larch, we are not justified in the assumption that there is a universal and direct proportion between the habitual or maximum rate of transpiration of leaves and the calibre of vessels or tracheides. And it may be remarked here that Ewart's experiments, so far as they register rates of flow under the influence of transpiration, merely record the rate at which the eosin ascended, and the approximate rate at which the water ascended, under the conditions of transpiration then prevailing; but they do not record the rate at which water could easily ascend under a higher rate of transpiration, nor do they demonstrate that there is any universal rule that the calibre of the wood vessels is directly proportional to the rapidity of the transpiration current of a tree. It is true that in climbing plants where the transpiration current is known to be very rapid, the tracheae are notoriously wide. Ambronn and Westermaier explain this as an expression of the principle that the resistance to the travelling of water up tubes decreases with increase of diameter of these. Decrease or increase in diameter of the conducting tubes is only one of several possible arrangements to decrease or increase the transpiration current.

Von Höhnel's prolonged experiments supply us with statistics for comparing the rate of transpiration and the width of the vessels.

In the subjoined table the first column gives the name of the tree. The second and third represent the number of grammes of water transpired in 1878 (June to November) and in 1879 (April to November) per gramme dry weight of leaf. The fourth and fifth columns record my calculations of the rate of transpiration per square centimetre during the same periods. The sixth and seventh columns record the maximum rates of transpiration (in the months of June, July, or August, 1879) attained, reckoned respectively per gramme dry weight and per square centimetre of leaf-surface. The eighth column gives the calibre attained by the wood vessels.

	Per gramme weight.		Per sq. cm.		Per grm. weight maxim.	Per sq. cm. maxim.	mm. Calibre.
	1878.	1879.	1878.	1879.			
<i>Betula alba</i> . . . . .	67.9	84.5	2.7	3.36	23.1	0.85	0.1-0.131
<i>Fraxinus excelsior</i> . . . . .	56.7	98.3	1.9	3.26	25.8	1.1	0.2
<i>Fagus sylvatica</i> . . . . .	47.2	85.9	1.3	2.4	55.3	2.2	0.057-0.076
<i>Acer Pseudoplatanus</i> . . . . .	43.6	61.8	1.4	2	24.7	0.67	0.1
<i>Carpinus Betulus</i> . . . . .	56.2	75.9	1.2	1.6	24.3	0.25	0.057
<i>Quercus Cerris</i> . . . . .	25	61.4	1	1.7	20.5	0.77	(0.35)
<i>Acer platanoides</i> . . . . .	46	51.7	1	1.17	12.1	0.27	0.07
<i>Quercus Robur</i> . . . . .	28.3	66.2	0.83	1.9	11.23	0.79	0.25(-3.5)

The figures giving the dimensions of the vessels are from Pedersen's pamphlet, except in the case of *Quercus Cerris*, which I measured.



Ranging these statistics in order, by reckoning the largest as 1 and so forth to 8, which is the smallest, the position taken by the respective trees as regards transpiration and calibre of vessels is as follows:—

	Transpiration per grm. dry weight.			Transpiration per square centimetre.			Calibre of vessel.
	1878.	1879.	Maximum.	1878.	1879.	Maximum.	
1. <i>Betula alba</i> . . . .	1	3	5	1	1	2	4
2. <i>Fraxinus excelsior</i> .	2	1	2	2	2	3	3
3. <i>Fagus sylvatica</i> . .	4	2	1	[4	3	1	7
4. <i>Acer Pseudoplatanus</i>	6	6	3	[3	4	4	5
5. <i>Carpinus Betulus</i> .	3	4	4	[5	7	6	8
6. <i>Quercus Cerris</i> . .	8	7	6	[6 (7)	6	5	1
7. <i>Acer platanoides</i> . .	5	8	7	[6 (7)	8	7	6
8. <i>Quercus Robur</i> . .	7	5	8	8	5	8	2

Where square brackets point to pairs of numbers these approach equality. According to calculations both by surface and by weight the three trees transpiring most rapidly are *Betula*, *Fraxinus*, and *Fagus*. Of these, only one, *Fraxinus*, has vessels which come within the first three for size. While the lowest four as regards transpiration include *Quercus Cerris* and *Q. Robur*, which have the largest wood vessels. When the larch is included in the series, the lack of proportion between diameter of the conducting tubes and rate of transpiration becomes still more obvious.

(c) WHY HAVE THE CONIFERAE PRESERVED THE TRACHEIDAL STRUCTURE OF THEIR WOOD?

As tracheae<sup>1</sup> have been evolved among Pteridophyta and occur in gymnospermous forms, we are not justified in assuming that the primitive Coniferae found it impossible to give rise to tracheae. The mechanism is such that it suffices not only for slowly transpiring leaves of evergreen conifers, but also for the rapidly transpiring ones of the larch.

There are, in fact, some reasons for believing that the tracheidal mechanism is better suited than would be a tracheal mechanism with wide vessels to normal coniferous habits (including the structure and slow working of the evergreen leaf and the root). Though it has been indicated above that we are not justified in believing that there is a universal proportion between rate of transpiration and calibre of tracheae, there is no doubt that in some cases the method of widening or narrowing the tracheae is adopted when the transpiration increased or decreased respectively. This may show itself in the different shoots of one individual, where one is a branch-spine with reduced foliage and the other is a foliated branch; for instance, in the spines of *Randia dumetorum* (Groom, '92) the vessels are narrower and scantier. Lothélier ('93) found the vessels and parenchyma less developed in spines than in foliated branches.

<sup>1</sup> In this paper the terms 'trachea' and 'tracheal' solely refer to wood vessels.

In different species of one genus at least some desert types show a decrease in the calibre of the tracheae when compared with mesophytic species. For instance, Volkens ('86) thus describes the wood of the small shrub *Convolvulus lanatus*: 'The wood-cylinder consists almost entirely of thick-walled libriform [fibres] and uniseriate medullary rays; and only after more close examination does one discover the few and narrow vessels.' Apparently, in a number of other desert species, the limitation of the water-conducting channel is accomplished rather by a diminution in the number of the vessels; for Volkens, describing the structure of the long, slender, poorly foliated branches of *Cocculus Leaeba*, states that the 'water-conducting system consists mostly of tracheides with bordered pits', and scattered at intervals among these are 'tracheae of unusual width'. It is evident that the tracheae in desert plants are not always very small, for Volkens describes those of *Zizyphus Spina Christi*, which has small caducous leaves, as being 'unusually wide'. But the descriptions of the wood of desert plants are generally too vague as regards number and size of vessels; moreover, comparisons in these respects with more mesophytic species are required.

Yet it is clear that, in many cases, in xerophytic desert types there is a tendency towards reduction in size or number of tracheae. But this conclusion, drawn from plants growing in the Egypto-Arabian deserts, seems to be at variance with results obtained by Cannon.

Cannon ('05) has given an account of the comparative abundance and size of the tracheae in irrigated and non-irrigated specimens of identical species growing in the desert near Tucson (North America). His surprising results, which are illustrated by micro-photographs, must be regarded as absolutely reliable as regards *facts*. He found that on equal measured areas of the cross section the non-irrigated plants had more numerous and often wider tracheae. But in *interpreting* these results care must be taken, as Cannon used for observation stems of approximately equal thickness. But the irrigated stems thickened more rapidly than the non-irrigated. Hence the relatively smaller number of tracheae in the irrigated specimens may have been due to an increase in the width of the zone of growth, which was not balanced by a corresponding increase in the number of wide tracheae. For instance, a specimen of *Quercus Robur* growing in a poor situation (say on dry soil), when compared with one growing in a good situation, shows a larger number of wide tracheae in any measured area, because its annual rings are narrow and yet have a very heavy percentage or approximately the full number of wide tracheae. Again, as regards the width of the tracheae, comparison was made by Cannon between younger irrigated and older non-irrigated plants; but we know that often at least, if not always, the width of the corresponding tracheae increases gradually outwards up to a certain limit in the successive annual rings. Hence the

younger age of the irrigated specimens might be responsible for the smaller calibre of the vessels.

It is thus possible that the absolute number of vessels in any corresponding zone of growth in thickness (in an annual ring, if present) is greater in the case of the irrigated plants, and that even the width of the large tracheae is greater.

But another consideration arises in connexion with Cannon's results, namely, that since comparison was made between stems of equal thickness but unequal age, we do not know which stem bore a larger leaf-surface, nor which shoot had a larger transpiration.

It will be interesting if more critical comparison of the two kinds of individuals does show what Cannon's results at present apparently suggest, namely, that when these xerophytic individuals modify their foliage in a mesophytic direction, there is a tendency for a reduction in the number and calibre of the tracheae.

Even then, however, we should not be justified in concluding that the change of structure in the individual under changed circumstances marks out the line of evolution of the species under like prolonged changes of environment.

Thus the consideration of desert plants so far gives us no safe guide as to the structure of wood in relation to xerophytic foliage. And the question now arises as to whether there is any relation between the structure of the wood of a temperate species and the evergreen habit.

Examining the three tall British woody evergreen species—box, holly, and ivy—which seem to be oecologically allied to evergreen north-temperate conifers, we find that two of the three are characterized by the small calibre of their tracheae.

*Buxus sempervirens* has tracheae that are no greater in calibre than are the spring-tracheides of the Scots pine. *Ilex Aquifolium* has vessels that are scarcely wider. In *Hedera Helix*, the tracheae are neither scanty nor minute; though they are narrower than those of *Bryonia* and *Clematis* they are broader than those of *Lonicera Periclymenum*. Eliminating the climbing plant, the two others, like a number of cold-temperate Coniferae, thrive best in damp situations, and show the nearest possible tracheal approach to the tracheidal wood of Coniferae. In fact, all the evergreen trees of cold-temperate Europe, whether coniferous or dicotylous, have approximately the same xylem mechanism in their older stems. As the smallness of the number of these dicotylous trees prevents us from drawing the conclusion that the evergreen habit is linked with the narrowness of the tracheae, it seemed possible that an examination of one genus with deciduous and evergreen species might throw light on the question.

Accordingly, I measured the maximum size of the vessels in American deciduous and evergreen species of *Quercus*. The specimens used were

from Hough's collection. To ensure uniformity of magnification, the vessels were drawn (with a camera lucida), and with the microscope *immovably fixed* at a focus giving a magnification of twenty diameters. In order to ensure impartial observation the actual drawings were made by Miss Beatrice Rhodes. For the measurements I am responsible. The resultant figures as to the actual diameter of the vessels were obtained by calculating the mean of the two diameters of two tracheae of each species.

In the subjoined tabular statement the statistics of the deciduous and evergreen species are given in parallel columns, viz. in the order of decreasing diameter of the tracheae.

<i>Deciduous Species.</i>			<i>Evergreen Species.</i>		
	<i>Diameters of two tracheae magnified 20 1 in mm.</i>	<i>Mean diameter in mm.</i>		<i>Diameters of two tracheae magnified 20 1 in mm.</i>	<i>Mean diameter in mm.</i>
1. <i>Q. Michauxii</i> . . .	{ 13 + 10 13.5 + 10.5	0.587	<i>Q. Engelmanni</i>	{ 7.5 + 6.5 7 + 5.25	0.328
2. { <i>Q. bicolor</i> . . .	{ 11 + 7.5 10 + 7.5	0.45	<i>Q. agrifolia</i> . . .	{ 7.25 + 5 7.25 + 6.5	0.325
{ <i>Q. alba</i> . . .	{ 9.5 + 9.5 9.5 + 7.5	0.45			
4. <i>Q. Muhlenbergii</i>	{ 10 + 7.5 10.5 + 7.5	0.444	<i>Q. virens</i> . . .	{ 7 + 5.5 7 + 5	0.306
5. <i>Q. aquatica</i> . . .	{ 10 + 7.5 10 + 7.5	0.438	<i>Q. hypoleuca</i> . . .	{ 7.25 + 5.5 6.5 + 4.75	0.300
6. <i>Q. palustris</i> . . .	{ 10 + 7 10 + 6.5	0.419			
7. { <i>Q. tinctoria</i> . . .	{ 9 + 7.5 9 + 7.5	0.412	<i>Q. Emoryi</i> . . .	{ 6.5 + 5 6.25 + 5.5	0.291
{ <i>Q. coccinea</i> . . .	{ 9 + 8.5 8.5 + 7	0.412	<i>Q. chrysolepis</i> . . .	{ 6.5 + 5 6 + 5.25	0.284
9. { <i>Q. rubra</i> . . .	{ 10 + 5.75 9.25 + 6	0.387			
{ <i>Q. Prinus</i> . . .	{ 9 + 6.75 9 + 6.25	0.387	<i>Q. Wislizeni</i> . . .	{ 6.5 + 4.25 6 + 4	0.259
11. <i>Q. californica</i> . . .	{ 9 + 6.5 8.5 + 6.25	0.378			
12. { <i>Q. Macdonaldi</i> . . .	{ 9.5 + 6 8.5 + 5.75	0.372	<i>Q. tomentella</i> . . .	{ 5.5 + 3.75 5 + 5	0.241
{ <i>Q. macrocarpa</i> . . .	{ 8.75 + 7 8.5 + 5.5	0.372			
14. <i>Q. obtusiloba</i> . . .	{ 9 + 6.5 8 + 5.5	0.362			
15. <i>Q. lobata</i> . . .	{ 9.5 + 5.5 8.5 + 5	0.356			
16. <i>Q. Garryana</i> . . .	{ 9 + 5 8 + 5	0.338	<i>Q. densiflora</i> . . .	{ 5.5 + 4.5 4.5 + 4.25	0.234

Sub-evergreen or sub-deciduous.

Truly evergreen.

There are several points worthy of notice in connexion with these results:—

1. The sixteen species with the widest tracheae are deciduous. And the leading six of these include the only four species that habitually grow in swampy places. The last of the series, *Q. Garryana*, is described by



Sargent ('05) as growing on dry gravelly slopes and as having leaves 'thick and firm or subcoriaceous'.

2. The nine species with the narrowest tracheae are all evergreen. But of these, the five with the widest tracheae are what I may term 'sub-evergreen' or 'sub-deciduous', as all their old leaves are cast off at the same time, or very shortly after, the new ones unfold in spring, while the four species having the narrowest tracheae are the only four that are typical evergreen.

Miss Beatrice Rhodes also measured the mean comparative diameters of the largest vessels of two to four-year-old twigs of three species of *Fagus*. They are less in the two evergreen South American species, *Fagus Cunninghami* (0.0155 mm.) and *F. betuloides* (0.0225), than in the deciduous *F. sylvatica* (0.03) and *F. obliqua* (0.043), the latter of which is also South American. But the wood of an older piece of *F. Cunninghami* from Nördlinger's series gave a diameter of 0.057, and that of an older stem of *F. sylvatica* a diameter of 0.052. Undoubtedly the twig of *F. Cunninghami* was correctly named, as it came from a plant growing at Kew: there is no equal guarantee that Nördlinger's specimen was genuine.

But just as in desert types there seem to be two alternative methods of reducing the conducting channels, namely, by decreased number or decreased calibre of the tracheae, so in temperate evergreens the same appears to be the case. For I find that in comparing the wood of *Prunus domestica* with that of *P. lusitanica* the tracheae are of approximately the same size in the two species, but they are far more scanty in the latter species.

That desert types and these evergreen species should show two different methods of bringing about the same result, namely, a limitation in the number of wide tracheae, suggests strongly that the phenomenon is not merely a case of inevitable correlation, but represents a structural change directly beneficial to the species.

The remarkably exact agreement between deciduous and evergreen habit and width of tracheae in *Quercus*, the evidence supplied by *Buxus* and *Ilex*, and the divergent case of *Prunus*, all suggest that even if the ancestors of the Coniferae had possessed deciduous leaves and tracheae, the assumption of the evergreen habit, coupled with the acquisition of small xerophytic leaves, would have been associated with a reduction in the number or calibre of the tracheae, until the wood more or less perfectly agreed in structure with that of existing Coniferae.

Thus the question arises: Is there any evidence of evergreen dicotylous woody plants having undergone such a process of reduction in their wood? The Magnoliaceae and Trochodendraceae supply examples whose true interpretation may perhaps be the one suggested. *Drimys*, the magnoliaceous genus characterized by having its water-conducting tubes consisting of tracheides only, is evergreen and in austral regions marks the limit of its

family as regards high latitude: it would be interesting to know if farther north the altitudinal limit is higher than that of other Magnoliaceae. The magnoliaceous *Zygogynum* also has wood of the same type, according to Van Tieghem ('00), but particulars concerning the exact habitat and evergreen or deciduous nature of the species seem to be lacking. The Trochodendraceae are composed of five genera. Of these *Trochodendron* and *Tetracentron* possess wood that is devoid of tracheae; while *Cercidiphyllum*, *Euptelea*, and *Eucommia* have true tracheae. Accordingly, we find that *Trochodendron* is evergreen: whereas *Cercidiphyllum*, *Euptelea*, and *Eucommia* are deciduous. Again, the evergreen *Trochodendron* with its tracheidal wood marks an extreme of distribution in the family as it occurs in the alpine region of Japan. *Tetracentron sinensis* is, however, deciduous, with leaves of hygrophytic structure. Yet its tracheides are much wider than those of *Trochodendron*, and at their dovetailing ends have very numerous pits with pit-membranes so extremely thin that the arrangement is the nearest approach to vessels with lattice-work perforations. It is interesting to note that the Trochodendraceae have their centre of preservation in China and Japan, where so many evergreen Conifers have been preserved: while *Drimys Winteri*, an extreme southern form, grows side by side with evergreen species of *Fagus*, austral Conifers, Myrtaceae, and Proteaceae, also species of *Berberis* and *Ilex*.

Degeneration of tracheal xylem to tracheidal xylem has certainly taken place in certain xerophytic species of *Tillandsia* (*T. usneoides* and others).

That this tracheidal structure of the evergreen north-temperate Coniferae is not a mere inevitable correlation phenomenon, but constitutes a mechanism that is best fitted to the evergreen Coniferae, is suggested also by the wide distribution and the survival of the Coniferae: and this question will be discussed later in the paper. Here I may merely indicate some possible advantages reaped by such temperate evergreen species through this tracheidal structure of the wood. It is easy to conceive that the acquisition of wider vessels would be a source of danger by facilitating a flow of water to the buds at times when available water was plentiful, and thus inciting the plant to produce a larger surface of foliage, and that of a less xerophytic type; such a change would bring with it the risk of injury or death from desiccation during either the physiologically dry or wet season. Again, we know that the rate of transpiration of a plant is greatly increased by increase in the rate of absorption and consequently of supply of water; it may therefore be that the narrowness of the tracheides (or tracheae) is itself a device for opposing resistance to the ascent of water, and for thus depressing transpiration. Strasburger ('91) demonstrated that more pressure is required to force water longitudinally through coniferous wood than through dicotylous wood possessing fair-sized vessels.



Finally, the narrowing of the tracheae involves a relative increase in the strength of the wood, and a consequent opportunity for economy of material.

(d) RATIONALE OF THE XEROPHYTISM OF THE CONIFERAE.

As already indicated, Schimper's theory, so far as water supply and water expenditure are concerned, explains the survival in north-temperate regions of the Coniferae which live in places where there is a physiologically dry season.

But, in seeking for a full explanation of the evolution and survival of Coniferae, it appears to me that one fundamental factor in their architecture has been overlooked, namely, the aggregate leaf-surface.

The amount of water emitted by a plant varies directly with the aggregate leaf-surface and the histological structure of the individual leaf. Now, if we imagine two plants, living within identical surroundings, to have equal powers of absorbing water, but one to have twice the leaf-surface of the other, then, if the one having the smaller leaf-surface just manages to retain sufficient water for its existence, it is obvious that the other will require more marked xerophytic structure in its foliage. Similarly, if there be two plants of equal leaf-surface, but unequal powers of absorbing water, and the one with the larger power of absorbing water contrives just to obtain and retain sufficient water, then the other will again have need of a more xerophytic type of leaf-structure. Such a type of xerophytism as is not evoked by edaphic or climatic agencies, but is dependent upon the organization of the plant itself, may be termed *architectural xerophytism*. And in any terrestrial community of plants, whether it be in an English meadow or a sub-tropical desert, one is apt to find a mixture of plants showing various grades of architectural xerophytism, mesophytism, and tropophytism; for instance, in a desert one can find growing side by side a deep-rooted plant with mesophytic foliage (e. g. the colocynth), a succulent tropophytic plant with delicate foliage (e. g. species of *Senecio*), and numerous typical xerophytes.

That the xerophytism of Coniferae is partly architectural in nature is demonstrated by several facts:—

1. Though the individual leaf is small *the aggregate leaf-surface of the conifer often greatly exceeds that of the dicotylous tree.*

In Table I, column 2, it will be seen that with specimens of equal age or size, the trees showing the largest aggregate leaf-surface are the Coniferae. (All the trees used by Von Höhnelt in 1878 are described by him as being five to six years old and about 70 centimetres high.) The trees with the largest leaf-surface were *Picea excelsa* (14,250 sq. cm.), *Abies pectinata* (12,400), *Pinus sylvestris* (5,300); and the dicotylous trees most nearly approaching these were *Acer platanoides* (4,500) and *Alnus campestris*

(4,000), while the other dicotylous specimens had considerably smaller aggregate leaf-surfaces.

2. Despite the low rate of transpiration of the single leaf or of a unit of its surface, at least *some north-temperate Coniferae expend and need as much water as do ordinary dicotylous trees.*

This is evidenced by Von Höhnel's results obtained in 1878, as these represent the nearest approach to a minimum (see Table I, columns 4 and 5).

3. The need for a certain amount of water on the part of certain Conifers (spruce, silver firs, and some pines, &c.) is demonstrated by the natural distribution of these trees, the experience of foresters, and by the failure to grow in Continental Europe certain Conifers that thrive when introduced to the more humid climate of the British Isles.

The conclusion to be drawn from these facts is that *such is the aggregate leaf-surface of cold-temperate Conifers that even with their xeromorphic and xerophytic leaves numbers of species succumb from desiccation or grow feebly in places where ordinary dicotylous forest trees can thrive.* It is therefore evident that were their foliage less xeromorphic or xerophytic, the result would be fatal unless the assimilating surface were reduced greatly. Such a reduction of the transpiring surface would involve a serious reduction in the assimilating organs and in the capacity of the tree to maintain itself as such.

As we have no comparative statistics in regard to the relative amount of assimilation and transpiration in different types of leaves within different surroundings, it is impossible to explain why the Conifer should have adopted the device of having a large aggregate surface with a greater degree of xerophytism. Yet a comparison with the oligotrophic small-leaved evergreen Ericaceae, the fact of the occurrence of various Coniferae on soils so poor that these species must be oligotrophic, and, finally, the existence of epiphytes with large leaves and xerophytic structure, cause one to hazard the guess that concurrent increases in the assimilatory surface and in the xerophytic devices generally increase assimilation in relation to transpiration.

(e) THE EFFICIENCY OF THE MECHANISM AND THE SURVIVAL  
OF CONIFERAE.

The statistics that I have given in regard to the width of the tracheae of the various species of *Quercus* might bear an interpretation different from the one stated above. It might be suggested that the evergreen species were the primitive ones, and that the evolution of the deciduous species was facilitated by the power of adding to the width of the tracheae. For, although the deciduous larch arose without any corresponding change in the water-conducting constituents, it is probable that increased width of the tracheae in more rapidly transpiring deciduous dicotylous trees is of use

either in decreasing the resistance to flow or in providing wider reservoirs that more readily part with the contained water.

Hence, it remains to inquire if the evergreen habit associated with narrow water-conducting tubes provides an efficient and successful mechanism.

In the genus *Quercus* the evergreen species show a wide range of habitat and general distribution. Evergreen oaks occur in dry habitats (Mediterranean and North America), ascend to 11,000 feet in the Himalayas, descend to the humid forests on the lower slopes of the Himalayas, and occupy truly tropical situations in the forests of Indo-Malaya. They therefore show a wide range of habitat and general distribution.

Evergreen northern Coniferae, which have more reduced individual leaves, vie with or even surpass dicotylous trees in range of habitat and distribution. It is well known to foresters that sundry Conifers, including the Scots pine, can thrive on soils too poor for the successful cultivation of dicotylous forest trees. In the Northern Hemisphere, not only in temperate but in tropical regions, they take possession of less favoured sites on mountains and hills. But on more favourable situations on mountains in Europe, North America, and Asia they can give rise to forests and defeat dicotyledons. In some instances at least, the Conifer defeats the dicotyledon in favourable sites; thus in the battle between two shade-enduring species, *Abies pectinata* and the beech, the former is sometimes the victor and drives its foe before it (for instance, in the Schwarzwald in Würtemberg). R. Gradmann ('00) has given an interesting analysis of the struggle between *Picea excelsa* and *Fagus sylvatica* in southern Germany. He shows that the victor below 400 metres is the beech, but above 1,000 metres the spruce; while the belt between 400 and 1,000 metres altitude is the region of a ceaseless struggle in which soil often decides the result, as the spruce is usually victorious on sandy soil and the beech on calcareous soil, but even on the latter soil heavy battalions of spruce may drive the beech on to the driest sites. Other examples of the defeat of dicotyledons (oak for instance) by spruce might be given. In India, *Pinus longifolia*, *P. Khasya*, and *P. Merkusii* form forests at altitudes at which tropical vegetation is wont to prevail.

Again, judged by range of the single species, the coniferous mechanism shows its efficiency. *Juniperus communis* in its ordinary form extends from arctic regions and alpine altitudes in the cold-temperate region down to the Mediterranean shores, where it reaches sea-level. As an oligotrophic plant it grows on sunny rocks, the driest dune-sands, dry heaths, and soaking sphagnum bogs; yet it can thrive on good soil and in less lighted places, and grow as underwood even in shady forests of silver firs and beech in northern Europe, or in moist misty country in southern Europe.

*Juniperus recurva*, occurring at an altitude of 16,000 feet on the Himalayas, yet thrives in Calcutta Botanic Gardens (but without flowering in the latter place, as Colonel Prain informs me). The Scots pine occurs in extremely varied habitats, as is notorious.

The genus *Pinus* shows a climatic range of distribution that I believe to be unequalled by any dicotylous arboreous genus. Occurring in arctic regions (at 70° N.), it extends southwards to the Equator (in Sumatra); and in southern Burma has one species, *P. Merkusii*, occupying a truly tropical position at only 500 feet above sea-level. *Pinus* includes species growing beyond the altitudinal and latitudinal limit of forest. Some species live in good soils in company with dicotylous trees and demand moist air; others occur in Mediterranean and Californian regions among sclerophyllous vegetation, often on particularly dry sites; still others grow in dry sterile sands or in swamps of various types (peat-bogs, wet sand, muddy swamp).

The mechanism that permits of such range of single species or genera must be an efficient one under very diverse circumstances, and must afford ample explanation of the survival of modern Coniferae. Certainly the plasticity of the coniferous evergreen foliage of one species is usually underestimated, first because its most obvious feature concerns differences in the length of the needles (which differences may exceed 1,000 per cent.), but the needles also show change of thickness and surface, and above all they exhibit great differences as regards longevity, so that the Scots pine, for instance, may prolong the life of some of its needles from the average 2-3 years to 8-9 years. The general impression that the evergreen north-temperate Conifer as a *working machine* is inferior to that of the deciduous dicotylous tree in the same region is, perhaps, a mistaken one. The two types of trees may represent rather two alternative mechanisms of approximately even efficiency when in normal action; and the low-tension coniferous machine in places outworks its high-tension tropophytic rival.

#### (f) THE VULNERABILITY AND SUPPRESSION OF CONIFERAE.

If then the coniferous mechanism in normal working really be as efficient as the dicotylous mechanism, it would seem possible that the defeat of the Coniferae by dicotylous trees and the suppression of many forms are due to the coniferous machine being more easily put out of order or being more exposed to injury.

In connexion with the second alternative, there is no doubt that the tree preserving its xerophytic leaves during the physiologically dry season, or in extremest conditions, is exposed to greater danger of desiccation than is a tree that is devoid of foliage during the same season. Though, as Von Höhnelt points out, we cannot rely upon his results as regards the amount of water given off by trees during winter (as the trees were kept under shelter in a room), the results sufficiently indicate the greater loss of water



by Conifers during that season. Yet despite of this, evergreen Coniferae advance into the extremest positions in the cold-temperate region. Although *Larix sibirica* reaches  $72^{\circ} 30'$  N. as a prostrate shrub, *Pinus sylvestris* and *Juniperus communis* reach  $70^{\circ}$  N., and *J. communis* var. *nana* goes at least as far north as  $71^{\circ}$  N. Again, while *Larix decidua* ascends to an altitude of 2,400 metres in Switzerland, this limit is equalled in the same country by *Pinus montana* and surpassed by *Juniperus communis* var. *nana*. Yet it is possible that in its tree-form *Larix* is better capable than any other conifer of enduring extreme cold, and we know that *Taxodium distichum* advances into an extreme habitat of another kind, namely swamp. Moss ('07) gives statistics suggesting that in north-temperate types certain deciduous species attain higher latitudes and altitudes than do allied evergreen species. In this connexion it may be pointed out that in the tall evergreen humid forests of Burma and the Malabar Province, the majority of the tallest trees are deciduous, and tower above the lofty trees which produce the main roof of the high forest: again, some of the few giant trees that are evergreen are only sub-evergreen, as they cast off the old leaves with the appearance of the new foliage. Yet in these forests, too, some of the tallest trees are evergreen, so that we cannot absolutely say that those exposed to the extremest condition are deciduous, though the tendency is in this direction.

There is one clear distinction between coniferous and dicotylous trees, namely, in their power of withstanding equal injurious influences that suddenly damage the tree. Coniferae succumb more readily, whether the injuries be wrought by chemical or cultural agencies, or by animals or plants.

Despite of the wide range of distribution of such species as *Juniperus communis*, Mayr arrives at the conclusion that *Conifers are less capable than are dicotyledons of acclimatizing themselves*. Mayr's view is probably correct, when we limit its scope to the relatively short periods during which attempts at acclimatization have been made and to the attempts made where the climate is continental.

In regard to chemical injuries, it is well known that Conifers, less sensitive than certain deciduous dicotylous trees to the action of sulphur dioxide, are, nevertheless, more easily killed by smoke. The cause here is triple. First, the leaves are exposed to the influence of smoke for a longer time; secondly, the aggregate leaf-surface is often larger than in the case of dicotyledons; thirdly, the sulphur dioxide of smoke kills leaves that required several years for their production.

In connexion with the last cause, it may be noted that complete defoliation, especially if repeated once or twice, of an evergreen conifer by insects, fungi, or other agencies, leads to the death or at least serious weakening of the tree; whereas a deciduous dicotylous tree may suffer defoliation

regularly each year (as in the case of the oak attacked by the leaf-roller moth) and continue to exist. The larch, being deciduous, rather resembles the deciduous dicotyledon in this respect, for repeated partial defoliation by the caterpillars of the little moth *Coleophora laricella* does not cause its death.

Bark-beetles easily cause the death of Conifers if the attack be severe or sustained; whereas birches, ash-trees, and elms can endure for many years severe attacks from the familiar species of bark-beetles that infest them. In this respect the larch seems rather allied to the evergreen conifers. Cortical injury to the larch by the canker-fungus, *Dasyscypha calycina*, varies in its result, but in numbers of cases the tree can resist this fungus for more than sixty years.

Even larvae tunnelling in the wood kill evergreen Conifers more readily than they do dicotylous trees.

Finally, Colonel Prain informs me that in Calcutta Botanic Garden coniferous trees thoroughly shaken by the wind, though not uprooted, seem to die more readily than dicotylous trees. To what extent this is due to the evergreen character in a climatically deciduous region or to tardy replacement of the injured roots it is impossible to say.

The causes of the greater vulnerability of the conifer are varied and perhaps partially obscure. When all the leaves of the tree are destroyed the evergreen Conifer loses what required years to produce and will require years to replace, and at the same time the tree is partially deprived of the assimilating organs required to manufacture the material for replacing the missing members. In addition, the coniferous plant is endowed with less power of replacing the missing leaves, as it has neither the wealth of dormant buds nor the power of emitting adventitious shoots possessed by a dicotylous deciduous tree. An exception to this rule prevails in the yew, which can withstand severe clipping, though this is fatal to most Conifers.

It is worthy to note that *evergreen Coniferae have a larger number of serious fungal and insect foes than have dicotylous trees* in north-temperate regions. This may be wholly, or only partially, another method of stating the proposition that the Conifers are more readily killed or injured.

To prove the statement I have drawn up lists of tree-attacking Fungi and insects from the recognized textbooks on the diseases of trees by Hartig, Von Tubeuf, and Judeich and Nitsche.

I give below a table showing the number of *Fungi* attacking forest trees, first as given in Hartig's 'Diseases of Trees', and thus as enumerating the ones that were so obviously important as to be investigated first, and secondly, as given in Von Tubeuf's 'Diseases of Plants'. In my lists I originally grouped fungal diseases into four classes:—

1. Those fatal to the tree.
2. Those severe, and causing much injury to the living tree.



3. Those that are less severe, and may be merely leaf-diseases from which the tree usually or always recovers.

4. Others, concerning whose precise significance we are not fully informed, but which are probably largely not of first-class importance.

In the table below, the first two classes are grouped together as severe and the last two as slight; the estimates of the severity or lenience of the diseases are my own.

The dicotylous trees are infected by 15 severe fungal diseases, of which 11 are limited to them, while the Coniferae are attacked by 25 severe fungal diseases, of which 21 are restricted to them: the remaining 4 affect dicotyledons and Conifers. Of the 15 attacking dicotyledons, 8 are wood-destroying Fungi that do not infect coniferous wood; while of the 25 serious fungal foes of Coniferae only 6 are limited to wood (there are others attacking both wood and cortex). As the Fungi attacking heart-wood can hardly be regarded as of first-class severity, if they were excluded from the lists the disparity between dicotyledons and Conifers would be further increased.

Species.	Number of parasitic fungal diseases.					
	Enumerated by Hartig.			Enumerated by Von Tubeuf.		
	Severe.	Slight.	Total.	Severe.	Slight.	Total.
<i>Abies pectinata</i> .	12	2	14	14-15	3-2	17
<i>Picea excelsa</i> . .	17-18	4-3	21	19	3	22
<i>Pinus sylvestris</i> .	10-11	4-3	14	18-20	6-4	24
<i>Larix decidua</i> . .	8-9	2-1	10	9-10	2-1	11
<i>Quercus Robur</i> .	10-11	3-2	13	10	14	24
<i>Fagus sylvatica</i> .	4-5	3-2	7	6	9	15
<i>Betula alba</i> . . .	2	4	6	3	14	17
<i>Carpinus Betulus</i>	1	2	3	2-3	7-6	9
<i>Alnus glutinosa</i> .	2-3	4-3	6	1-3	12-10	13
<i>Fraxinus excelsior</i>	1	0	1	1-3	6-4	7

Similarly, in regard to insect foes, I have drawn up lists of the various insects attacking forest trees in Europe, using as the source of information 'Lehrbuch der Forstinsektenkunde', by Judeich and Nitsche: the results are given in the columns 2-7 of the succeeding table. These diseases I have ranged into several classes: (a) severe, and attacking young plants (seed-beds and nurseries, &c.); (b) slight, and attacking young plants; (c) severe, and attacking older trees; (d) slight, and attacking older trees. There is a certain vagueness in the classification because I have had to use my judgement, first, as to whether the disease is severe when it does attack the tree, and secondly, as to whether it is sufficiently common on the tree to be reckoned as a menace; but diseases caused by insects that rarely attack the species I have reckoned as 'slight'. In order to check my estimate I have drawn up the list of insect foes that attack the same species of forest trees

and are admittedly of such importance as to be included in the small work on 'Forest Protection' by Fürst; the results are given in column 8 of the same table. The results of both methods of estimating agree in showing the larger number of insects menacing the existence of coniferous species.

NUMBER OF SPECIES OF INSECTS ATTACKING.

Species.	Young trees.		Old trees.		Grand Total (young and old).		No. of species of prime importance attacking.
	Severe.	Total.	Severe.	Total.	Severe.	Total.	
<i>Abies pectinata</i> . . . . .	3	20	6	27	9	47	16
<i>Picea excelsa</i> . . . . .	21	64	10	57	31	121	30
<i>Pinus sylvestris</i> . . . . .	33	67	20	67	53	134	32
<i>Larix decidua</i> . . . . .	8	14	7	37	15	51	20
<i>Fraxinus excelsior</i> . . . . .	2	2	0	18	2	20	6
<i>Fagus sylvatica</i> . . . . .	14	17	8	46	22	63	19
<i>Betula alba</i> . . . . .	5	13	1	50	6	63	13
<i>Carpinus Betulus</i> . . . . .	0	3	0	16	0	19	6
<i>Alnus glutinosa</i> . . . . .	5	6	4	42	9	48	5
<i>Ulmus campestris</i> and <i>U. montana</i>	0	0	2	37	2	37	9

(The number of slight diseases is obtainable by subtracting the 'severe' from the 'total' in the respective cases. The total number of insects listed was 385.)

Columns 6 and 8 are worthy of special comparison.

Of the insects mentioned here as attacking young trees, 102 species attack the Conifers only, 18 attack the Conifers and dicotyledons, and 15 species attack the dicotyledons only. Thus, on the average, each Conifer has 25.5 exclusive foes or 30 inclusive foes, while each dicotyledon has 2.5 exclusive foes or 5.5 inclusive foes. Similarly, attacking old trees, 121 are confined to the Conifers, 8 damage the Conifers and dicotyledons, while 131 are confined to the dicotyledons. On the average each conifer is attacked by 30.25 exclusive foes or 32.25 inclusive foes, and each dicotyledon by 21.8 exclusive or 23.1 inclusive foes.

Thus dicotylous trees (at least in north-temperate climes) may owe their victory over Coniferae in the majority of favourable sites largely to their power of resisting or repairing injury caused by sudden hostile influences, including animal and fungal foes. It is possible, too, that in secular changes of climate Coniferae suffered more than dicotyledons, though certain coniferous genera, such as *Pinus*, betray no signs of inability to secular acclimatization. So far as the chief forest trees are concerned, insect-pollination appears to have played but a small part in aiding the north-temperate dicotyledons.

## SUMMARY.

1. The northern evergreen Coniferae are *architectural xerophytes* in which the extensive surface exposed by the evergreen leaves as a whole renders it necessary for the individual leaves to be xeromorphic in form and xerophytic in structure. This type of structure enables these Coniferae to live in regions where there is a season of physiological drought, in situations varying from dry dunes to moist forests, and from arctic and alpine situations to tropical sites.

2. The tracheidal structure of the wood of these conifers is well suited to their xerophytic evergreen leaves; and a similar type of wood is apt to occur in north-temperate and austral-temperate dicotyledons that have evergreen xerophytic leaves, as is shown by American species of *Quercus*, *Trochodendron*, and *Drimys*. The tracheidal structure of the wood is not a bar to progress and to the adoption of the deciduous habit, for in the larch a rapid transpiration current flows through it and the leaves transpire rapidly. The tracheidal structure of the wood more probably provides the conifer with a safety mechanism that is a defence against extinction.

3. Conifers are more easily deranged and killed by sudden injuries, and are attacked by a larger number of serious fungal and insect foes, than are dicotylous trees. To their greater vulnerability and smaller powers of repairing injuries we may at least partially attribute the defeat and extinction of many Conifers in past ages.

In conclusion I beg to express my thanks, first to Lt.-Col. Prain, F.R.S., Director of the Royal Botanic Gardens, Kew, for permitting me to perform the physiological experiments at the Jodrell Laboratory, and for some of the material used in this investigation; and secondly, to Messrs. James Veitch & Sons for twigs of *Tetracentron*.

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# Vegetative Reproduction in Metzgeria.

BY

ALEXANDER W. EVANS.

With sixteen Figures in the Text.

THE organs of vegetative reproduction in *Metzgeria* are so conspicuous that they attracted the attention of observers at a very early date. They consist of thalloid structures, usually but one cell thick throughout, and easily become detached at maturity. Each one arises from a single cell of the parent thallus, either from an alar cell or from one of the cortical cells of the costa. The position of the reproductive bodies on the thallus varies in different species. In some they are always marginal, while in others they are borne on the upper, or antical, surface of the wings. In still other species, where they are produced on strongly specialized branches, their position is much more indefinite, any superficial cell being apparently able to assume the reproductive function. It has been customary to designate these reproductive bodies as 'adventive branches' when they arise from marginal cells of the thallus, and as 'gemmae' or 'bulbils' when they arise elsewhere. It has already been shown, however, by Schostakowitsch ('94, p. 350) and others that there is no essential difference between them, either in their development or in the way in which they become detached from the parent plant. In the present paper, therefore, they will be spoken of as 'gemmae', whatever their position. The term 'adventive' may then be retained for branches which arise from the postical surface of the costa, and in the development of which the internal costal cells take part. Strictly speaking, such branches are not reproductive structures at all, although they may eventually give rise to new individuals through the progressive dying away of the old thallus. The differences between adventive branches of this character and gemmae are usually well marked, but they sometimes become less definite on poorly developed individuals.

Apparently, Hooker ('16, pl. 55) was the first one to describe the gemmae of *Metzgeria* clearly. He studied them in what he called *Fungermannia* [now *Metzgeria*] *furcata*  $\gamma$  *aeruginosa*, a plant characterized by a peculiar bluish-green colour and by specialized gemmiparous branches. The next writer who added substantially to our knowledge was Nees von



Esenbeck ('38, p. 488), who redescribed Hooker's variety *aeruginosa* under the name *M. furcata*  $\delta$  *gemmaipara* and called attention to the most striking peculiarities of the gemmiparous branches. He also described, as  $\epsilon$  *prolifera* and  $\zeta$  *ulvula*, forms of *M. furcata* in which the gemmae are marginal. A few years later Naegeli ('45) gave a full description of the way in which these marginal gemmae develop, and emphasized the fact that each one arises from a single cell. Further details were added by Hofmeister ('51, p. 22) and by Kny ('64, p. 76), the latter stating that the separation of the gemmae was brought about by the destruction of tissue.

Up to this time the gemmae had been of interest more particularly to students of plant morphology; but Lindberg showed, in his *Monographia Metzgeriae* ('77), that they deserved the attention of taxonomists as well. Unfortunately he failed to emphasize this fact very strongly, although he went far enough to refer different types of gemmae to definite species, and to imply by omission that they did not occur in the other species which he recognized. He stated that marginal gemmae occurred not only in *M. furcata*, but also in his newly described *M. myriopoda*, and that specialized gemmiparous branches were to be observed in *M. conjugata*, var. *violacea*, and *M. furcata*, var. *fruticulosa*, the latter being identical with Hooker's variety *aeruginosa*. He also called attention, apparently for the first time, to those gemmae which arise on the surface of the wings, and noted their occurrence in *M. Liebmanniana*, *M. dichotoma*, *M. crassipilis* (published here as a sub-species of *M. furcata*), and *M. linearis*. According to his statements gemmae of this character are always borne on the postical surface of the wings, but the writer has found them to be invariably antical in origin. Apparently, Lindberg's mistake was due to the fact that gemmae, after becoming separated, often attach themselves to the postical surface of an overlying thallus and begin their germination in this position.

Subsequent references to the gemmae of *Metzgeria* are very much scattered, but describe a few new observations of interest. Miss Boatman ('92), for example, notes the production of marginal gemmae in *M. conjugata*, while Schiffner ('00, p. 60) and Pearson ('02, p. 465) say that they are sometimes to be seen in *M. hamata*. Specialized gemmiparous branches have been studied by Ruge ('93, p. 304) in an unnamed species from Ecuador, and by Goebel in the South American *M. adscendens* ('93, p. 427), as well as in plants which he referred to *M. conjugata* ('98a, p. 275). According to Ruge, the gemmiparous branches grow out from the antical surface of the costa, but Goebel states that they are simply the prolongations of ordinary branches of the thallus. Except for a short note by the writer ('09, p. 189) on the antical gemmae of *M. crassipilis*, there are apparently no allusions to reproductive bodies of this type in the recent literature. Stephani, in his revision of the genus ('99), makes no mention whatever of gemmae, and

consequently does not connect the specialized branches studied by Ruge and Goebel with processes of reproduction. He attaches but slight importance to these branches (from the standpoint of the taxonomist), and implies that they probably occur in many species where they have not yet been detected. He suggests that their development may perhaps be due to heliotropic stimuli, but makes no further attempt to account for them.

The study of an abundant material of *Metzgeria*, mostly from North America, soon made it evident to the writer that the gemmae showed a much greater variety in form and structure than had been supposed. It also became evident that many of their peculiarities were specific in character, and that they often afforded a convenient means for distinguishing between closely related plants. The individual peculiarities of the gemmae have scarcely been noticed in earlier publications. This, however, is not surprising, because it is only within comparatively recent times that gemmae in other genera of the Bryophytes have been at all adequately described. Perhaps another reason why *Metzgeria* has been especially neglected in this respect is because the gemmae sometimes undergo reversion to a greater or less extent, and under these circumstances may fail to show their normal characteristics. This phenomenon has already been discussed by Goebel ('98 *b*) in the case of *M. furcata*, and allusion will again be made to it in the following pages.

#### DESCRIPTION OF GEMMAE.

In the present paper the gemmae of twelve species are described, and fall naturally into three groups according to their position on the thallus. In the first group, including five species, the gemmae are marginal; in the second group, including six species, they arise from the antical surface of the wings; in the third group, including but a single species, they are indefinite in position. The gemmiparous branches in the first and second groups are very similar to normal branches, but in the third group they are much more specialized. In a few other species gemmae have also been noted, but they are not present in sufficient number for careful investigation. The remaining species examined seem to be entirely destitute of gemmae. Since these include both *M. conjugata* and *M. hamata*, where gemmae have been indicated by other observers, it would appear either that their statements were based on incorrect determinations or that the gemmae in these two species required very exceptional conditions for their development. Since several of the gemmiparous species discussed are apparently new, their diagnoses will follow the descriptions of their gemmae.

#### *Metzgeria uncigera*, sp. nov.

A gemmiparous branch in this species tends to be narrower than a normal branch and to develop a less highly differentiated costa. When

gemmae are produced in abundance the growth of the branch soon comes to an end and the gemmae become crowded in the apical region (Fig. 1). In many cases, however, a branch will develop gemmae for awhile and then continue its growth normally. The gemmae arise in no definite order, large and mature individuals being often scattered among those which have scarcely begun their development. There is no evidence therefore that they are formed in acropetal succession.

When a gemma is to be produced one of the marginal cells projects beyond its neighbours. In doing this the outer wall of the cell is ruptured, but the protruding protoplast is not naked, being covered over by a thick layer of transparent gelatinous substance, which perhaps represents a modification of the inner portion of the original outer wall (Fig. 2, A). The deposition of this substance takes place prior to the rupture of the wall.

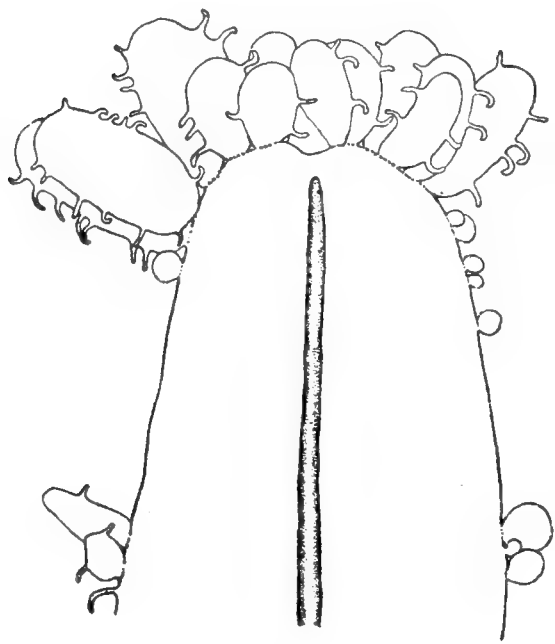


FIG. 1. *M. uncigera*. Apex of a gemmiparous branch.  $\times 40$ .

Upon the inner surface of the gelatinous layer a very thin new wall is soon secreted. The projecting cell thus formed does not represent the mother-cell of the future gemma, except in very rare instances. It almost invariably divides by a periclinal wall into two cells, the outer of which becomes the actual mother-cell. The periclinal wall is sometimes at right angles to the surface of the thallus, but is more likely to be inclined to it in such a way that the mother-cell of the gemma lies partly over the antical surface of the inner cell. The first wall in the mother-cell extends obliquely outwards from about the middle of the periclinal wall. A second

oblique wall meets the first (Fig. 2, B) and thus forms at the tip of the young gemma a wedge-shaped cell, which proceeds to function as a two-sided apical cell, and to give rise to two rows of segments in the way so often described for *Metzgeria furcata*. The segments soon undergo further divisions (Fig. 2, C), and usually one of the two cells first cut off from the mother-cell divides as well, thus making the base of the gemma three cells wide (Fig. 2, D). In the early stages the gelatinous substance which covered over the original projecting cell becomes stretched out into a thinner and thinner layer at the apex of the gemma, and before long disappears completely (Fig. 2, C and D).

The separation of the gemmae is brought about by the splitting of the walls between the two or three basal cells and the cell of the thallus cut off by the original periclinal wall. At the time of separation they vary considerably in length, a condition which is doubtless due to the fact that germination sometimes begins prematurely. What may be considered an

average gemma is represented in Fig. 2, E. It consists of a flat strap-shaped thallus about eight cells broad and narrowing somewhat towards the base. At the rounded apex the single apical cell, still active in cutting off segments, can be distinguished. Such a gemma is about 0.6 mm. long and 0.25 mm. wide. Along the margin are scattered hairs, each about twice as long as one of the ordinary cells of the gemma and almost always hooked at the apex. The hairs are often strictly marginal, but in some cases are slightly displaced to one surface. The gemma shows no signs of dorsiventrality; even when the hairs are displaced they appear on either surface indiscriminately. There are also no signs of cell-differentiation except for the marginal hairs.

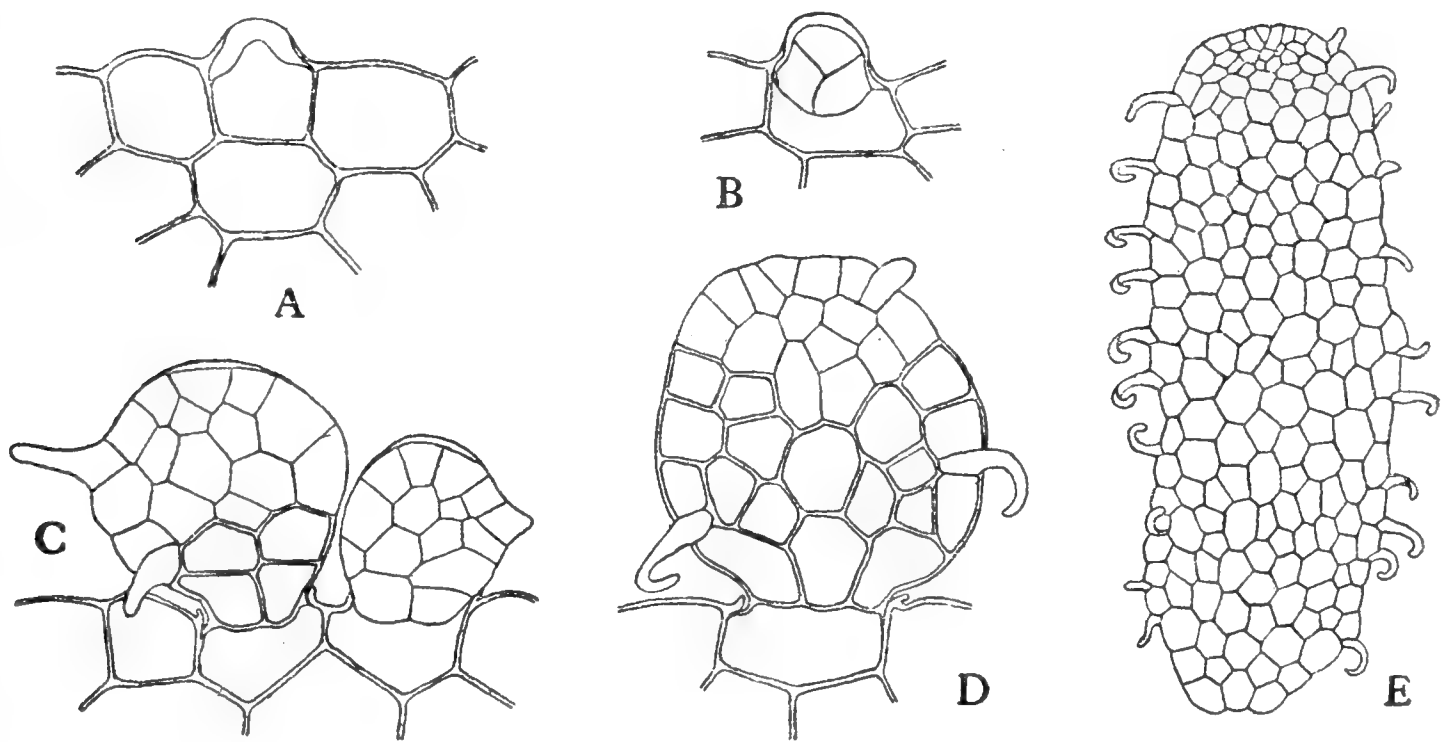


FIG. 2. *M. uncigera*. A-D. Gemmae in various stages of development.  $\times 300$ . E. A gemma at time of separation.  $\times 80$ .

The germination of a gemma is a comparatively simple process, although its course is liable to be affected by external changes. The apical cell of the gemma begins to function at once as the apical cell of the young thallus. In fact it is quite impossible to discern where the gemma merges into the young plant to which it gives rise. As development proceeds the young thallus gradually becomes broader and the new hairs formed tend to be straight instead of hooked and to act as rhizoids. Before long the median cells of the thallus become arranged in two longitudinal rows and begin to divide by walls parallel to the surface, thus giving rise to a rudimentary costa. With the appearance of the costa the evidences of dorsiventrality become more distinct. The marginal hairs, for example, when displaced from the margin, always appear on the lower, or postical, surface; the costa itself begins to give off hairs, most of which function as



rhizoids; and slime papillae make their appearance in the region of the growing point, always on the postical surface. With the further development of the costa the differentiation of the thallus becomes complete, and the characteristic branching by forking soon begins. The young plant is strongly subject to the phenomenon of reversion. Even after the costa has become distinct it is not unusual for a thallus to continue its development as a simple layer of cells. It is also very prone to develop secondary gemmae (Fig. 3), and it sometimes does this before it has begun to show any signs of differentiation. In fact, secondary gemmae sometimes arise

on a primary gemma which is still attached to the parent thallus. The secondary gemmae are essentially like the primary, passing through the same course of development and becoming separated in the same way.

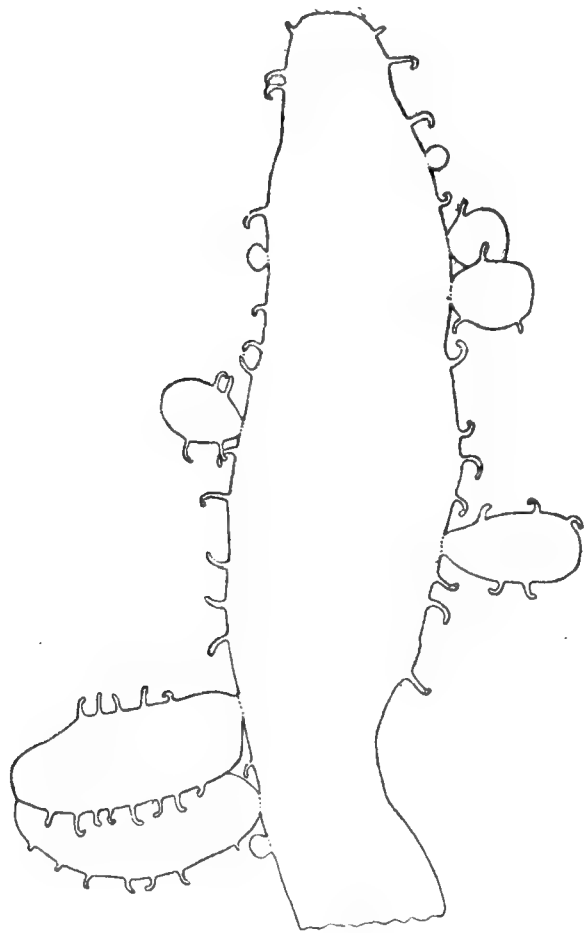


FIG. 3. *M. uncigera*. Apical portion of a thallus developed from a gemma and bearing new gemmae along its margin.  $\times 40$ .

*Metzgeria uncigera*, sp. nov. Pale green, growing in depressed mats: thallus prostrate, repeatedly dichotomous, well-developed branches about 1.2 mm. wide and from 1.5 to 2.5 mm. long between two successive forks, plane; costa bounded both antically and postically by two rows of cortical cells; wings usually from fifteen to twenty cells broad, the cells thin-walled throughout, averaging about  $28 \times 21 \mu$  and not varying much in size in different parts of the thallus; hairs few and irregularly scattered, restricted to the margin and to the postical surface of the costa, the marginal hairs averaging about  $70 \mu$  in length, usually straight but sometimes hooked at the apex, occurring singly, sometimes truly marginal, but often slightly

displaced to the postical surface: inflorescence dioicous: ♀ branch broadly orbicular-obovate, about 0.15 mm. long, with scattered marginal hairs: gemmae marginal, ligulate, one cell thick throughout, without a distinct stalk, apical cell single, hairs marginal or slightly displaced to one surface, hooked at the tip: remaining parts not seen.

On trunks of trees. Porto Rico: Mount Morales, near Utuado, March 19, 1906 (Howe, No. 1128).

The present species is closely related to *M. furcata*, although the costa has only two rows of cortical cells on the postical surface instead of four. It is distinguished by its smaller cells and by the absence of surface hairs on the wings of the thallus. Its gemmae, also, are less differentiated and fail to show a costa even when large and well developed. The relationship of *M. uncigera* to *M. hamata*, where the costa is built up on essentially the

same plan, is much more remote. In this species the thallus is strongly convex, the marginal hairs are longer and occur in pairs, while the leaf-cells are considerably larger, averaging about  $57 \times 37 \mu$ .

*Metzgeria furcata*, (L.) Dumort.

The gemmiparous material examined of this common and widely distributed species came from the following localities:—Zurich, Switzerland (Foerster); Cumberland, Maine (Chamberlain, No. 904); Jackson, New Hampshire (A. W. E.); Onteora Mountain, New York (Miss Vail, No. 26). The plants which develop the gemmae are usually more slender and less highly differentiated than normal plants. The hairs, for example, are less numerous and are sometimes absent altogether, the cortical cells along the postical surface of the costa are often reduced to two rows, while the elongated internal cells of the costa are reduced in number and sometimes not developed at all. The thallus, in other words, undergoes a reversion to a more or less juvenile condition, as Goebel ('98 *b*) has already noted. The gemmae are borne without definite order, and the gemmiparous branch usually continues its growth for a considerable period.

The early development of a gemma is similar to that just described for *M. uncigera*. One of the marginal cells projects in the same way, secretes a gelatinous substance on the inside of the projecting wall, ruptures the outer wall, and then forms a thin new wall on the inner surface of the gelatinous layer. In contrast to *M. uncigera*, however, the projecting cell becomes at once the mother-cell of the future gemma without undergoing a preliminary division. It proceeds to divide in the characteristic way, already described in detail by Naegeli ('45) and other observers. With regard to the separation of the gemmae at maturity, this is doubtless brought about in some instances by a rupture across the base, in the way described by Kny. But this method of separation is often premature and is apparently abnormal. In normal cases the basal cells simply split away from the adjacent thallus cells, and the process is unaccompanied by any destruction of tissue. The remains of the ruptured wall of the original projecting cell may often be distinguished after the gemma has been set free (Fig. 4).

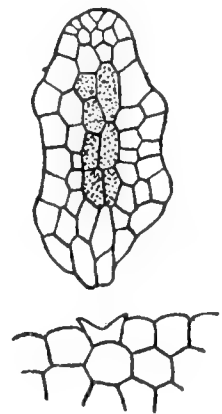


FIG. 4.  
*M. furcata*. A small gemma just separated from the parent thallus.  $\times 80$ .

At the time of separation the gemmae vary greatly in length, but are usually more highly differentiated than in the other known members of the genus. They quickly form the beginnings of a costa, even the basal cells often dividing by walls parallel with the surface. The existence of dorsiventrality is also shown very early by the development of slime papillae on one surface of the gemma, the first papilla sometimes appearing only a few



cells away from the base. Except for these papillae the gemmae are usually destitute of appendages, but in some cases hairs are produced early, and even the first ones commonly show a slight displacement to the postical surface in the way characteristic for the mature thallus. The gemmae just described may be considered typical, but reversions to a more embryonic condition are likely to occur in a greater or less degree. Under

these circumstances a gemma, as fully described by Goebel, may be simply a cell-row or a cell-layer variable in width, and frequently shows no signs either of differentiation or dorsiventrality. The germination of a gemma, so far as observed, exhibits no features of especial interest. It simply gives rise to a thallus, which gradually becomes wider and more differentiated as its growth proceeds, and there is no line of demarcation between the gemma and the thallus. The production of new gemmae on the young plant sometimes begins very early, especially in the var. *ulvula*.

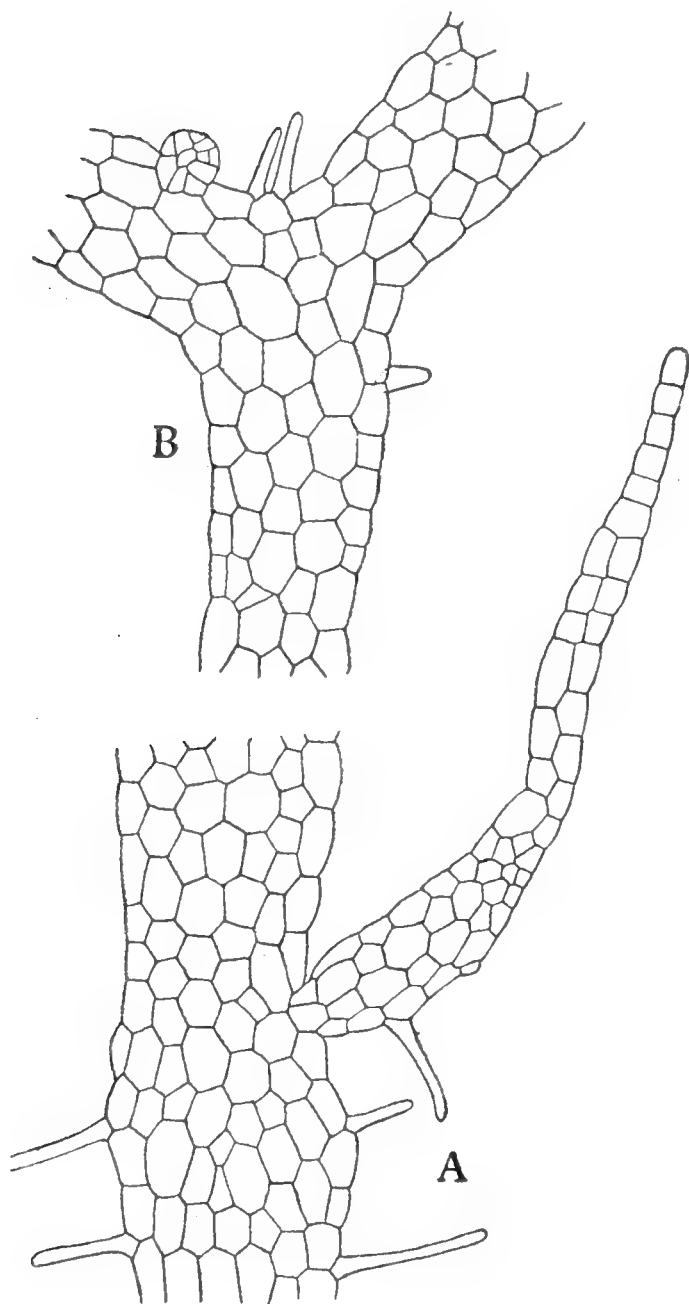


FIG. 5. *M. quadriseriata*. A. Portion of a thallus developed from a gemma; the new gemma on the right shows marked indications of reversion.  $\times 80$ . B. Another portion of the same thallus, showing dichotomous branching.  $\times 80$ .

*Metzgeria quadriseriata*, Evans.

This species is still known from a single locality in Japan, Ioki-mura, Tosa, where it was collected in 1903 by Yoshinaga.<sup>1</sup> The gemmiparous branches are more slender than normal branches, but show no further differences. The gemmae are usually sparingly produced and arise without definite order. They bear considerable resemblance to the gemmae of *M. furcata*, but show no signs of a costa and tend to be much narrower.

Occasionally, they bear a very few short and straight marginal hairs, but they are more frequently quite undifferentiated. Although their width, through reversion, may be reduced to only one or two cells (Fig. 5, A), they are normally from six to eight cells wide. Their length, however, varies within wide limits at the time of separation. When a gemma germinates the thallus to which it gives rise usually continues narrow and

<sup>1</sup> Cf. Evans: Proc. Wash. Acad. Sci., viii, 142, pl. 6, figs. 1-5, 1906.

undifferentiated for a long distance. It may even show a branching, by dichotomy, while it still consists of a single layer of cells, a condition which has not been observed in either of the preceding species (Fig. 5, B). It may also give rise to new gemmae at a very early stage. Except for the difference in position the distinction between gemmae and adventive branches is not always clearly marked in *M. quadriseriata*. In typical cases the branches show a definite costa from the beginning, but they are sometimes in the form of narrow band-like structures only one cell thick throughout. Such a branch takes its origin from a single cortical cell of the costa, and is perhaps to be considered an example of regeneration rather than a true adventive branch. The close connexion between regeneration and the production of gemmae will be discussed later.

*Metzgeria myriopoda*, Lindb.

This species, although first described in 1874, is still incompletely known. It has been collected in several of the Southern States and also in Brazil and Argentina. The gemmiparous material studied came from Sanford, Florida (Rapp, No. 8), and from Opelousas, Louisiana (Langlois); it agrees closely with the specimens distributed by Drummond and by Sullivant, both of which are quoted by Lindberg. The branches which bear the gemmae are a little narrower than normal branches, but are otherwise scarcely modified.

The gemmae are irregularly scattered and are sometimes abundant, but their development does not seem to affect the growth of the branch in any marked degree. The early stages in the development of a gemma are

similar to those described for *M. furcata*, the original projecting cell becoming at once the mother-cell of the gemma. In most cases the gemmae maintain a width of six cells (about 0.12 mm.), but they may be still narrower or even a little broader (Fig. 6, A). Their length varies greatly and sometimes attains 1.5 mm. before separation takes place. The gemmae are usually but one cell thick throughout, but in rare cases a few of the cells at the base undergo a division parallel to the surface, thus forming an exceedingly rudimentary costa, which may or may not bear a very few postical hairs. This condition, however, apparently never persists, and the gemma as it continues its growth quickly becomes reduced to a simple cell-plate. Marginal hairs make their appearance very early, but usually remain short. Although some of them are truly marginal, the majority are slightly displaced to one surface, thus showing the first

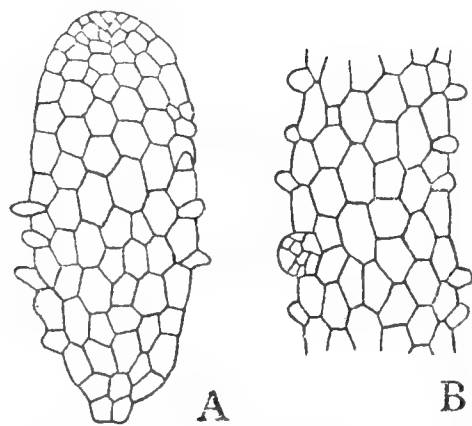


FIG. 6. *M. myriopoda*. A. A young gemma.  $\times 80$ . B. Portion of a longer gemma, still attached to the gemmiparous branch, but showing a secondary gemma at the left.  $\times 80$ .

indications of dorsi-ventrality. The gemmae often give rise to secondary gemmae while still attached (Fig. 6, B), and their method of separation from the parent plant is similar to that described for *M. furcata*. The germination of the gemmae has not been observed.

*Metzgeria oligotricha*, sp. nov.

The gemmae in this species are rather sparingly produced and are borne without definite sequence on normal vegetative branches. In the

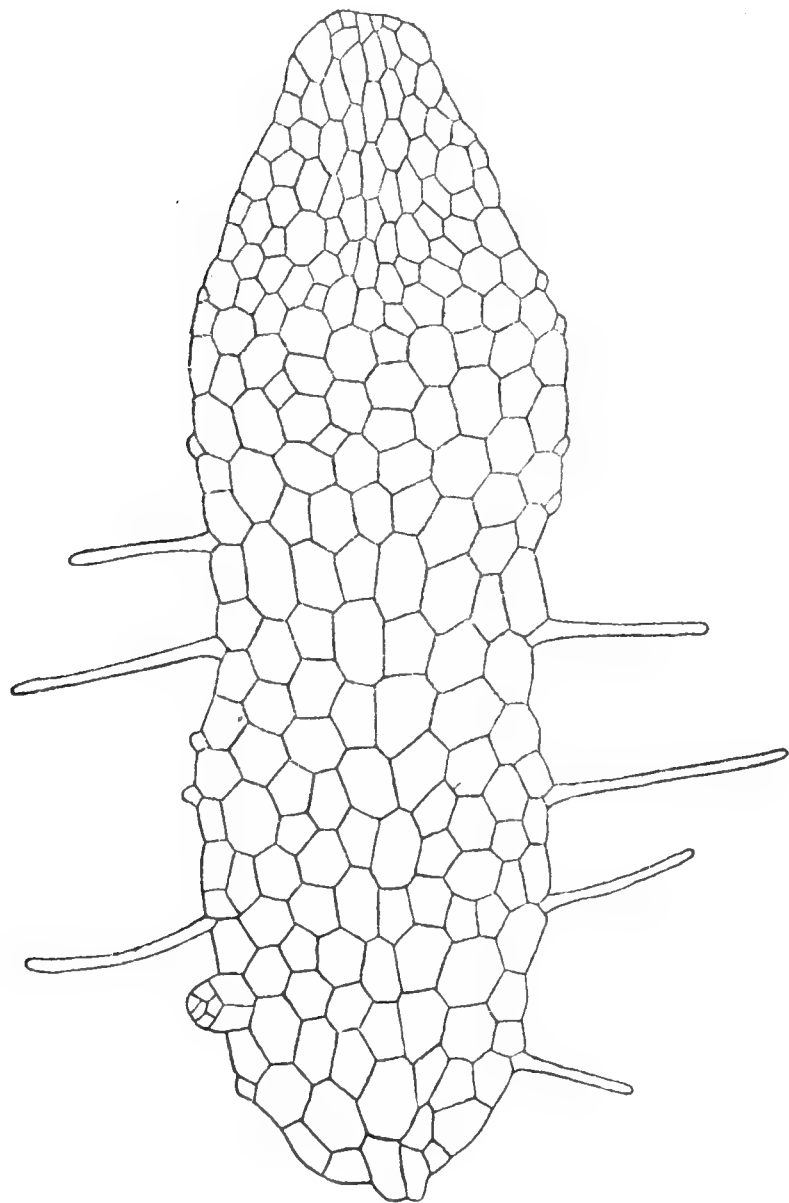


FIG. 7. *M. oligotricha*. A gemma about ready to separate, showing a secondary gemma at the left. x 80.

majority of cases their occurrence is intimately associated with the death of the apex of the branch, or else with the passage of the apical cell and the youngest segments into permanent tissue; under the latter circumstances the growth of the branch is limited even if its cells remain alive. Here again, as in *M. furcata*, the original marginal cell, which projects, becomes at once the mother-cell of the gemma. The gemmae are exceeding variable, not only in size, but also in the degree of differentiation which they show, in the length of time during which their apical cells continue functional, and in the number of secondary gemmae which they produce. A typical gemma is a flat and broad thallus, abruptly broadening out from the two basal cells. It is about ten cells (or 0.4 mm.) wide and usually from two to four times as long, and develops a variable number of long straight marginal hairs (Fig. 7). In some cases the hairs appear as mere rudiments and they may even be absent altogether. Although a gemma is usually but one cell thick, a rudimentary costa occasionally begins its development close to the base. The presence of such a costa, however, does not necessarily indicate dorsiventrality because it rarely develops either postical hairs or slime papillae.

The germination of a gemma seems to follow a normal course under favourable circumstances. It gives rise at once to a thallus which gradually

becomes broader, the costa appears or increases in complexity, postical hairs and slime papillae are formed, and the differentiated thallus soon shows the normal branching by dichotomy. The course of development, however, is usually affected by two tendencies, both of which seem to be especially potent in the present species. One of these is the tendency towards reversion, which has already been discussed under some of the preceding species; the other is the tendency which the apical region shows of acquiring the characteristics of permanent tissue, thus bringing the growth of the young thallus to an end. The cessation of growth and division in the apical cell may take place in any stage of differentiation, and is apparently associated with an early development of secondary gemmae.

*Metzgeria oligotricha*, sp. nov. Pale green, growing in depressed mats: thallus prostrate, repeatedly dichotomous and frequently giving off adventive branches from the postical surface of the costa, well-developed branches about 1.5 mm. wide, up to 4.5 mm. long between the forks, plane or nearly so; costa bounded both antically and postically by two rows of cortical cells; wings usually from ten to fifteen cells broad, the cells thin-walled throughout, averaging about  $55 \times 37 \mu$  and not varying much in size in different parts of the thallus; hairs few and irregularly scattered, restricted to the margin and to the postical surface of the costa, the marginal hairs often from 150 to 300  $\mu$  long, slender, straight or irregularly curved, but never hooked at the apex, usually truly marginal, but sometimes displaced to the postical surface: inflorescence dioicous: ♀ branch broadly orbicular-obovate, about 0.23 mm. long, with numerous long hairs on the margin and postical surface; calyptra clavate, about 1 mm. long, sparingly pilose: ♂ branch not seen: sporophyte (not quite mature) showing an oval capsule about  $35 \times 28 \mu$  and a stalk about 30  $\mu$  long: gemmae marginal, broadly ligulate, usually one cell thick throughout, stalk distinct, apical cell single, hairs marginal, not displaced, scattered, long and straight.

On trunks of trees. Cuba: without definite locality (Wright, Hep. Cubenses, distributed as *M. furcata*, mixed with *Dendroceros Breutelii* and other Bryophytes). Jamaica: Morce's Gap, July 12, 1903 (A. W. E., No. 37, mixed with *M. hamata*). Porto Rico: Mount Morales, near Utuado, March 19, 1906 (Howe, No. 1118). The specimens from Jamaica may be considered the type.

In the structure of its costa *M. oligotricha* agrees with both *M. uncigera* and *M. hamata*. It differs from *M. uncigera* in its larger size and larger alar cells, and in its longer marginal hairs which are never hooked at the apex even on the gemmae. The latter are also distinguished by their early development, the original projecting cell not undergoing a preliminary division before functioning as the mother-cell of the gemma. It differs from *M. hamata* in its plane thallus and in its sparse marginal hairs which are never geminate. From *M. furcata*, with which it is also allied, it differs in the structure of the costa, which is bounded postically by two instead of



by four cortical cells. It differs further in its larger alar cells, in its longer marginal hairs which are slightly, or not at all, displaced to the postical surface, in its lack of postical hairs on the wings, and in its broader and less differentiated gemmae.

*Metzgeria crassipilis*, (Lindb.) Evans.

The antical gemmae found in *M. crassipilis* and its allies are on the whole more highly specialized than the marginal gemmae just described.

Although they are sometimes borne in great abundance many of the plants fail to produce them altogether. The range of *M. crassipilis*, so far as known, extends from Vermont to North Carolina and Tennessee. The present study is based almost entirely on material collected by J. K. Small, on Blowing Rock Mountain, North Carolina, in 1892 (No. 31). The same material has served for the accompanying figures. The gemmiparous branches differ very slightly from ordinary branches and show no marked indications of having their growth limited. The only important modification which they show is an increase in the number of cortical cells along the antical surface of the costa. On normal branches these cells are arranged in two rows; on gemmiparous branches the number of rows varies from two to four (Fig. 8, A and B). The gemmae are not developed in definite order

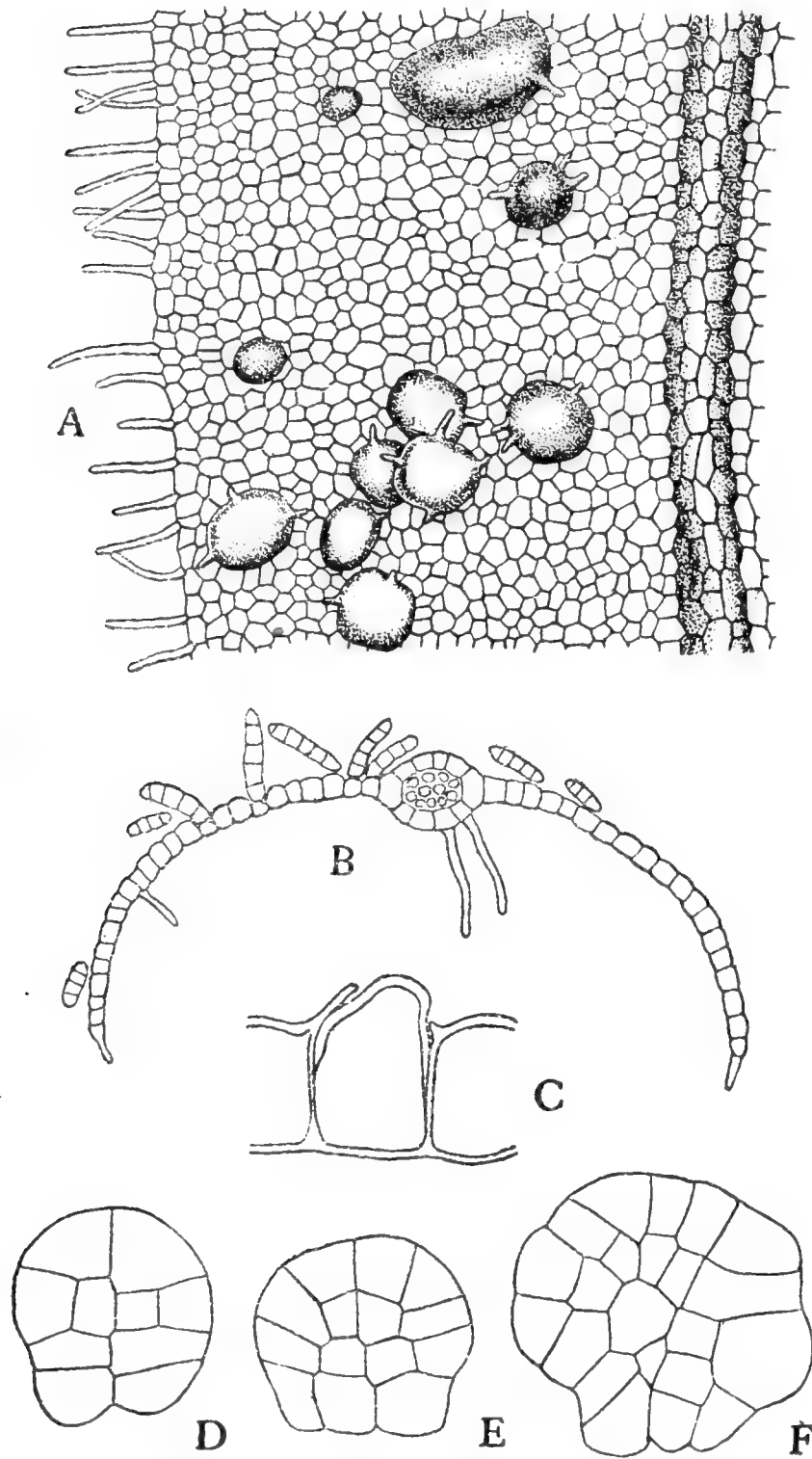


FIG. 8. *M. crassipilis*. A. Portion of a gemmiparous branch.  $\times 50$ . B. Cross section of a gemmiparous branch.  $\times 50$ . C-F. Early stages in the development of gemmae.  $\times 300$ .

and sometimes occur so close together that they overlap as their size increases. Each one arises from a single alar cell which first of all projects above the surface of the thallus. In this process the protoplast of the cell

secretes a gelatinous layer, just as in *M. uncigera*, and this is followed by a rupture of the outer wall (Fig. 8, C). The projecting cell then divides by a wall approximately parallel to the surface of the thallus, and the upper cell thus cut off becomes the mother-cell of the future gemma. In some cases the projecting cell divides first by a perpendicular wall, and then, when the horizontal wall is formed, the rudiment of the gemma consists of two cells. Even when it consists of a single mother-cell, a condition which seems to be the more typical, it is quickly divided by a longitudinal wall

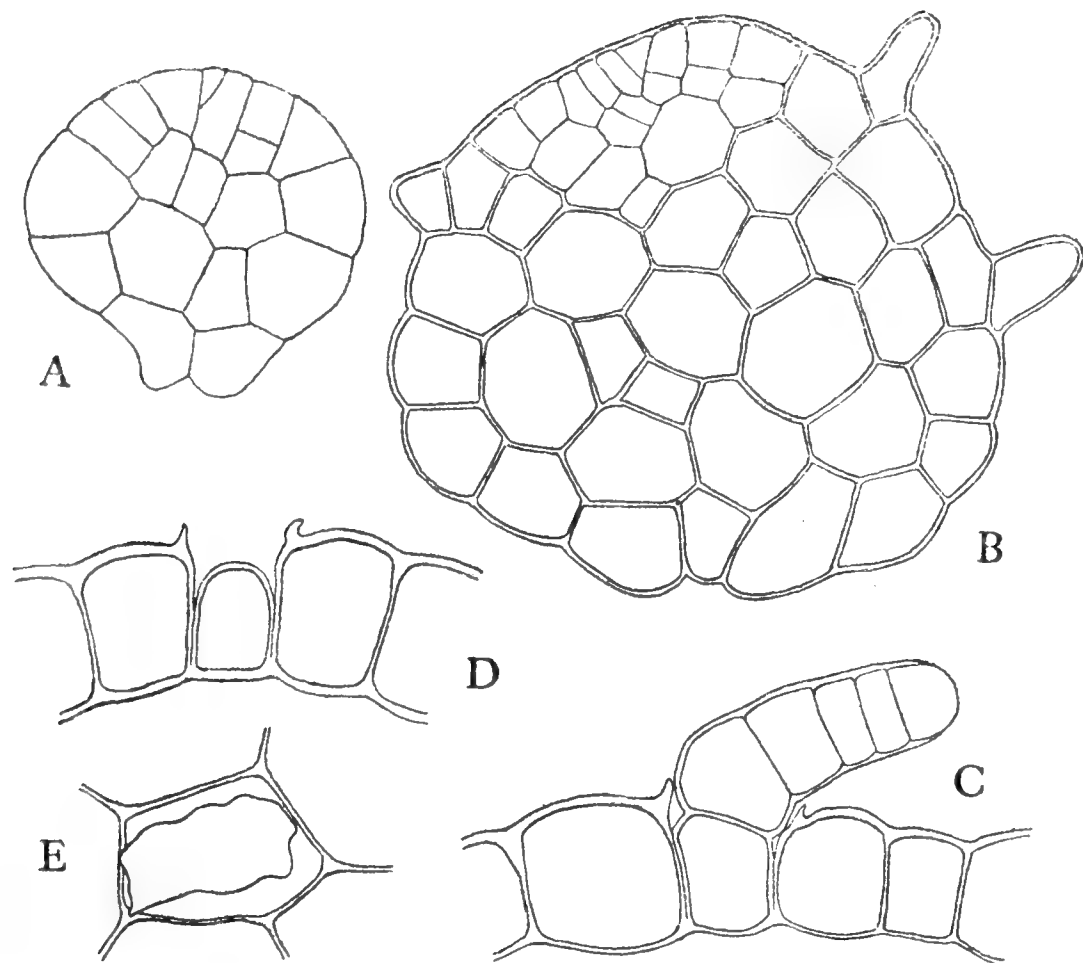


FIG. 9. *M. crassipilis*. A. A very young gemma with two apical cells.  $\times 300$ . B. A typical gemma about ready to separate.  $\times 300$ . C. Section through a young gemma and adjacent cells of thallus.  $\times 300$ . D. Section through a similar region after the gemma has escaped.  $\times 300$ . E. Surface view of a ruptured cell.  $\times 400$ .

into two cells. Beyond the two-celled stage the development of the gemma is subject to further variation. The two cells first divide two or three times by cross walls, thus giving rise to a rounded structure composed of two rows of cells side by side. Some of these cells then divide by periclinal walls (Fig. 8, D and E), and before long one or two cells acting as two-sided apical cells make their appearance (Figs. 8, F, and 9, A). The apical cells are derived from the terminal cells of the original two rows, and it usually happens, even when two are formed, that only one continues to function. The result is that the mature gemma, except in very rare instances, has a single apical cell. At the time of separation the gemmae vary considerably in size, but the example represented in Fig. 9, B, may be regarded as fairly typical. Such a gemma is in the form of an orbicular and flat plate



of cells, averaging about 0.15 mm. in diameter and measuring from six to eight cells across. Along the margin are scattered a few rudimentary hairs. The gemmae are set free by a splitting of the walls at the base (Fig. 9, C), leaving behind the single cell (or the two cells) cut off by the original

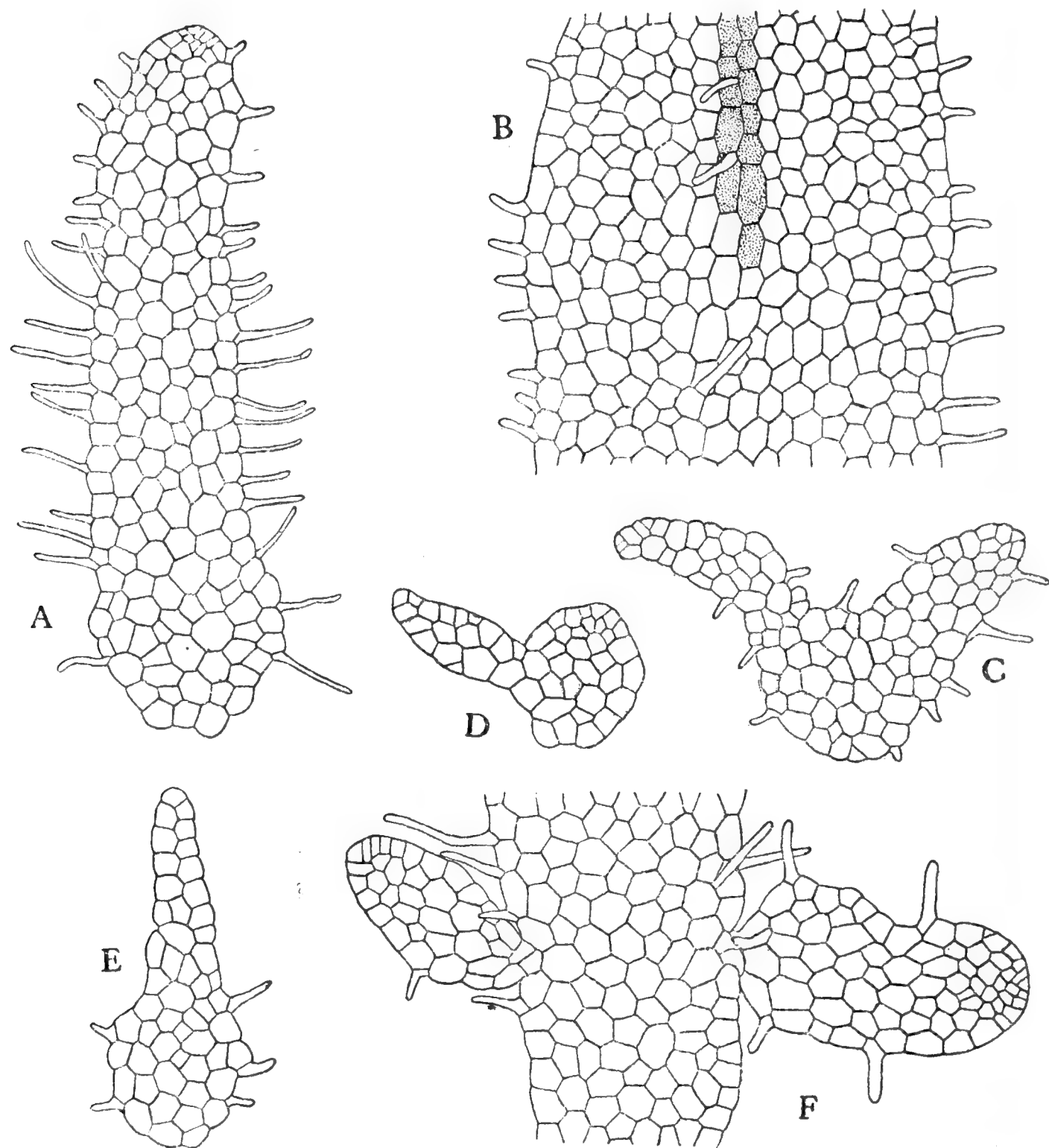


FIG. 10. *M. crassipilis*. A. A germinating gemma.  $\times 80$ . B. Portion of a young thallus developed from a gemma, showing the first indications of a costa.  $\times 80$ . C. Germination of a gemma with two apical cells.  $\times 80$ . D. A germinating gemma, showing the young thallus developed from the margin instead of from the apex.  $\times 80$ . E. A germinating gemma, the young thallus showing indications of reversion.  $\times 80$ . F. Portion of a young thallus with new marginal gemmae.  $\times 80$ .

horizontal wall. The cell thus left is lower than the neighbouring thallus cells, and the remains of the ruptured wall of the original projecting cell may often be demonstrated (Fig. 9, D and E). The two or three basal cells of the gemma become rounded off after separation and can usually be distinguished from the other marginal cells.

In rare cases the germination of the gemmae begins while they are still attached to the parent thallus, but the process is usually deferred until they have become separated. In normal cases, such as the one represented in Fig. 10, A, the apical cell of the gemma renews or continues its activities, and gives rise to a strap-shaped thallus which is at first but a single cell thick and about six cells wide. The margin bears numerous scattered hairs, but the young plant shows no further evidences of cell-differentiation and no signs of dorsiventrality. As its growth proceeds scattered hairs appear on one surface (destined to become the postical), the median cells become arranged in two longitudinal rows, and before long divide by walls parallel to the surface, thus giving rise to a rudimentary costa (Fig. 10, B). The further differentiation of the thallus pursues the normal course. In the rare cases where two apical cells are present and functional the gemma gives rise to two diverging thalli (Fig. 10, C). Another rare case is shown in Fig. 10, D, where the young thallus does not arise from the apical cell at all, but from one of the other marginal cells of the gemma. The process of germination is sometimes modified by reversion, as illustrated in Fig. 10, E, where the young thallus is only two cells wide. It may also be complicated by the formation of new gemma, which invariably arise on the margin of the young thallus instead of on its surface. In Fig. 10, F, where two such marginal gemmae are shown, their formation was apparently induced by the death of the apical cell of the thallus, but they sometimes occur while the apical cell still continues active. The marginal gemmae thus produced differ from the antical gemmae of the species, and bear a marked resemblance to the marginal gemmae described for some of the preceding forms.

*Metzgeria vivipara*, sp. nov.

The gemmae, as in *M. crassipilis*, are borne upon branches which show few or no signs of specialization. When they are produced in great abundance the growth of the gemmiparous branch tends to be limited, but this tendency is not very apparent and seems to be easily overcome. The projecting alar cell which is to form a gemma first divides by several longitudinal walls before the transverse walls which mark off the young gemma make their appearance. As a result of this the gemma upon separating leaves behind a patch of three to five small thallus cells instead of a single larger cell. The mature gemmae are very similar to the marginal gemmae of *M. uncigera*. Each one is a flat strap-shaped thallus with a single apical cell and numerous hooked marginal hairs (Fig. 11, A). It measures about 0.6 mm. in length by 0.15 mm. in width and is usually from six to eight cells across. Towards the base there is a gradual tapering, and the three to five basal cells, which form a short and poorly defined stalk, are commonly arranged in two layers. Sometimes the two-layered

condition persists for a short distance in the basal region (Fig. 11, B), but except for this the gemma is only one cell thick throughout its entire extent. The hooked hairs are sometimes truly marginal, but are more likely to be displaced to either surface. They usually occur singly, but are occasionally found in pairs. The gemmae show no evident indications of dorsiventrality.

The germination of a gemma exhibits no unusual features. It gives rise to a flat thallus which gradually becomes broader, the new marginal

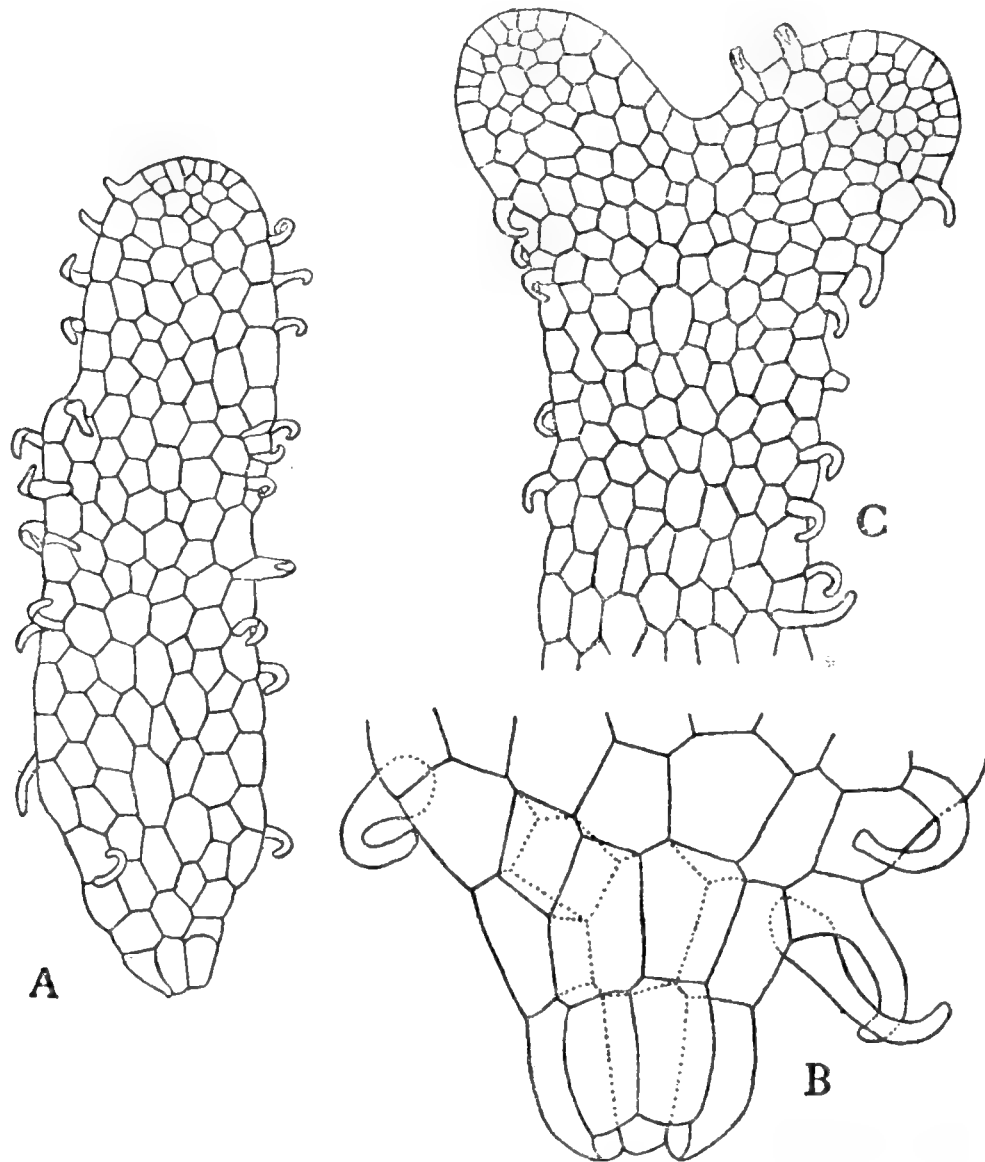


FIG. 11. *M. vivipara*. A. A gemma about ready to separate.  $\times 80$ . B. Base of a gemma.  $\times 300$ . C. Portion of a young thallus developed from a gemma, showing dichotomous branching.  $\times 80$ .

hairs tend to be straight instead of hooked, and many become displaced to what may now be considered the postical surface, other hairs arise on the same surface at some distance from the margin, and finally the costa appears and increases more and more in complexity. In some cases the development of postical surface hairs is deferred until the rudiments of the costa have become evident. A tendency to dichotomy frequently becomes manifest, not only in the gemma itself, but also in the young thallus while it still consists of a single layer of cells (Fig. 11, C). A similar dichotomy has already been described in *M. quadriseriata*, where, however, it occurs as a rather unusual exception. Both the gemmae and the young plants

which develop from them are subject to reversion. The production of new gemmae on a young and undifferentiated thallus is very unusual. When they do occur they are invariably marginal.

*Metzgeria vivipara*, sp. nov. Yellowish or brownish green, growing in depressed mats: thallus prostrate, repeatedly dichotomous, well-developed branches about 1.5 mm. wide and from 1.5 to 3.5 mm. long between the forks, plane or nearly so; costa bounded above by two rows of cortical cells and below by four rows (rarely only two or three); wings mostly from twenty to twenty-five cells broad, the cells thin-walled throughout or with very minute trigones, averaging about  $35 \times 25 \mu$ , slightly smaller towards the margin; hairs restricted to the margin and to the postical surface of both wings and costa, the marginal hairs often absent altogether, when present usually few and scattered, up to  $150 \mu$  long, straight or nearly so, sometimes truly marginal, but often slightly displaced to the postical surface, alar surface hairs and costal hairs usually more numerous than the marginal hairs, but sometimes more sparingly developed: inflorescence dioicous: ♀ branch orbicular-obovate, about 0.25 mm. long, sparingly pilose on margin and postical surface: ♂ inflorescence and sporophyte not seen: gemmae numerous, arising from the antical surface of the wings, ligulate, one cell thick except at the very base, stalk poorly defined, apical cell single, hairs marginal, short, hooked at the apex, usually single, but occasionally twinned, sometimes displaced to one surface.

On trunks of trees. Porto Rico: Barceloneta, April 19, 1887 (Sintenis, No. 144, distributed as *M. furcata*); Utuado to Adjuntas, March 21, 1906 (Britton and Cowell, No. 1242). The second specimens may be considered the type.

In its vegetative structure, *M. vivipara* shows a close relationship to *M. furcata*, the costa being built up according to a similar plan and the distribution of the hairs being much the same. It may be at once distinguished, however, by its antical gemmae with their slight cell-differentiation and hooked marginal hairs. Among other species with antical gemmae its closest ally is apparently *M. crassipilis*, which has a similar costa, and also bears hairs on the postical surface of the wings. The gemmae will serve at once to separate the two species; in *M. vivipara* these are ligulate and bear hooked hairs, while in *M. crassipilis* they are orbicular and the hairs are straight.

*Metzgeria Liebmanniana*, Lindenb. & Gottsche.

According to Stephani the range of this species extends from Mexico to Chile and eastward into Brazil. The specimens here described were collected in Contreras, Mexico, on October 15, 1908, by Barnes and Land (No. 430). They bear gemmae in great abundance. The gemmiparous branches, as in the two preceding species, are very slightly modified. In some cases a tendency towards a decrease in the number of cortical cells along the antical surface of the costa becomes apparent. On normal

branches, for example, these cells are arranged in from four to six rows, while on gemmiparous branches there are often only two rows present. An opposite tendency has already been noted in *M. crassipilis*, where the number of rows is often increased from two to three or four. It is possible, however, that this difference between the two species is not of very great significance, because it may be due to factors which are not concerned in the production of the gemmae. The development of the gemmae in *M. Liebmanniana* is very much the same as in *M. crassipilis*. Their

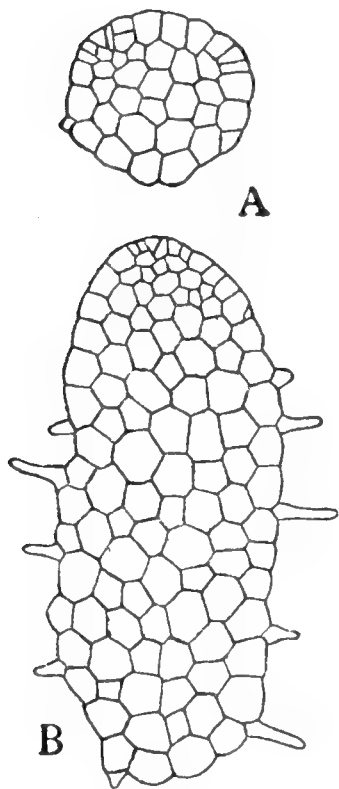


FIG. 12. *M. Liebmanniana*. A. A gemma about ready to separate.  $\times 80$ . B. A germinating gemma.  $\times 80$ .

structure at the time of separation is also similar, except that they are usually less differentiated. A mature gemma is in the form of a flat or concave plate of cells, orbicular in form and measuring about 0.12 mm. across (Fig. 12, A). It consists throughout of a single layer of cells and does not have a stalk, although the two basal cells can usually be distinguished. Very young gemmae sometimes show two apical cells, but it is exceedingly rare for more than one to persist. Marginal hairs are sometimes absent altogether and sometimes appear, in very small numbers, as short rudiments. If the gemma is plane it shows absolutely no indications of dorsiventrality; if it is not plane the concave surface represents what is morphologically the postical surface. Even here, however, the dorsiventrality is probably not stable at the beginning.

In germination (Fig. 12, B) the rudimentary hairs develop further and assume the function of rhizoids. New marginal hairs also make their appearance and tend to be slightly displaced to one surface. In cases where the gemma is concave, the hairs are always displaced to the concave surface, thus emphasizing its postical nature. The apex of the gemma soon gives rise to a new thallus which consists at first of a strap-shaped plate of cells bearing a few marginal hairs. Since these are truly marginal, the young plant is quite without evidences of dorsiventrality, even if the gemma from which it grew was concave. It is only later, as the differentiation of the thallus proceeds, that the dorsiventrality again becomes apparent. The steps of the process are essentially the same as in previously described cases. In rare cases the young thallus forks while it still consists of a single layer of cells.

#### *Metzgeria dichotoma*, (Swartz) Nees.

The present species was originally collected in Jamaica by Swartz and is apparently not uncommon in the Blue Mountains. Lindberg records it also from Cuba, Mexico, and Brazil. The gemmiparous material described



below was collected on Mount Morales, near Utuado, Porto Rico, on March 15, 1906, by E. G. Britton and D. W. Marble (No. 498). Its determination as *M. dichotoma* must be considered somewhat doubtful, since the Jamaican specimens examined by the writer are entirely destitute of gemmae. Lindberg, however, notes the occurrence of surface gemmae in this species although he fails to mention any of their peculiarities. The gemmiparous branches in the Porto Rican plants are scarcely if at all modified, and there seems to be no marked tendency for their growth to be limited. The gemmae arise in considerable abundance, but without definite order. An alar cell which is to give rise to a gemma first bulges in the

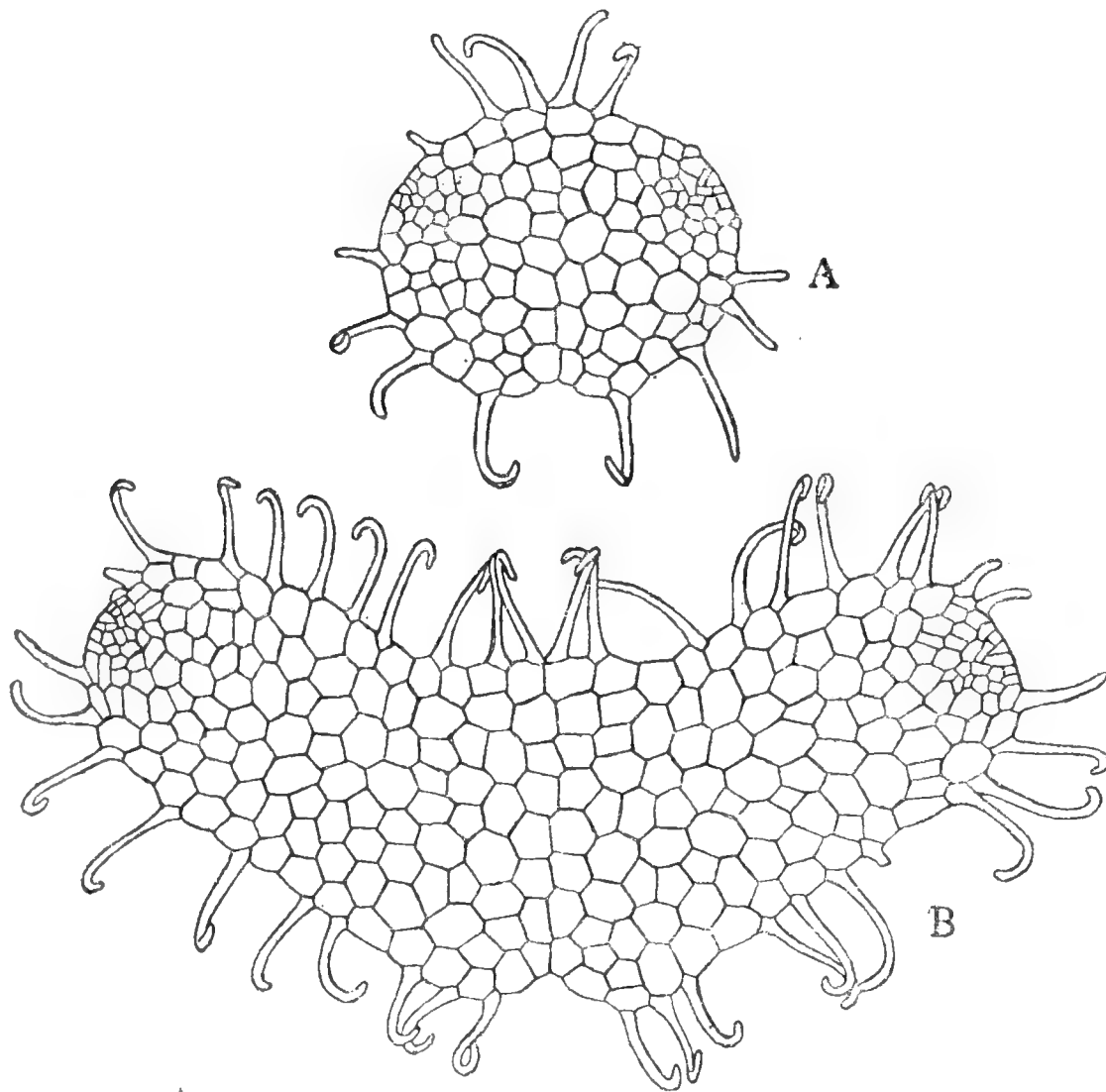


FIG. 13. *M. dichotoma*. A. A gemma at time of separation.  $\times 80$ . B. A germinating gemma showing the two young thalli.  $\times 80$ .

usual way and then divides by a longitudinal wall before forming the transverse wall which marks off the future gemma. The result is that the gemma leaves behind two small thallus cells when it becomes separated. The gemmae are very remarkable from the fact that each of the two cells, of which the young rudiment consists, develops regularly an apical cell after a few divisions, and the two apical cells persist and continue their activities until the gemma is ready to separate. The gemma is accordingly symmetrically developed, and the condition which is found in *M. crassipilis* as a rare exception is here the rule. A mature gemma (Fig. 13, A) is a circular or reniform plate of cells, perfectly plane and showing no



indications of dorsi-ventrality. Where the two basal cells are situated the margin is either truncate or more or less retuse. The numerous hairs are all truly marginal and always occur singly. They are about three times as long as the cells of the gemma and are hooked at the apex. The two apical cells are situated in the outer portions of the symmetrical halves. A typical gemma is about 0.2 mm. long at the time of separation and measures about eight cells across the middle.

When a gemma germinates normally each of the apical regions develops a strap-shaped thallus one cell thick and about eight cells wide (Fig. 13, B). These two thalli diverge at a wide angle, and continue to produce an abundance of scattered hooked hairs along the margin similar to those on the gemma. In some cases only one of the apical cells continues its divisions, and under these conditions only one thallus is developed, the germinating gemma thereby losing its symmetrical appearance. A similar result is produced when one of the young thalli grows more rapidly than the other. Unfortunately, the later stages of germination could not be observed.

*Metzgeria disciformis*, sp. nov.

The plants which bear the gemmae are scarcely modified, and apparently show no marked tendency to limit their growth. The gemmae, as in

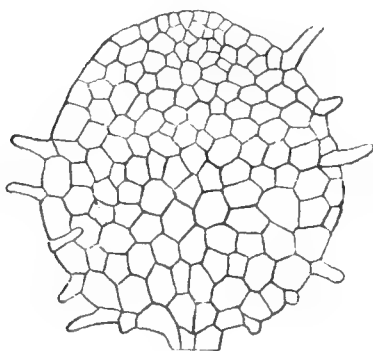


FIG. 14. *M. disciformis*.  
A gemma about ready to separate, postical view.  $\times 80$ .

the preceding species, arise without definite order, and are sometimes fairly abundant. In the early development of a gemma one of the alar cells projects above the surface in the usual way, but it becomes at once the mother-cell of the gemma without undergoing one or more preliminary divisions. In this respect *M. disciformis* differs markedly from most of the other species which bear antical gemmae, but agrees with most of those which bear marginal gemmae. When a gemma separates it leaves behind an empty cell which appears like a perforation of the thallus. The gemmae are often more or less strongly tinged with blue, especially when young, a condition which seems to be due to a pigmentation of the cell-walls. A mature gemma (Fig. 14) is in the form of a concave and circular plate of cells, about 0.2 mm. in diameter and measuring about twelve cells across. In the basal region it shows a short but distinct stalk, composed of two or three cells, and the single apical cell is also clearly apparent. As in *M. Liebmanniana*, the concave surface of the gemma represents the postical surface, and the dorsi-ventrality of the structure is even better marked. All around the margin are numerous short and straight hairs, some of which are truly marginal, although the majority are slightly displaced to

the postical surface. The hairs sometimes spread, but are more likely to be at right angles to the surface of the gemma. The convex antical surface is occasionally destitute of hairs, but usually bears a few near the centre, and these antical hairs are considerably longer than the marginal.

The germination of a gemma was observed in a single instance. The marginal hairs had grown longer, and assumed the function of rhizoids, holding the gemma firmly to the surface of a leaf. The apical end of the gemma had grown out into a short thalloid structure which had produced a number of scattered postical hairs also acting as rhizoids. The majority of these hairs were situated close to the margin. Further differentiation of the thallus had also taken place, a rudimentary costa having made its appearance. It will be seen that the dorsiventrality of the gemma here persists in the young thallus, whereas in *M. Liebmanniana* it apparently disappears.

**Metzgeria disciformis**, sp. nov. Pale green, more or less tinged with blue, growing in depressed mats: thallus prostrate, repeatedly dichotomous, well-developed branches about 1.2 mm. wide and from 1 to 2 mm. long between the forks, plane or slightly convex; costa bounded antically by two rows of cortical cells and below by four rows (rarely only two or three); wings mostly from fifteen to twenty cells broad, the cells with slightly thickened walls, but without evident trigones, averaging about  $25 \times 21 \mu$ , and not varying much in size in different parts of the thallus; hairs restricted to the margin and to the postical surface of both wings and costa, sometimes few, but usually abundant along the costa, marginal hairs averaging about  $90 \mu$  in length, straight or nearly so, occurring singly, slightly displaced to the postical surface: inflorescence not seen: gemmae arising from the antical surface of the wings, in the form of circular concave disks, one cell thick throughout, abruptly contracted at the base into a short stalk composed of two or three cells, apical cell single, antical hairs few or wanting, borne near the centre of the convex surface, marginal hairs numerous, but much shorter, usually displaced to the postical surface.

On leaves. New Zealand: without definite locality or date (Colenso, No. 1997). The specimens were communicated to the writer under the name *M. australis*, Steph., a species which is now considered a synonym of *M. nitida*, Mitt. They differ, however, very markedly from this species.

Except for its small cells and peculiar colour the present species agrees pretty closely with *M. furcata* in its vegetative structure, the costa being built up according to the same plan, and the distribution of the hairs being much the same. The antical gemmae will at once serve to distinguish it. Their marked dorsiventrality is perhaps their most striking characteristic. It should also be noted that the antical surface of the thallus bears a few scattered hairs in rare instances, a condition which should doubtless be regarded as abnormal.

*Metzgeria linearis*, (Swartz) Aust.

The original material of this peculiar species was collected by Swartz on the island of Santo Domingo. It has since been found in Cuba by Wright and in Jamaica by E. G. Britton. The account of the gemmae is largely based on Wright's specimens, which have also served for the figures. *M. linearis* is distinguished from the other known species of the genus by the fact that the marginal cells are much elongated and have strongly thickened walls,

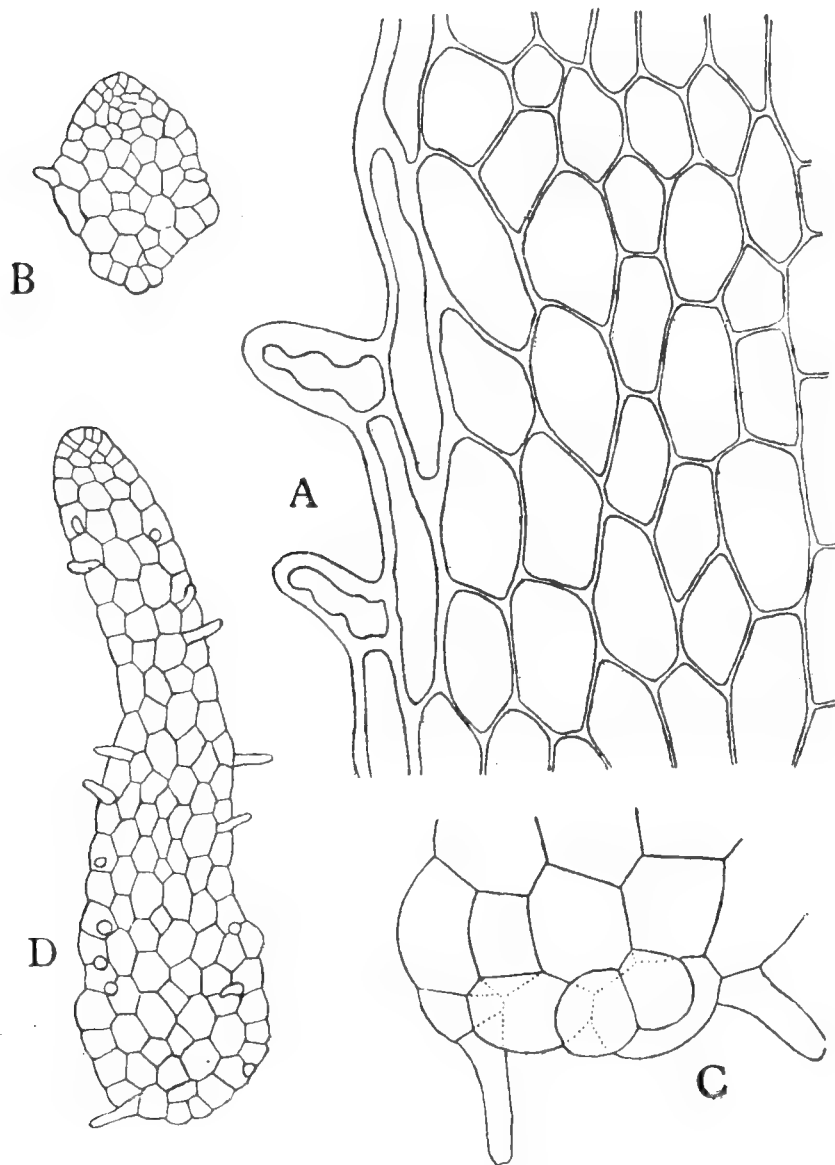


FIG. 15. *M. linearis*. A. Portion of a normal thallus, showing the differentiated margin.  $\times 300$ . B. A gemma at time of separation.  $\times 80$ . C. Base of a gemma, showing the two stalk-cells in a different plane.  $\times 300$ . D. A germinating gemma.  $\times 80$ .

thus forming a distinct border to the thallus (Fig. 15, A). The marginal hairs also have thickened walls, except when they assume the function of rhizoids, and are frequently sharp-pointed. The gemmiparous branches tend to be a little narrower than usual, and to be more or less tinged with blue. In other respects they are essentially like normal branches, and show no marked indications of being limited in their growth. The gemmae arise in no definite order. Each one begins as a single projecting alar cell, which becomes at once the mother-cell of the gemma, thus agreeing with *M. disciformis*. At the time of separation the gemma consists of an oval or circular plate of cells (Fig. 15, B), which is plane or nearly so. It averages about 0.12 mm., or six cells, wide. At the base of the gemma two stalk-cells, usually lying in a different plane, may be distinguished (Fig. 15, C), while the opposite end, with its single apical cell, is often narrowed to a very blunt point. The hairs produced are short, thin-walled, and few. They are sometimes truly marginal, but are more likely to be displaced to either surface indiscriminately. Under these circumstances they are often situated at the inner edge of the marginal cells, and thus appear at some little distance from the margin. Occasionally, in fact, some of the internal cells give rise to hairs. The gemmae present no evidences of dorsiventrality.

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The germination has been observed only in the early stages, and agrees essentially with what has been described for most of the preceding species. The young thallus, which results from the activity of the apical cell, is usually narrower than the gemma (Fig. 15, D), although the change from one to the other is often gradual. The narrow condition persists for a variable distance, and then the young thallus becomes broader again. Scattered hairs, like those on the gemma, continue to be produced, and a forking of the thallus is sometimes to be observed. Although in some cases the young thalli had attained a length of a millimetre or more, no further differentiation and no evidences whatever of dorsiventrality had become apparent. In all probability the peculiar marginal cells and hairs of the mature thallus mark a very late stage of development.

*Metzgeria fruticulosa*, (Dicks.) comb. nov.

Since most recent writers have considered *M. fruticulosa* to be nothing more than a form or variety of *M. furcata* its geographical distribution is very incompletely known. It has been recorded in Europe from a number of scattered localities, but the only American station which the writer can quote at the present time is near Aberdeen, Washington, where the plant was collected on February 18, 1909, by Foster (No. 944). The description of the gemmiparous branches (with the accompanying figures) is drawn largely from these specimens, which were kindly supplied by Miss Haynes. Material from Salem in Baden, Germany, collected by Jack (Hep. Europ. No. 357), and from Cherbourg, France, collected by Corbière, has been used for comparison. In contrast to the species already described *M. fruticulosa* has strongly specialized gemmiparous branches. Other species with similar branches have already been alluded to in the introduction, but unfortunately none of these have been available in sufficient quantity for detailed study.

The normal branches in *M. fruticulosa* are prostrate and divide by forking. The costa is usually bounded both antically and postically by two rows of cells (Fig. 16, A), although the number of postical rows is sometimes three or even four. The number of internal costal cells averages about ten in cross section. The wings are plane or slightly convex and attain a width of perhaps ten cells. On the postical surface of the costa and along the margin are numerous straight hairs which often act as rhizoids. Scattered hairs on the postical surface of the wings are sometimes present as well. Most of the marginal hairs occur singly, but they are sometimes geminate. They are apparently never displaced to the postical surface. In the vicinity of the apex slime papillae of the usual type may be demonstrated. According to most writers the fresh plants are green, but they often become tinged with blue after being dried.

When a branch becomes gemmiparous, it gradually curves away from

the substratum, and may in time assume a position at right angles to it. The hairs become fewer and fewer, and before long cease to be developed at all, and the slime papillae also fail to appear. The wings become strongly convex, and at the same time grow more and more narrow until in some cases the thallus becomes reduced to the costa. The first change to be noted in the costa is a reduction in the number of internal cells (Fig. 16, B). As growth continues the number of these cells again becomes larger (Fig. 16, C). With their increase in number they also increase in size, thus approaching in appearance the cortical cells, but they can always be

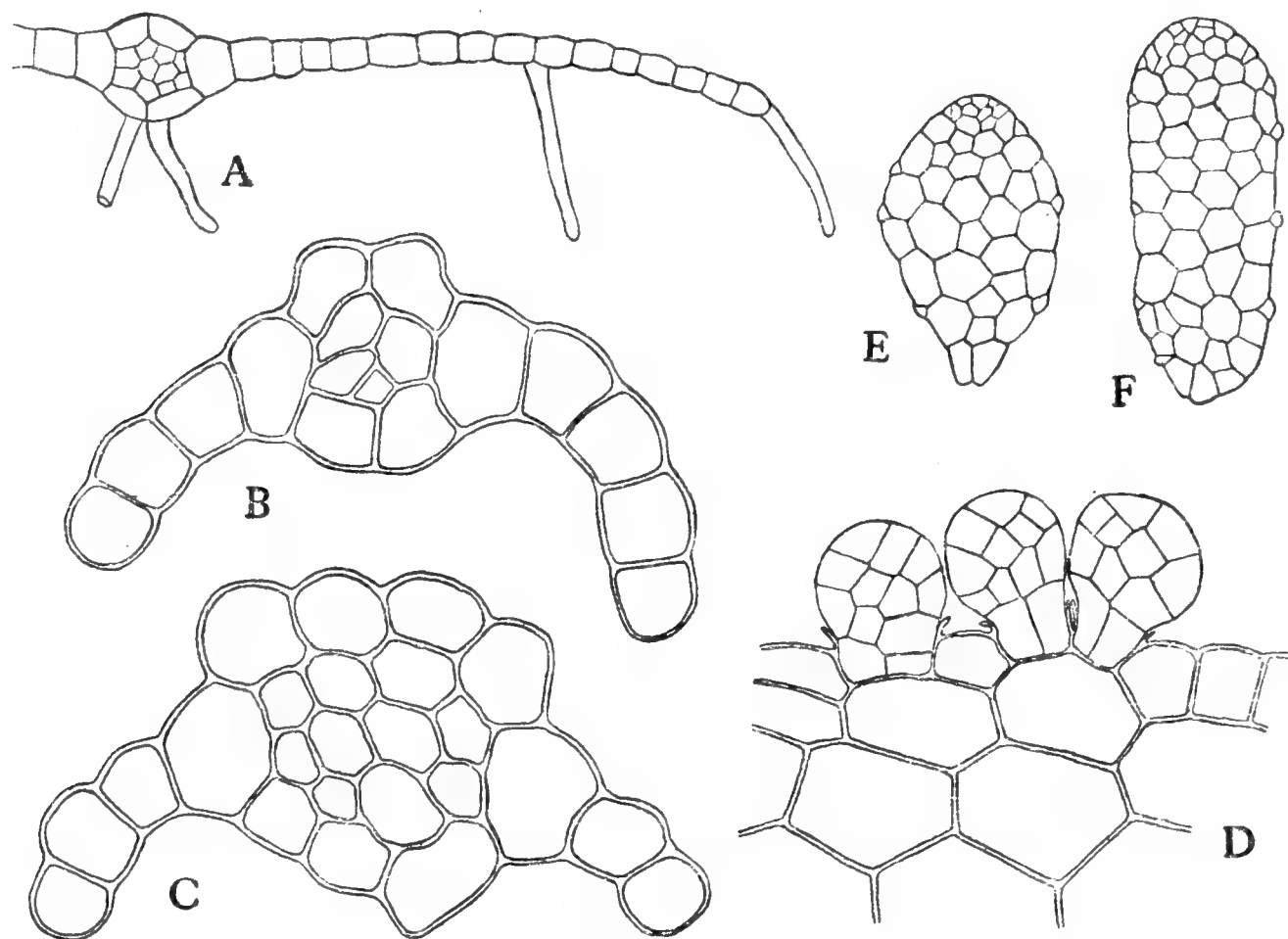


FIG. 16. *M. fruticulosa*. A. Cross section of a normal thallus.  $\times 80$ . B. Section through the lower portion of a gemmiparous branch.  $\times 300$ . C. Section through the upper portion of a gemmiparous branch.  $\times 300$ . D. Young gemmae.  $\times 300$ . E, F. Gemmae about ready to separate.  $\times 80$ .

distinguished by their somewhat greater length. The cortical cells first remain in four rows, but are more bulging than in a normal costa. Later on they become more numerous, forming perhaps four rows both antically and postically, but as they increase in number their arrangement becomes irregular so that the rows are no longer distinct. If the wings become reduced to the cells which bound the internal tissues of the costa laterally, then the gemmiparous axis exhibits a condition of radial symmetry, as Goebel ('98 *a*, p. 275) has described for *M. conjugata*. The radial portion of the shoot is always very short because its growth comes to an end soon after extreme specialization has been reached. Very often, in fact, the growth stops before the radial condition has been attained.



The first gemmae formed are marginal in position. Sometimes they appear on branches which are just beginning to show signs of specialization, and under these circumstances the branch may recover, as it were, and continue its growth normally. In most cases, however, the formation of gemmae does not begin until the modification of the branch is well under way. They become more numerous as the growth of the branch continues, and before long arise not only from the alar cells, but from the cortical cells as well. When the thallus ceases to develop wings, any surface cell seems to have the power of producing a gemma. When a gemma is to be formed, whatever its position, the projecting cell becomes at once the mother-cell of the gemma (Fig. 16, D). At the time of separation the gemmae are small circular to oblong plates of cells about 0.12 mm. wide (Fig. 16, E and F). They taper more or less distinctly towards the basal end and show a rounded apex with a single apical cell. Except for the scattered rudiments of marginal hairs they are quite undifferentiated. They also show no distinct signs of dorsiventrality, although they are sometimes slightly concave. It will be seen that these gemmae are among the simplest that have been described.

The germination of a gemma, when following a normal course, shows few peculiar features. The marginal rudiments of hairs become elongated and assume the function of rhizoids. The apical region then grows out into a thallus which gradually becomes broader. Although consisting at first of a single layer of cells it soon produces marginal and surface hairs. The latter are restricted to the surface turned towards the substratum, which at the same time becomes concave, so that the dorsi-ventrality of the young plant is established at an early stage. The marginal hairs usually occur singly, but occasionally appear in pairs as differentiation proceeds. They may be truly marginal or slightly displaced to the postical surface, a condition which tends to become less and less frequent. With the differentiation of the costa, which takes place in the usual way, the postical hairs become more numerous along its course and less numerous on the postical surface of the wings. Forking occasionally takes place before the costa has made its appearance.

The germination, however, often follows an aberrant course. This is sometimes due to reversion, by means of which the thallus becomes narrower or less differentiated as it advances in length. But it is much more frequently due to the development of new gemmae and to the modifications associated with their production. The new gemmae may arise on the original gemma itself or on the young thallus in any stage of its differentiation. When the production of gemmae is abundant the growth of the gemmiparous axis soon comes to an end, and this may take place while the young plant still consists of a single layer of cells. If the costa is already differentiated before the new gemmae appear the



axis acquires the form and structure of the specialized gemmiparous branches described above. Except under the last conditions the new gemmae are always marginal and show no peculiar features.

It follows from the above description that the development of gemmae in *M. fruticulosa* is often associated with a more or less incomplete differentiation of the gemmiparous plant ; in other words, that the growth of the plant is concluded while it is still in an embryonic or juvenile condition. Even the prostrate thallus, which has been spoken of as normal, presents certain juvenile features, and apparently never reaches the stage of development in which sexual branches can be produced. These facts naturally bring the validity of the plant, as a species, into question, and it seems possible that it may simply represent an immature stage of some other member of the genus. If the known range of *M. fruticulosa* is taken into consideration, it will be seen that three other species, *M. pubescens*, *M. furcata*, and *M. conjugata*, have a very similar distribution. With the very distinct *M. pubescens* it can hardly have any close connexion, but with the other two species it shares numerous characters in common. In *M. furcata*, var. *ulvula*, however, another plant is met with in which the thallus usually fails to develop beyond a certain stage. Since this variety *ulvula* is entirely different from *M. fruticulosa* in appearance and method of growth, it is scarcely probable that they can both represent juvenile conditions of the same species. The fact that the marginal hairs are not displaced, and that they are occasionally geminate, indicates an approach to *M. conjugata*, but its identity with this species could only be proved by culture experiments. It should be noted, however, that the gemmiparous plants which Goebel ('98 a, p. 275) refers to *M. conjugata* are apparently what is here described as *M. fruticulosa*, but Goebel does not state that he demonstrated the connexion between his plants and undoubted *M. conjugata*. Until this is done it seems allowable to keep the two species distinct. If it becomes necessary to unite them in the future the plant should still bear the name *fruticulosa*, since this has a priority of over eighty years. The history of *M. fruticulosa* and the various views which writers have held concerning it are clearly shown by the following synonymy, taken mostly from Lindberg's Monograph:—

***Metzgeria fruticulosa***, (Dicks.) comb. nov.

*Riccia fruticulosa*, Dicks., Pl. Crypt. Brit., i, 8. 1785.

*Fungermannia fruticulosa* Smith, Engl. Bot., xxxv, pl. 2514. 1813.

*Fungermannia furcata*, var. *aeruginosa*, Hook., Brit. Jung., pl. 55. 1816.

*Fasciola violacea*, Dumort., Comm. Bot., 114. 1822 (not *Fungermannia violacea*, Ach.).

*Echinogyna violacea*, Dumort., Syll. Jung. Eur., 84. 1831.

*Echinomitrium violaceum*, Corda, Deutschl. Jung. (in Sturm's Flora), 81, pl. 22. 1832.

*Metzgeria violacea*, Dumort., Recueil d'Obs. sur les Jung., 26. 1835.

*Metzgeria furcata*, var. *gemmifera*, Nees, Naturg. der europ. Leberm., iii, 488. 1838.

*Metzgeria furcata*, var. *violacea*, Dumort., Hep. Europ., 139. 1874.

*Metzgeria furcata*, var. *aeruginosa*, Moore, Proc. Irish Acad., 2nd Ser., ii, 665. 1876.

*Metzgeria furcata*, var. *fruticulosa*, Lindb., Acta Soc. Faun. Fl. Fenn., i, 40. 1877.

By some of the earlier writers *M. fruticulosa* was supposed to be identical with *Jungermannia violacea*, Ach.,<sup>1</sup> a species based on specimens collected at Dusky Bay, New Zealand, in 1773, by Sparrman. These specimens were examined by Lindberg, who pointed out that they were distinct from the European plant. He referred them to *Metzgeria conjugata*, as a variety *violacea*, although he knew the typical *M. conjugata* only from the Northern Hemisphere. Judging from Lindberg's description the variety *violacea* bears specialized gemmiparous shoots and is deserving of further study. In Stephani's monograph of the genus *Metzgeria* neither *M. fruticulosa* nor *M. violacea* is mentioned.

#### GEMMAE OF METZGERIA AND OF OTHER BRYOPHYTES COMPARED.

Vegetative reproduction by means of gemmae is a phenomenon of widespread occurrence among the Bryophytes, and has been observed in all the orders except the Sphagnales and the Andreaeales. The various types of gemmae which have been found in the Bryales are fully discussed by Correns ('99), and the gemmae of the Hepaticae have been similarly, but more briefly, treated by Cavers ('03). According to Correns (p. 446) the power to produce gemmae (and similar reproductive bodies) is inherent in certain species, but absent from others, so that it ought to be regarded as a definite specific character. In most of the gemmiparous species of the Jungermanniales the gemmae are in the form of minute unicellular or bicellular bodies, but in the remaining species they are multicellular and sometimes show a greater or less degree of cell-differentiation. In a few cases such gemmae form solid masses of cells, as in *Blasia* and *Cavicularia*, but it is much more usual for them to be in the form of flattened thalloid structures, similar to those just described for *Metzgeria*. Gemmae of this type are by no means confined to genera in which the adult plant is a leafless thallus. They occur also in a number of leafy genera belonging to the Porelleae, Raduleae, and Jubuleae. In all of these sub-orders, however, as Goebel ('89, p. 16) and others have pointed out, the spore in germinating first gives rise to a thallus, upon which the leafy shoot after-

<sup>1</sup> In Weber and Mohr's Beiträge zur Naturkunde, i, 76, pl. 1, figs. 1-3, 1805.

wards develops, so that the gemmae here, as in *Metzgeria*, show certain of the characteristics of embryonic plants.

Thalloid gemmae are now known in nine genera of the leafy Jungermanniales, and have been carefully studied in *Radula*, *Cololejeunea*, *Metzgeriopsis*, and *Cyclolejeunea*.<sup>1</sup> They usually arise on the leaves themselves, but are occasionally borne on the margins of other gemmae or on young plants which are still in the thalloid condition. In all the known cases the gemmae are but one cell thick throughout. Each one takes its origin in a single cell, just as in *Metzgeria*, and in all cases that have been described the cell first projects and then divides into two cells by a wall perpendicular to its long axis. The outer cell thus formed represents the mother-cell of the future gemma, while the inner cell may be regarded as a poorly defined stalk. The conditions are essentially the same as in *M. uncigera* and *M. crassipilis*. The separation of the gemma is schizolytic, and is brought about by a splitting of the walls between its basal cells and the stalk-cell. Although the gemmae in all of these leafy genera are very similar to those described for *Metzgeria*, the resemblance is especially close in the tropical genus *Cyclolejeunea*, of which about six species are at present known. In this genus there are two types of gemmae, one with a single apical cell (which does not always persist until maturity) and the other with two. The first type is apparently the more frequent, the second being known in the single species *C. angulistipa*. Gemmae of the first type are essentially like those of *Metzgeria crassipilis*, *M. linearis*, &c., while those of the second type are duplicated in *M. dichotoma*. The resemblance is made still more striking by the presence of hairs, formed by the elongation of small cells. These hairs sometimes function as rhizoids, but often fail to do so, the true rhizoids being independently produced.

According to Lindberg ('75) the marked similarity between the various thalloid gemmae indicates a true genetic relationship, and in his classification of the Hepaticae he separates *Metzgeria* from the other thallose Jungermanniales and includes it in his group Anomogamae, to which he refers also *Frullania*, *Lejeunea* (in its broad sense), *Radula*, *Porella*, and *Pleurozia*. His views on the subject, however, have not met with much favour, most writers preferring to retain *Metzgeria* in the group to which the other thallose genera are referred. It should be remembered in this connexion that the species producing thalloid gemmae all grow in places where it is difficult for young plants to gain a foothold, such, for example, as rocks, the bark of trees, or the surface of living leaves. The thalloid form is especially effective in enabling the gemmae (as well as the young plants developing from spores) to hold themselves in place. It seems perfectly reasonable, therefore, to associate the similarity in the gemmae with the similarity in environment.

<sup>1</sup> Cf. Goebel ('87), Schiffner ('93), and Evans ('04).

Among the thallose Jungermanniales the closest relative of *Metzgeria* is apparently *Riccardia* (*Aneura*), and this is the only genus in which the gemmae are at all similar. Even here the resemblance is much less striking than in the leafy forms just described, largely because the gemmae are still very rudimentary structures at the time of their separation. They are wholly undifferentiated and are in the form of minute oval bodies, each consisting usually of only two cells. Each gemma arises from one of the surface cells of the thallus, which becomes at once the mother-cell of the gemma without undergoing a preliminary division. The contents of the cell separate from the wall, surround themselves by a new wall of cellulose, and then divide. The free outer wall of the mother-cell is then ruptured and the gemma escapes. According to Goebel ('82, p. 338), the escape is effected by a swelling of the inner layers of the wall of the mother-cell. This process is comparable with the deposition of the gelatinous layer in the projecting cell of *Metzgeria* which is to give rise to a gemma, except that in the latter case the gelatinous substance does not completely enclose the protoplast of the cell, but only that portion of it which would have been exposed upon the rupture of the outer wall. In *Riccardia* further development is deferred until after the gemma is set free, while in *Metzgeria* the development is continued until a multicellular gemma is formed. In other respects the conditions are much the same in the two genera, as Goebel ('98 a, p. 275) has already emphasized.

The large and complicated gemmae in the Marchantiales, known only in *Marchantia* and *Lunularia*, are totally distinct from the gemmae of *Metzgeria*, while the gemmae of the Anthocerotales are still too incompletely known to make comparison profitable. The gemmae in the Bryales, which have been so thoroughly studied by Correns ('99), are almost always set free by a rhexolytic process, in which a specialized stalk-cell, or tmema, is torn across. Among those in which the separation is schizolytic the only ones which at all resemble the gemmae of *Metzgeria* are found in *Tortula papillosa* and *T. latifolia*, both of which grow on the trunks of trees. In these two species the gemmae are irregular oval bodies, each consisting of about twelve cells and showing only a slight differentiation. A single leaf-cell gives rise to a number of these gemmae in succession, and when they germinate they first develop a branched protonema as in all other mosses.

#### CONDITIONS UNDER WHICH GEMMAE ARE PRODUCED.

The gemmiparous species of *Metzgeria* do not produce gemmae under all circumstances, and the same is true of other Bryophytes. Apparently the conditions which induce the formation of gemmae are similar to those which induce regeneration. As Goebel (cf. '98 a, p. 277) has pointed out more than once almost any liverwort cell has the power of regenerating, that is, of giving rise to an entire new plant. In doing this, if it has



acquired the characteristics of maturity, it first goes back into an embryonic condition, and then undergoes the necessary cell divisions. The new plant formed in this way does not at first show the peculiarities of an adult individual, but begins its life in one of the juvenile or embryonic stages of the species. In those Lejeuneae, for example, where the mature plant is a leafy shoot with underleaves, the regenerated plant is very often a leafy shoot without underleaves or even a thallus, upon which a leafy shoot subsequently develops. Under conditions which are considered normal for the growth of a species, the mature cells are in some way prevented from exercising their latent power of division, and regeneration does not take place. This is apparently due to a kind of antagonism which exists between the apical region and the other parts of the plant. In other words, the apical region, where active growth is normally going on, exerts an inhibitory influence upon the other cells, preventing or making difficult their independent development. It is probable that this influence is connected with nutritive processes in such a way that all the food available for growth passes to the apical region, leaving none for the other cells.

The influence just described can easily be removed by dissecting off a leaf or a mature piece of a thallus. If the leaf or thallus fragment is then placed under conditions favourable for growth, regeneration ought to be induced. Among those who have carried on successful experiments along this line, Vöchting ('85) and Schostakowitsch ('94) may be especially mentioned. Vöchting confined his attention to *Marchantia* and *Lunularia*, but Schostakowitsch selected his material from all groups of the Hepaticae. Regeneration is sometimes brought about in nature by a very similar process. In certain leafy species, for example, the separation of some of the leaves from the axis may be considered a perfectly normal occurrence. This is seen especially well in various tropical Lejeuneae, such as *Cheilo-lejeunea decidua* and the species of *Rectolejeunea* recently described and figured by the writer ('06). The deciduous leaves, under suitable conditions for growth, give rise to new plants by regeneration.

In some of the gemmiparous species of *Metzgeria*, a similar antagonism between the apical region and the cells capable of developing gemmae is evident. When this is the case no gemmae are produced so long as the apical growth continues vigorous. It is only when the apical cell dies or when its activities are lessened or stopped altogether that the formation of gemmae begins. The death of the apical cell apparently takes place regularly in *M. oligotricha*, and brings about not only the production of gemmae, but also the formation of postical adventive branches, which seem to require similar conditions for their development. The gradual diminution in the divisions of the apical cell, leading eventually to complete suppression, is a regular process in the specialized gemmiparous thalli of *M. fruticulosa*, but it also takes place in gemmiparous branches which are not specialized,

such as those described for *M. uncigera*. In all these cases the inhibitory influence of the apical region is either destroyed completely or else diminished to such an extent that the thallus cells are able to overcome it and develop gemmae. In other words, some or all of the food available for growth is distributed among the cells capable of forming gemmae.

A similar connexion between the limitation of growth in the gemmiparous shoot and the production of gemmae may also be observed in many of the leafy Hepaticae. It is especially marked in species with unicellular or bicellular gemmae, such as *Odontoschisma denudatum*, *Sphenolobus Hellerianus*, and *Calypogeia Trichomanis*. In these species the gemmiparous shoots curve upwards until they are erect instead of prostrate, their leaves diminish more and more in size, and the shoot as a whole becomes more and more nearly radial. The gemmae are at first limited to the leaf-margins, but eventually, when growth in length has come to an end, the whole apex of the shoot becomes a mass of gemmae. In species with discoid gemmae the connexion is rarely so marked, but in *Cyclolejeunea convexistipa* the gemmae are frequently borne on short branches with reduced and specialized leaves. The modifications exhibited by these gemmiparous shoots with limited growth are comparable with those seen in *Metzgeria fruticulosa*.

Just why the normal activities of the apical region are lessened in these cases and finally brought to an end is by no means clear. In some instances the result is perhaps due to poor nutrition, bringing about an enfeeblement of the whole plant, but this cannot be the effective cause in all cases, because a limitation of growth often takes place in plants which are robust. Under these circumstances the plant is probably able to control the apical growth, perhaps by diverting the currents of food to other regions. Apparently something of the same sort takes place in such species as *M. dichotoma*, where the growth of the gemmiparous branch continues for an indefinite period. The power of the plant to regulate the distribution of the nutritive materials, and thus to weaken or destroy the inhibitory influence exerted by the apical region upon the cells capable of producing gemmae, may be considered a specific characteristic.

In certain species of *Metzgeria*, notably in *M. fruticulosa*, the strength of the inhibitory influence seems to increase as the plant grows more differentiated. In other words, a plant which is passing through an embryonic or juvenile stage is more likely to produce gemmae, and to have its growth brought to an end, than a plant with well-differentiated wings and costa. The same tendency is also strongly marked in *M. furcata*, especially in the variety *ulvula*, and may be observed in a less degree in several of the other species. As the thallus of *M. furcata* becomes more and more differentiated, the apical growth gains so strong a supremacy that its



influence can only be overcome with difficulty. The result is that a robust and mature thallus usually produces no gemmae whatever. Whether the species in which gemmae are unknown are able to produce them before they have completed their embryonic life is an interesting problem which can only be solved by experiment. It has already been suggested that *M. fruticulosa* may perhaps represent a juvenile stage of *M. conjugata*. If this is proved to be the case, then the influence of the apical region in this remarkable plant must be regularly overcome before the complete differentiation of the thallus has been reached.

Some writers recognize a second antagonism between the production of gemmae and the development of sexual organs, based on the fact that gemmae are usually more abundant on sterile individuals. Correns ('99, p. 449) has shown, however, that this antagonism is more apparent than real in the Bryales, and the same thing seems to be true of other Bryophytes. The development of sexual organs, as he notes, is associated with a late stage in the differentiation of the gametophyte, while gemmae, as has just been shown, are often produced before the late stages are reached, and sometimes cease to be formed afterwards. It follows from these observations that the sexual organs make greater or more special demands upon the gametophyte than the gemmae. The sterility of gemmiparous plants is therefore due to the fact that they often fail to reach the stage of development which can meet the requirements necessary for the development of sexual organs. The occasional occurrence of both gemmae and sexual organs on the same individual also indicates that there can be no strong antagonism between them.

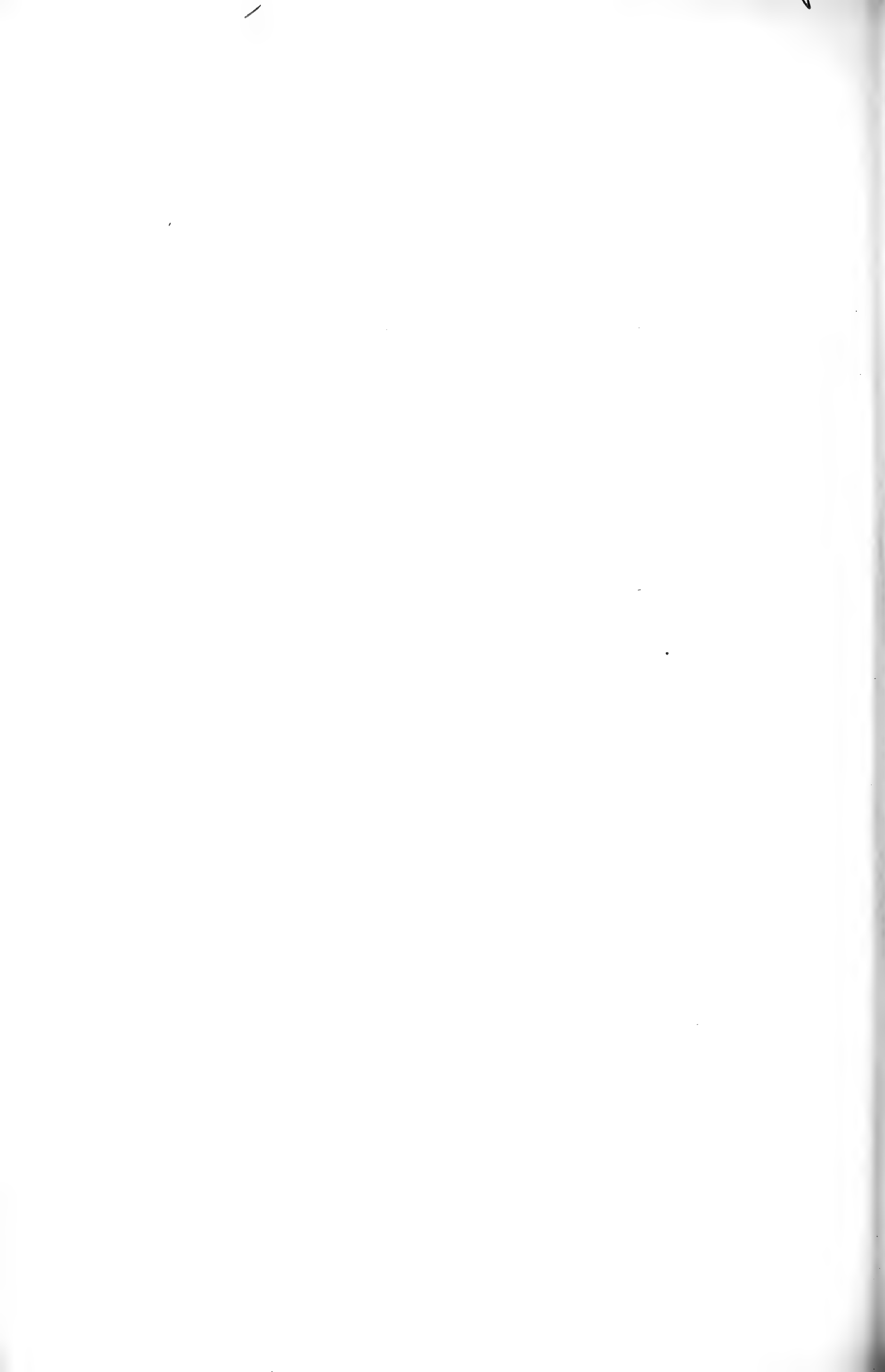
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# The Structure of *Podocarpus spinulosus*, (Smith) R. Br.

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With Plate XXI.

INTRODUCTION.

THE following account of the structure of *Podocarpus* is based mainly upon material of *Podocarpus spinulosus*, (Smith) R. Br., which was obtained from the Botanic Gardens, Sydney, New South Wales. The material was sent to Professor Seward by Mr. Maiden, and was handed over to us for investigation.

Material of *Podocarpus alpinus* obtained from the Royal Gardens, Kew, and of one or two other species of which only a limited stock was available, were examined at the same time for the sake of comparison.

Pilger (13) in his monograph of the Taxaceae places *Podocarpus* and four other genera—*Pherosphaera*, *Microcachrys*, *Saxegothaea*, *Dacrydium*—in the sub-family Podocarpoideae, the other sub-families of the group being the Phyllocladoideae, containing the single genus *Phyllocladus*, and the Taxoideae, comprising *Taxus*, *Torreya*, *Cephalotaxus*. Pilger evidently then considers the Podocarpoideae to be very closely related to the Taxoideae. Later work, however, would appear to necessitate a modification of this view.

The systematic position of *Podocarpus* and closely allied genera is discussed in the latter part of this paper.

The genus *Podocarpus*, as described in Pilger's monograph, contains no less than sixty-three species, the large majority of which are found in the Southern Hemisphere. New Zealand, Australia, and the Malayan islands are at present the home *par excellence* of the genus, while smaller centres of distribution are such different regions as East Africa (São Thomé), Cape Colony and Natal, Assam, Japan, China, Central America, West Indies, and Chili. Where the genus is present within the tropics it is generally

found on mountain ranges at a considerable elevation. Just as *Pinus* may be considered the dominant Coniferous genus of the Northern Hemisphere, so *Podocarpus* occupies a similar position among the Conifers of the Southern regions.

In regard to the distribution of the genus in geological times, most of the specimens referred to *Podocarpus* or closely allied genera have been determined from leaves only, and it is well known that leaf determinations are frequently untrustworthy. In this connexion mention may be made of the striking similarity in external features between the leaves of some species of *Agathis* and those of the *Nageia* section of *Podocarpus*. Seward and Ford (16) found, on cutting sections of a species originally referred to *Dammara Motleyi*, that the plant so named is really a species of *Podocarpus*.

Assuming that some at least of the specimens are correctly determined, then *Podocarpus*, like *Araucaria*, must have been widely distributed in the North temperate zone during the later Mesozoic and Tertiary times. Pilger states that the leaves from the Potomac or later Mesozoic flora of the United States are to be referred to the *Nageia* section of the genus on account of the plurinerved form of venation, while leaves from the Eocene and Miocene strata of Europe belong to the uninerved sections of the genus.

*Podocarpus* shows a considerable range of habit among its many species. Several of them are large trees; e. g. *P. amarus* attains a height of sixty metres in Java, and *P. dacrydioides* forms practically pure forests in New Zealand. On the other hand, *P. alpinus*, found on mountains in Tasmania, is a low shrub, and *P. nivalis*, on exposed places in the alpine regions of New Zealand, becomes a dwarf shrub with prostrate branches.

#### VEGETATIVE ORGANS.

##### Stem.

The structure of the wood of *P. spinulosus* is very similar to that of *P. macrophyllus* described by Penhallow. Resin canals are absent in the wood, but there is a ring of them present outside the phloem. Their place in the wood is taken by resin cells, which are present in considerable numbers. The bordered pits of the secondary tracheides are uniseriate; no trace of the Araucarian type of pitting could be discerned, such as has been described by Gothan (8) for *Dacrydium* spp. and for *Saxegothaea* by Beust (3), Gothan (8), and Stiles (17). The medullary rays are invariably uniseriate, and are from one to three cells deep. The cells of the rays contiguous to the pith frequently contain resinous material, but apart from this all the cells of the rays are alike, there being no tracheidal cells such as are met with in the rays of *Pinus* and some other Conifers. There is a small central pith which consists mainly of resin cells and groups of stone cells; the latter are very conspicuous. Resin cells are also extremely abundant in the cortex.

### Leaf.

The leaves of *P. spinulosus*, as indeed is the case in the large majority of the species, are arranged spirally on the stem axis. It is interesting to note, however, that in the *Nageia* section of the genus the leaves have a decussate or subdecussate arrangement. The leaf of *P. spinulosus* is about 4-6 cm. long by 3-4 mm. broad, and ends in an acute point.

The anatomy of the leaf of *P. spinulosus* is very similar to that of *P. chilinus* described by Worsdell (29), and that of *P. neriifolius* and *P. falcatus* examined by Bernard (1). As in all the narrow-leaved species of the genus, there is a single vascular bundle; this is accompanied on its phloem side by a resin canal. Placed laterally to the vascular bundle is the transfusion tissue, which is extremely well developed. The transfusion tracheides show numerous reticulations on their walls when the leaf is cut at right angles to the midrib.

On the side of the transfusion tissue remote from the bundle, there is a layer of parenchymatous cells, beyond which is the 'accessory transfusion tissue', such as is described by Worsdell for *P. chilinus*. This tissue runs outwards almost to the margin of the leaf. This 'accessory transfusion tissue' of Worsdell corresponds to the 'hydrostéréome transversal' of Bernard. The cells composing this tissue are long and lignified, and, as Worsdell points out, they have the appearance of stone cells rather than of tracheides. Their function presumably is primarily that of strengthening the lamina of the leaf.

### REPRODUCTIVE ORGANS.

With regard to the reproductive organs *Podocarpus* is generally dioecious, though very rarely monoecious. This distribution of the sexes is the same as that in *Dacrydium* and in *Microcachrys*, but *Saxegothaea* is monoecious. The reproductive organs in *Taxus* are also dioecious, and in *Araucaria* and *Agathis* they are generally dioecious.

#### (1) Male Cone.

The male cones are borne either singly on a very short axis which arises in the axil of an ordinary foliage leaf, or more often they are arranged in clusters of two to four which are borne on a similar abbreviated axis, each cone being attached by means of a short stalk. The male cone is from 1 cm. to 1.5 cm. long and about 2.5 mm. in diameter. At the base of each male cone are a few tiny bracts which are spirally arranged along the short stalk of the cone. The axis of the cone bears a number of spirally arranged sporophylls, each one of which consists of a pedicel which becomes flattened distally to a lamina on either side. Each sporophyll bears on its under surface two ovoid microsporangia.



The axis of the cone, as seen in transverse section, contains a ring of collateral vascular bundles of the usual structure. No trace of centripetal xylem could be found, such as is present in the axis of the male cone of *Saxegothaea* (17). Each vascular bundle is accompanied by a single resin canal outside the phloem, but it seems to be the general rule that these resin canals are not functional, for in most of them no distinct epithelial layer could be seen. On the other hand, resin cells are frequent throughout the fundamental tissue. A single vascular bundle, consisting of a few tracheides and phloem elements, passes out to each sporophyll. It arises by division of one of the axial bundles, the plane of division being radial; the two derived bundles lie side by side before one of them passes out to the sporophyll. The tracheides in the sporophyll are somewhat scattered, and some of them may represent centripetal xylem. The phloem dies out before the xylem, as it ordinarily does in the termination of the vascular bundles of the leaves. A small resin canal, which appears to be non-functional, accompanies the single vascular bundle in its passage from the cone axis, but this canal dies out very quickly. In *Saxegothaea*, on the other hand, the resin canal of the sporophyll is a much more conspicuous structure, and passes out practically as far as the xylem elements extend.

The microsporangia of our material were either ready for dehiscence or else dehiscence had already taken place. Remains of two layers of cells within the sporangium wall could be made out. The wall of the sporangium is very much like that of *Saxegothaea* (10, 17) and *Araucaria* (16). It is one cell wide except on the outside, and the vertical cell-walls are very thick, and have numerous strengthening bands on them running at right angles to the surface (cf. Pl. XXI, Fig. 1). The line of dehiscence of the sporangium is oblique, as can be seen by an examination of Pl. XXI, Fig. 2. Pilger (13) states that the dehiscence of the sporangium in the Taxaceae is longitudinal, but this is without doubt incorrect for *P. spinulosus*. Thibout (20) correctly figures the dehiscence of the microsporangia of several species of *Podocarpus* as being oblique. In *Saxegothaea* (17) the line of dehiscence is transverse.

## (2) Pollen-grains.

As is well known, the pollen-grains of *Podocarpus* are winged like those of *Pinus*. The material of *P. spinulosus* available contained only pollen-grains which were mature. The material had been fixed in a dilute solution of formalin, and apparently had all been collected at one time. Several observers have recently related the occurrence of as many as eight prothallial cells in the pollen-grains of different species of *Podocarpus*. Thibout (20) describes the presence of several prothallial cells in *P. polystachyus*. Coker (5) mentions the division in *P. coriacea* of the two original prothallial cells, but he considers that these divisions are abnormal.

Jeffrey and Chrysler (9) found that in two other species, *P. ferrugineus* and *P. dacrydioides*, there were as many as eight prothallial cells present, and that the generative cell also had divided. They and Miss Young (31) also record several prothallial cells in species of *Dacrydium*. Jeffrey and Chrysler incline to the view that these divisions of the prothallial cells and the generative cell cannot be regarded as a primitive feature. Burlingame (4) reports the occurrence of a similar group of prothallial cells in *P. totarra Hallii*. Norén (10) records a similar complex of prothallial cells in *Saxegothaea*, and Thomson (23) describes the occurrence of the same in *Microcachrys*. The latter (21) has also found that in *Agathis* as many as thirty to forty prothallial cells may be present.

It was thought advisable to compare the structure of the male gametophyte of *P. spinulosus* with that of other species of *Podocarpus* previously described. Microtome sections of male cones were cut whose microsporangia had not dehisced, and were stained with Heidenhain's Iron Alum Haematoxylin with a background stain of Orange G. It will be seen on examination of Pl. XXI, Figs. 3-8, that the contents of the pollen-grains are almost exactly the same as those figured by Jeffrey and Chrysler for *P. ferrugineus* and *P. dacrydioides*. In Figs. 3 and 4 both prothallial cells have divided in a plane at right angles to that of the section, but in Figs. 5 and 6 divisions in the first and second prothallial cells respectively have not occurred in this plane. From an examination of Fig. 7, which is a section of a pollen-grain cut tangentially at its prothallial end, it is apparent that there may be another plane of division of the prothallial cells at right angles to that indicated in Figs. 3 and 4, &c. Thus as many as eight prothallial cells may be present. The generative cell is generally conspicuous with its aggregation of protoplasm around the central nucleus. It is evident from Fig. 5 that the generative cell sometimes divides before the liberation of the pollen in a manner which is probably the same as that described for the first time by Jeffrey and Chrysler (9) as occurring in the pollen of *P. ferrugineus* and *P. dacrydioides*. The tubenucleus is the largest nucleus present in the pollen-grain. All the nuclei of our material were in the resting condition, as indicated by the large nucleolus and absence of a reticulum. After a time the walls of the prothallial cells break down, and the nuclei come to lie free in the cytoplasm, as will be seen on examination of Fig. 8. Jeffrey and Chrysler record a similar phenomenon in *P. polystachyus*.

It is apparent then from this examination of *P. spinulosus*, and from the *résumé* of work done on other species given above, that the multicellular condition of the male gametophyte of *Podocarpus* must be considered a characteristic of the genus, especially as the species investigated come from widely different regions (New Zealand, Australia, Cuba, Java).

The fact that a similar condition of the male gametophyte exists in

*Dacrydium*, *Microcachrys*, and *Saxegothaea* is another indication that the Podocarpeae are a natural group.

It is impossible, with our present meagre knowledge of the inter-relationships of the Conifers, to say whether this multicellular condition of the male gametophyte of *Podocarpus* is primitive or derived. Jeffrey and Chrysler (9) consider that it is not a primitive feature, but it seems to us that data for deciding the question are not at present available. The fact that a similar prothallial complex is present in *Saxegothaea* and *Microcachrys*—two genera which on other grounds are considered to be relatively primitive—is a point in favour of considering the prothallial complex of *Podocarpus* primitive. The fact that the divisions of the nuclei of the prothallial cells are karyokinetic is in keeping with the interpretation that the occurrence of several such cells is a normal feature for the genus. The presence of wings in the pollen-grains of *Podocarpus* does not point to any necessary relationship with the Abietineae, a group from which we consider the Podocarpeae to be considerably removed, for in the Podocarpeae the wings on the pollen-grains appear to have been evolved within the group.<sup>1</sup> Jeffrey and Chrysler (9) consider, on the other hand, that the ground plan of the male gametophyte of *Podocarpus* is very similar to that of the Abietineae, but we cannot gather from their statements what adequate grounds they have for this conclusion. Jeffrey and Chrysler bring the proliferation of the prothallial cells in Araucarieae into line with the peculiar protosiphonogamic method of fertilization in this group described by Thomson (22). But this does not account for the occurrence of several prothallial cells in *Podocarpus*.<sup>2</sup> On examining our material it was seen that the pollen-grains alight in the micropyle, and so presumably the growth of the pollen-tubes is a normal one through the nucellus.

### (3) Female Fructification.

Of the female fructification we had material of three species, *Podocarpus spinulosus*, *P. alpinus*, and a third species. We had the first in largest quantity, so this species will be described more fully and the others compared with it.

The female fructification is borne laterally on the stem in the axil of a bract about six millimetres long, resembling a small foliage leaf. In the axil of the bract is the peduncle, about six or seven millimetres long and nearly a millimetre wide, and somewhat flattened in the vertical plane at right angles to that containing the main axis and the peduncle. At its upper end it bears three pairs of bracts decussately arranged (cf. Pl. XXI, Fig. 9). The lowest pair are small, about one and a half millimetres long, and are recurved and more or less leaf-like. They lie in the plane containing both stem and pedicel. The other two pairs of bracts,

<sup>1</sup> Cf. Thomson (23).

<sup>2</sup> Cf. Burlingame (4).

which are inserted close together, appear more or less swollen and fleshy; they are about five or six millimetres long, and fused together except at the tip. The two lower bracts are about a millimetre longer than the upper pair; each bears, just above the point where the four bracts become free from one another, a single stalked anatropous ovule. Occasionally one of the two lower bracts is sterile, while the upper pair of bracts in all cases examined were both sterile. Generally one of these is developed more strongly than the other. Pilger (13) notes a good deal of variation in the bracts of *P. spinulosus*. Sometimes the lowest pair may be missing, while the other four may vary a great deal in size relatively to one another. The following account of the internal structure is of the normal case where the two lowest bracts are present, and the next two bracts are fertile and of approximately equal size, while the uppermost pair are also fairly well developed.

The peduncle contains in transverse section a somewhat elliptical ring of endarch collateral bundles which anastomose somewhat. Each bundle is accompanied on its phloem side by a single resin canal.

A section through the base of the fused bracts shows a similar structure (Pl. XXI, Fig. 10). The bundles divide occasionally and reunite, the resin canals sometimes dividing as well as the bundle; in other cases the canal arises *de novo* outside one of the bundles, and occasionally it may be absent altogether from one of the bundles. This behaviour points to the canals being functionless, and there is no epithelium properly developed. Resin cells are extremely abundant throughout the whole of the parenchyma of the bracts.

At a higher level the ring of bundles widens out in one plane, and the bundles then come to arrange themselves in two groups of three at opposite sides of the wider diameter with two bundles between them (Pl. XXI, Figs. 11 and 12).

The two groups at each end belong to the fertile scales and ovules, the bundles between them belonging one to each of the sterile scales. This state of affairs is intermediate between that figured by Van Tieghem (27) for *Podocarpus sinensis*, where the sterile bracts also contain each a group of three bundles, and that figured by Strasburger (18) for *Podocarpus chinensis*, Wall., apparently the same species as that described by Van Tieghem, where the sterile bracts have no vascular supply. In one place the stronger of two sterile bract bundles had two small groups of xylem elements some little distance away from it on the xylem side (Pl. XXI, Figs. 12 and 19); these apparently correspond to the two inner bundles of one of the groups in Van Tieghem's figures. As the bundles of the sterile bracts die out, some transfusion tracheides appear.

Of the group of three bundles serving the fertile scale, the middle one passes out into the bract subtending the ovule, the other two approach



one another and fuse by their margins to form a V-shaped bundle with its xylem and phloem inversely orientated with regard to the xylem and phloem of the subtending bract bundle (Pl. XXI, Fig. 13). This V-shaped bundle next straightens itself out (Pl. XXI, Fig. 14) and begins to divide into three (Pl. XXI, Fig. 15), so that as the chalazal end of the ovule is approached there are found three bundles, each, as a rule, with a small non-functional resin canal (Pl. XXI, Fig. 16).

At the chalazal end of the ovule the two lateral bundles bend round through about a right angle and descend, one on each side of the ovule, for a short distance towards the micropyle; as seen in transverse section they lie on the diameter of the ovule (Pl. XXI, Fig. 17). The central bundle continues its course over the top of the ovule, and in so doing divides into two bundles (Pl. XXI, Fig. 18) which descend a short distance towards the micropyle (Pl. XXI, Fig. 17). All these descending bundles soon die out, and all show a tendency to amphivasal structure (Pl. XXI, Fig. 20).

The nucellus of the ovule is surrounded by two integuments. The inner is fused for about half its length with the nucellus. The outer integument or epimatium is free from the inner for a short distance at the micropyle end, and on the side remote from the ovular stalk; on the other side it is either not present or is completely fused with the stalk.<sup>1</sup>

Where free, the innermost layer of the inner integument often consists largely of resin cells. These are apparently continued into the part of the ovule where the inner integument and nucellus become fused, and so apparently mark the line of division between these. A zone containing larger and more numerous resin cells also seems to mark the line of division between the inner and outer integuments, but these do not extend far enough towards the chalaza, and are not definite enough to enable one to state whether the descending bundles belong to any particular integument or to the nucellus.

In a species of *Podocarpus* grown in the Cambridge Botanic Garden, the structure was found, on the whole, to be similar. The peduncle is borne in the axil of an ordinary foliage leaf. In the stage examined the peduncle is about six millimetres long and nearly a millimetre broad, and, as in *P. spinulosus*, is flattened in a vertical plane at right angles to that containing the peduncle and the branch bearing it. In the few specimens available for examination the lowest pair of leaf-like bracts were not present, and the whole fructification consisted of two pairs of bracts fused together, of which one or both of the lower pair bore ovules. The arrangement of the bundles is practically the same as in *P. spinulosus*. One bundle of the stem passes into the subtending leaf, while the two bundles on each side of it pass into the peduncle. These almost immediately divide up into a ring of bundles. At the base of the fused bracts

<sup>1</sup> See Postscript.

two sets of three bundles are present; these belong to the fertile scales. One of these bundles on each side divides, and so gives rise to the bundles of the two sterile scales. Additional small bundles may be present on the inside of the sterile scale bundles as in *P. spinulosus*. The xylem of the bundles tends to be semicircular in shape, more or less enclosing the phloem on three sides. The resin canals, of which there is one on the phloem side of each bundle, are much larger than in *P. spinulosus*, and as a rule the resin duct divides after the division of the bundle to which it belongs. The ovular bundle, as in *P. spinulosus*, divides into three; when on a level with the nucellus, transfusion tissue begins to appear both centripetally and laterally, and the division into three bundles is no longer obvious. These bundles die out at the chalaza, and do not descend on the sides of the ovule as in *P. spinulosus*. For the last part of their course they are composed of transfusion-like tracheides only.

In *Podocarpus alpinus*, material of which was obtained from the Royal Gardens, Kew, the peduncle is very short, being less than a millimetre long. The lowest pair of bracts are extremely small and scale-like, although Pilger (13) states they are absent altogether; of the next pair only one bears an ovule, while of the upper sterile pair one is often aborted altogether, and the other is small compared with those of the lower pair.

The vascular system at the base of the fused bracts consists of four bundles arranged along the sides of a square; of these one serves the fertile scale and two the ovule it subtends, the fourth serving the opposite sterile scale. The upper sterile scale has usually no vascular supply.

The two ovular bundles fuse as in the other species described, but at first almost the whole of the xylems of the two bundles come in contact, so that the resulting bundle is almost concentric; further up, however, it straightens itself out. Nearer the chalaza it divides into two bundles which curve over the top of the ovule and descend a little way towards the micropyle on the sides of the ovule in much the same way as the lateral bundles do in *P. spinulosus*. In *P. alpinus* the resin canal accompanying each of these two bundles extends down to the level of the nucellus where it occurs on the inner margin of the outer integument, to which these bundles would appear to belong. Other canals also appear on the inner margin of the outer integument; these presumably correspond with the middle bundle of *P. spinulosus*, which bends over the ovule and then divides into two. On the whole, it would seem from their behaviour in *P. alpinus* that these bundles all belong to the outer integument, but the evidence at present is insufficient to decide this point.

As far as the female fructification is concerned, the Podocarpeae obviously fall into two divisions; one in which the megasporophylls are aggregated into cones, and which comprises the monotypic genera *Saxegothaea* and *Microcachrys*, the other in which the fertile megasporophylls



are mostly either solitary or associated in pairs, and which includes the genera *Dacrydium* and *Podocarpus*. A remarkably different condition exists in two species of *Podocarpus*, *P. andinus* and *P. spicatus*, in which the megasporophylls bearing ovules occur at intervals along the fertile branch. In all a single ovule is present on the upper surface of the megasporophyll, and is borne in a more or less reversed position.

Assuming that the Podocarpeae form a natural group, a conclusion which from general considerations seems inevitable, there appears to be every reason to suppose that the state found in *Dacrydium* and *Podocarpus* is derived from that in *Saxegothaea* and *Microcachrys* by reduction in the number of scales in the cone, from an indefinite number to two or three pairs decussately arranged in *Podocarpus*. Here very occasionally both scales of two pairs may contain ovular bundles (27); in some species normally both bracts of the lower pair are fertile, while in many species, e. g. *P. alpinus* and *P. elatus*, only one scale of the whole fructification is fertile. The female fructification is then to be regarded as a very reduced cone. In *P. andinus* and *P. spicatus* it would appear that the axis, instead of becoming very much shortened, has lengthened out a good deal, and the sporophylls are now separated by comparatively long internodes. The insertion of the ovule differs markedly from that found in *Saxegothaea* and *Microcachrys*. In these genera the single ovule is sessile on the upper surface of the megasporophyll, whereas in *Podocarpus* it is borne on a stalk which apparently arises from the upper surface of the bract which bears it. *Dacrydium* exhibits transitions between the two cases, for in some species the ovule is borne on the upper surface of the scale, in others it is more or less as in *Podocarpus*. The internal structure of the megasporophylls of the Podocarpeae also shows a similar series of stages. In *Saxegothaea* one bundle leaves the axis of the cone and gives off the ovular supply by branches arising from it; in *Microcachrys* the two bundles are presumably separate from the cone axis, but very close together (30);<sup>1</sup> in *Podocarpus* one bundle of the peduncle passes up into the megasporophyll, while two others, one on each side of the sporophyll, join up and supply the ovule.

It seems fairly clear that *Saxegothaea* is primitive for the Podocarpeae, and the state of things found in *Podocarpus* is derived from that found in *Saxegothaea*. The much smaller amount of centripetal xylem and the loss of function of the resin canals in some species support this view.

The independence of the vascular supply of the ovule from the vascular bundle of the subtending bract can be easily, and it seems to us reasonably, explained as due to basipetal evolution of the vascular system, as the ovule has become more and more important in relation to the subtending bract.

<sup>1</sup> But Thomson (24) states that the sporangium bundle arises in the same way as that of *Saxegothaea*, by branching from the sporophyll bundle.

In *Saxegothaea*, where the ovule is a small appendage of the bract, the bundles supplying it arise by branching from the bundle of the sporophyll. As the ovule has become relatively of more importance than the bract, its vascular supply has also become of more importance, until in *Podocarpus* the vascular supply of the ovule is carried down into the peduncle independently of the supply of the megasporophyll. This point of view has also been emphasized by Thomson (24), in regard to the megasporophyll of *Dacrydium*, in some species of which the behaviour of the bundles is very similar to that of *Podocarpus*.

With regard to the branching of the ovular bundles, Favre (7) has rather vaguely described their behaviour in *Podocarpus sinensis*, where he says the ovular bundle divides a little below the summit of the organ into a large number of branches which extend downwards in the plane of separation of the integuments and so form a vascular cupule which is prolonged up to the point where the nucellus becomes free. In *P. spinulosus* and *P. alpinus* the (presumably) integumentary bundles are much less developed, and in *Podocarpus* sp. they are practically not developed at all.

How the ovular bundles are to be homologized with those found in the ovules of other Gymnosperms (11) there are not at present sufficient data to decide. Favre's species would apparently throw light on this question, but his description is too vague to be of much help. Moreover Strasburger's description of *P. chinensis* (19), which is apparently the same species as *P. sinensis*, is almost identical with our description of *P. spinulosus*.

In *P. spinulosus* and *P. alpinus* there seems to be an outer integumentary system arising from the chalazal bundles. Whether there is an inner integumentary system as well cannot yet be decided, but what facts there are available point against it.

#### GENERAL CONSIDERATIONS.

The relationships of *Podocarpus* have already been discussed at some length in the course of this paper. The genus seems to be a recently modified type with close relationships to *Dacrydium*, *Microcachrys*, and *Saxegothaea*. Its present wide geographical range, the wings on the pollen-grains, the frequently functionless nature of the resin canals, and the peculiar structure of the female fructification are all considerations which point to this conclusion. *Microcachrys* and *Saxegothaea* are undoubtedly more primitive than *Dacrydium* and *Podocarpus*. *Saxegothaea* would appear to be a connecting link with the Araucarieae, and the complex of prothallial cells in the pollen-grains of the Podocarpeae generally can be brought into relation to the numerous prothallial cells described for the pollen-grains of the Araucarieae.

Miss Robertson (14) has pointed out that the somewhat isolated genus *Phyllocladus* has definite points of resemblance to *Podocarpus*, on the one

hand, and relationships less marked, on the other, to the Taxeae. It would seem to us that the differences between *Phyllocladus* and the Taxeae are greater than Miss Robertson supposes—two of her four points of resemblance between them being of an unsatisfactory nature. Thus the markings of the centripetal wood of *Phyllocladus* are practically the same as those in the transfusion tracheides of *Podocarpus*, and the presence of a certain amount of centripetal wood in *Phyllocladus* is not sufficient evidence of phylogenetic relationship.

There are considerable differences in the two groups between the structure of the male cones, and these differences become no less apparent when we consider the male gametophytes. The only point of resemblance between the female fructifications of the two groups is the generally fleshy consistency of the ripe seeds. *Phyllocladus* is certainly near the Taxeae in its female fructification, because its symmetrical arillus, like that of the latter group, only develops at a late stage. There seem to be no striking resemblances in anatomical characters between the Podocarpeae and the Taxeae.

Thus the points of similarity between the Podocarpeae and the Taxeae do not seem to us to be very striking, though, as has been supposed in the past, the two groups may perhaps be connected by *Phyllocladus*.

With regard to the relationships of the Podocarpeae with the Abietineae, there appears to be no evidence of any recent phylogenetic connexion between the two groups. The structure of the male gametophytes in the two groups has already been discussed, and the anatomical characters both of the female reproductive structures and of the vegetative organs are very different in these two orders of Coniferae.

#### SUMMARY.

1. The structure of the stem and leaf of *Podocarpus spinulosus* is similar to that of other species described respectively by Penhallow and Worsdell.
2. The structure of the microsporangium is similar to that of *Saxegothaea* and *Araucaria*, but the line of dehiscence is oblique.
3. Several prothallial cells are present in the pollen-grains of *P. spinulosus*, as in other species of the Podocarpeae investigated. The walls of the prothallial cells break down at a later stage and their nuclei come to be free in the cytoplasm.
4. The generative cell of the pollen-grain sometimes divides in the same way as that described by Jeffrey and Chrysler as occurring in *P. ferrugineus* and *P. dacrydioides*.
5. The course of the vascular bundles in the female fructification is described in detail. One bundle from the peduncle passes into the megasporophyll, while a pair of bundles, one on each side of the sporophyll trace, unite and serve the ovule. The bundle thus formed has its xylem and phloem inversely orientated as compared with the megasporophyll

bundle. At the chalazal end of the ovule it divides to form ultimately about four bundles which soon die out.

6. *Podocarpus* is regarded as a modified genus derived from a type similar to *Saxegothaea*.

7. The Podocarpeae are a natural group with no very definite connexions with the Taxeae, though *Phyllocladus* may form a connecting link. There is no evident relationship between the Podocarpeae and the Abietineae. It is likely that the former are connected with the Araucarieae by means of *Saxegothaea*.

BOTANY SCHOOL, CAMBRIDGE,  
October, 1809.

POSTSCRIPT.—Since this paper was sent in for publication we have received Tison's second paper on *Saxegothaea* (26). This author considers the epimatium and ovular stalk in *Podocarpus* as together equivalent to the epimatium in *Saxegothaea*. He also refers to two figures of the ovule of *Saxegothaea* published by one of us (17), which both show an epimatium 'qui enveloppe complètement l'ovule et son tégument à la façon de celui des *Dacrydium* et des *Podocarpus*.' One of these figures was a diagram to show the ovular supply arising as a branch from the sporophyll bundle and was not intended to show details of ovular structure. Nevertheless, inasmuch as it represented the epimatium as present on the under side of the ovule, it was incorrect. The other figure showed a section of the ovule cut more or less parallel to the cone scale, and so showed the epimatium present on both sides; it did not show an epimatium completely surrounding the ovule. Moreover, only on Tison's view can it be considered as proved that the epimatium completely surrounds the ovule in *Podocarpus*.

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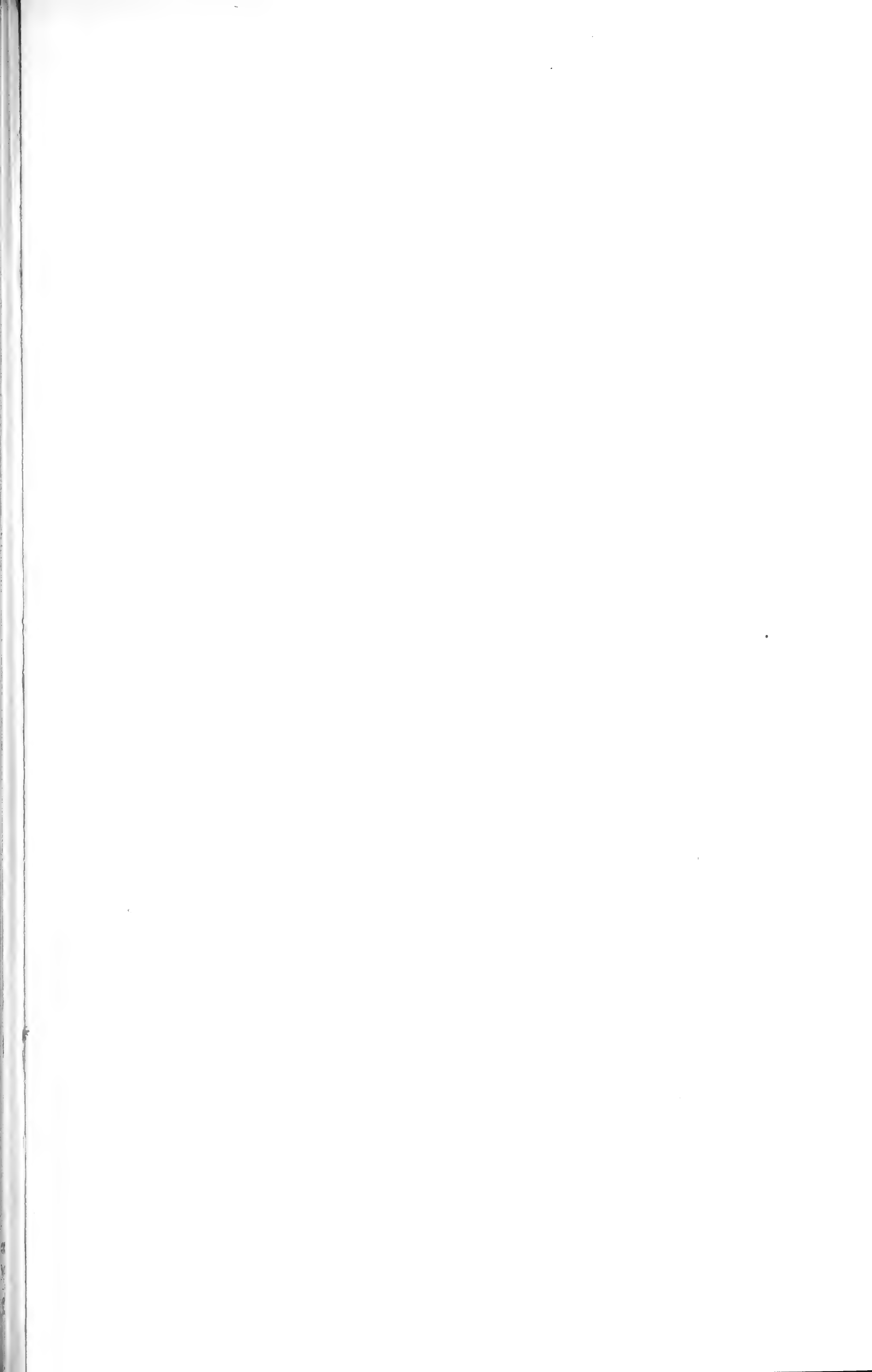
## EXPLANATION OF PLATE XXI.

Illustrating Messrs. Brooks and Stiles's Paper on *Podocarpus*.

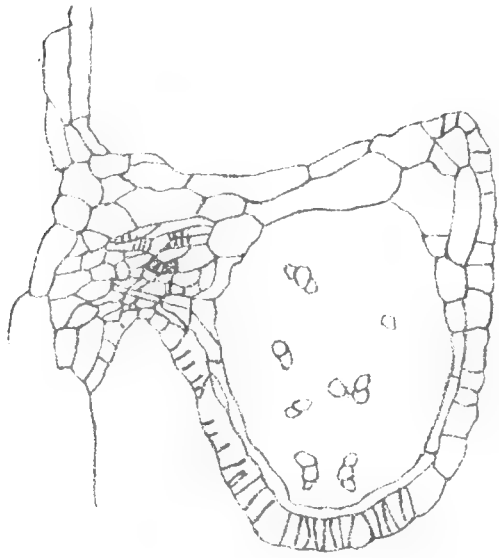
All the figures are of *Podocarpus spinulosus*.

- Fig. 1. Microsporophyll as seen in radial section through the cone. × 63.
- Fig. 2. Microsporophyll showing oblique dehiscence of sporangia. × 63.
- Figs. 3-8. Sections of pollen-grains showing contents. Stained with Heidenhain's Iron Alum Haematoxylin and Orange G. × 725.
- Fig. 9. Diagram of female fructification indicating the position of the sections shown in the following diagrams.
- Figs. 10-18. Diagrams representing sections through the female fructification at different levels.
- Fig. 19. Transverse section of the vascular bundle of a sterile scale of the female fructification showing two small isolated groups of xylem outside the main bundles. × 300.
- Fig. 20. Transverse section of, presumably, an integumentary vascular bundle showing a tendency to amphivasal structure. × 300.

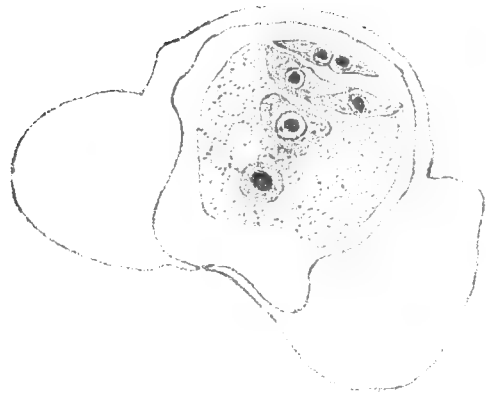




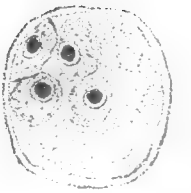




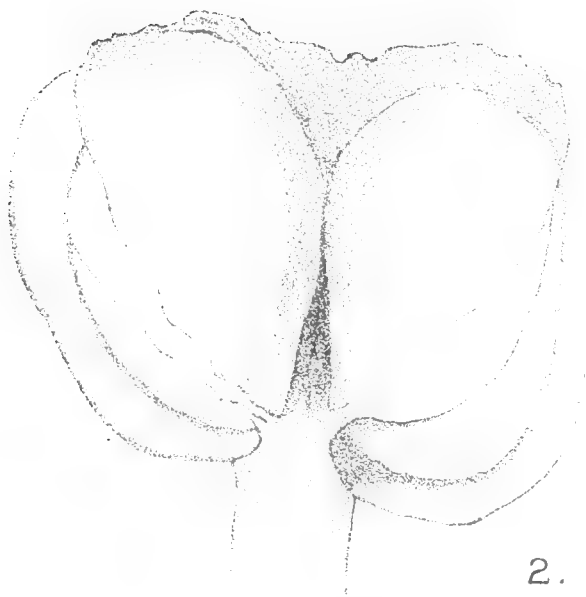
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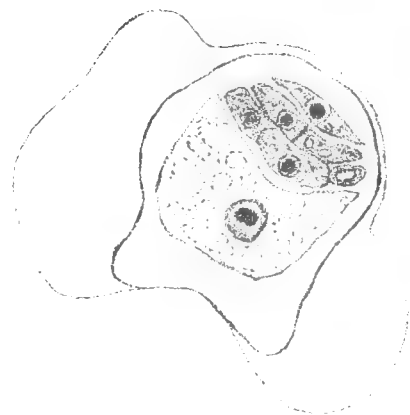
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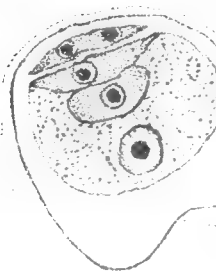
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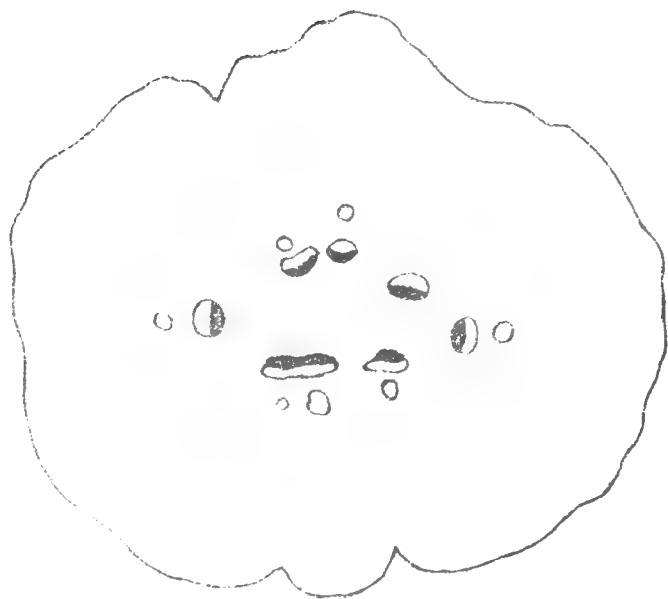
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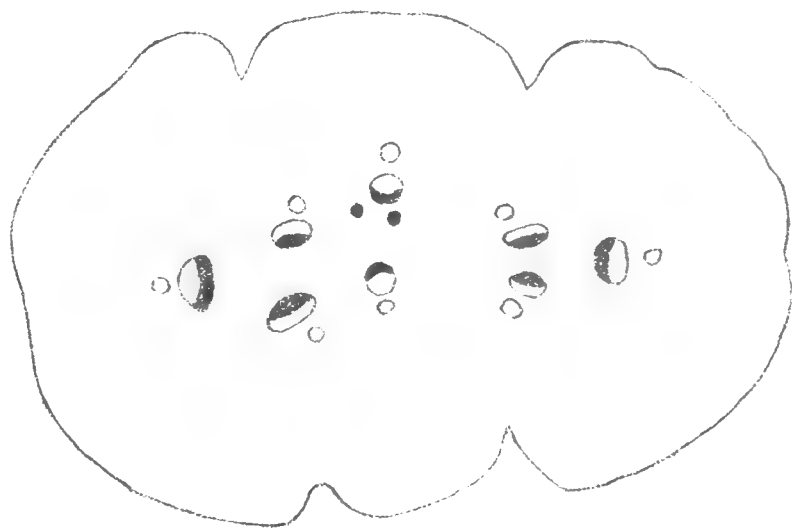
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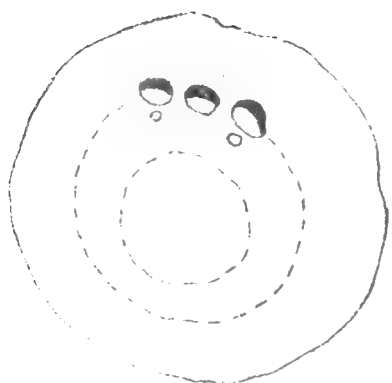
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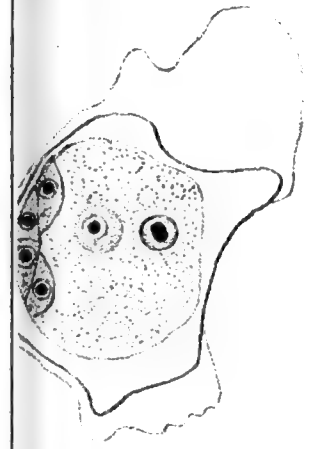


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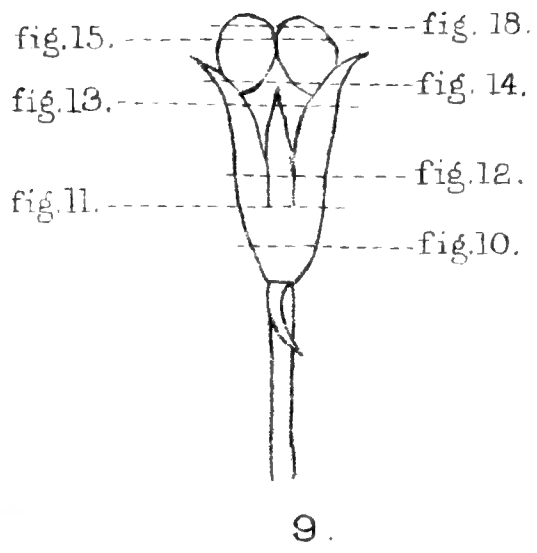
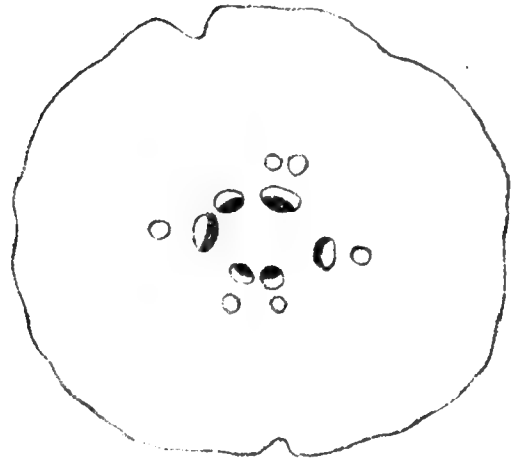
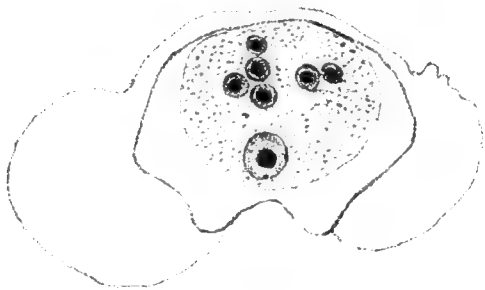


fig. 15. ----- fig. 18.  
fig. 13. ----- fig. 14.  
fig. 11. ----- fig. 12.  
----- fig. 10.

9.



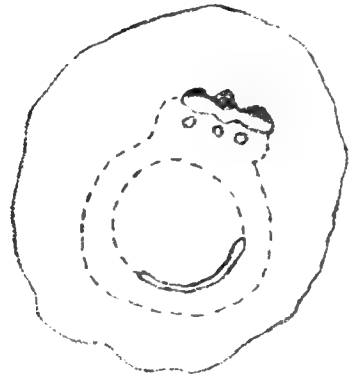
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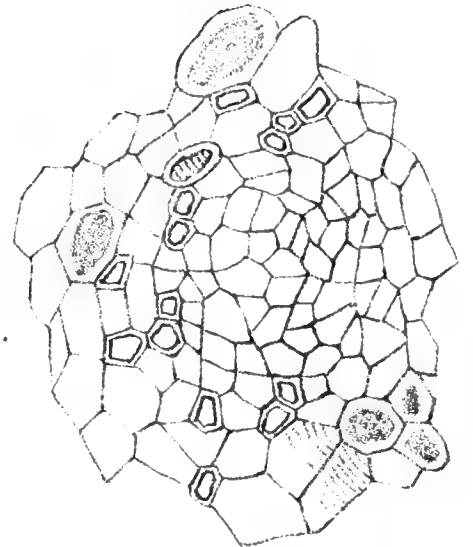
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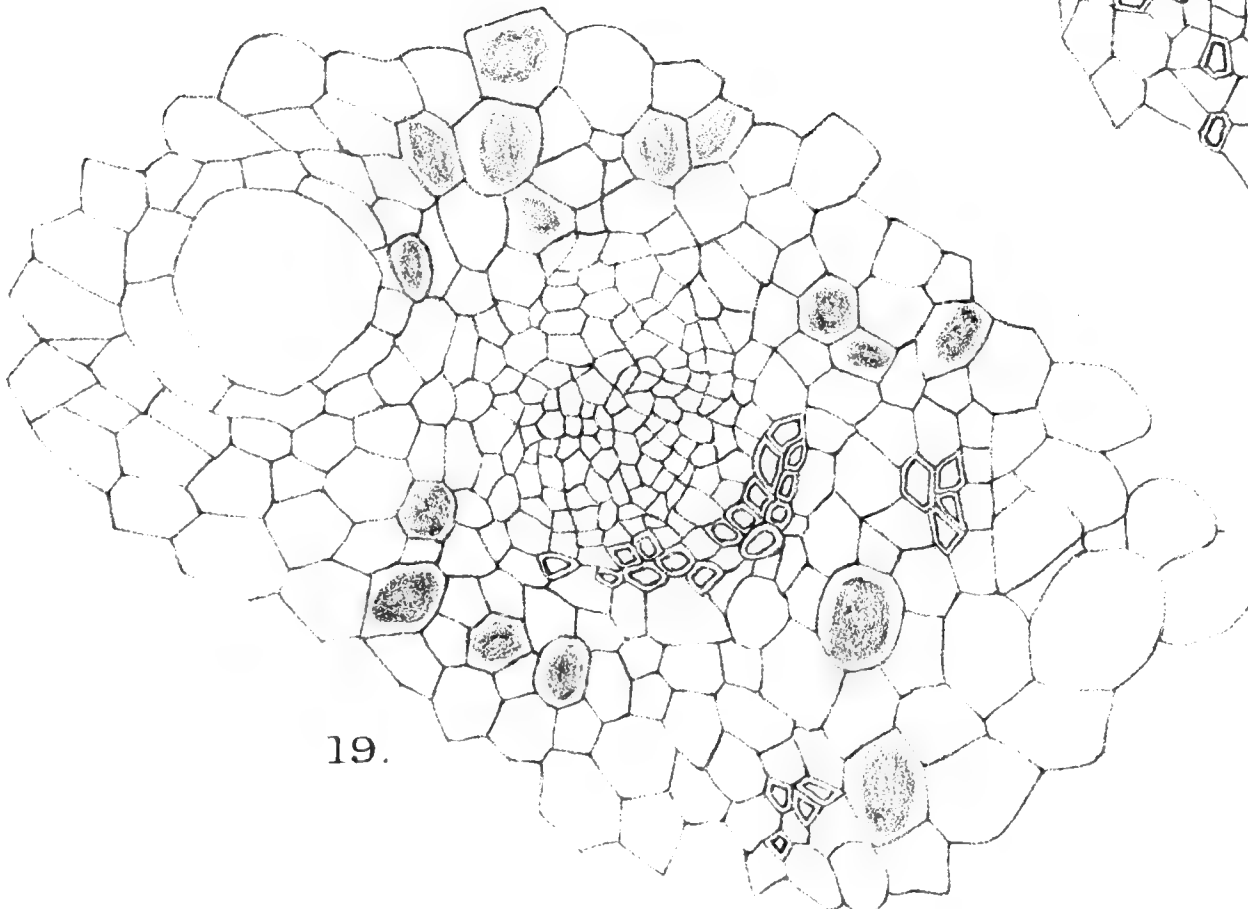
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13.



20.



19.

18.



# On the Seedling Structure of Gymnosperms. IV.

BY

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AND

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With Plates XXII and XXIII, and three Figures in the Text.

GNETALES.

EPHEDRA.

THE germination of the seeds of *Ephedra* has been described by Strasburger<sup>1</sup> and Bower<sup>2</sup>; the latter author does not enter into the structure of the seedlings, but Strasburger draws attention to their morphology and the course of the cotyledonary and plumular bundles; he also describes briefly the transition to root-structure, and remarks upon the close resemblance, in the transition phenomena, between *Ephedra* and *Araucaria*. Passing on to our own observations, the seedlings of the species of this genus examined, *E. distachya*, *E. fragilis*, *E. campylopodia*, and *E. altissima*, are indistinguishable one from the other, and are dicotyledonous, epigeal, and linear in shape (Pl. XXIII, Figs. 6, 7, and 8). At first the cotyledons are short, but ultimately they grow to a much greater length; thus seed-leaves ten centimetres long are not uncommon in *E. fragilis*. On the other hand the hypocotyl does not elongate to a corresponding degree, and it is very slender.

The structure of the seed-leaves is very simple and calls for but little comment. The epidermis is covered with a cuticle which is very thin over the ordinary epidermal cells, but over the stomates, which are sunken below the general surface level of the leaf, it is considerably thicker.

<sup>1</sup> Strasburger: Die Coniferen und die Gnetaceen (Jena, 1872).

<sup>2</sup> Bower: The Germination and Embryology of *Gnetum Gnemon* (Q.J.M.S., xx, 1882).

The mesophyll is homogeneous and consists of normal parenchymatous cells; the intercellular space system is not highly developed.

Each cotyledon contains two vascular bundles which are collateral in structure and do not exhibit any vascular rearrangement within the seed-leaves. Transfusion tracheides, so far as has been seen, are absent, and no fibrous elements, abutting directly on to the soft bast, such as occur in so many species of *Pinus*, &c., have been observed (Pl. XXII, Fig. 1). *E. altissima*, however, does possess a number of fibrous elements, with thickened, but apparently unligified walls, scattered throughout the mesophyll of the seed-leaves and in the cortical ground-tissue of the hypocotyl.

The orientation of the bundles of a cotyledon is peculiar; they are arranged in such a manner that the plane of the bundles is at right angles, or nearly so, to the dorso-ventral plane of the leaf; the protoxylem of one bundle thus is directed towards the same tissue of the strand on the other side (Diagram 1, Fig. 1).

In all cases a well-marked cotyledonary tube is formed (Diagram 1, Fig. 2).

#### Transition.

*E. distachya*. The orientation of the bundles of the cotyledons just remarked upon obtains throughout the whole length of the seed-leaves, and in this condition they enter the hypocotyledonary axis; thus there are four bundles in the hypocotyl arranged in two well-defined pairs (Diagram 1, Fig. 3). During the downward passage the two strands from each cotyledon approach one another and, concurrently, a rearrangement of the elements of the wood takes place, which leads finally to the fusion of the protoxylem elements (Diagram 1, Fig. 4). The approachment continues so that the xylem-masses of the bundles of each pair come into continuity, with their protoxylems in an exarch position (Diagram 1, Fig. 5). The four groups of phloem elements still retain their identity, and continue so to do through the greater length of the root of young seedlings (Diagram 1, Fig. 6); indeed, we have seen no absolutely certain case in which the phloem, which, in this genus, generally is very poorly defined when viewed in transverse section, forms two well-marked strands as would be expected. This fact is curious, and may be compared with the similar state of affairs which occurs in certain Cactaceae.<sup>1</sup>

The transition-phenomena in *E. campylopodia*, *E. fragilis*, and *E. altissima* are precisely similar to those obtaining in *E. distachya*.

Attention may now be drawn to a rather interesting feature occurring in the hypocotyl of all the seedlings of the species of *Ephedra* examined.

At a level just below the cotyledonary node a shallow band of interfascicular cambium occurs which joins the fascicular cambium of the opposing bundles of each cotyledonary pair (Diagram 1, Fig. 3). This

<sup>1</sup> See de Fraine, On the seedling structure of Cactaceae (Ann. Bot., xxiv, Jan., 1910).

meristem is bounded on its inner side by a number of short tracheides having an appearance which strongly recalls that usually associated with

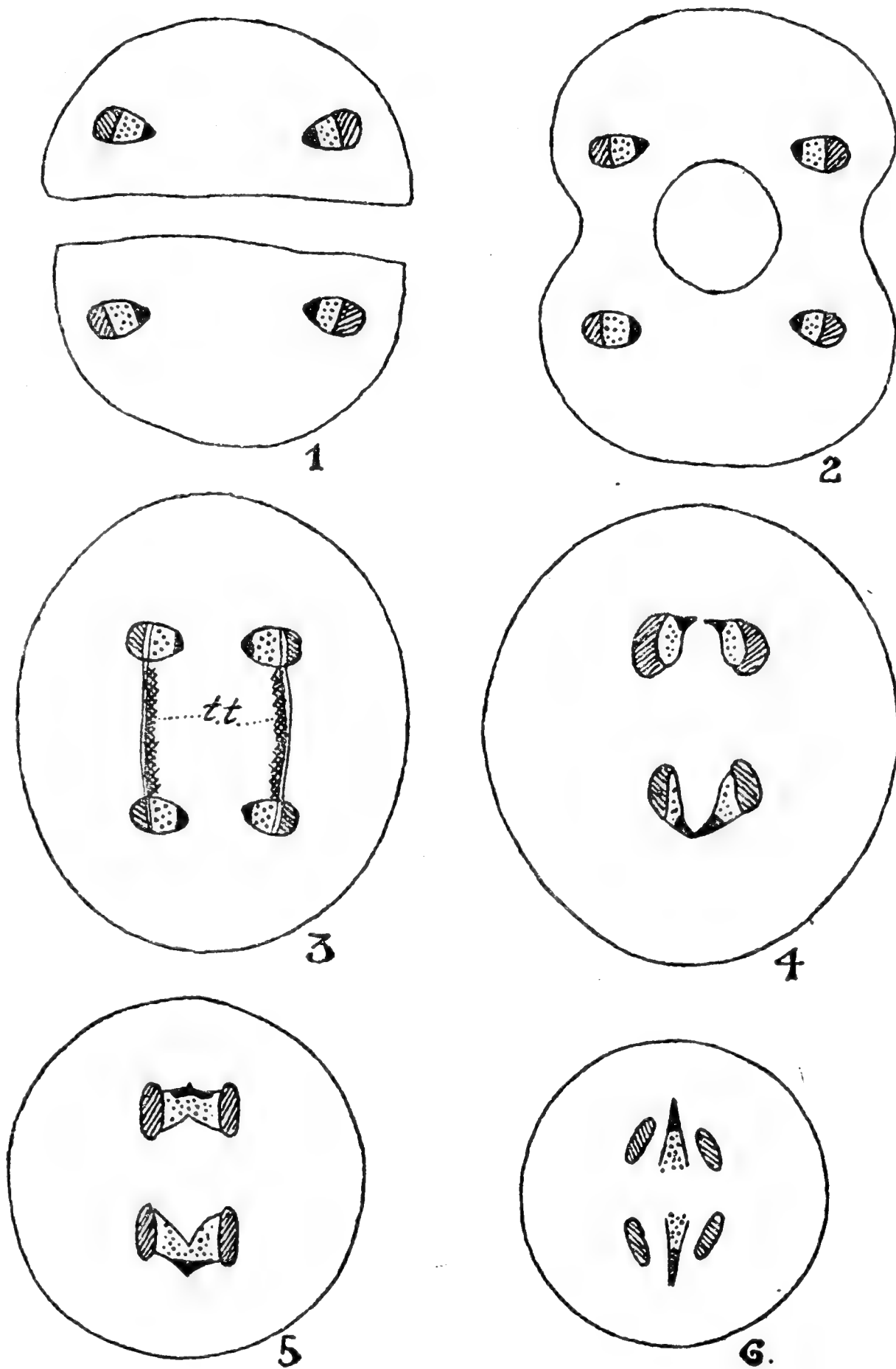


DIAGRAM I. *Ephedra*.—In this and the following text-figures, the protoxylem is represented by black areas, the metaxylem by dots, the phloem by diagonal shading, and the transfusion elements by cross hatching.

many transfusion tracheides of the cotyledons of other Gymnosperms. These elements, in *Ephedra*, form a bridge connecting the xylem-masses of the two corresponding bundles.



The cambium does not form, as far as has been seen, any phloem, so that the appearance is not altogether that of normal secondary thickening (Pl. XXII, Figs. 2 and 3).

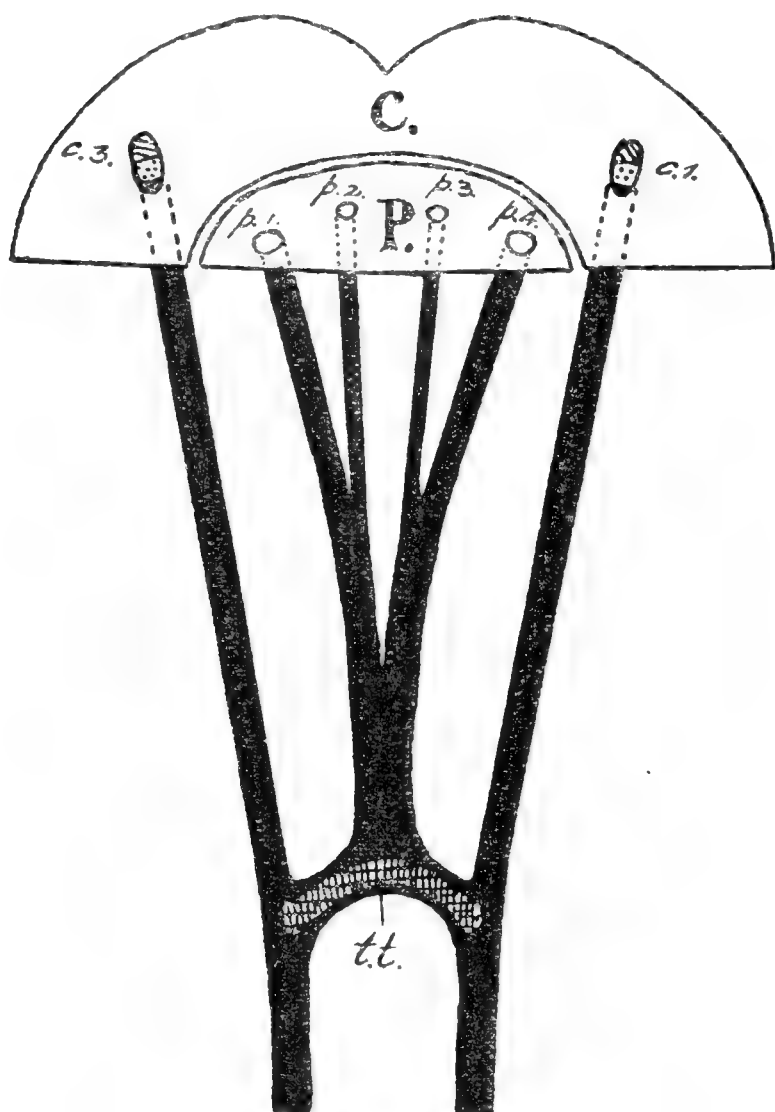


DIAGRAM 2.

It is in this region that the plumular strands effect a junction with the cotyledonary traces, and the short tracheides are formed before the epicotyledonary bundles are differentiated. In older seedlings, with the plumular vascular tissue developed, these tracheides become more abundant, their position is on the inner side of the protoxylem, and they serve as a bridge for the insertion of the epicotyledonary strands. A similar transverse girdle of tracheides occurs at an early stage in the nodes of the shoot and joins the two leaf-trace bundles together.<sup>1</sup>

This tissue may be looked upon as a special development, differentiated in order that the

means of communication between the different traces may be rendered more efficient. The accompanying figure, Diagram 2, illustrates the course of the bundles on one side: *c. 1* and *c. 3* represent seed-leaf-traces; *p. 1*, *p. 2*, . . . indicate the plumular strands; *C.* is the cotyledonary tube; *P.*, the epicotyledonary axis; and *t.t.*, the region of the special tracheides referred to above.

#### WELWITSCHIA.

*Welwitschia mirabilis*, Hook. f. The morphology and structure of the seedlings of this plant have been fully described by Bower,<sup>2</sup> whose account we are able to corroborate in all essential features; but, for the sake of completeness, it is desirable to draw attention to the more important facts bearing directly on the present work.

The seedling is illustrated in Plate XXIII, Figs. 9, 10, and 11, from which it may be seen that the epigeal cotyledons are two in number and relatively

<sup>1</sup> Strasburger, loc. cit.

<sup>2</sup> Bower: On the Germination and Histology of *Welwitschia mirabilis* (Q.J.M.S., xxi, 1881).

large ; just above the cotyledonary node they fuse together to form a tube. The lower end of the hypocotyl bears on one side a spade-like projection, the foot or sucker, which remains embedded within the endosperm, from which it absorbs the stored nourishment. The foot consists entirely of parenchyma, and contains no vascular tissue ; it resembles pretty closely the hypocotyledonary outgrowth which occurs in a similar position in certain Angiosperms, e. g. *Mirabilis multiflora*.

The epidermis is covered by a cuticle which is very thin except over the guard-cells of the stomates, which are sunken slightly below the general level of the epidermis and occur on both surfaces of the leaf. The mesophyll is but feebly differentiated into palisade and spongy parenchyma, and the air spaces are but poorly developed. Some of the mesophyll elements are practically devoid of contents ; these cells occur more especially along the lateral edges of the cotyledons and around the larger bundles. Our material was insufficient to enable us to examine older cotyledons for these particular cells ; in the young seed-leaves they appear rather like immature fibrous elements (Plate XXII, Fig. 4, a).

The number of bundles in each cotyledon varies ; usually there are four larger strands with a number of much smaller traces between, the venation being parallel. The smaller bundles freely anastomose, and towards the base of the seed-leaves, they fuse on to the larger traces, so that four bundles from each cotyledon enter the hypocotyledonary axis.

The structure of these vascular strands is quite normal ; they are endarch and collateral throughout their whole length, and there is some cambial activity ; the tracheae frequently have very thick walls, and in transverse section present an appearance much like some of the metaxylem elements of certain Cactaceae (Plate XXII, Fig. 4).

#### Transition.

From each seed-leaf four strands enter the axis so that, at the top of the hypocotyl, there are eight vascular bundles arranged in four pairs (Diagram 3, Figs. 1 and 2). The members of each pair very quickly fuse together, and rotation immediately begins. The xylem-masses of each of the now two bundles, derived from one and the same cotyledon, face each other in a manner which strongly recalls the appearance presented by the corresponding structures in *Ephedra* (Diagram 1, Fig. 2, and Diagram 3, Fig. 3). Each strand now gradually rotates outwards, a movement which ultimately brings the four protoxylem groups into the exarch position. At the same time the protoxylems tend to become diffuse, and new protoxylem elements may make their appearance in the ground tissue ; these, at first, are not in continuity with the same tissue derived from the cotyledons (Plate XXII, Fig. 5). At about this level the cambium is very conspicuous, and extends in such a manner as partly to enclose the phloem.

The appearance of the bundle throughout the greater length of the hypocotyl is represented in Diagram 3, Fig. 5. The foot, which is situated in the cotyledonary plane, although relatively large, has no vascular supply ;

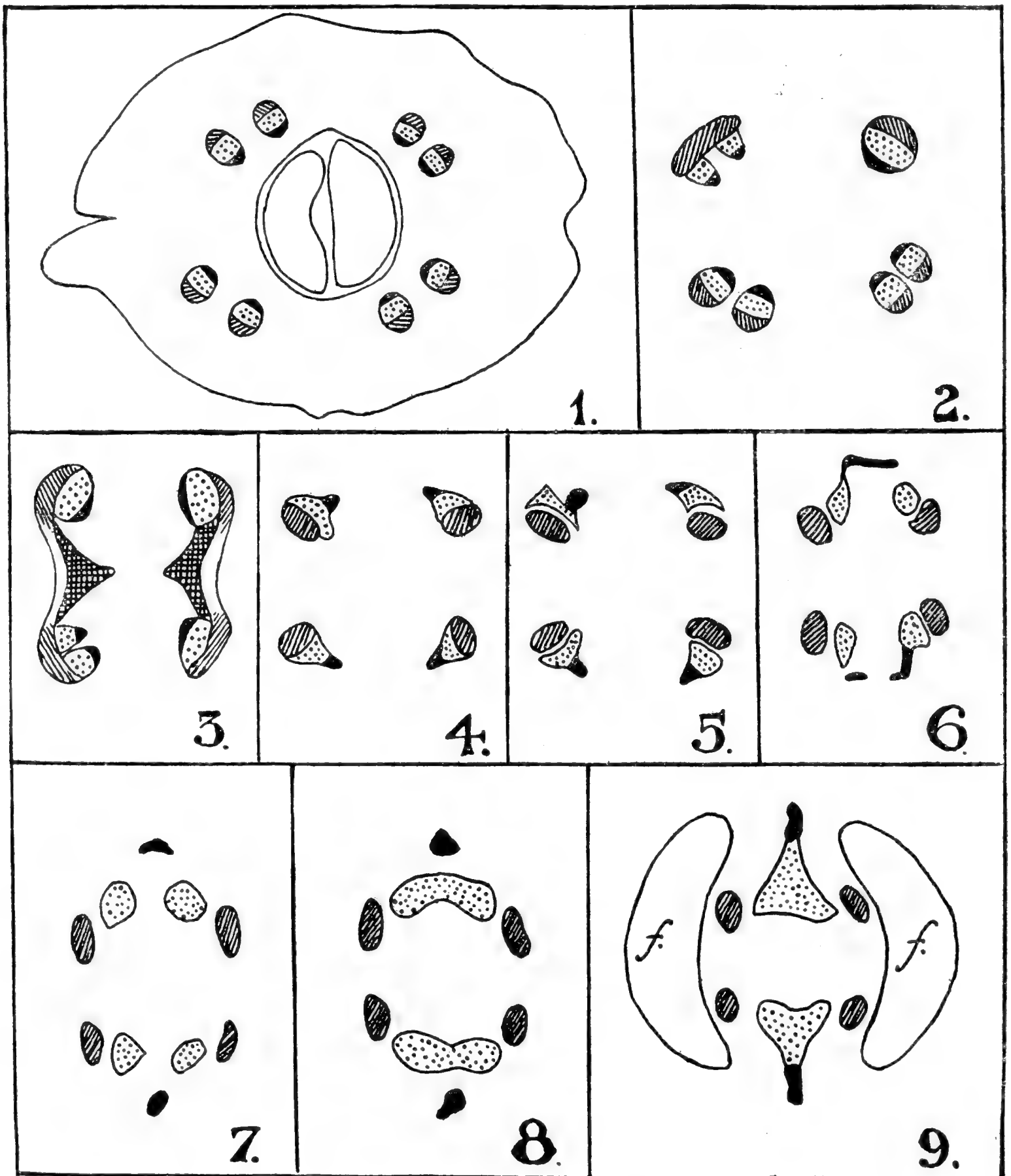


DIAGRAM 3. *Welwitschia*.

but the two bundles on that side of the axis gradually curve outwards towards it, and, when immediately opposite, increase in size. This increase is especially noticeable in the xylem, which, in area, is about twice as extensive as the same tissue of the bundles distal to the foot, but at the same level.

Below the sucker the transition to root-structure rapidly proceeds; the four bundles undergo a centripetal displacement; at the same time, the individuals of each pair of strands come more closely together, and, at a slightly lower level, the corresponding groups of protoxylem elements effect a junction. Thus there are four masses of phloem, four of metaxylem, and two collections of protoxylem elements (Diagram 3, Figs. 7 and 8).

While these changes are being effected two broad bands of thick-walled fibrous tissue are differentiated on the outer sides of the phloem elements (*f.* Diagram 3, Fig. 9).

At first the two isolated groups of protoxylem elements are tangentially elongated, but a rearrangement soon takes place, resulting in the formation of two rounded masses (Diagram 3, Fig. 8). Then the corresponding metaxylems fuse, and, at a lower level, the metaxylems and protoxylems effect a junction (Diagram 3, Figs. 8 and 9). The four groups of phloem elements retain their identity for some considerable distance downwards, as in the case of *Ephedra* described above. Fusion does take place ultimately, we believe, although it is difficult to say with absolute certainty because the soft-bast in the root is very poorly differentiated; indeed, in transverse section it is hardly distinguishable.

The endodermis is first visible at a level just below the region of the foot, appearing at the same time with the fibrous tissue already alluded to. At lower levels the endodermis may be two or three cells in thickness.

The occurrence of a band of a form of transfusion tracheides in the hypocotyl of *Ephedra* has already been described above. *Welwitschia* also shows them, and in this plant they are very prominent and have a disposition exactly the same as in *Ephedra*, although in the former plant they also show an extension into the central ground-tissue (Diagram 3, Fig. 3). Abutting on to the margin of these two bridges are a number of cambiform cells, and possibly also some phloem elements, but no sieve-tubes have been observed. The presence of these tracheides is not due in the first place to the presence of a cambium, but rather to the differentiation of the ground-tissue elements. A cambium, however, may soon put in an appearance, and thus lead to their increase. The material at our disposal was too young to show differentiated epicotyledonary strands, but it is clear from Bower's account and illustrations, taken in conjunction with our own observations, that there is a wonderfully close agreement between *Ephedra* and *Welwitschia* in the inter-relationships between the plumular and cotyledonary bundles and these transfusion tracheides.

It may be remarked in this particular connexion that Bower's account and ours appear to differ: this is due to the fact that Bower's seedlings were older, with the plumular traces developed; and as these mask the presence of the transfusion tracheides, it would be impossible to realize their nature and significance without younger stages.

## GNETUM.

The seedlings of *Gnetum Gnemon* have already been considered by Bower,<sup>1</sup> and as our results are in general agreement with his, it is necessary for us only to draw attention to the main features connected with the transition-phenomena. Unlike *Welwitschia*, the seeds of which germinate in about two weeks, the seedlings of *Gnetum* do not appear above ground much before three months, at the earliest, after sowing; the majority take eight months to a year.

The general morphology of the seedlings of *Gnetum Gnemon*, Linn., have already been described and illustrated,<sup>2</sup> but for the sake of completeness the figures referred to are reproduced here. Plate XXIII, Fig. 12, shows the split seed-coat and a very short primary root; the hypocotyl is seen to be curved with its tip still embedded within the prothallus. The hypocotyl soon straightens and is withdrawn from the prothallus, but the seed-leaves are still quite small and scale-like, which condition they retain for some time: this stage is illustrated in Plate XXIII, Fig. 13, in which example the prothallus has been partly cut away in order to show the sucker or foot embedded within it. The next figure is that of an older seedling, and shows the rod-like foot entirely freed from the tissues of reserve; it will be observed that the cotyledons have increased in size, and that a few lateral roots have arisen, but the main root still remains stunted, a feature figured by Roxburgh as occurring in *Gnetum scandens*.<sup>3</sup>

After a time the primary root elongates considerably and the seed-leaves increase greatly in size (Plate XXIII, Figs. 15 and 16), and ultimately resemble the foliage leaves very closely; but for some time they are, in *G. Gnemon*, unequal in size, a feature which also obtains in *G. scandens*.

The seedling of *Gnetum scandens*, Roxb., judging from one example, very closely resembles *G. Gnemon*; on the other hand, that of *G. moluccense*, Karst., the seed of which is much larger than that of either *G. scandens* or *G. Gnemon*, shows some points of difference. One only of several seeds of *G. moluccense* germinated, and this example, owing to the fact that it was planted the wrong way or possibly was disturbed and replaced improperly, showed the root and shoot much curved. A lateral view of this seedling is illustrated in Plate XXIII, Fig. 18; the chief points of interest are the small size of the seed-leaves, the early development of a cotyledonary bud, and the relatively advanced state of the epicotyl, although the foliage leaves are still rudimentary. Fig. 20 is an end-on view of the same seedling, in which *f* indicates the region of insertion of the sucker, and, finally, Fig. 19 illustrates a dissection which shows the sucker.

<sup>1</sup> Bower: The Germination and Embryology of *Gnetum Gnemon* (Q.J.M.S., xx, 1882).

<sup>2</sup> Hill, T. G.: The Germination of *Gnetum Gnemon* (Journ. Roy. Hort. Soc., xxxiv, Pt. 1, 1908).

<sup>3</sup> See Bower, loc. cit.



Inasmuch as Bower has described the structure of the foot and has considered its morphology very fully, there is no need for us further to deal with it in detail; it, however, may be remarked that we look upon this structure as an outgrowth produced expressly to effect the removal of the reserve food materials, and that it has no phylogenetic importance.

A short cotyledonary tube occurred in all examples of the species examined.

With regard to the structure of the seed-leaves there is nothing of importance to remark upon. The young cotyledons have very little lamina and a well-marked midrib containing five vascular bundles, which in their structure resemble those of *Welwitschia*. The strands are endarch and collateral throughout the length of the cotyledon, and at the base they tend to fuse together, so that in *G. scandens* and *G. moluccense*, as far as has been seen, and in some examples of *G. Gnemon*, five strands may enter the axis from one cotyledon, and four from the other; in other seedlings of *Gnetum Gnemon* five bundles enter the hypocotyl from each seed-leaf. Within the axis the bundles tend to fuse together, the extreme lateral strands of one cotyledon joining with the corresponding bundles of the other seed-leaf, but this again is not constant in all cases.

#### Transition.

At a level just below the cotyledonary node the bundles anastomose freely, and some, at any rate, of the xylem elements of these connexions are differentiated from the ground-tissue, and resemble the transfusion tracheides remarked upon above as occurring in the hypocotyl of *Ephedra* and *Welwitschia*. In the case of *G. moluccense*, the anastomoses between the bundles of the cotyledons, the cotyledonary bud, and the plumule were of the greatest complexity, and the special tracheides were extensively developed. After these general connexions between the various traces have been made, six large bundles obtain in the upper part of the axis together with a few small intervening ones, ill developed and small at this level, but rapidly increasing in size and differentiation lower down; also the larger strands branch and new ones may come into being between the pre-existing traces. Thus the number of bundles is increased, and throughout the greater length of the hypocotyl a varying number, about twelve to fifteen, occur in the species examined.

In that part of the axis situated immediately above the foot, a reduction in the number of bundles is brought about by the union of neighbouring structures; several of those on the foot side of the axis enter the upper part of the sucker, and so supply it with vascular tissue as described by Bower. After having passed to the extremity of the foot these strands return along the lower side, and thus regain the axis. Of these bundles, the two last, on their arrival near the central region of the hypocotyl, are seen



to be in a state of transition, their protoxylems being directed towards each other and outwards. The completion of this rearrangement forms one pole of the primary root. The bundles on the other side of the axis continue their course straight downwards; the protoxylem of the two strands situated in the cotyledonary plane, at the upper level of, or immediately above, the sucker, turn towards each other and outwards, and thus form the second pole of the root-structure, which is organized before that on the foot-side of the axis.

In seedlings of the stage illustrated in Pl. XXIII, Fig. 14, isolated protoxylem elements may be found at some distance above the foot, at which higher levels they are few in number and separated one from the other; but lower down they increase in number, and ultimately become attached to that protoxylem pole of the root situated on the side of the axis opposite the foot. These isolated elements have not been observed in the corresponding position on the sucker side of the axis. This peculiarity, in all probability, is due to the elongation of the axis after the rearrangements of the vascular tissues have been accomplished.

The remaining changes take place on normal lines; the phloem-masses of the strands directly concerned in the transition pass to one side and join with the bast of the bundles situated in the intercotyledonary plane, which close up and so produce a proper root-structure which, in the species examined, *G. scandens*, *G. Gnemon*, and *G. moluccense*, is diarch.

Attention may now be drawn to a few minor points in the structure of the hypocotyl. In seedlings of about the stage indicated in Pl. XXIII, Fig. 14, a lignification of the elements of the central ground-tissue occurs in the lower regions of the axis; this alteration takes place centripetally, but immediately above the foot the thickening is not nearly so extensive as at higher levels. In the same parts of the axis, and at about the same time, an interfascicular cambium arises and also a phellogen which is epidermal in origin. The periderm formation below the sucker, i. e. in the root region, is internal.

It is clear that the transition-phenomena in the seedlings of *Ephedra*, *Welwitschia*, and *Gnetum* are essentially the same; each pole of the diarch root-structure being formed by the rearrangement of the vascular tissue of two seed-leaf-traces. In the case of *Gnetum* this similarity is obscured by the larger number of cotyledonary bundles and by the numerical increase of these in the hypocotyledonary axis. In all cases the transition to root-structure occurs at the lower end of the hypocotyl. On the other hand, there are some differences, the chief of which are in the number of vascular bundles in the cotyledons. In *Ephedra* each seed-leaf has two strands which retain their identity until the transition is accomplished; in *Welwitschia* the number of traces in the blade of each cotyledon is eight or more, but just before entry into the axis, these fuse together; thus

four are produced and are arranged in two pairs, the units of each of which join together in the hypocotyl, so that, from this level downwards, the appearance is remarkably like that of *Ephedra*; finally, in *Gnetum*, the bundles are very numerous in the blade of each seed-leaf; the petiole, however, contains five which, by fusion, may be reduced further, so that either four or five bundles enter the axis.

Comparing the Gnetales with other Gymnosperms, it is seen that the transition-phenomena exhibited by these plants resemble those of the Podocarpeae<sup>1</sup> and the Araucarieae,<sup>2</sup> the similarity between which two groups has already been remarked upon. *Ephedra* is practically identical with *Podocarpus*, the only difference being that in the former plant the transition is slower, taking place in the lower region of the hypocotyl. The same observations apply equally well to *Welwitschia*, although the resemblance is masked by the larger number of cotyledonary traces; still more so is this the case in *Gnetum*.

A comparison with the Araucarieae shows, with regard to the features under discussion, that *Welwitschia* is very like indeed to *Araucaria Cunninghamii*. The resemblance between *Ephedra* and *Gnetum* on the one hand, and the Araucarias on the other, is, on a superficial examination, less well marked owing to the fewer number of seed-leaf-traces in *Ephedra*, and to the numerical increase and anastomoses, within the hypocotyl, of these structures in *Gnetum*. The comparison of the critical stages, however, reveals the similarity between these plants. There is also a resemblance between *Welwitschia* and *Ginkgo*, and *Gnetum* and certain Cycads, as regards the number of bundles in the cotyledons, but the behaviour of these strands in the transition region is, however, not the same.

The present paper concludes the statement of our observations on the seedling structure of the Gymnosperms, and incomplete though it be, it would have been impossible to have considered the subject even thus fully without the co-operation of many in supplying us with material.

To the following we wish to render thanks and to express our appreciation of their kindness:—

The Directors of the Botanic Gardens of Brisbane, Buitenzorg, Kew, Peradeniya, and Sydney; Mr. Boodle, Mr. de Fraine, Prof. H. H. W. Pearson, Dr. D. H. Scott, Mr. Tansley, and Miss Thomas.

More especially are we indebted to Mr. Hales, the Curator of the Old Physic Garden, Chelsea; and him we desire particularly to thank, not only for material, but also for the trouble and care he has taken in the germination of our seeds.

Our general conclusions will appear later on, when our work on certain Angiosperms is completed.

*A correction.*—In Part III, p. 434, it is stated that Sprecher does not

<sup>1</sup> Part I (Annals, xxii, 1908).

<sup>2</sup> Part II (Annals, xxiii, 1909).

remark upon the presence of four vascular bundles in the apex of the cotyledons of *Ginkgo*. The author cited does comment upon this feature, and in making this correction we desire to express our regret to Dr. Sprecher for the misrepresentation.

## SUMMARY.

### COTYLEDONS.

1. The plants examined, viz. *Ephedra distachya*, *E. fragilis*, *E. campylopodia*, *E. altissima*, *Welwitschia mirabilis*, *Gnetum Gnemon*, *G. scandens*, and *G. moluccense*, have dicotyledonous and epigeal seedlings.

2. The cotyledons of *Ephedra* are linear in shape; at first they are small, but with increasing age they elongate greatly. The seed-leaves of *Welwitschia* are large and leaf-like, and persist relatively for a long time; those of *Gnetum* to begin with are scale-like and their growth is slow, finally they attain a large size, and closely resemble the foliage leaves.

3. In all cases a short cotyledonary tube is formed.

4. The number of cotyledonary bundles varies; in all the *Ephedras* examined there are two traces in each seed-leaf; in *Welwitschia* there are in the base of each seed-leaf four strands arranged in two pairs; in *Gnetum* four or five bundles occur in the lowest region of the cotyledons.

5. The seed-leaf-traces are endarch and collateral in structure.

### HYPOCOTYL.

6. The upper part of the hypocotyledonary axis shows a stem-like structure; the chief bundles, in the material examined, are those derived from the cotyledons; of these strands there are four in *Ephedra*, eight or less in *Welwitschia* which quickly become reduced to four, and a variable number, about twelve or fifteen, occur throughout the greater length of the hypocotyl in *Gnetum*.

7. In *Gnetum* and *Welwitschia* a parenchymatous outgrowth occurs at the base of the hypocotyl; this is the foot or sucker, which is rod-like in the former plant and spade-like in *Welwitschia*. The foot functions as an organ of absorption, and remains embedded in the prothallus. The sucker in *Welwitschia* has no vascular supply, but in *Gnetum* the bundles in the foot are numerous and well differentiated.

A foot has not been observed in *Ephedra*.

This structure is not considered to have any phylogenetic importance.

8. Short tracheides, closely resembling the transfusion tracheides obtaining in the cotyledons of other Gymnosperms, occur in all the plants examined in the region of the insertion of the plumular bundles on to the cotyledonary traces. These tracheides form a bridge between the corresponding bundles of the seed-leaves.

## Transition.

9. The transition to root-structure takes place in the lower region of the hypocotyl; in *Welwitschia* and *Gnetum* at the level of and immediately below the foot.

10. Each pole of the root-structure is formed from two cotyledonary bundles which rotate towards each other and outwards, and so bring their protoxylems into an exarch position. These groups of elements become more compact, and the corresponding metaxylem-masses come into continuity. The groups of phloem elements pass towards the intercotyledonary plane, but their fusion may be delayed considerably.

11. In all cases the primary root is diarch.

12. In *Gnetum* the primary root remains short and peg-like for some time; later, it elongates after two or three lateral roots have been formed. In *Ephedra* and *Welwitschia* there is no delay in the growth of the primary root.

## EXPLANATION OF FIGURES IN PLATES XXII AND XXIII.

Illustrating Mr. T. G. Hill's and Miss de Fraine's paper on the Seedling Structure of Gymnosperms.

Abbreviations used:—*f.*, region of insertion of foot: L.S., longitudinal section: *mx.*, metaxylem: *ph.*, phloem: *px.*, protoxylem: *s.*, foot; T.S. transverse section.

## PLATE XXII.

Figs. 1-5 × 240.

Fig. 1. *Ephedra distachia*. T.S. of part of cotyledon.

Fig. 2. *E. fragilis*. T.S. hypocotyl, showing the transfusion tracheides.

Fig. 3. *E. fragilis*. The same in L.S.

Fig. 4. *Welwitschia mirabilis*. T.S. of part of cotyledon.

Fig. 5. *W. mirabilis*. T.S. hypocotyl, showing a vascular bundle and isolated protoxylem elements.

## PLATE XXIII.

Figs. 6-20 natural size.

Fig. 6. *Ephedra distachia*. Young seedling.

Fig. 7. *E. fragilis*. Older seedling.

Fig. 8. *E. campylopodia*. Still older seedling, showing the much elongated seed-leaves and the plumule.

Fig. 9. *Welwitschia mirabilis*. Seedling.

Fig. 10. The same with seed-coat removed.

Fig. 11. The same with the prothallus removed in order to show the spade-like foot.

Figs. 12-17. Different stages in the growth of the seedlings of *Gnetum Gnemon*.

Fig. 12. Very young seedling, showing short primary root and the curved hypocotyl, the tip of which is still enclosed within the prothallus.

Fig. 13. An older example, partly dissected to show the foot embedded within the prothallus; the cotyledons are still very small.

Fig. 14. An older specimen from which the prothallus has been entirely removed to show the rod-like foot (*s.*). The seed-leaves, although relatively larger, are still small, and the primary root is still stunted and bears three lateral roots.

Fig. 15. In this seedling the root has elongated and the cotyledons have increased in size.

Fig. 16. An older seedling, showing the seed-leaves much further developed. Their difference in shape and size may be noted.

Fig. 17. The top part of a young plant, showing the first pair of foliage leaves and, below them, the seed-leaves, which are partly represented in outline.

Fig. 18. *G. moluccense*. Seedling, showing the split seed-coat, the scale-like cotyledons, and the epicotyledonary axis.

Fig. 19. *G. moluccense*. A dissection to show the sucker.

Fig. 20. *G. moluccense*. An end-on view of the seedling represented in Fig. 18. *f.* indicates the insertion of the sucker.

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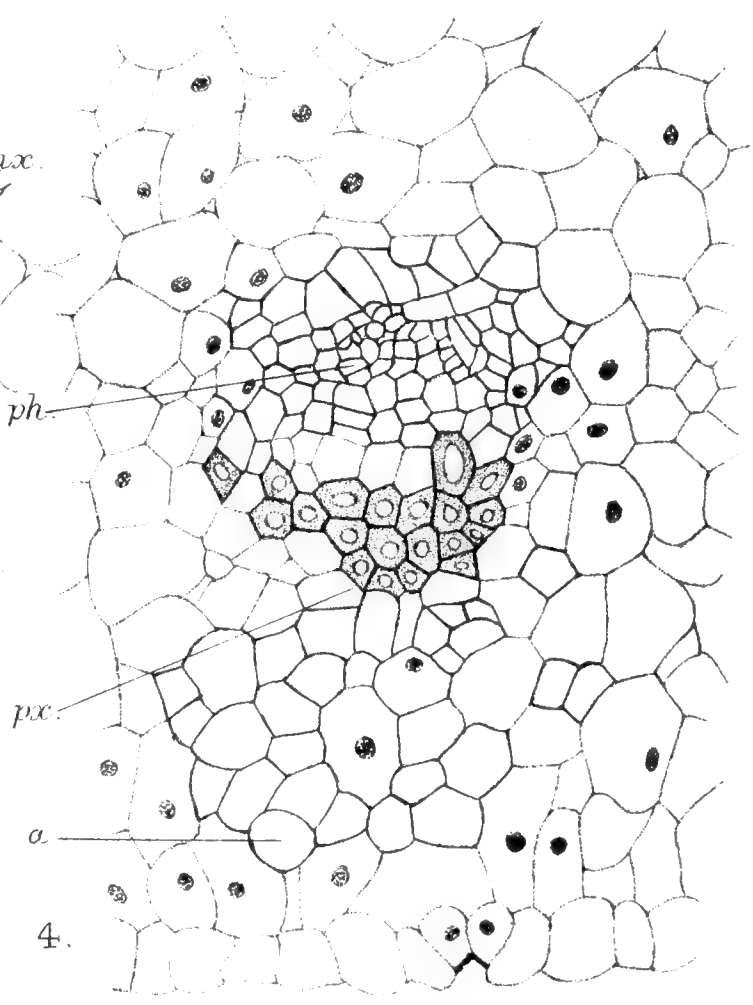
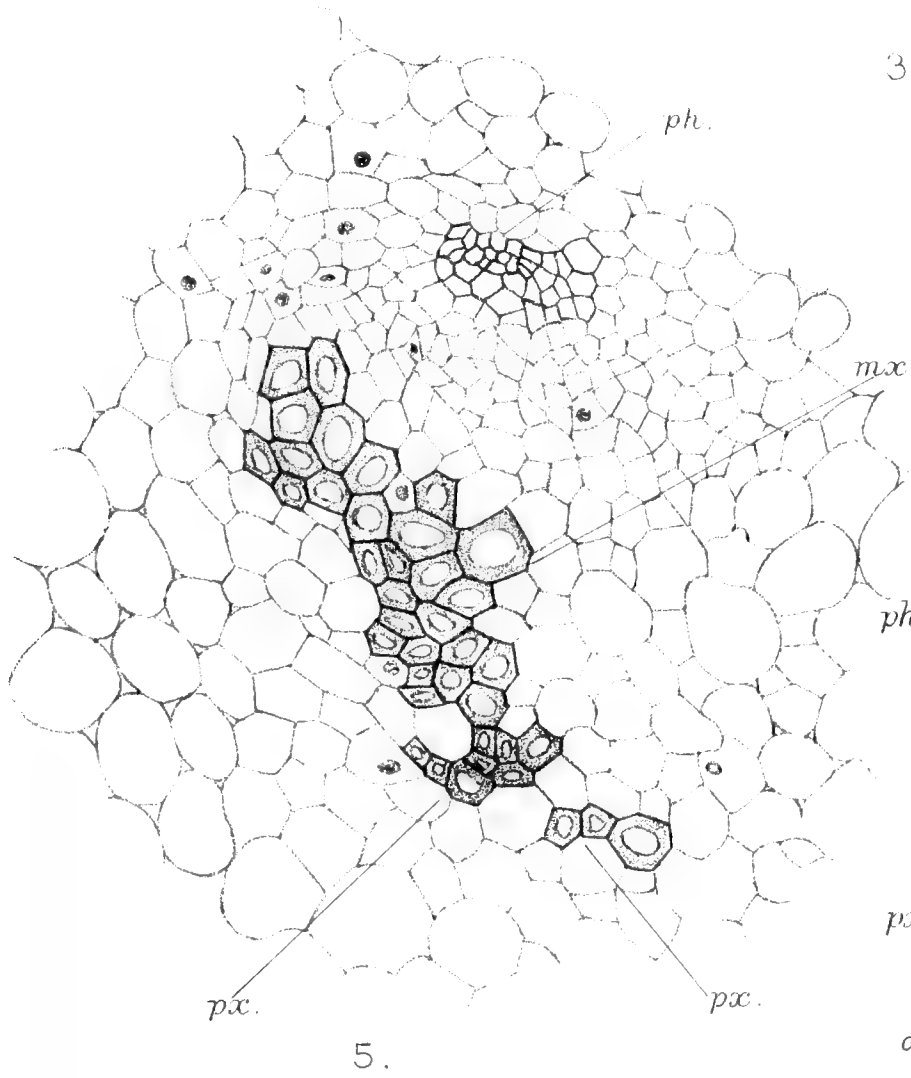
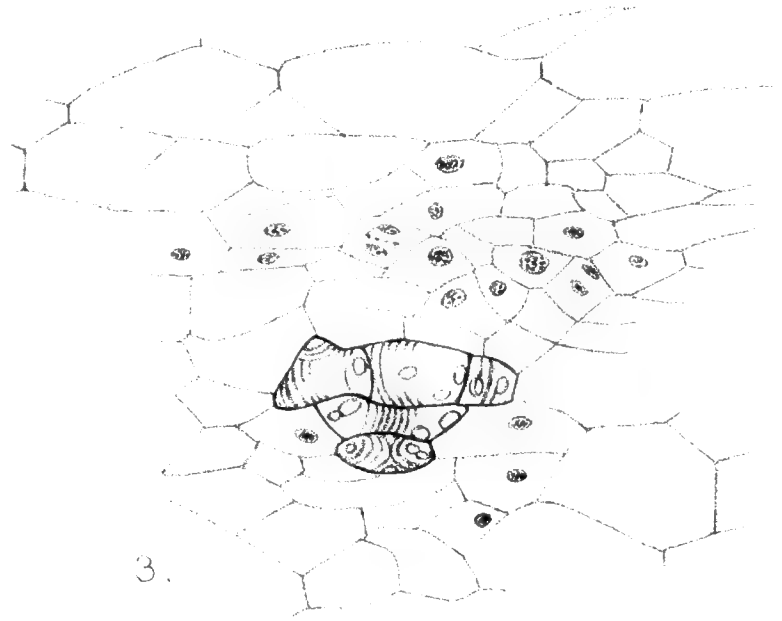
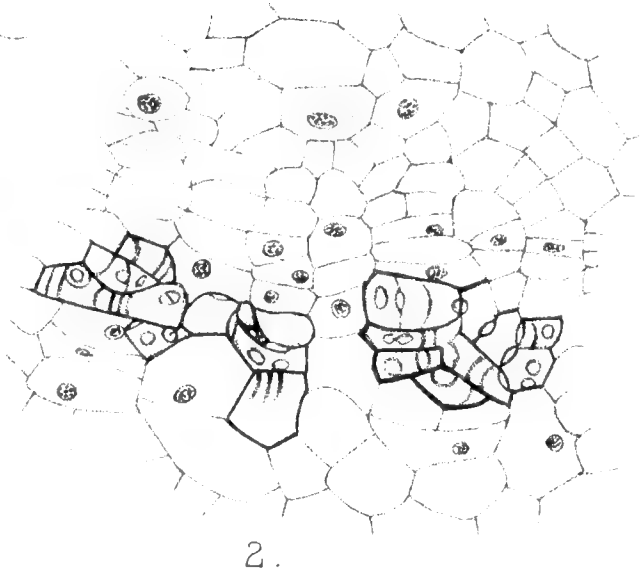
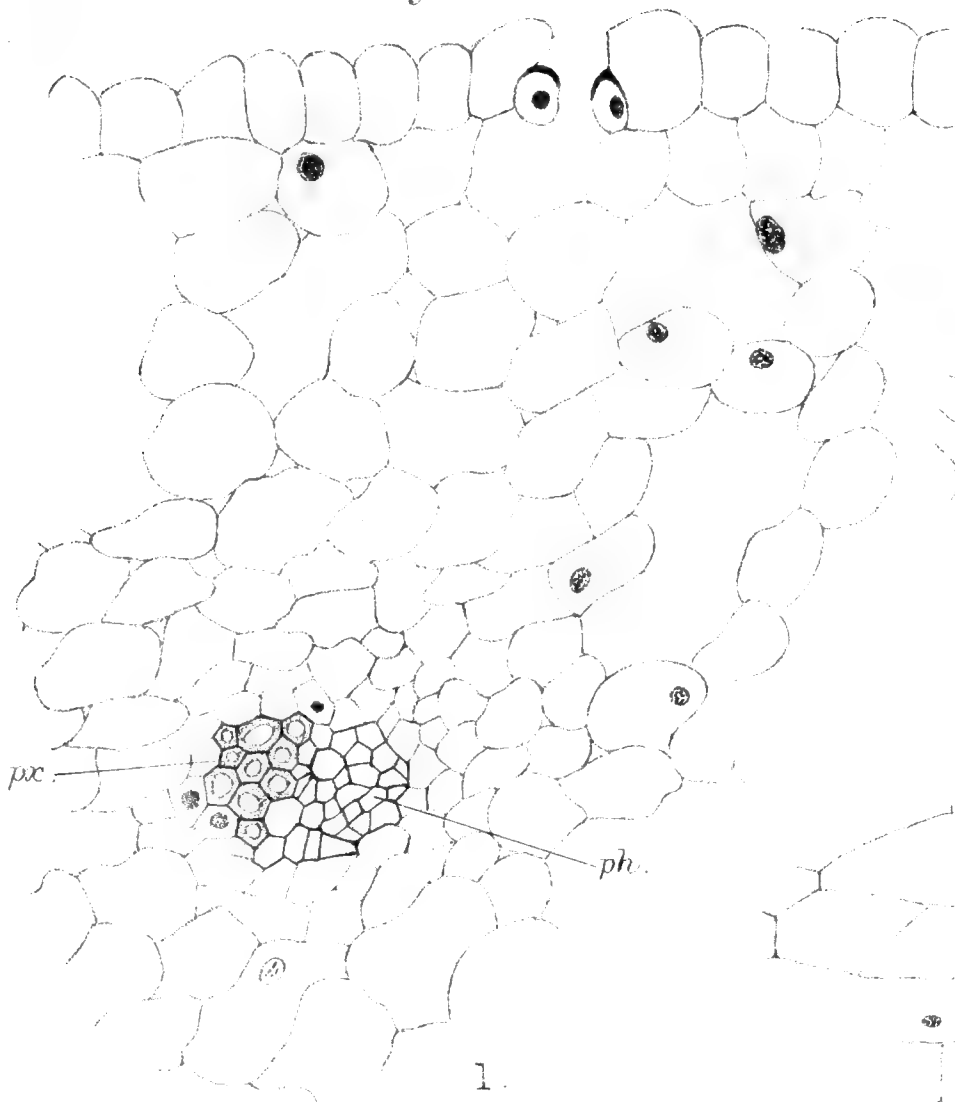
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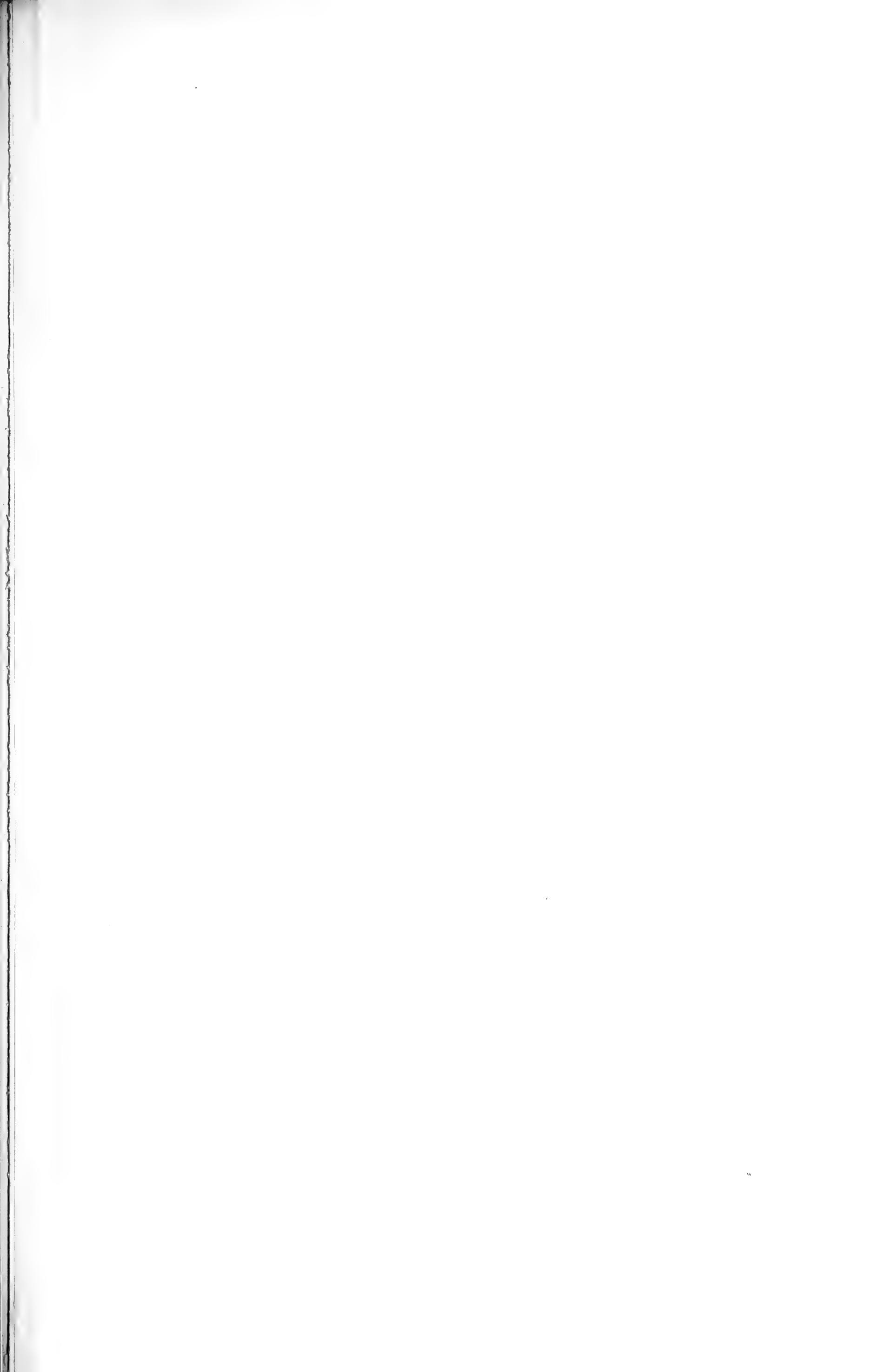


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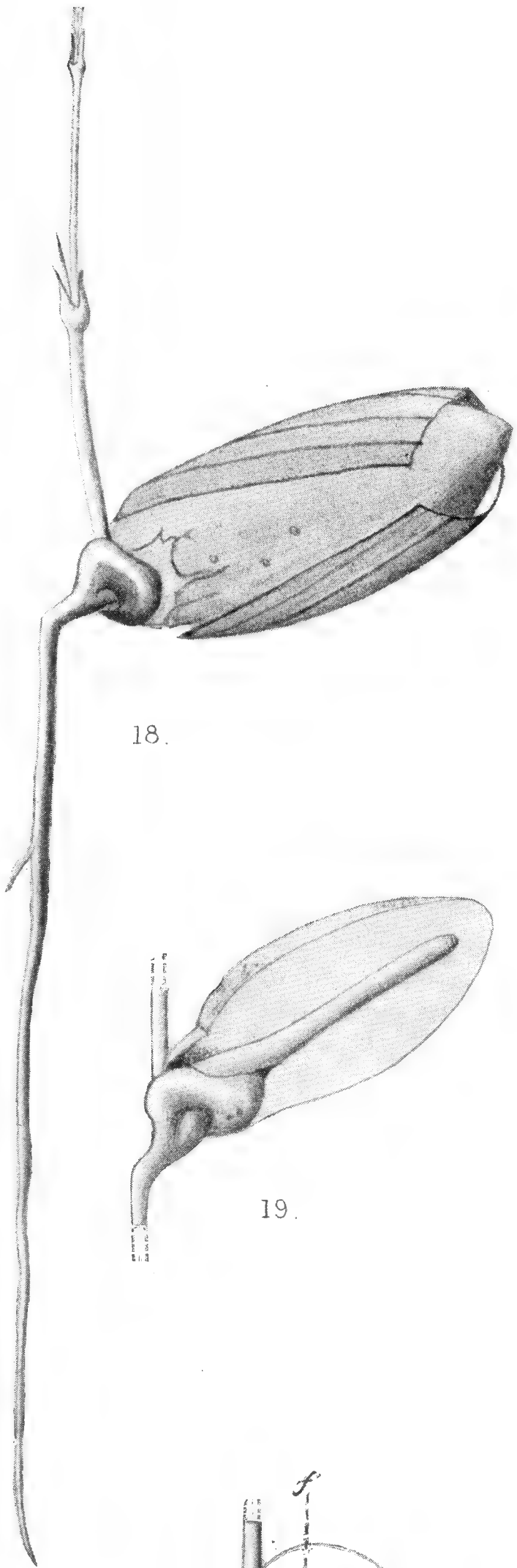








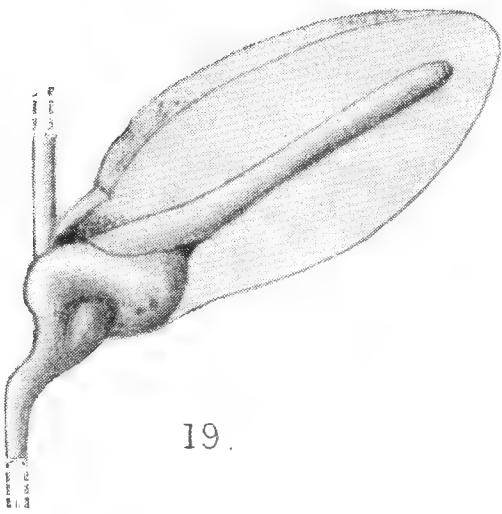
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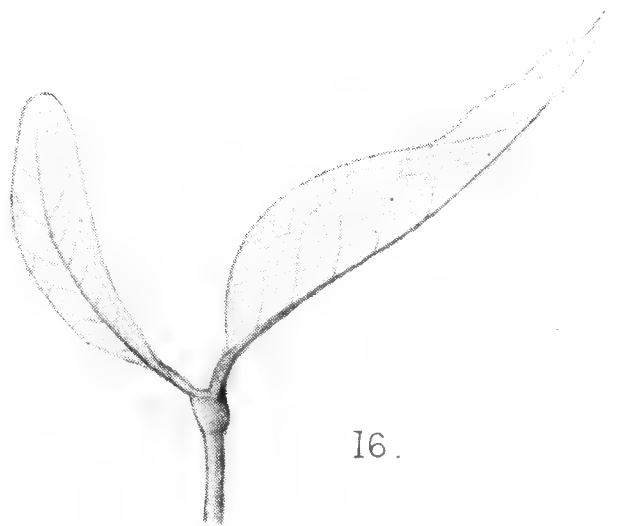
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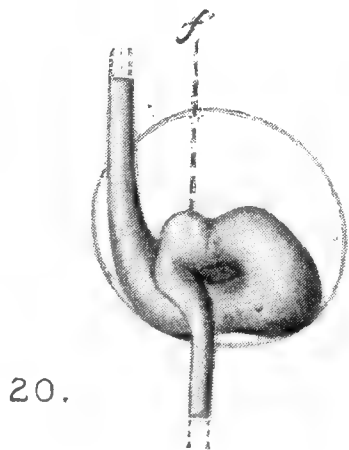
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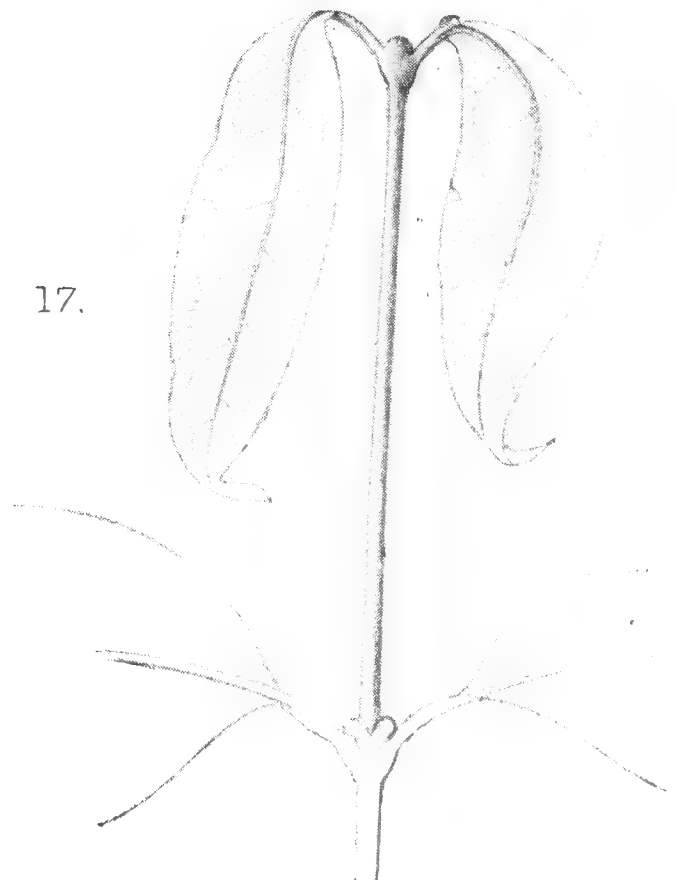
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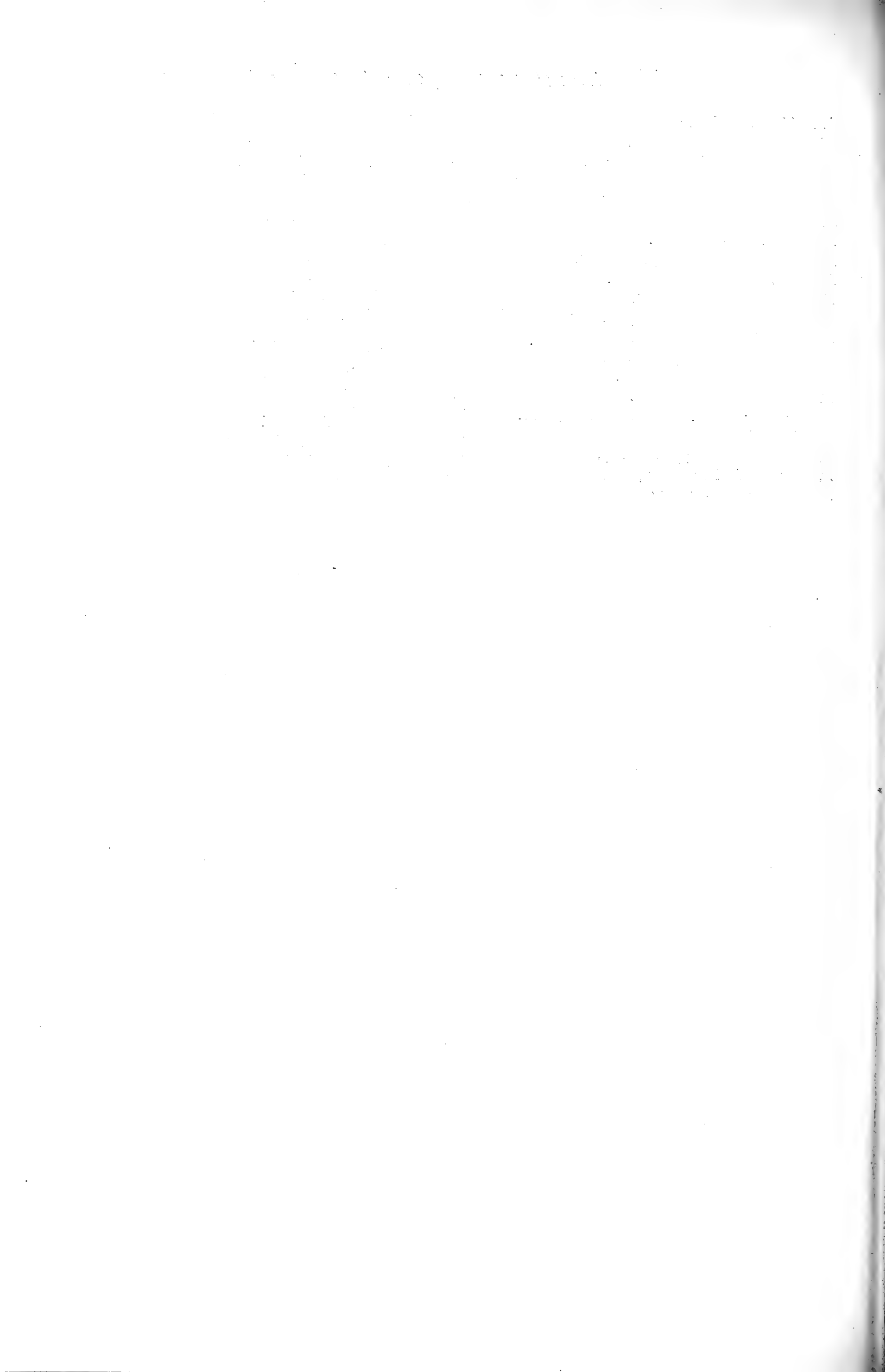


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# The Morphology of *Phylloglossum Drummondii*.

BY

H. F. WERNHAM, B.Sc.

With eight Figures in the Text.

*PHYLLOGLOSSUM* was first described in 1843 by Kunze, in the *Botanische Zeitung*; its morphology was investigated in great detail by Bertrand in 1885 (5). Other contributors to our knowledge of the plant will be referred to as we proceed; they are, notably, Bower (1), Mettenius (9), Treub (12), and Thomas (13), who has given a detailed description of the gametophyte.

The observations recorded in the present paper were made on serial micro-tomed sections of two plants which, in their external features, showed no important points of dissimilarity; on the other hand, they were not precisely the same as regards their anatomy: the differences will be referred to in the description which follows.

## EXTERNAL MORPHOLOGY.

A small plant (Fig. 1), about 1 to 1½ in. high; on the under side of the thickened lower part two tubers are borne, each upon what appears to be a relatively long stalk. Immediately above the insertion of these stalks, borne laterally to the thickened part, are the roots, which are horizontal, or almost so.

This thickened part, which is the stem, is much compressed vertically. On its upper surface it bears five or six subcentric fleshy leaves, each

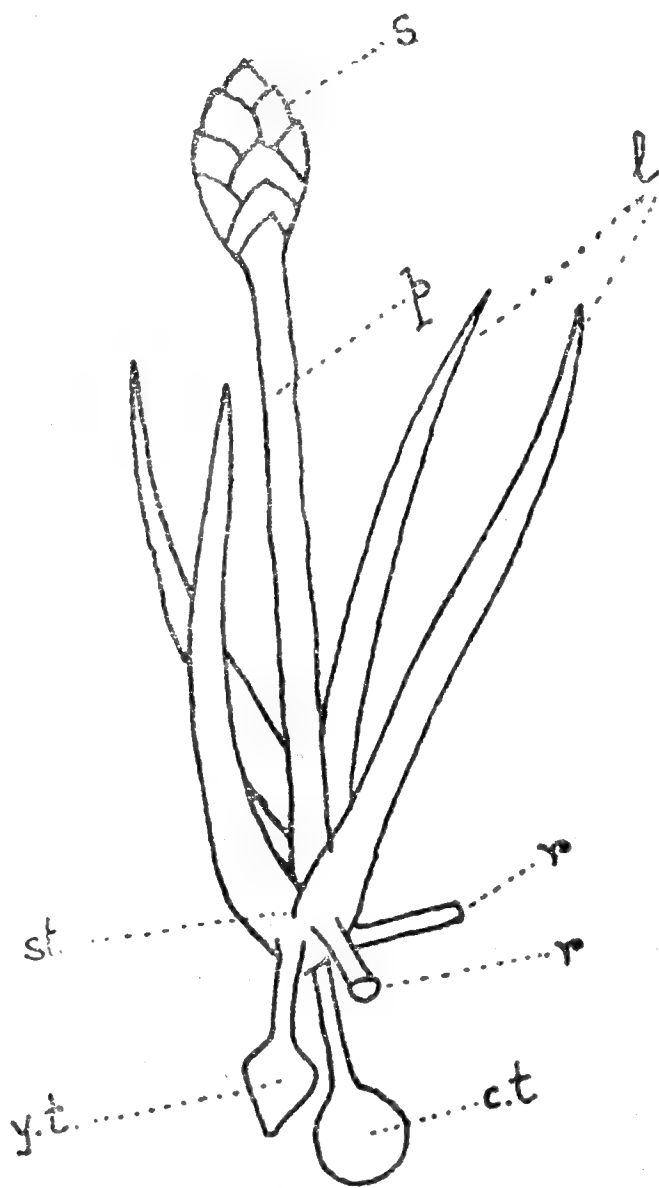


FIG. 1. General appearance. *y.t.*, young tuber; *c.t.*, current season's tuber; *st.*, stem region; *r, r*, roots; *l*, leaves; *s*, strobilus; *p*, pedicel of strobilus.

tapering to a point. From the midst of the leaves arises the long peduncle, bearing terminally a small ovoid strobilus with closely overlapping bracts, spirally arranged.

#### INTERNAL ANATOMY.

The internal anatomy of *Phylloglossum* has been exhaustively described by Bertrand (5); in the present paper only the broader features will be considered.

The succeeding description will follow the microtomed sections from below upwards; the first structure which presents itself is:—

1. *The Young Tuber.* At the extreme tip this consists solely of an aggregate of parenchymatous cells, with well-marked nuclei; but at a very short distance above, this begins to be clearly differentiated from the peripheral layer, which is of regular, more or less radially elongated, empty cells. This peripheral layer persists throughout the entire length of the tuber; followed upwards, it presents a more or less strong thickening of the exterior cell-walls. Sections of the older tuber, at a higher level, also reveal a well-marked thickening on the radial walls of the peripheral cells.

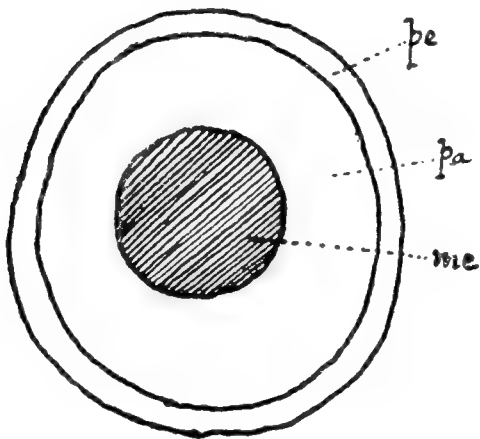


FIG. 2. Diagram of transverse section of young tuber; *pe*, peripheral layer; *pa*, storage parenchyma; *me*, merismatic tissue.

Ascending, the sections show a gradual aggregation of the nucleated cells towards the centre, these being surrounded by a broad zone of homogeneous parenchyma, in which intercellular spaces are noticeably absent. The latter zone is doubtless destined to form the food-storage tissue, the nucleated cells being probably merismatic (Fig. 2).

Proceeding upwards, the central merismatic zone becomes smaller and smaller, ultimately breaking loose from the surrounding parenchymatous zone, and coming to lie in a cavity.

In the meantime the radial walls of the peripheral cells have acquired a well-pronounced thickening, particularly in the centre, so that they present a resemblance to the appearance often associated with an endodermis.

The next twelve sections in ascending order show simply a central cavity—indicating the existence of a rather long central channel (Fig. 4) in the 'stalk' of the tuber. This is borne out by the longitudinal sections, as shown in the diagram (Fig. 3).

One or two of the nucleated cells persist for a time around the edge of the central cavity as the transverse sections are followed upwards, so that the meristem tapers to a fine point (Figs. 3, 4); the segmentation

observed in the longitudinal and transverse sections does not, however, justify the conclusion that this meristem focuses upon a single apical cell.

In the meantime the sections have passed through the older tuber (Fig. 4). This latter, it will be seen, is really the tuber of the current season, the younger one, described above, being the tuber for the following season.

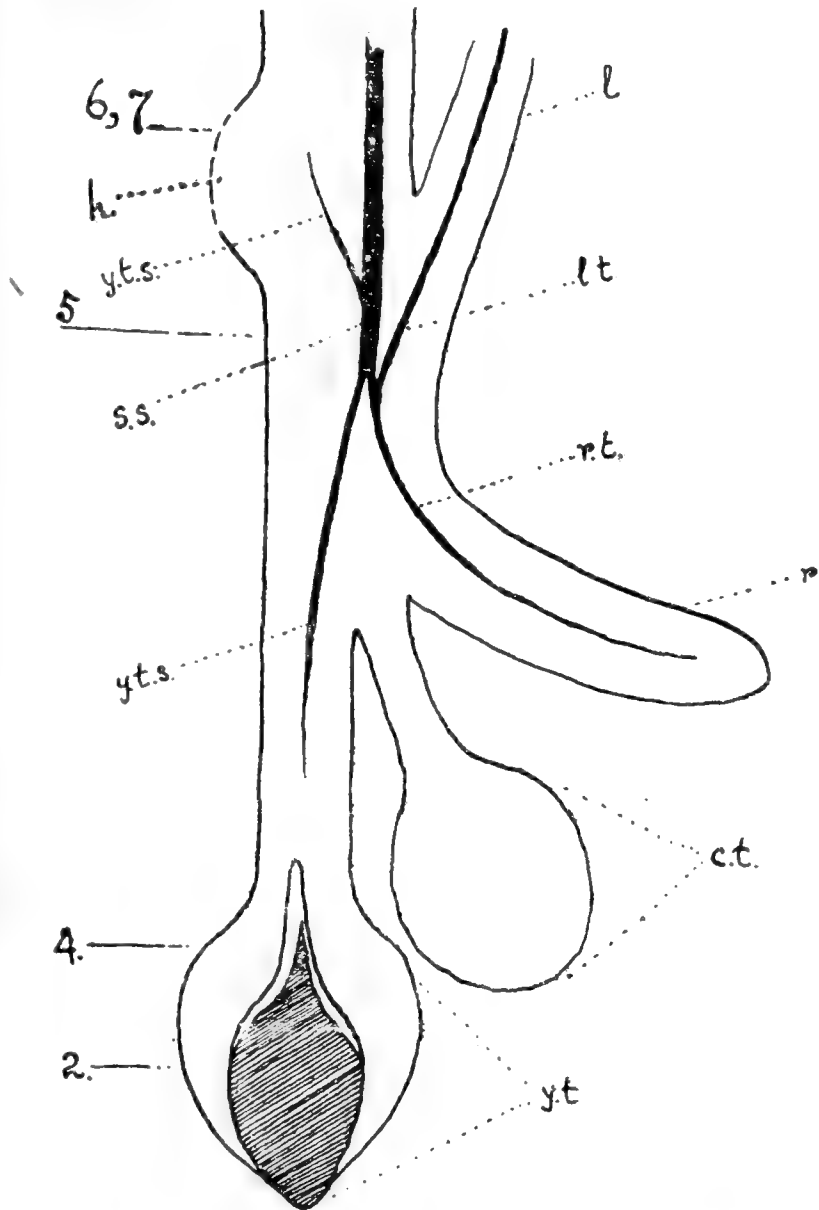


FIG. 3. Diagram of median longitudinal section. *y.t.s.*, strand of young tuber; *s.s.*, stem-stele; *r.t.*, *l.t.*, root- and leaf-trace; other letters as in Fig. 1. The numbers 2, 4, 5, 6, 7 indicate the levels at which the transverse sections shown in Figs. 2, 4, 5, 6, 7, respectively, were taken. The shaded portion denotes the meristematic tissue of the growing tuber. Xylem in thick black lines.

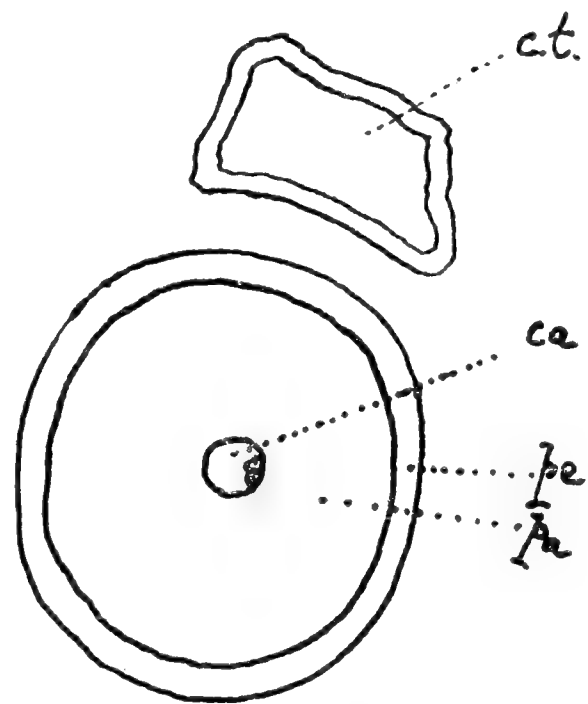


FIG. 4. Transverse section of young tuber, showing central cavity *ca*, with two meristematic cells at its edge. The older tuber (*ct.*) also appears in section.

The older tuber presents a highly lacunar appearance below. This would be significant, but it is doubtful whether this is not due to indifferent preservation; there is no indication of lacunar structure in the parenchymatous tissue further up in the tuber—the aeration of the stem and tuber being provided, apparently, chiefly by the loose packing of the parenchyma cells.

Returning to the younger tuber, a few wood-elements appear, shortly above the extremity of the 'channel', in the centre of the sections (see Fig. 3). At no level do these exceed about a dozen in number; and in

many of the sections they are seen to be undoubtedly mesarch. The wood-elements are thus confined in this specimen to the stalk of the tuber; the body is entirely destitute of vascular tissue.

One or two notable differences were observed, however, in the second specimen examined: first, the bundle of the young tuber divided into two strands, which united further up, before joining the vascular system of the stem; second, a ring, more or less continuous, of somewhat degraded xylem was observed surrounding the central merismatic tissue of the young tuber. This ring, however, disappears before the stalk-region of the tuber is reached.

In the second specimen, moreover, three tubers appeared in section; one corresponding to the younger tuber, and two older ones—and this corroborates Thomas's statement in the Proc. Roy. Soc., xix, p. 285.

These differences in the two specimens will be referred to later.

The two sections—old and young tubers—are now seen to approximate as we follow the sections successively upwards; the older begins to be cut somewhat obliquely, and two root-traces appear in longitudinal section. The latter evidently bend up rapidly at this stage (Fig. 3), as they are seen, three

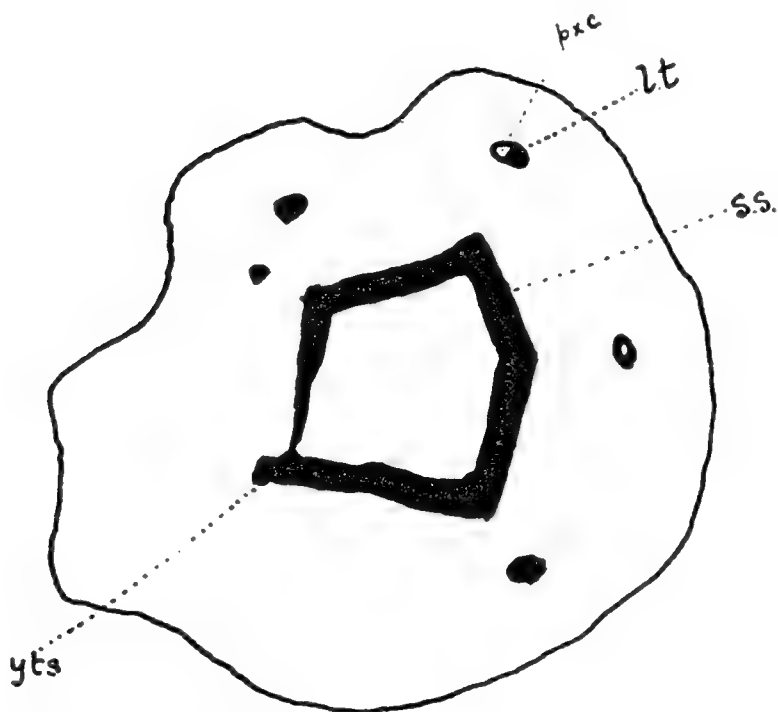


FIG. 5. Transverse section, lower part of stem. Parenchyma left blank, xylem black. The young tuber strand, *y.t.s.*, has joined the main stele (*s.s.*). Five leaf-traces appear, two with included protoxylem cavities (*pxc*).

in number, in clear transverse section shortly after the two tuber sections have merged. The roots are distinctly monarch, and resemble Stigmarian rootlets closely; a well-marked endodermis is present, and the ground tissue presents a highly lacunar appearance.

Further up, these root-traces are found somewhat nearer the centre of the section, having become connected laterally by other wood-elements running, at first, almost horizontally; at this point we are entering the lower part of the compressed stem.

2. *The Stem.* Proceeding as before with the sections in ascending order, leaf-traces begin to be 'pinched off' the stele thus formed, and these leaf-traces occupy the same relative positions previously taken by the root-traces. Leaf- and root-traces are thus continuous, a fact pointed out by Bower twenty-five years ago. The mode of exit of these leaf-traces is that typical of the Lycopsida, there being no disturbance of the stele. The

leaves of *Phylloglossum* are thus anatomically microphyllous, so to speak, and may be compared usefully with *Isoetes* in this regard—the leaves in both being large as compared with the stem, although detailed examination reveals them, in both cases, to be characteristically microphyllous in their behaviour to the stele.

At a level where the three leaf-traces, corresponding to the three root-traces first observed, have progressed outwards to about midway in the cortex, the transverse section presents the appearance shown in Fig. 5. The leaf-traces are mesarch, the protoxylem in many cases being represented by cavities (see *postea*).

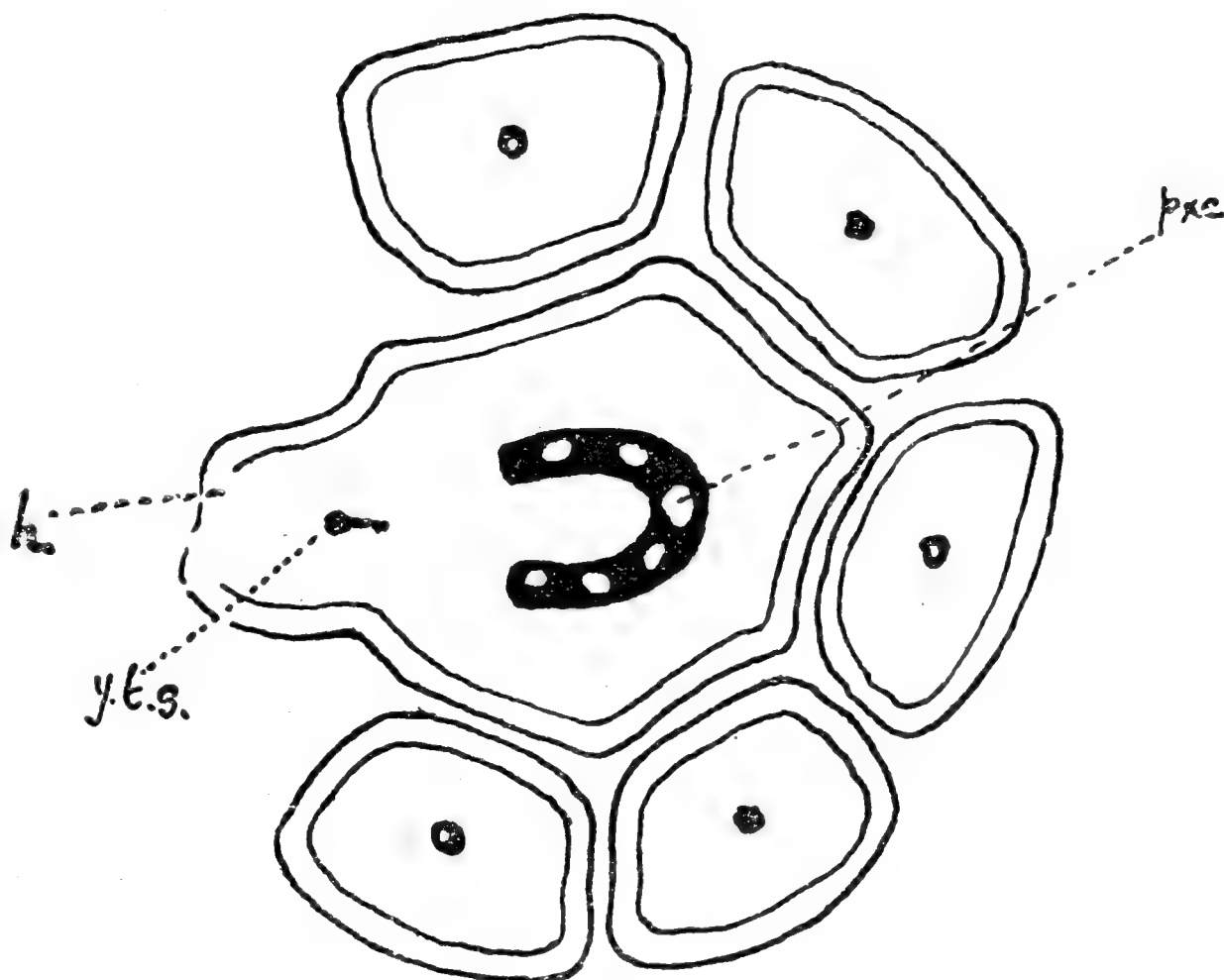


FIG. 6. Showing the U-shaped stele, with protoxylem-cavities. The strand of the young tuber has broken away from the stele, and is dying out. Five leaves appear, each with a single, small central vascular strand. There are no leaf-traces in this region: no more foliage leaves emerge.

The central stele is here similar to that seen in many of the Lycopodiales. The xylem-strand of the young tuber stalk has joined this main stele, which is a more or less continuous band of xylem enclosing a parenchymatous 'pith', and is roughly pentagonal. This is noticeably like *Psilotum* and many of the fossil *Lepidodendra*.

This condition, however, does not persist for any appreciable length of the stem; a little further up there is a distinct break in the xylem-band, on the side occupied, in sections at a lower level, by the young tuber. The stele thus becomes horseshoe-shaped; and although there seems to be a tendency to bridge over this gap, at first, by weakly developed tracheides, the gap may be said, approximately speaking, to persist until the base of



the peduncle is reached, at which point, we shall see, the stele breaks up into the vascular strands of the peduncle.

As we ascend, the original xylem-strand of the young tuber breaks away from the main stele; it ultimately disappears. In Fig. 6, taken across the lower part of the leaf region, we have a distinctly U-shaped stele, with the gap facing the former site of the young tuber. Five leaves are seen in section; and it will be well, perhaps, at this point, to digress with a brief description of the anatomical structure of—

3. *The Leaf.* The outline of the transverse section is roughly circular; a distinct peripheral layer is differentiated from the parenchyma which

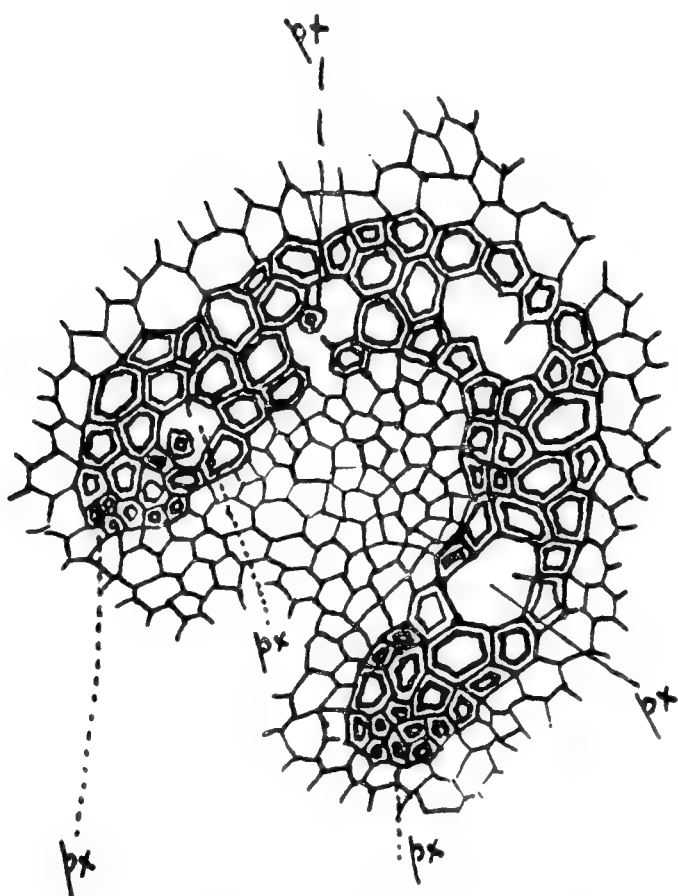


FIG. 7. The stele in detail. Protoxylem-groups appear at the free ends, and degraded groups form cavities in the body of the stele. A protoxylem-element, *px*, is seen clinging to the edge of one of the cavities.

occupies the greater portion of the section. In the centre is a single very small xylem-strand, consisting of six or seven wood-elements at most. In one case the xylem-strand was observed to be surrounded by a zone of tissue which was possibly phloem, although no sieve-tubes could be recognized.

Stomata occur evenly distributed over the epidermal layer, and leading into large air-spaces. The parenchyma seems to be traversed throughout by intercellular spaces; and the presence, in many of the sections, of radially elongated parenchymatous cells near the periphery suggests the existence of a rudimentary palisade tissue.

Let us now return to a closer examination of—

4. *The Stem-stele.* The investigation of the structure of the U-shaped

vascular band yields some interesting results. It is surrounded by markedly homogeneous parenchyma, which is continuous into its concavity.

There appear to be several protoxylem-groups. One is observed situated in an exarch position at each free end; but what is more remarkable, several cavities are present in the body of the stele (Figs. 6, 7); and the presence in some cases of one or two small tracheides clinging to the sides of the cavities indicates that the latter represent degraded protoxylem-groups, comparable with those seen in *Equisetum*. These cavities appear in Bertrand's figures, and he expresses the opinion that they represent degraded protoxylem-groups. The xylem is thus, of course, mainly mesarch in development.

The position of the phloem in this stele would be of the most significant interest, but the closest examination has failed to reveal its presence in the stem.

5. *The Peduncle of the Strobilus.* This follows in the transverse sections in a continuous ascent from the stem region. The xylem of the latter breaks up, very soon after entering the peduncle, into isolated strands, very irregularly disposed; not in a circle, as has apparently been the general impression hitherto (Fig. 8).

Each strand encloses a protoxylem-cavity and is thus clearly mesarch in development. One or two small wood-elements cling to the edge of the cavity; and here the resemblance to the carinal canals of *Equisetum* is much more striking than in the stem-stele.

6. *The Strobilus region.* As we approach the base of the strobilus, one or two of the strands move outwards to the periphery of the section; these, we shall see, go to supply sporophylls. The first indication of the latter in the sections is the appearance of a tangentially elongated mass of parenchyma, entirely isolated from the section of the main axis, and destitute of vascular tissue. This is obviously the down-pointing free 'dorsal lobe' of the lowest sporophyll<sup>1</sup> (*dl*, Fig. 8). Opposite this, in the axis section and near its periphery, is the xylem-strand (*t*) which is going to supply the sporophyll.

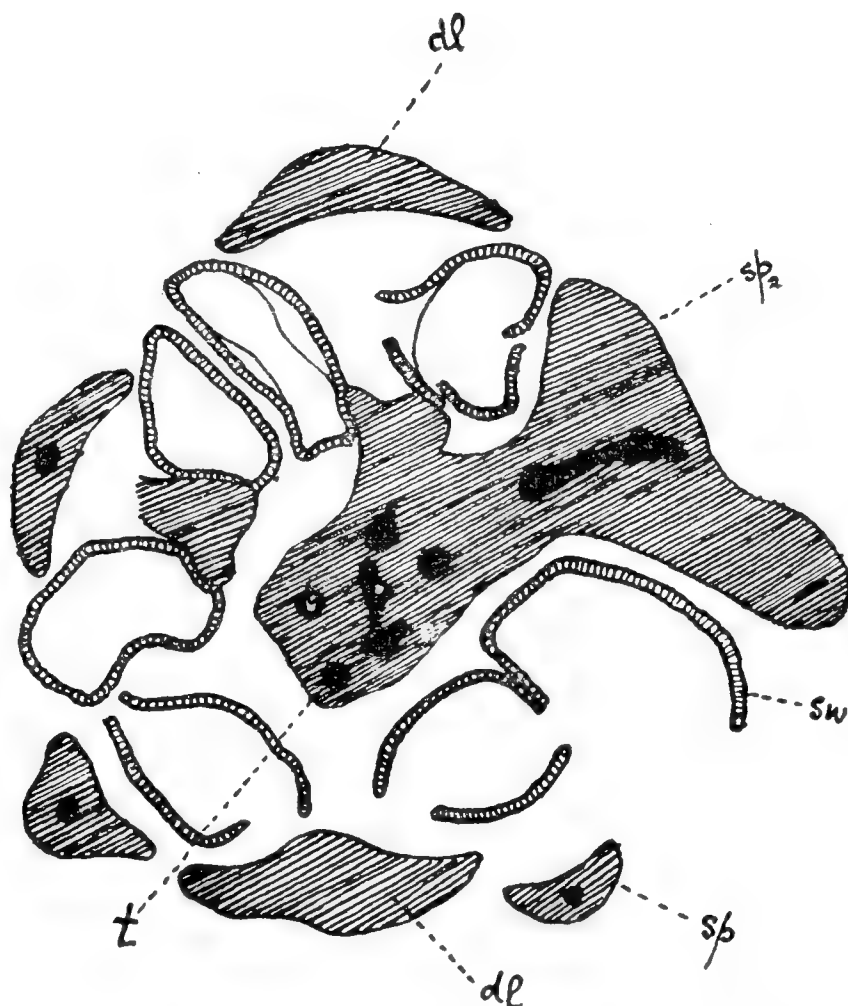


FIG. 8. Transverse section of strobilus. The walls of seven sporangia are seen in section, *sw*. The section passes through the base of one sporophyll, before it has become free of the main axis. Xylem black, parenchyma shaded.

Fig. 8 shows the general appearance seen in a transverse section taken about midway across the strobilus. Dorsal lobes (*dl*), sporophylls (*sp*), and sporophyll traces are seen in section at different levels. The sporophylls, like the leaves, are seen to possess a small central vascular strand, the latter originating from the isolated bundles of the axis; one is shown, in partial longitudinal section, in the act of passing out into its sporophyll.

<sup>1</sup> Sykes (11).

The vascular system of the strobilus-axis thus appears to be an aggregation of sporophyll-traces; but at the extreme tip, above the level of departure of the highest sporophyll, a few xylem-elements are still to be seen in section, so that the system is in reality cauline in origin.

#### SUMMARY OF THE INTERNAL STRUCTURE.

The vascular system is embedded in closely packed parenchymatous tissue. The parenchyma is remarkably homogeneous in tuber, root, stem, and in the peduncle and axis of the strobilus; the sole differentiation is in the peripheral layer of empty, radially elongated cells, with thickened walls; the parenchyma, moreover, which is in immediate association with the stele, is more compact and of smaller cells than that of the main body of the cortex. The latter is traversed by large intercellular spaces. This peripheral layer is fairly constant in sections taken at all levels, and in all parts of the plant.

There is no indication of an endodermis, except in the root.

The vascular tissue consists of xylem only (except perhaps in the leaf). This is, for the most part, of typically scalariform tracheides.

The general course of the vascular system is briefly as follows: That of the lower portion of the stem is a medullated protostele, which breaks up below into the strands which supply the roots on the one hand, and the young tuber on the other. The leaf-strands are continuous with those of the roots, and leave the stele without causing any disturbance in its continuity.

The xylem on the side of the ring which faces the tuber rapidly thins out in an upward direction, and a level is soon reached at which there is a definite gap in the stele, so that 'pith' and cortex become continuous.

Before this gap is reached, a xylem-strand, corresponding in position to that occupied by the strand of the young tuber at a lower level, leaves the stele and takes a central course into a hump of tissue (*h*, in Figs. 3 and 6), which seems to represent what Bertrand calls the 'organ of Mettenius' (5); both hump and strand, however, soon disappear from the transverse sections as they are followed upwards.

The U-shaped stele breaks up above into the isolated peduncle-strands, each of which goes to supply a sporophyll. One or two, however, appear in transverse section above the level of the last sporophyll.

#### GENERAL DISCUSSION OF THE DETAILS OBSERVED.

1. The noticeable U-form of the upper part of the stem-stele is, perhaps, the most striking feature; it has no parallel in other Lycopodiaceae. It suggests, rather, an analogy to the upper part of the stem in *Tmesipteris*, in which the original protostele is disturbed by the outgoing leaf. As Bower points out, a similar appearance is seen in *Ophioglossum Bergianum*,

the U-shaped stele of which is remarkably like that now under discussion. This analogy to *Tmesipteris* is enhanced by the presence of what appear to be imperfectly developed tracheides bridging over the gap.

If there be any significance in this analogy, and the shape of the stele demands some such explanation, we must suppose that the gap indicates the presence, in an ancestral form, of a leaf, typically 'megaphyllous' in its behaviour to the stele. In this case we have a complete analogy to *Tmesipteris*, the lower leaves of which are clado-, and the upper phyllo-siphonic; and *Phylloglossum* presents yet another link between the Pteropsida and Lycopsida of Jeffrey.

The leaf-member which causes the gap must be regarded as entirely suppressed in the modern form, and there is no indication of the presence of a branch in its axil; the structure is, in fact, typically Filicinean. The gap never closes above; the U-shaped stele breaks up directly into the isolated strands of the strobilus pedicel.

It must be mentioned at this stage, however, that Jeffrey (8) denies that the gaps in the stele of *Tmesipteris* are foliar, and he points his denial by a strict definition of the term 'leaf-gap'; he emphasizes the closeness of the relation between the gap and the outgoing leaf. More than one botanist of distinction is less certain than Jeffrey upon this point; but, whatever may be the true case in *Tmesipteris*, the gap in the stele of *Phylloglossum* certainly complies with Jeffrey's criteria of a leaf-gap. In his paper (8) he evades this point by assuming, without offering any discussion upon the matter, that the opening in the stele of *Phylloglossum* is caused by the exit of the bundle which supplies the young tuber; the fact that this opening is found to face the site occupied by the latter at a lower level seems to lend some support to this contention. In this problem, however, we may derive considerable assistance from the stalked tuberous structures met with in certain Monocotyledonous plants, and described by Miss Robertson (10) in the case of the genera *Tulipa* and *Erythronium*. These 'stalked bulblets' or 'droppers' are the precise analogues of the *Phylloglossum* tuber, and serve the same fundamental purpose, namely, the exploration, so to speak, of the soil; the analogy is further enhanced by the fact that more than one tuber may be formed during a season<sup>1</sup>—so that the tuber of *Phylloglossum* is essentially a means of vegetative reproduction; this consideration seems to lessen very considerably the fundamental phylogenetic importance which has been assigned to it.

Like the tubers we have described above, the stalks of the droppers are tubular, and bear small bulblets at the tips. Miss Robertson points out that the dropper is actually a continuation of the base of the leaf; and we may therefore suppose that the *Phylloglossum* tuber is a continuation of the suppressed leaf which we have postulated above; this contention is sup-

<sup>1</sup> See *supra*, p. 338, and Thomas (13).



ported by the relative positions of the gap and of the young tuber. Further, the morphology of the dropper is described as being partly foliar and partly axial; there is a vascular ring derived from the leaf; and this is represented in *Phylloglossum* by the ring observed in the tuber of the second specimen examined.<sup>1</sup> The 'axial' portion of the anatomy of the latter is, of course, represented by the central vascular strand (*y.t.s.*, Fig. 3).

Reasoning from analogy is admittedly dangerous; but the remarkable similarity in the structure and function of the tubers under discussion and the 'stalked bulblets' described in Miss Robertson's paper seems to lend no little weight to the supposition that a megaphyllous leaf existed in the ancestors of *Phylloglossum*, while the position and rudimentary nature of the 'organ of Mettenius' is very suggestive; it might very well represent the vestige of a megaphyllous leaf.

The habit of the plant further favours this suggestion; like the Monocotyledons, it is typically geophilous; and, as Bower has pointed out,<sup>2</sup> this habit conduces to reduction in the number of leaves and enlargement of the individual leaf—in other words it conduces to megaphylly.

2. The geophilous habit of *Phylloglossum* recalls some interesting parallels in other Pteridophyta. The adoption of this habit results in general reduction, as witness, for example, the modern pigmy descendants of the giant Calamariæ; while there is no doubt whatever that *Phylloglossum* is extremely reduced, however primitive may have been the condition of its Lycopod ancestors; of this primitiveness we shall, however, have something to say further on. The latter may have been 'permanently embryonic Lycopods'—in which case it is not improbable that their remains would be as lost to us as are the embryonic stages of the ancient *Lepidodendra*; but their modern descendants are highly specialized in accordance with their special habitat. This is described in Cheeseman's Flora of New Zealand as 'barren clay hills' in the North island,<sup>3</sup> and Thomas states that the plant flourishes best on a hill-top.

The thickening of the epidermal cell-walls is doubtless a xerophytic adaptation, designed for the purpose of providing a tegument to serve as a protection against the environmental conditions. Sclerenchyma is, however, entirely absent—a feature not surprising in so small a plant.

Similar degradation of the protoxylem occurs in many other forms which occupy special habitats—notably the Equisetales, *Sphenophyllum insigne*, the leaf of *Isoetes*, and many aquatic flowering plants.

3. The indefiniteness of the vascular system is also in accord with the habitat of the plant; and there is a striking resemblance in this regard between *Phylloglossum* and the Isoetaceæ. In both cases we have a geophilous habit. The storage tuber of *Phylloglossum* is analogous to the thick tuberous stem of a form like *Isoetes Hystrix*, the storage parenchyma

<sup>1</sup> *Supra*, p. 338.

<sup>2</sup> Bower (4), pp. 231, 479.

<sup>3</sup> Cheeseman (6).

in the former being paralleled in the latter by the strong development of secondary cortex. We are reminded, in this connexion, of the notably strong development of secondary cortex in many fossil forms.

The leaves, again, of the two forms are noticeably alike in shape, at least in the upper and greater part of their length; while in both, the leaves are large relatively to the stem, in spite of their 'microphyllous' anatomy.

The presence of phloem in the stem is much more problematical in *Phylloglossum* than in *Isoetes*; indeed it may be said to be entirely absent. True phloem probably exists, as we have seen, in the leaves of the former, the single leaf-bundle being concentric.

The mesarchy seen in the lower part of the leaf of *Isoetes* is reflected in *Phylloglossum*, in which plant, however, this condition is much more general, as it occurs throughout the leaf, in the upper part of the stem, in the peduncle and axis of the strobilus, and even in the strand of the young tuber.

In both *Isoetes* and *Phylloglossum* the vascular system is cauline, and the leaf-trace bundles leave the stele—a protostele in both cases—undisturbed by their exit.

The protoxylem cavities, so prevalent in *Phylloglossum*, occur constantly in the leaf of *Isoetes*.

The roots in both are monarch in structure.

In fact the external and internal morphology of the vegetative organs in the two plants are remarkably alike in fundamental points, suggesting additional evidence of the Lycopodinean affinities of *Isoetes*.

There seem at first sight, on the other hand, to be important differences in the structure of the reproductive organs. This feature, however, is mainly due to the adoption of the heterosporous habit of *Isoetes*. With this are probably connected the ligule and the 'velum'—structures which have been paralleled with those found in the integumented sporangia of *Miadesmia*; many may consider, in fact, that *Isoetes* has advanced beyond mere heterospory; it has made the first step towards seed-production. We may suggest that the homosporous *Phylloglossum* could not proceed so far, at least in this direction; but in specialization for spore dispersal it is a decided advance upon *Isoetes*, as its sporangia are aggregated into a very definite and pedicellate cone. It shares, of course, with *Isoetes* the constant Lycopod character of one sporangium to each sporophyll.

The structure and development of the individual sporangia have been exhaustively described by previous authors; it will suffice to recall here the fact that the sporangium is typically Lycopodinean, and of the less specialized, *Urostachya*, type.<sup>1</sup>

4. Let us now proceed to consider the question of the primitiveness, or otherwise, of *Phylloglossum*.

<sup>1</sup> Sykes (11).



The striking similarity of the development of this plant to the embryonic stages of *Lycopodium* has led many to regard it as a 'permanently embryonic form of Lycopod'. This has led to the conclusion that *Phylloglossum* is a relatively primitive form, and this receives a certain amount of support from the gametophytic characters; but the fact that more than one tuber may be formed in a season, with the consequence that the tuber is probably a merely adventitious organ of vegetative reproduction, seems to militate very forcibly against such a conclusion.

The anatomy, moreover, seems to point to extreme specialization. The latter is, doubtless, largely biological in significance, and comparatively recent in descent; but the gap in the upper part of the stem-stele can scarcely be regarded as other than an ancestral character, and one of considerable importance.

If the gap is a leaf-gap, as seems by no means unlikely, then *Phylloglossum* is no more primitive than *Tmesipteris* or *Ophioglossum Bergianum*; in fact, it is less primitive, for reduction has led to the complete suppression of the megaphyllous leaf in *Phylloglossum*.

This suppression is not without parallel in the Pteridophyta. In recent *Equiseta* the leaves are extremely reduced, but the gaps in the stele are now generally admitted to be leaf-gaps.<sup>1</sup> In *Ophioglossum simplex* we have an exact parallel with the suggested condition in *Phylloglossum*, for, although leaf-gaps are present in the stele of this species, the megaphyllous sterile laminae which cause them, so to speak, are altogether absent; and as Bower points out, 'it is thought that *O. simplex* forms the end of a series of reduction of the vegetative system, consequent on . . . habitat: that as *O. intermedium* . . . shows . . . only a reduced lamina, so in *O. simplex* the reduction having proceeded further has resulted in the complete elimination of the sterile blade.'

It is suggested that *Phylloglossum* stands at the end of a similar reduction series, of which the transitional forms have been lost. If this be so, we can no longer regard it as a primitive form, at least, so far as the vegetative organs are concerned; while in specialization for spore-dispersal it stands as high as any of its Lycopodinean allies.

We may summarize our conclusions briefly thus:—

1. In view of its anatomical structure, *Phylloglossum*, like *Tmesipteris*, is 'microphyllous' in its lower portion, and 'megaphyllous' in the upper—thus occupying a position intermediate between Pteropsida and Lycopsida.
2. The general degradation of the vascular system, coupled with the geophilous habit, suggests that *Phylloglossum* has undergone considerable reduction recently in descent.

<sup>1</sup> Gwynne-Vaughan (7); but Jeffrey (8) is a notable exception to this statement.

3. This reduction has resulted in the complete suppression of the megaphyllous leaves, a condition comparable with that presented by *Ophioglossum simplex*.

4. The similarity in respective habits and structure of *Phylloglossum* and *Isoetes* go to support the Lycopodinean affinities of the latter.

5. *Phylloglossum*, far from being a primitive form, is highly specialized.

I wish to express my sincere thanks to Mr. T. G. Hill, not only for placing at my disposal his preparations, which were made from plants brought from Australia by Professor J. P. Hill, of University College, but also for his very valuable advice and criticism throughout the investigation.

It should be stated that the present investigation was carried out in the Botanical Department of Goldsmiths' College, University of London.

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# On Androgynous Receptacles in *Marchantia*.

BY

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With five Figures in the Text.

IN some material of *Marchantia* which had been collected for class-work, a number of archegoniophores were found which differed considerably from those of *Marchantia polymorpha*, the stalk ending in a well-marked disc bearing a variable number (6-12) of short lobes, and it was observed that some of these archegoniophores bore on the lower surface a prominent outgrowth (Figs. 1, 2, and 3) rather suggestive of an antheridial lobe.

A few hand sections were cut of one of these abnormal receptacles, and it was at once noticed that antheridia were borne in great abundance on the apparently under side of the outgrowth. There were also indications of the presence of archegonia in other parts of the sections. The material was not examined any further by means of hand sections, but the microtome sections which were afterwards cut from this and other of these abnormal receptacles have shown that archegonia are also present on them.

The material had been originally obtained from Mr. Williams, of Avery Hill, Eltham, from whom I learnt that the species was *Marchantia palmata*; that he had obtained the original specimens from the Chelsea Physic Gardens, and that the receptacles with the disc shape, already shortly described, were commonly formed on some of the plants, and on others archegoniophores similar to those of *Marchantia polymorpha*, but more robust and with longer stalks. He also said that the plants had, unfortunately, all died, but he kindly placed at my disposal all that remained of the material, which had been preserved in alcohol. There was, unfortunately, very little of this and it was in a fragmentary condition; so that it was seldom possible to trace more than one inflorescence to any individual thallus: indeed I have only been able to do this in the case of the antheridiophores and of the ordinary 'polymorpha' type of archegoniophores. All of the 'disc'-type of receptacles were unattached.

There were present two kinds of archegoniophore, the disc-type with short processes, and the 'polymorpha' type with long processes; and two kinds of antheridiophore—one with only a slightly crenate edge and equal lobes, and one that was asymmetric and in which the lobes were free for the greater part of their length. In the last mentioned the number of lobes varied greatly.

A microscopic examination of the thallus and gametophores revealed the fact that at least three different types of pore were present in the material, and this fact, as well as the presence of different types of gametophores, makes it more than probable that two or more species had somehow got mixed.

I intended to try to get a further supply from Chelsea in the hope that more androgynous receptacles might be found, and that experiments might be tried in order to find the factors that regulate the appearance of this condition in this species, and to find out whether it can be inherited. As both Schiffner (17) and Stephani (18) regard *Marchantia palmata* and *Marchantia emarginata* as synonyms, and as two very characteristic species under these names are grown at Chelsea, both of these were collected during this year: neither of them, however, showed the androgynous condition. The *Marchantia palmata* formed archegoniophores like those with the long processes found in the Avery Hill material, but no disc-shaped ones. The *M. emarginata* formed very few gametophores during the year and none that I had were adult, but Mr. Hales, the Curator of the Gardens, informs me that the adult shape is in the form of a disc similar to that of the androgynous receptacles described above.

Mr. Gepp, of the Natural History Museum, has very kindly shown me the Herbarium specimens of *M. palmata* and *M. emarginata*. They seem very similar and both are evidently very variable, but neither of them resembles either the Chelsea specimens or my own: nor does Stephani's description of *M. palmata* (syn. *M. emarginata*) agree with either the Chelsea specimens or my material. The latter, unfortunately, is in too fragmentary a condition for the separation of the constituent species and the determination of the specific name of the androgynous specimens.

As I have failed to find any androgynous gametophores on the Chelsea specimens, and as the androgynous material at my disposal was insufficient for the determination of the species, it was determined to publish the results that have so far been obtained.

The disciform receptacle has a wide, slightly concave upper surface, and its margin is produced into 6–12 short, blunt protuberances which at times show indications of a slight apical depression (Figs. 1, 2, and 3). Between two of the protuberances on each receptacle is a very deep cut reaching in some cases almost to the centre of the disc-like upper surface. This cut indicates the first dichotomy of the shoot which gave rise to the

inflorescence, and the organ is rendered bilaterally symmetrical on account of it. On the under surface is to be found a dense mass of rhizoids, &c., and amongst these are often to be seen a number of sporogonia, thus showing that the archegonia are fertile. On some of these gametophores there are one or more irregular masses of tissue attached by a short stalk to the under surface (Fig. 5). The stalk quickly widens out from above downwards, and ends in an almost flat downwardly-directed disc of irregular shape and slightly crenate outline (Figs. 1 and 2). The disc, when looked at from below, is seen to resemble a lobe or a few lobes of the antheridiophore, and the surface, which is directed downwards,



FIG. 1. Side view of androgynous receptacle of *Marchantia* sp., showing a male outgrowth.  $\times$  circa 2.

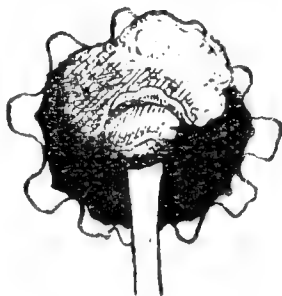


FIG. 2. Under surface of an androgynous receptacle, showing two male outgrowths one of which has branched.  $\times$  circa 2.

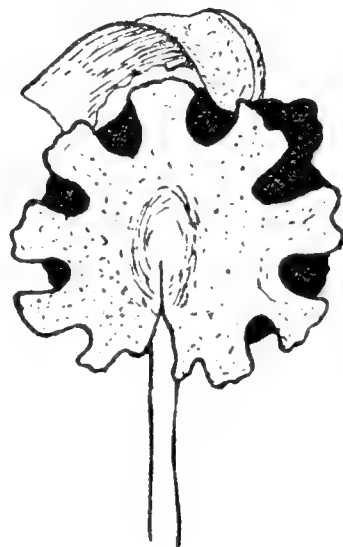


FIG. 3. An androgynous receptacle, view from above.  $\times$  circa 2.5.

is covered with minute punctations which, from a comparison with the sections, are evidently caused by the presence of antheridia. As many as three such masses have been noticed on some of these abnormal gametophores.

A macroscopic examination does not show whether these antheridia-bearing lobes correspond to any definite lobe of the hermaphrodite receptacle or not. Sections, however, show very clearly that they are formed as outgrowths from a portion of the under surface of a female branch. In cases so examined it was found that archegonia were present at the base of the stalk of the outgrowth. This is clearly shown in Fig. 4. Here on the right hand we have a male outgrowth, and at the base an archegonium is shown, and a little to the right of this the involucre of the branch. The other sections in the series had other archegonia in a similar position; one only is put in the drawing for the sake of clearness. It would not be unlikely for the entire archegonia-bearing portion of a branch to grow out into a protuberance, but no such case



has been seen. In the *Marchantia* here described we have, therefore, a male outgrowth from one or more branches that have not only been formed in the manner characteristic of female branches, but which also bear archegonia. The outgrowth, as has been mentioned above, may form only one branch (Fig. 1), or it may divide again to form an asymmetric branch-system; as many as three branches have been seen in one such outgrowth.

The structure of the antheridia and of the branch which bears them is perfectly normal, with the usual amphigastria and rhizoids. One very remarkable feature, however, was noticed. Although the gametophores



FIG. 4. Longitudinal section through an androgynous receptacle, showing hermaphrodite branch on right side. ♀ = archegonia, ♂ = antheridia, s = stalk, and i = involucre.  $\times 15$ . Semidiagrammatic.

were provided with a long stalk and gave other evidence of being fairly adult, the majority of the antheridia had not yet discharged their contents. In the older parts of the male outgrowths empty antheridia were found, so that the fact that the majority were full evidently was not caused by their not having the power of opening. It seems, on the other hand, to suggest that the male outgrowth was formed secondarily as a kind of proliferation, and is not a mere replacement of the normally female branch. This point will be discussed later.

Only about half of the disc-shaped gametophores bore male outgrowths, but the latter easily fall off, and it is certain that many have lost them. It is probable, however, that some few of them were purely female, as no sign of a broken surface was seen on them.

The other type of female receptacle and the two types of antheridiphore have already been shortly described. They are perfectly normal in structure, and a detailed account of them is not given because it is certain that the material contained at least two species and it is impossible to separate them. It is probable, however, that the asymmetric type of antheridiphore belongs to the same species as does the disc-shaped

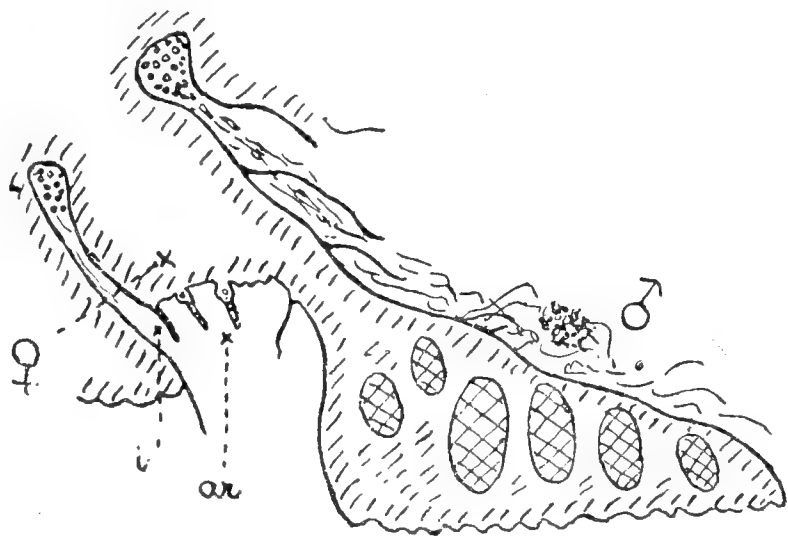


FIG. 5. Hermaphrodite branch from a gametophore. *i* = involucre, *ar* = archegonia, part, and ♂ = male part. × 15. Diagrammatic.

gametophore; the male outgrowth of the latter much resembling the lobes of the former.

#### HISTORICAL.

The first record of androgynous gametophores in the Marchantiaceae seems to have been made by Taylor (20), who in 1834 writes of them as occurring in *Dumortiera irrigua* (*Hygropyla irrigua*, Tayl.). In 1836 he writes of *D. irrigua* (*Hygropila*, Tayl.) in Mackay's *Flora Hiberniae* (21), 'The fructification is commonly dioecious, sometimes monoecious, and not rarely androgynous as observed in *Marchantia androgyna*.' This last-mentioned plant is now known as *Preissia commutata*. In his paper 'De Marchantieis' (20), published two years previously, an account is given of *Marchantia androgyna* (*Preissia commutata*), but this peculiarity is not mentioned. Since then androgynous receptacles of *Preissia commutata* have been found and described by Goebel (9), Leitgeb (12), and Miss Townsend (22).

Goebel found that the front portion of the fructification bore antheridia on the upper surface and the back portion archegonia on the lower surface. He compared the androgynous condition to the state of affairs noted by him (8) in *Isoetes lacustris*, where a vegetative bud was found in the position in which a sporangium usually occurs. He does not think that the androgynous receptacle need necessarily be explained as a reversion to a primitive, monoecious arrangement of the sexual organs.

Leitgeb (12) confirms Goebel's results, and also points out that the organ has four rays as usual, and that two of these are male, two female. He also grew plants which were producing androgynous fructifications, and in the following year another crop of androgynous receptacles was formed—an extremely interesting observation, the significance of which will be discussed later. Leitgeb also mentions that Schmidel and Bischoff have observed androgynous *Preissias*. He holds the opinion that the androgynous condition is caused by the sexual differentiation being delayed until the formation of the branches which bear the sexual organs instead of taking place in the vegetative portion of the thallus. He gives some interesting facts concerning the distribution of male and female gametophores in *Reboulia* in support of this view. In this genus the male and female receptacles bear a relationship to each other similar to that of the male and female branches on the *Preissia* androgynous gametophores. Mention is also made of the relationship of the gametophores to the ordinary vegetative branches in *Marchantia*.

Miss Townsend was not aware of the work already done on *Preissia commutata*. She expresses the opinion that the gametophore of the androgynous *Preissia* was primarily an archegoniophore, and it is to be presumed that she therefore thinks the development of the antheridia on the structure to be secondary. She does not record any correlation between the lobes and their sex. The gradual development of complexity in the arrangement of the sexual organs in the Marchantiales is described, and the question whether the hermaphrodite condition recorded in *Preissia* is to be regarded merely as abnormal or as a reversion to an earlier type is discussed. It is suggested that the latter is the more likely explanation, and the different arrangements of the gametophores in the *Vaucherias* are brought forward in support of this theory.

Ernst in 1907 published a preliminary note (6) on androgynous receptacles in *Dumortiera velutina*, Schiffn., and *D. trichocephala*, (Hook.) N. ab E., and in 1908 a very full and interesting paper (7), entitled 'Untersuchungen über Entwicklung, Bau und Verteilung der Infloreszenzen von *Dumortiera*'. Androgynous receptacles would seem to be very common in these species, and were found abundantly in specimens gathered from many different localities. The nature of the disturbance of the usual arrangement in these cases is very much more complicated than in the case of *Preissia*, the proportion of the female to the male portion varying within wide limits. As in *Preissia*, the antheridia are borne on the upper surface, and the whole of the branch is male. Pure male and female gametophores are very commonly found on thalli which bear androgynous receptacles.

## DISCUSSION.

The androgynous specimens of *Marchantia* described above differ from any androgynous fructifications yet described, in that the male portion not only arises from a branch after this branch has been definitely differentiated as female and has grown with the configuration of such and with a portion of its morphologically upper surface turned downwards, so that the morphologically upper surface of the male outgrowth is likewise turned downwards, but also is capable of continuing its growth and giving rise to a series of branches, resembling, in general outline, the arrangement seen in the asymmetric antheridiophores of some of the *Marchantias*, e. g. in *M. chenopoda*. In this respect it resembles a proliferation<sup>1</sup> of the tissues of the female branch rather than a replacement of it, and this suggestion is made even more likely when we remember that archegonia are usually, if not always, formed before the female branch gives rise to the male outgrowth. The distribution and number of the male outgrowths is irregular, agreeing in this respect with *Dumortiera* rather than with *Preissia*.

Leitgeb was of opinion that the androgynous nature of his *Preissia* receptacles was due to a delaying of the sexual differentiation; granting this, the reason for the delay still remains to be discovered. The fact that a clump of thalli, probably derived from one or a few thalli by vegetative reproduction, was found by Leitgeb bearing androgynous receptacles, and that individual thalli selected from these continued to bear such in abundance the following year, strongly suggests that their formation does not depend on external conditions, but on the inherent nature of the thallus. Ernst reports that *Dumortiera* may bear male, female, and androgynous receptacles in any combinations,<sup>2</sup> but this does not negative the view given above, more especially as *Preissia* itself is often monoecious. It must also be remembered that in some of the monoecious species amongst the *Marchantiales* the male and female gametophores are borne quite close to each other, and yet androgynous receptacles have not been recorded in them.

In the absence of living plants of the androgynous *Marchantia* it is not possible to decide whether the condition in this species is governed by external or internal factors. It is very interesting, however, to find a bisexual species in a genus which has always been regarded as strictly dioecious.

The experiments of Kny, in which the gemmae of *Marchantia*

<sup>1</sup> Similar vegetative proliferations have been noticed by Lindberg (13) in archegoniophores of *Dumortiera*, by Leitgeb (12) in those of *Dumortiera* and *Marchantia*, and by Okamura (16) in the antheridiophores of *M. cuneiloba* and *M. geminata*.

<sup>2</sup> I cannot find whether this is so in *Preissia* or not, but as it is often monoecious it would seem that this is very likely to be so.



*polymorpha* on germination always gave rise to thalli of the same sex as the parent plant, are quoted by Blakeslee (3) in support of his view that the sexual tendencies are separated at spore-formation in this species, and the experiments of Strasburger (19) on *Sphaerocarpus*, in which two female plants and two male plants came from each tetrad, point more strongly to a similar conclusion in this case. Also the experiments of E. and E. Marchal (14 and 15), in which regenerated portions of the gametophytes of certain dioecious mosses always gave rise to plants of the same sex as the experimental plants and a regenerated stalk of a sporogonium to monoecious plants, seem to prove this for certain of the mosses also. Both Harper (11) and Strasburger (19) have pointed out that this coincidence between sexual differentiation and the reduction-division is by no means a general one, so that even in the same genus the sexual differentiation may take place at different points in the life-history.

In the higher plants the gametophyte is always unisexual, but the sporophyte often bears both mega- and microspores, and the sex of the plants arising from these is determined even before spore-formation. The interesting case of *Salix*, which is usually dioecious, but occasionally monoecious, shows that plants usually forming only one kind of spore—and this giving rise only to one kind of gametophyte—are capable, under certain unknown conditions, of forming both, and thus giving rise to both gametophytes. The power of forming both of these kinds of spore—and through them both kinds of gametophyte—was present in the plant, but the formation of one kind of spore was inhibited by some factor or factors.

A similar case is to be seen in *Lychnis dioica*, in which the ordinary plants are strictly 'dioecious'. The macrosporangiate form, when attacked by a smut fungus, forms microsporangia as a result of a stimulus caused by the fungus. A similar result has never been obtained by artificial stimulation, so that without the fungus we should not have known that the 'female' plants possessed the power of forming anthers. On several ordinary bisexual fern-prothallia we can inhibit the formation of either or both of the sexual organs, and the gametophyte of *Equisetum*, usually described as unisexual, can be made to bear either antheridia or archegonia or both by altering the external conditions (10). In many other cases it is possible that the plant contains the factors necessary for the formation of both sexual organs, but that one of these factors is obscured by some other internal or external condition. The case of *Lychnis* suggests that this factor, even if an external one, may be difficult to find or even to imitate when found. From this point of view it may be seen that many plants regarded as strictly unisexual may yet contain the factor necessary for the formation of the other sex.

Any underlying phenomena that may exist in the inheritance of sex can only be arrived at by thoroughly investigating each separate case of sex-inheritance. The efforts to get at a general theory of the subject

have often been premature, and it is certain, as has been pointed out by Bateson (1 and 2), that the results obtained in one field of observation often differ from those in others. It may also be, as he has suggested, that the inheritance of sex is differently arranged in different species.

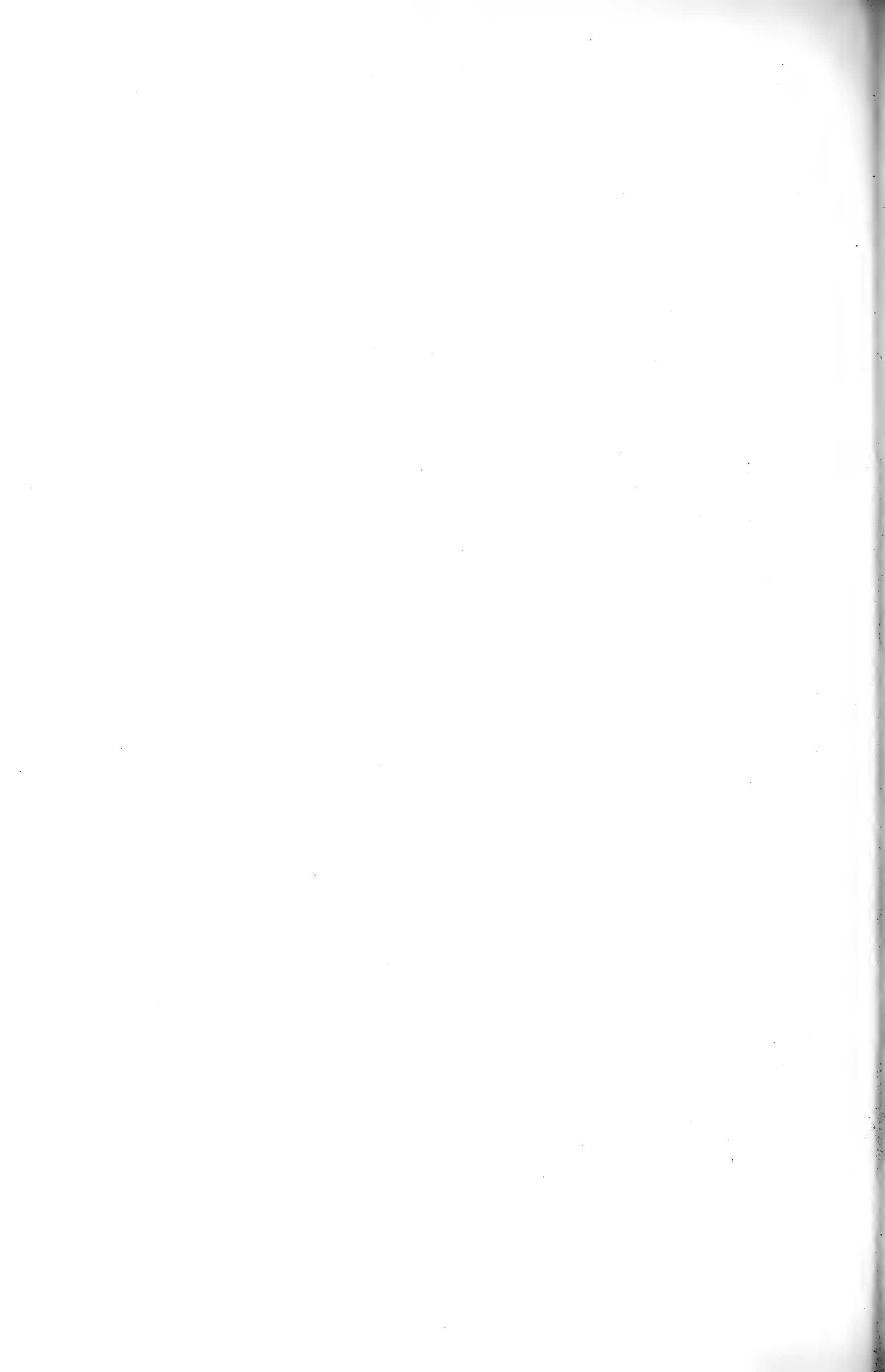
UNIVERSITY COLLEGE, LONDON,  
December, 1909.

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# Researches on the Life-history of Parasitic Fungi.

BY

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With Plate XXIV.

## I. CLADOSPORIUM HERBARUM, Link.

THE genus *Cladosporium* is a comparatively old one, having been founded by Link in the year 1791. The species *Cladosporium herbarum* was described by the same observer in the following terms:—‘atrovirens, densum, effusum, floccis septatis.’

The species is of somewhat wide occurrence; almost everywhere the dead remains of plants can be seen to be covered with dark, sometimes almost black patches formed of its conidiophores. The conidiophores are olive and are arranged in tufts which are frequently large and dense. The conidia are olive, continuous at first and becoming later typically once septate; they measure on the average  $18-20 \times 8-10 \mu$ , and are produced at the tip of the conidiophore in short chains which sometimes remain attached so that they may become larger and twice or even thrice septate. *Cladosporium herbarum* in its life-history will be shown to be connected with another conidial form belonging to the form-genus *Hormodendron*; this form *Hormodendron* will be shown to be parasitic on several plants, causing disease, and it is proposed first to treat with this disease and after to deal with the relations of the conidial form to *Cladosporium herbarum*.

### The disease caused by the form *Hormodendron* and the evidence of its parasitic nature.

For some time past the green foliage of many different species of plants has been seen to be suffering with a disease which takes the form of large, irregular holes in the lamina of the leaf. The damp summer of last year seems to have been particularly favourable to the spread of the disease. Of the species which have frequently been found to be affected in this way the following are given:—

*Brassica oleracea* (Cabbage), *Cucumis sativa* (Cucumber), *Cucumis Melo* (Melon), *Arctium Lappa* (Burdock), *Catalpa bignonioides*, *Althaea rosea*,

*Malva sylvestris*, *Circaea lutetiana* (Enchanter's Nightshade), *Funkia* spp., *Digitalis purpurea* (Foxglove), and *Phlox* spp.

Since it attacks such plants as Cucumber, Melon, and Cabbage, the fungus is one of economic importance; the assimilating surface of plants attacked may be considerably reduced, and further, in the case of Cabbage, the disease may render the 'heads' quite unsaleable. At times the disease has taken the form of an epidemic in Cucumber houses in this country. When a leaf is attacked small perforations make their appearance; these increase in size and frequently run together so that they eventually become large, irregular holes. The largest holes which have been recorded measured about three-fourths of an inch. Surrounding each hole there is usually a small, brownish area formed of the dead tissues of the leaf; the remaining parts appear to be green and healthy. Along with the rest of the tissues the smaller veins perish; the larger veins, however, as a rule remain. It is interesting to note that in its progress this disease differs markedly from the so-called 'leaf-spot' diseases; in the latter the tissues of isolated spots are killed and fall away, leaving holes in the leaf, whereas in the former the tissues appear to be eaten away and the holes continue to increase in size. An examination of the tissues of the leaf bordering on the hole shows that the cells are apparently exhausted of their contents and dried; it might be thought that the formation of the holes and their increase in size were due to the falling away of these dead cells, but this is apparently not so in this case. From the very commencement of the disease a perforation appears, not a dried spot from which the dead tissues fall away leaving a hole; in fact, it seems as if the tissues of the leaf are not merely killed but devoured. This mode of action is most unusual for a fungus and resembles that of an animal; it would point to the occurrence of ferments of the cytase, protease, and diastase classes, and it is hoped that further work on the ferments present in this parasite will throw some light on its action on the tissues of the leaf.

The mycelium of the fungus is most abundant in that region which is in close proximity to the holes; further away from the holes the tissues contain little or no mycelium. The hyphae run through the cells; they are colourless when young, become darker in colour as they grow older, are variously branched, measure about  $4\mu$  in width, and are without haustoria. The conidiophores arise from the hyphae in the tissues of the leaf bordering on the holes; they are short, lateral branches of the hyphae, are simple, almost hyaline, continuous or sparingly septate, and bear at their tips branched chains of conidia. The branched chain arises thus:—from the tip of the conidiophore three conidia are usually formed; each of these bears two conidia of a second order, and each conidium of the second order can bear one or two of a third order of branching, and so on. The conidia are hyaline or almost hyaline, continuous, elliptical, and measure  $8-9 \times 4\mu$ .

From the structure and arrangement of the conidia the fungus was found to correspond with the form-genus *Hormodendron*.

An account will now be given of the infection experiments which show that the form *Hormodendron* is the cause of the disease described above. Before actually carrying out the infections a pure culture of the fungus was made in order to ensure the exclusion of any spores other than those of the form *Hormodendron*. First, some of the conidia were taken from an infected leaf of *Catalpa bignonioides* and were transferred to pure water in a 'hanging-drop'; they were thus kept in water in a chamber which was constantly moist. In from one to two days they were found to have germinated. Each emitted one hypha, as a rule, which became septate and branched; the absence of food-material prevented any further growth of the hyphae. A cultural medium composed of 4 per cent. of the expressed juice of fresh grapes and 6 per cent. of gelatine was found to be favourable to the growth of the fungus. This nutrient gelatine was sterilized by heating in a steam-oven for four hours each day on three successive days. Five Petri dishes and a boiling-tube, into which a plug of cotton-wool had been inserted so as to close its mouth, were sterilized by heating in an air-oven at 100° C. for five hours each day on two successive days. Some of the nutrient gelatine was transferred to the boiling-tube, and to this were added some conidia taken from a previously made, impure culture; the needle used for transferring the conidia had been previously heated to a red heat and allowed to cool. The liquid in the boiling-tube was shaken, and some of it was poured into a Petri dish. The remainder of the liquid in the boiling-tube was then diluted by one-half, it was shaken and some was transferred to another Petri dish; this process of diluting, shaking, and transferring some of the fluid to a Petri dish was repeated three times. Care was taken to keep the boiling-tube closed as much as possible, and before removing the plug of cotton-wool the part of the plug which was exposed to the air was slightly burnt on each occasion so as to prevent any spores which had fallen on it from the air from dropping into the liquid in the tube. The last Petri dish was found to contain a pure culture derived from the growth of a single conidium. The growth and development of the mycelium in the pure culture was carefully followed. The hyphae when young were colourless, but as they grew older they assumed an olive colour; they were septate at fairly short intervals and were variously branched. From them there arose abundant lateral branches which grew above the surface of the nutrient gelatine and bore branched chains of conidia. The conidia in their structure, mode of formation, and arrangement corresponded with those observed on the infected leaves and described above.

Conidia taken from the pure culture were used for infecting living plants. Two methods were made use of for transferring them to the leaves;

in the one a sterilized needle was used, in the other they were mixed with sterilized water in a spraying-apparatus which had been previously washed out with absolute alcohol and allowed to dry. Six young Cabbage plants were selected as being healthy and intact and were arranged in two lots of three each. In one experiment the leaves of three plants were sprayed with the conidia; in the other experiment the conidia were transferred to definite spots on the leaves of the other three plants and the spots were moistened with distilled water. Each lot of three plants was covered with a bell-jar of which the inner surface had been cleaned with absolute alcohol. In from four to five days the leaves thus treated were found to have perforations in them; the leaves of those plants which had been sprayed showed numerous perforations, while in the others the perforations were limited to the spots on which the conidia had been placed. The perforations increased in size and soon became large holes. A microscopic examination showed that the conidiophores of the fungus were arising from the tissues of the leaf surrounding the holes and were producing conidia abundantly. The symptoms developed by these plants which had been artificially infected were similar to those observed naturally on the infected plants. All of the six plants yielded the same results, while a control in the form of a young Cabbage plant kept under a sterilized bell-jar and to which no conidia had been transferred showed no signs of infection.

Another series of experiments was made for the purpose of determining with what degree of facility the fungus could be transferred from one host to another. For this purpose excised leaves were used; the leaves were placed on filter papers in Petri dishes and the filter papers were moistened with distilled water, the Petri dishes and filter papers having been previously sterilized by heating in an air-oven at 100° C. for five hours each day on two successive days. Conidia were taken from an infected leaf of one species and were placed on definite spots of a leaf of another species in the Petri dish; the spots were moistened with distilled water. The experiments showed that conidia from one species would grow on another with apparent facility. The fungus on Cabbage could be transferred to *Catalpa*, that on Melon to Cabbage and vice versa, that on *Catalpa* to Mallow and vice versa, and that on *Catalpa* to Melon. It is concluded that the form *Hormodendron* has not adapted itself closely to any one host, at least in the case of the species experimented with. Further, it may be mentioned that the disease caused by this form would be particularly difficult to exterminate, since the fungus attacks many species of plants and can apparently pass from one species to another, and this apart from its connexion with *Cladosporium herbarum*, which is so common everywhere on the dead leaves of plants.



### The origin of *Cladosporium* from *Hormodendron*.

An examination of the old cultures of *Hormodendron* showed that the production of conidia of *Hormodendron* had ceased. The mycelium remained the same, but from some of the hyphae lateral branches had been produced which bore pale olive conidia at their tips. These fertile hyphae were thicker than those which had borne conidia of *Hormodendron* and were three or four times septate. The conidia were borne in short chains and were typically once septate; sometimes they were twice or thrice septate. They were similar in their structure and mode of origin to those of *Cladosporium herbarum*. Some of them were seen to have germinated in the medium of the culture; each emitted two hyphae, as a rule, which were septate at somewhat short intervals and were usually unbranched. At the tips of the hyphae were borne short chains of conidia of *Cladosporium*. The examination of the cultures, therefore, showed that the mycelium of *Hormodendron* after having produced conidia of *Hormodendron* gave rise to conidia of *Cladosporium*, which after germination in the medium of the culture reproduced themselves.

### The production of *Hormodendron* from *Cladosporium*.

The origin of *Cladosporium* from *Hormodendron* shown above led to a careful examination of the leaves of plants which were attacked by *Hormodendron*, for the purpose of finding out whether *Cladosporium* occurred on them. On the living leaves no *Cladosporium* was observed, but the waning or dead leaves showed *Cladosporium* to be present in abundance. Whereas from these leaves *Hormodendron* had evidently disappeared, tufts of the conidiophores of *Cladosporium* could be seen growing around the holes previously made by *Hormodendron* as well as on other parts of the leaves which showed no signs of having been affected with *Hormodendron*. The dead or waning leaves of all the species which had been attacked by *Hormodendron* showed *Cladosporium* on them.

Conidia of *Cladosporium* were taken from the leaves and were mounted in 'hanging-drops' in a weak solution of cane sugar. Whether they were continuous or once, twice, or thrice septate the conidia were found to be capable of germinating; it is, therefore, interesting to note that the mature conidium is not necessarily septate, that is if the power of germinating is to be taken as the significance of maturity. Each conidium emitted usually two hyphae; the hyphae grew, branched, and on the lateral branches were borne branched chains of conidia of *Hormodendron*. Sometimes a hypha was observed to bear conidia of *Hormodendron* when quite short and unbranched. The results of the germination of conidia of *Cladosporium* taken from different species of plants were the same; in the experiments conidia of *Cladosporium* were taken from the leaves of Cabbage, Oak, *Catalpa*, *Funkia*, Melon, and other plants.



The action of different media was tried for the purpose of ascertaining whether the composition of the medium in which germination took place would have any effect on the production of conidia of *Hormodendron*. The following media were employed:—

1. Distilled water.
2. A solution of mineral salts in water consisting of equal weights of potassium phosphate, calcium chloride, and magnesium sulphate, and amounting to 2 per cent. total salts.
3. A 2 per cent. solution of pure cane sugar.
4. A decoction of dried leaves of *Catalpa* and Cabbage made by grinding the leaves in a mortar with the addition of a little water, filtering through muslin and adding more water to make up to a 4 per cent. solution of the expressed juice of the leaves. In these media the germination of conidia of *Cladosporium* taken from leaves of Cabbage, *Catalpa*, Oak, and *Funkia* was carried out in 'hanging-drops'. It was found that whatever medium was employed the same result attended the germination of the conidia, viz. the formation of conidia of *Hormodendron*.

The conidiophores and the small masses of cells produced by the mycelium of *Cladosporium* were also found to be capable of giving rise to conidia of *Hormodendron*. When some of the conidiophores of *Cladosporium* were mounted in a 'hanging-drop' in a 2 per cent. solution of cane sugar it was found that occasionally from the broken end of a conidiophore there arose a hypha which branched, and at the tips of the branches were borne conidia of *Hormodendron*. The small masses of cells produced by the mycelium of *Cladosporium* were also found to be capable of giving rise to hyphae which bore conidia of *Hormodendron*.

These experiments showed that not only can conidia of *Hormodendron* be produced by *Cladosporium*, but also that there is a great tendency on the part of *Cladosporium* to give rise to conidia of *Hormodendron*, and that this tendency has made itself manifest, in the case of the species of plants used as the source of *Cladosporium*, independently of the nutrient medium employed for germination.

By making use of the method described above for the preparation of a pure culture, it was found possible to obtain a pure culture of *Cladosporium*. The medium used consisted of 4 per cent. of the extracted juice of fresh grapes and 6 per cent. of gelatine. In the pure culture the growth and development of the mycelium were followed. The hyphae were at first colourless and became later olive in colour. The general nature and appearance of the mycelium and of its component hyphae were similar to those of the mycelium and hyphae in the cultures of *Hormodendron* described above. From the hyphae there arose lateral branches which grew above the surface of the nutrient gelatine and produced conidia of *Hormodendron*. The production of conidia of *Hormodendron* was not

abundant, and was much less than in the cultures of *Hormodendron*. Soon conidia of *Cladosporium* began to be formed; they were found to arise in a similar way to those described above in the cultures of *Hormodendron*. On germination these conidia gave rise to conidia of *Cladosporium* again.

The following conclusions are based on the comparison of the cultures of *Cladosporium* and *Hormodendron*:—the characters of the mycelium and of the component hyphae are similar in both cases; there is a production of conidia of *Hormodendron* which is more abundant in the latter case than in the former; this is followed in both cases by the formation of conidia of *Cladosporium*, and these conidia of *Cladosporium* on germination reproduce themselves; of the two conidial forms, *Cladosporium* and *Hormodendron*, each can give rise to the other and each can also reproduce itself; the tendency on the part of *Hormodendron* in an artificial matrix to pass to *Cladosporium* is clear.

#### The effect of a lower temperature on the germination of conidia of *Cladosporium*.

In the experiments described above, in which 'hanging-drops' were employed, the conidia of *Cladosporium* had been germinated at the temperature of the laboratory, i. e. at 60° F. and above, and it has been shown that they always gave rise to conidia of *Hormodendron*. Further experiments were conducted for the purpose of ascertaining whether a lower temperature would in any way influence the formation of conidia of *Hormodendron*. The hosts used were Cabbage, *Catalpa*, and *Funkia*; one or two conidia from each were mounted in a 'hanging-drop' in a 2 per cent. solution of cane sugar. The 'hanging-drop' cultures were placed on a box outside of the building, and near to them were placed a maximum and a minimum thermometer. During the space of five days the thermometer recorded the following temperatures:—

<i>Date.</i>	<i>Maximum Temperature.</i>	<i>Minimum Temperature.</i>
November 10	49° F.	35° F.
„ 11	50° F.	32° F.
„ 12	55° F.	33° F.
„ 13	54° F.	30° F.
„ 14	56° F.	31° F.

The highest temperature attained was 56° F. on the last day, and the lowest temperature was 30° F. on the fourth day; the average temperature for the five days was 42° F. All of the conidia germinated, and each gave rise to hyphae which were septate at somewhat short intervals, were sparingly branched, and bore conidia of *Cladosporium*. In some cases not only were the conidia borne at the tip of the hypha, but the hypha became converted

into a chain of conidia. Other 'hanging-drop' cultures were prepared as controls, and were kept at the temperature of the laboratory; in these the production of conidia of *Hormodendron* was found to take place in the ordinary way. At the lower temperature, then, the formation of conidia of *Hormodendron* was completely suppressed.

#### The microsclerotia of *Cladosporium*; their growth and development.

Each tuft of conidiophores of *Cladosporium* springs from a cellular mass produced by the approximation and repeated division of the hyphae. When the conidiophores fall away this mass of cells remains and forms what has been called a microsclerotium. The microsclerotium is somewhat rounded and measures, when fully formed, about 160  $\mu$  across. Its surface is corrugated, and there can be seen projecting from the main mass a few outlying cells which are more or less transparent. The main mass of the microsclerotium is black or of a very dark brown colour, and is composed of cells which are more or less rounded, dark brown in colour, and have rather thicker walls than the hyphae from which they were formed. It is conceivable that the microsclerotia do not necessarily always arise at the base of a tuft of conidiophores; the hyphae in the tissues, in their later stages at any rate, show a tendency to form masses of cells, and these probably also develop into microsclerotia. The microsclerotium is regarded as a resting-stage of *Cladosporium*, or rather as a stage which is capable of enduring a period of inactivity.

The growth of microsclerotia taken from leaves of Oak, *Catalpa*, Cabbage, *Funkia*, Plantain, and other plants, was observed; they were allowed to germinate in 'hanging-drops' in water. Each superficial cell by the rupture of its thick wall was found to be capable of emitting a hypha; the hypha grew, became septate, and seldom branched. From its tip there arose a short chain of conidia of *Cladosporium*. The microsclerotium was thus seen to be capable of producing conidiophores which bore conidia of *Cladosporium*, and this was found to occur in the case of all of the species of plants used. Some of the smaller masses of cells had been seen, as was stated above under the section dealing with the origin of *Hormodendron* from *Cladosporium*, to give rise to conidia of *Hormodendron*, but these are not regarded as fully formed, true microsclerotia.

Some of the conidia of *Cladosporium* derived from microsclerotia were germinated in 'hanging-drops' in a 2 per cent. solution of cane sugar; they were found to give rise to conidia of *Hormodendron*. This was observed in the case of conidia produced by microsclerotia which had been taken from all of the species mentioned above. It is, therefore, concluded that this is the means by which *Hormodendron* is naturally regenerated in the spring.

Before discussing the relations of the two conidial forms, *Cladosporium* and *Hormodendron*, to each other, it may be well to describe briefly the experiments which were conducted on the infection of living leaves with conidia of *Cladosporium*. For this purpose excised leaves were used, and the experiments were conducted in a similar way to those described above in which the leaves were placed on filter papers in Petri dishes, and the conidia transferred to definite spots on them by means of a needle. The conidia were taken from the leaves of *Catalpa* and of Cabbage. After four days an examination of the spots showed that conidia of *Hormodendron* were being produced; later on, the leaves died and commenced to rot, and the conidia of *Hormodendron* ceased to be formed; conidia of *Cladosporium* were then found to be present in abundance. The infection of the leaves of Cabbage and *Catalpa* yielded similar results, and led to the following conclusions:—

Conidia of *Cladosporium* placed on living leaves give rise to conidia of *Hormodendron* which are capable of infecting living leaves; direct infection by conidia of *Cladosporium* is not possible when the leaves are living; when the leaves die, *Hormodendron* disappears, and *Cladosporium* then makes its appearance.

#### The relations of *Cladosporium* and *Hormodendron* to each other and the nature of the life-cycle.

It has been shown above that if healthy leaves are infected artificially with conidia of *Hormodendron*, they develop symptoms of disease which are similar to the symptoms observed in the case of leaves which have been infected naturally with *Hormodendron*. It is, therefore, concluded that *Hormodendron* is capable of living as a parasite on the green leaves of plants and of causing disease.

*Hormodendron* attacks many plants which exhibit no close relationship to each other; in its range it is not confined to species of one family or even of closely allied families, and it can be transferred from one species of host to another without any difficulty. This shows that it is only weakly parasitic, in the sense that it represents no highly developed form of parasitism.

The cultural experiments have shown that the same mycelium which had previously formed conidia of *Hormodendron* gives rise later to conidia of *Cladosporium*, while observations on the leaves of plants attacked by *Hormodendron* in nature have shown that when the leaves commence to wane and die *Hormodendron* disappears, and *Cladosporium* makes its appearance. It is inferred that *Cladosporium* occurring on waning or dead leaves has its origin from *Hormodendron* which attacks the leaves when they are living. Also, since on the dead leaves *Hormodendron* passes



to *Cladosporium* and the same change occurs in an artificial medium, *Hormodendron* is considered to require a living matrix for its further continuance.

The occurrence of *Cladosporium* on dead leaves, its production in an artificial, cultural medium, and its tendency to reproduce itself in such a medium show that it is to be regarded as a saprophytic form. In the life-history of many fungi when two forms are concerned, the one a parasite and the other a saprophyte, it is customary for the saprophytic form to be ultimately produced in an artificial medium and to continue to reproduce itself in such a medium. In the genus *Ustilago*, for example, the secondary spores produced by the so-called *Ustilago*-spores continue to reproduce themselves in an artificial medium, and it is only when they are transferred to the host-plant that they give rise to the parasitic form from which the *Ustilago*-spores are again produced.

Conidia of *Cladosporium*, when germinated at a temperature of 60° F. or above, give rise to conidia of *Hormodendron*. This is considered as a means by which *Hormodendron* is produced for the purpose of infecting living leaves during the summer; there are, then, two sources from which *Hormodendron* is derived during the summer, namely, conidia of *Cladosporium* and conidia of *Hormodendron*. A lower temperature has been shown to suppress the production of conidia of *Hormodendron* and to favour the formation of conidia of *Cladosporium*. This points to *Hormodendron* being a summer form and to *Cladosporium* being a later form.

The fully formed microsclerotia have been shown to produce conidiophores which bear conidia of *Cladosporium*, and these conidia of *Cladosporium* have been shown to give rise to conidia of *Hormodendron* after germination. This is considered to afford the means by which *Hormodendron* is regenerated in the spring of each year. The microsclerotia are formed in late autumn, after the conidiophores of *Cladosporium* have disappeared, and exist through the winter in a state of inactivity. On the recurrence of spring they germinate to give rise to conidia of *Cladosporium*, from which conidia of *Hormodendron* are produced, and so can infect the living leaves of plants during the summer.

From the experimental results and from the conclusions based on them, the life-history of *Cladosporium herbarum* is considered to include two conidial forms, the one a parasitic form, *Hormodendron*, and the other, *Cladosporium*, a saprophytic form. The latter form is capable of existing in an inactive condition through the winter. Such a life-history, which is made up of two conidial forms only, is unusual among the Fungi. Each form has been shown to be capable of giving rise to the other, and an attempt has been made to elaborate the conditions under which one form tends to pass into the other. The life-cycle appears to be complete, and the intervention of any other spore-form would be unnecessary.

The species of *Cladosporium* dealt with in this article has been regarded as *Cladosporium herbarum*; hitherto it has been customary to regard the species occurring on *Quercus*, *Platanus*, *Populus*, and a few others, as *Cladosporium epiphyllum*, Nees, and that occurring on the dead remains of plants generally as *Cladosporium herbarum*, Link. It is considered to be advisable to include these two hitherto distinct species under one specific name, partly because the differences in the nature of the conidia and conidiophores are not thought to be sufficiently great to warrant their separation as two distinct species, and also because the conidia taken from *Quercus*, or from any of the other species of plants worked with, have yielded similar results; the name of the older form, *Cladosporium herbarum*, Link, is accepted as the name of the species.

#### Previous work on *Cladosporium herbarum*.

*Cladosporium herbarum* has afforded material for the work of several authors; reference will now be made to a few of these. M. Janczewski obtained a giant and a dwarf form of *Hormodendron* from *Cladosporium herbarum*; the same observer has also obtained an ascigerous stage which he has named *Sphaerella Tulasnei*. M. Laurent obtained *Hormodendron cladosporioides*, Sacc., *Dematium pullulans*, De Bary, and a *Fumago* form, all being forms of *Cladosporium herbarum*. M. Berlèse, on germinating conidia of *Cladosporium herbarum* taken from a number of species of plants, always obtained the form *Hormodendron*, which he regards as essentially the saprophytic form of *Cladosporium herbarum*. Other observers have obtained several other forms.

In the life-history given above the only other form mentioned is the form *Hormodendron*; this was the only other form obtained in the cultures or observed on the leaves of plants examined, except in one instance which will now be mentioned. On germinating conidia of *Cladosporium* taken from the leaves of *Eucalyptus globulus* in a 2 per cent. solution of cane sugar in 'hanging-drops', there was produced, in addition to the small form *Hormodendron*, a larger conidial form of which the conidia measured  $18 \times 8 \mu$ . The conidia were borne in branched chains not only at the apex of the conidiophore, but also at other points of its axis. In their method of origin, therefore, the conidia of the larger form differed from those of the small form which were borne only at the apex of the conidiophore. In both the smaller and larger forms, however, the conidia differed somewhat from those of the form *Hormodendron* occurring on other species of plants, in that instead of being elliptical they were nearly circular in shape. It must be borne in mind that *Eucalyptus globulus* is an exotic species, and more work is needed to determine whether the species of *Cladosporium* occurring on it is a different species from *Cladosporium herbarum*.



The work of the authors mentioned above would show that *Hormodendron* is a saprophytic form, while the experiments described in this article would point to its being a parasitic form. With reference to the ascigerous stage, *Sphaerella Tulasnei*, mentioned above, it may be said that there is no record of its occurrence in Britain, and, further, that the life-history of *Cladosporium herbarum* has been shown to be complete without the intervention of an ascigerous stage.

*Cladosporium herbarum* has been described by several observers as, in certain circumstances, a parasite. Prilleux and Delacroix consider it to be parasitic on Apple trees and Raspberry bushes, Cavara on Raspberry, Cycads, and other plants, Sorauer on Peas, Lopriore on Wheat, and Ritzema Bos on Oats.

On the whole the work of various authors tends to show that there are several races or species included under the name *Cladosporium herbarum* which are separated from each other, not by the characters of conidia, conidiophores, or hyphae, but by virtue of the other spore-forms which they can give rise to.

The following is given as a brief summary of the life-history as worked out in the above pages :—

1. The life-cycle is composed of two conidial forms, *Hormodendron* and *Cladosporium*.

2. *Hormodendron* is a parasitic and summer form; in the summer it produces a disease of the green leaves of several species of plants. When the leaves die it passes to the other form, *Cladosporium*.

3. *Cladosporium* is a later form and exists as a saprophyte on the dead leaves which have been previously attacked by *Hormodendron*. It can give rise to *Hormodendron* if the temperature be moderately high; but at a lower temperature it reproduces itself.

4. In the winter *Cladosporium* exists in the form of microsclerotia which on the advent of the spring germinate to produce conidia of *Cladosporium*; these conidia of *Cladosporium*, after germinating, give rise to conidia of *Hormodendron* which serve to continue the disease caused by *Hormodendron* during the summer.

A list of the species which are regarded as synonymous with *Cladosporium herbarum* is appended.

*Cladosporium epiphyllum*, Nees. Das Syst. der Pilze und Schwämme.

*Cladosporium nigricans*, Fr. Syst. Myc., iii, 370.

*Cladosporium solutum*, Link. Spec., i, 39.

*Acladium herbarum*, Link. Obs., i, 10.

*Byssus caespitosa*, Roth. Fl. Germ., iv, 566.

*Byssus herbarum*, DC. Fl. Fr., vi, 11.

*Byssus nigricans*, Roth. Catal., i, 126.

*Dematium articulatum*, Sow. Fung., T. 400, F. 8.

*Dematium Brassicae*, Pers. Syn. Fung., 699.

*Dematium conicum*, Schum. Fl. Saell., ii, 445, Nr. 2171.

*Dematium epiphyllum*, Pers. Syn. Fung., 695.

*Dematium fungorum*, Pers. Ibid., 699.

*Dematium graminum*, Pers. Myc. Eur., i, 16.

*Dematium herbarum*, Pers. Dispos. Fung., 75; Alb. et Schw. Consp.

1104.

*Dematium Hippocastani*, Pers. Syn. Fung., 698.

*Dematium vulgare*, Pers. Sec. Steud.

My thanks are due to Lieut.-Col. Prain, C.I.E., F.R.S., Director, Royal Botanic Gardens, Kew, for kindly permitting the work in connexion with this article to be carried out in the Jodrell Laboratory, and also to Mr. G. Masee, F.L.S., for his kind assistance.

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————— : Les Périthèces du *Cladosporium herbarum*. Comptes-rendus de l'Acad. des Sc., 1893.

LAURENT : Annales de l'Institut Pasteur, Dec. 1888.

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PRILLEUX and DELACROIX : Note sur le parasitisme du *Botrytis cinerea* et du *Cladosporium herbarum*. Bull. de la Soc. Myc. de France, vii.

SACCARDO : Sylloge Fungorum IV.

SORAUER : Handbuch der Pflanzenkrankh., 1886.

TUBEUF and SMITH : Diseases of Plants, 1897.

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#### DESCRIPTION OF PLATE XXIV.

Illustrating Mr. Bancroft's Paper on *Cladosporium herbarum*.

Fig. 1. A tuft of hyphae of *Cladosporium herbarum* springing from a mass of cells and bearing conidia.

Fig. 2. A diseased leaf of *Catalpa bignonioides*, showing the effects of the disease caused by the form *Hormodendron*.

Fig 3 a. A conidium of *Cladosporium* which has germinated, and is producing conidia of *Hormodendron*.

Fig. 3 b. Another conidium of *Cladosporium* giving rise to *Hormodendron* after germination.

Fig. 4. Production of conidia of *Hormodendron* from a conidiophore of *Cladosporium* in a 'hanging-drop'.

Fig. 5. Early stages in the germination of conidia of *Hormodendron*.

Fig. 6. Hyphae of the mycelium of *Hormodendron* producing conidia in a culture in nutrient gelatine.

Fig. 7. Later production of conidia of *Cladosporium* from the same mycelium as in Fig. 6.

Fig. 8. Germination of a conidium of *Cladosporium* in a culture in nutrient gelatine with formation of conidia of *Cladosporium*.

Fig. 9 a. Production of conidia of *Cladosporium* by a conidium of the same form which has germinated at a low temperature.

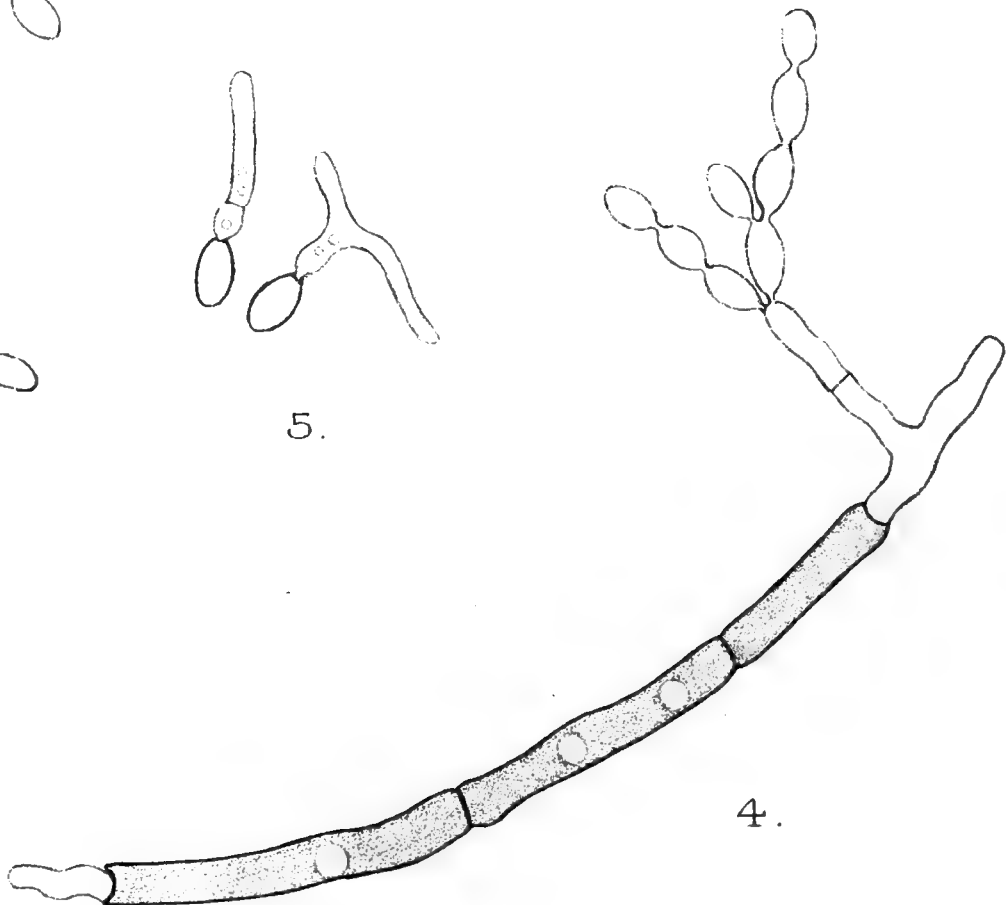
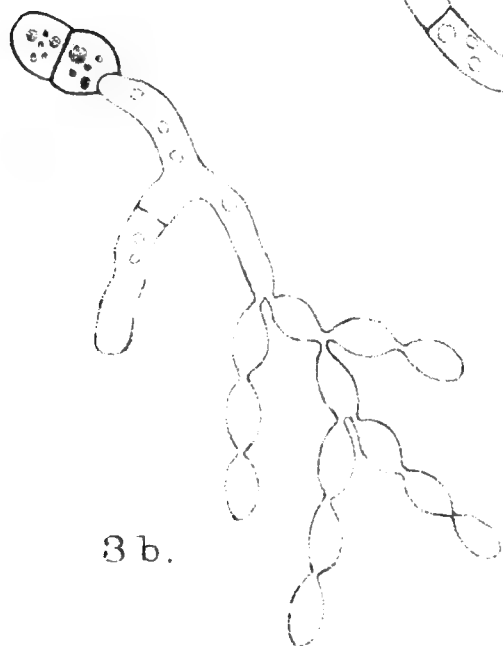
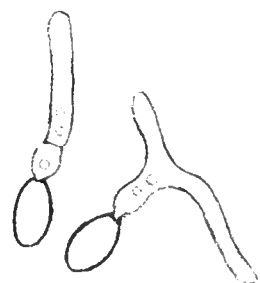
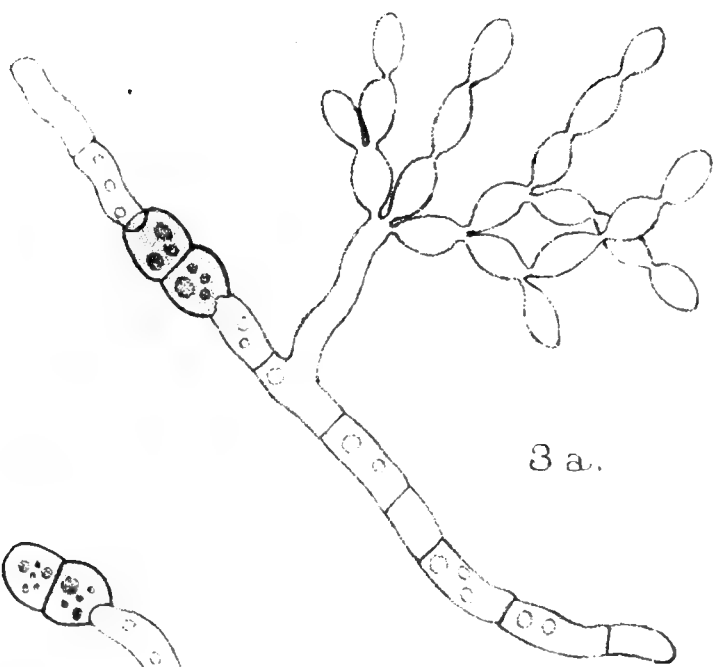
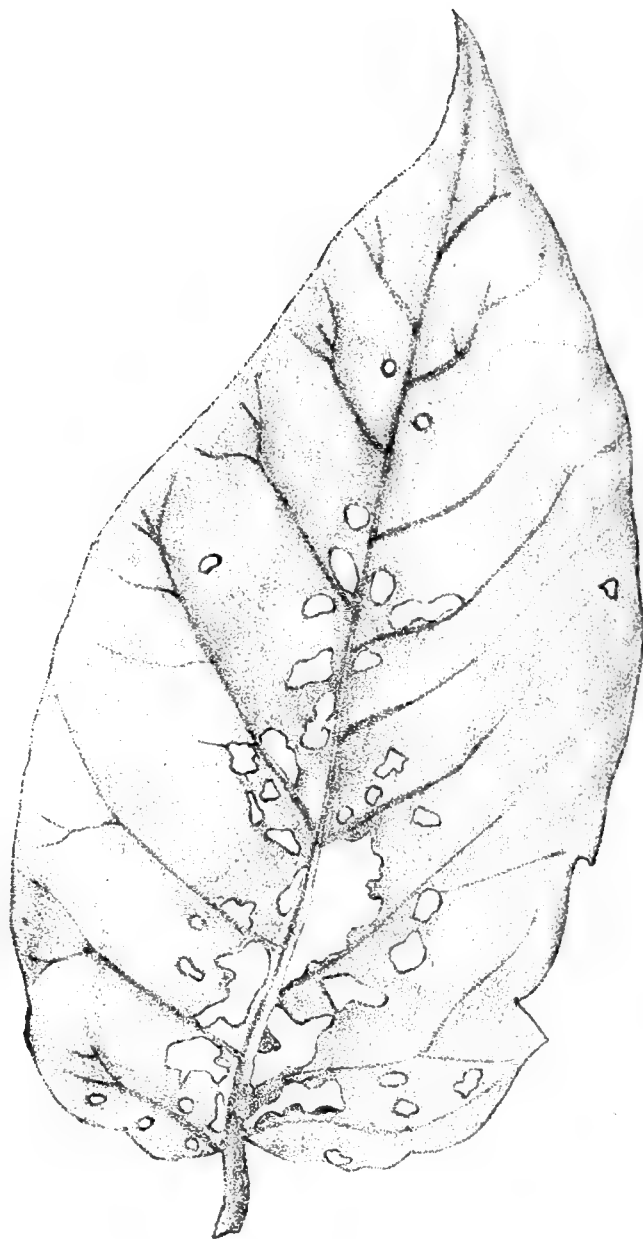
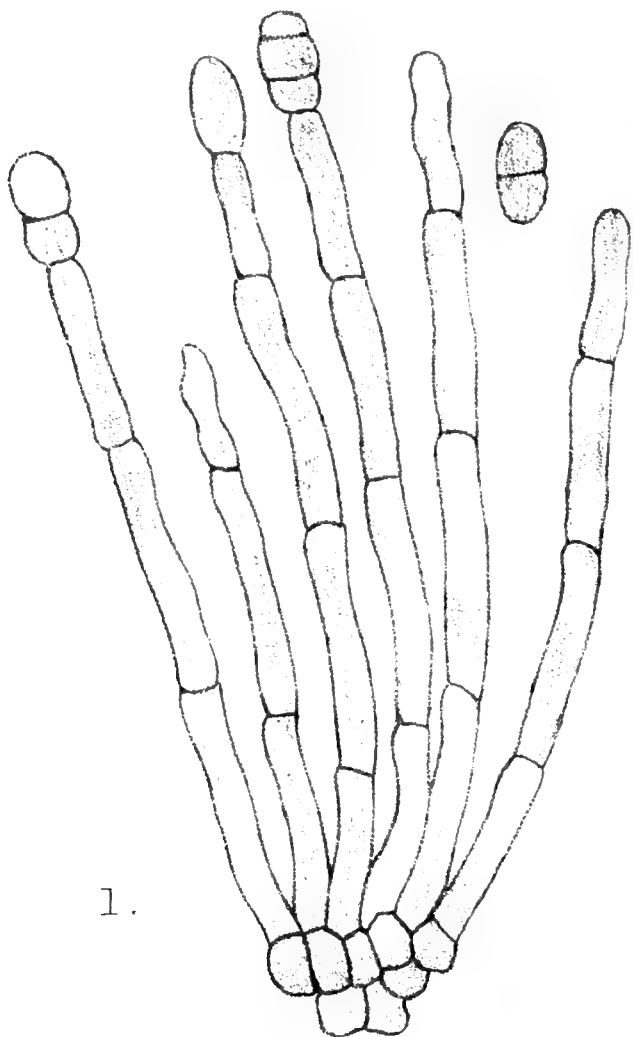
Fig. 9 b. The same as in Fig. 9 a.

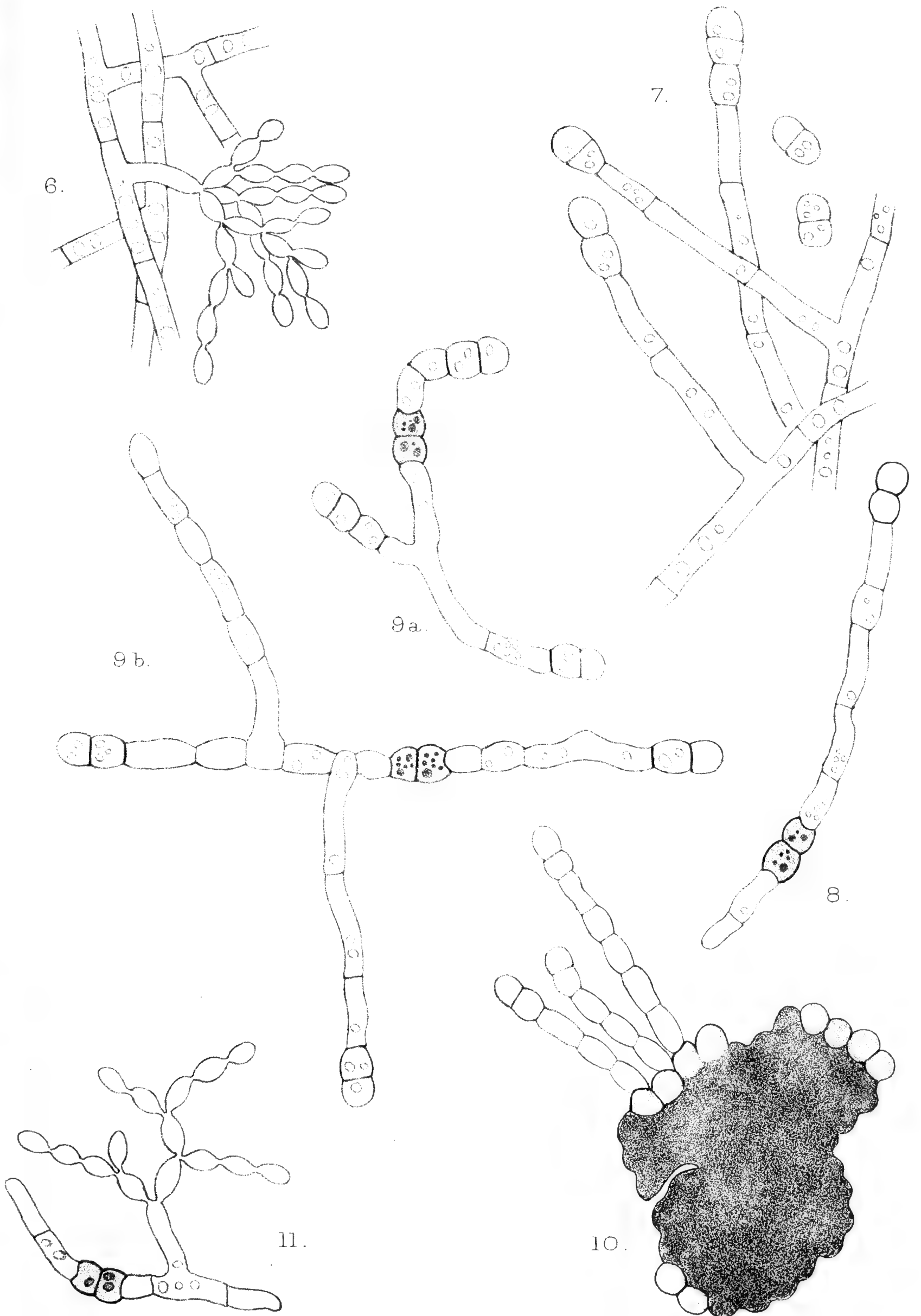
Fig. 10. Germination of a microsclerotium.

Fig. 11. A conidium produced by a microsclerotium giving rise to conidia of *Hormodendron* after germination.

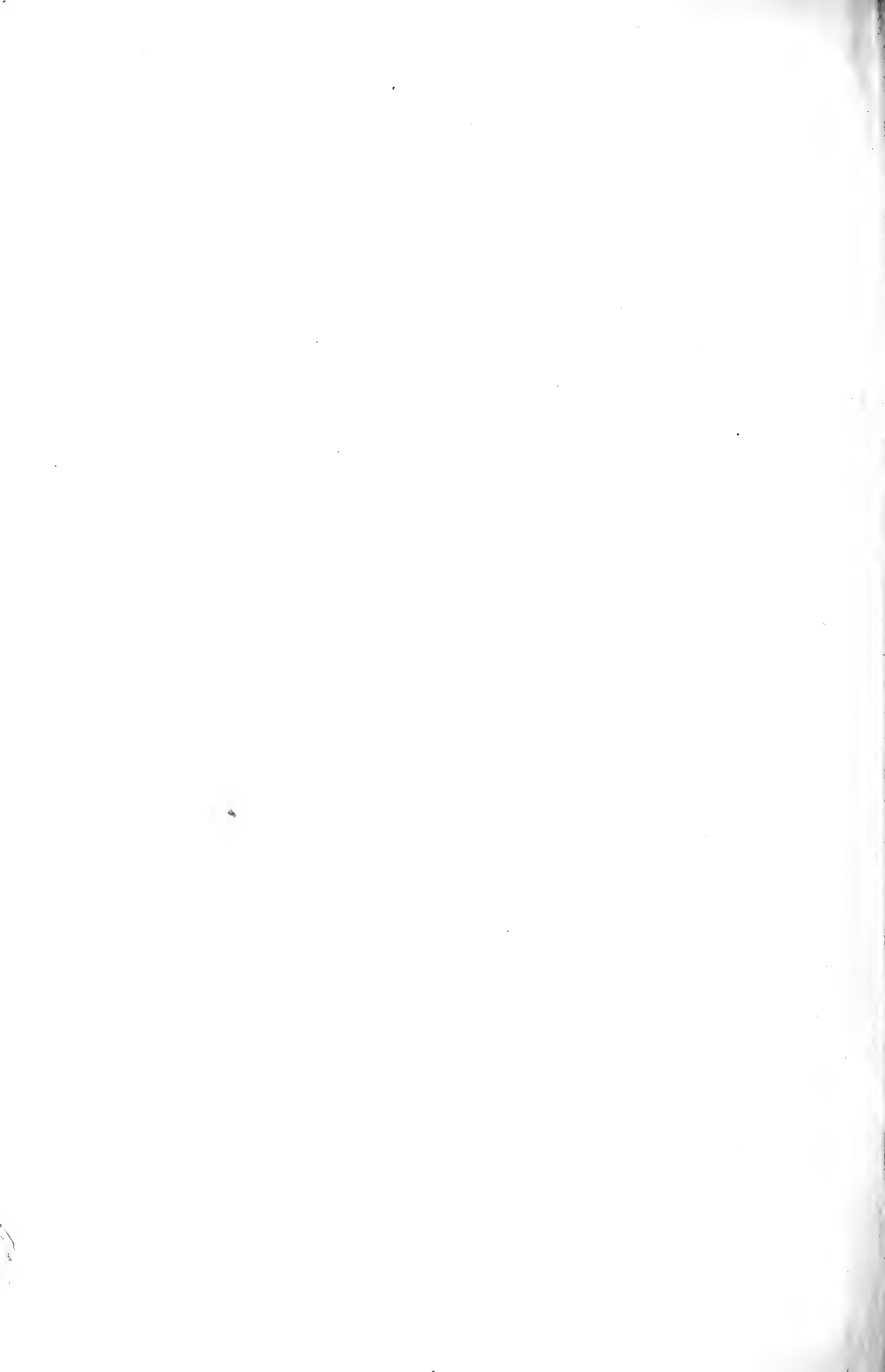
Fig. 2 natural size; Fig. 10 magnified  $\times 400$ ; the others magnified  $\times 600$  (about).











# The Structure of the Aerial Shoots of *Psilotum flaccidum*, Wall.

BY

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With Plate XXV.

## INTRODUCTION.

THE genus *Psilotum* is generally regarded as comprising two species : *Psilotum triquetrum*, Sw., and *P. flaccidum*, Wall. The two species differ chiefly in the form of the aerial stem ; in *P. triquetrum* this is multi-angular in the lower parts and triquetrous in the ultimate branches, while in *P. flaccidum* the lower part is triquetrous and the upper part flattened in one plane. Each species includes a variety with more slender aerial shoots which is by many authorities regarded as a distinct species ; *P. Capillare*, Bl., is the variety of *P. triquetrum*, and *P. complanatum*, Sw., the variety of *P. flaccidum*. In the case of the latter *P. complanatum* is sometimes regarded as the type and *P. flaccidum* as the variety. From Baker's<sup>1</sup> description the material here described is referable apparently to *P. flaccidum*, but the distinction between this and *P. complanatum* does not seem to be very clear.

As regards geographical distribution, according to Bertrand,<sup>2</sup> who regards *P. complanatum* and *P. flaccidum* as distinct species, the former is found in Jamaica, Mexico, Philippine Islands, Society Isles, and Sandwich Isles, while *P. flaccidum* occurs in Java and the Philippine Islands. The material on which the following account is based was, however, collected by Professor Stanley Gardiner in the Seychelles, on the summit of Mount Sebert at an elevation of 1,800 feet, and was preserved in methylated spirit. This material consisted of the complete parts of five aerial stems, but unfortunately none of the rhizome was present. Some laboratory material of unknown origin, and which had been preserved in formalin, was also available for examination, but consisted of the upper aerial branches only. In both this material and that from the Seychelles sporangia were numerous.

<sup>1</sup> Baker, J. G. ('87), p. 30.

<sup>2</sup> Bertrand, C. E. ('83), p. 11.

To Professor Seward my thanks are due for handing over the material to me for examination. I would also thank him and Mr. Tansley for their advice given with regard to the work and for their interest in its progress. To Mr. H. H. Thomas I am indebted for advice and help with the photomicrographs.

#### EXTERNAL MORPHOLOGY.

The sporophyte of *Psilotum flaccidum* consists of a branched rhizome, certain branches of which grow out towards the surface of the substratum, and ultimately become aerial stems. As in all the Psilotaceae, there are no roots. The plant is epiphytic and of pendulous habit; its rhizome apparently grows in the humus attached to trees, from which its aerial branches hang down in clusters.<sup>1</sup>

Our knowledge of the rhizome is practically limited to the references to it in the monographs of Bertrand,<sup>2</sup> Solms-Laubach,<sup>3</sup> and Pritzel,<sup>4</sup> from which it would appear to be very similar to that of *P. triquetrum*. The branches of the rhizome which are destined to become aerial shoots pass through a transition region before reaching the surface of the substratum.

All my five specimens of aerial shoots are from 15 to 20 centimetres long, and in each case at its very base the aerial stem is cylindrical and of a dark colour. Higher up this stem loses its dark colour and becomes wider, and soon passes over into a triquetrous region, which persists for a shorter or longer distance, and itself gradually changes into the flattened form characteristic of the greater part of the aerial branches (Pl. XXV, Fig. 1). The triangular lower part and flattened upper part are clearly shown in the early figure of *P. complanatum* published by Swartz<sup>5</sup> in 1806.

In *Psilotum triquetrum* the branching is apparently dichotomous, the planes of successive dichotomies being at right angles. This would seem to be the case in *P. flaccidum* so long as the stem is triangular<sup>6</sup> (Fig. 1), but where the stem is flattened the branching takes place all in one plane.

The stem bears two kinds of appendages, leaves and sporophylls. The former are small scale-like structures about a millimetre long and lanceolate in shape. The sporophylls are forked, the lower part below the two prongs being extremely short, so that the sporophyll has much the appearance of two leaves closely connate at their base. Between the two lobes of the sporophyll is the sporangiophore. This consists of a very short axis bearing at its apex the three confluent sporangia. Bilocular synangia are also by no means uncommon.<sup>7</sup>

<sup>1</sup> Pritzel, E. ('00), p. 610.

<sup>2</sup> Bertrand, C. E. ('83).

<sup>3</sup> Solms-Laubach ('84).

<sup>4</sup> Pritzel, E., l. c.

<sup>5</sup> Swartz, O. (1806), pp. 188, 414, t. 4, Fig. 5.

<sup>6</sup> Branching in the triquetrous region of the stem is rare, and my specimens were few in number, so that this point could not be definitely ascertained.

<sup>7</sup> Cf. *Psilotum triquetrum*, Miss Ford ('04), p. 591.

The leaves are borne on the ridges of the stem so that on the lower parts the leaf insertion is tristichous, and on the upper flattened parts distichous.<sup>1</sup> The sporophylls occur mostly on the upper branches, and are inserted just in the same position as the ordinary leaves. Among them, however, ordinary leaves may be scattered, while on the other hand they are not confined to this region, and are sometimes found on the lower branches. The aerial shoot, like that of *P. triquetrum*, thus exhibits the 'Selago' condition of Bower.<sup>2</sup>

The branching of the stem is apparently dichotomous, but Prantl<sup>3</sup> showed that in *P. triquetrum* a leaf always occurred below the fork of the stem, and he supposed that one of the branches of the apparent dichotomy was really axillary. Solms-Laubach, on the other hand, thought that in *P. flaccidum* the leaf insertion pointed to a true dichotomy, for the alternate distichous arrangement is continued at first on the two branches taken as a whole, as if no dichotomy had occurred, while the distichous arrangement on each branch is assumed higher up.<sup>4</sup> In my material of *P. flaccidum* there was constantly a leaf associated with the branching, although this was sometimes carried up, presumably by later intercalary growth, on to one of the branches. This point will be referred to subsequently.

A single cylindrical stele traverses the stem and bifurcates with the latter. In many cases a vascular bundle is given off from the stem-stele to the leaves, though this is not always so. This would appear also to be much the same as in the case of *P. triquetrum*,<sup>5</sup> though, as many writers have stated that the leaves are without any vascular supply whatever in the latter species, leaf-traces are apparently of very much rarer occurrence in it. In *P. flaccidum*, as in *P. triquetrum*, the sporophyll is always served with a vascular supply.

#### INTERNAL STRUCTURE.

1. *Stem.* At the lowest parts of the stems of which my material was composed, in what Miss Ford<sup>6</sup> describes for *P. triquetrum* as the intermediate region between rhizome and aerial stem, the branch is circular in transverse section and externally smooth and of a dark colour. The single stele is circular or somewhat elliptical in transverse section, and is surrounded by an endodermis which, although not very well differentiated, is yet clearly visible when suitably stained, its radial walls taking a lignine stain in some degree. The xylem consists of a band-shaped mass, generally with smaller protoxylem-groups at each end of the band, though sometimes these protoxylem-groups are not obvious. The whole of the xylem between the two protoxylems is composed of tracheae with scalariform

<sup>1</sup> Cf. Solms-Laubach ('84), p. 164.

<sup>3</sup> Prantl, K. ('76), p. 92.

<sup>5</sup> Pritzel, E. ('00), p. 616.

<sup>2</sup> Bower, F. O. ('08), pp. 165, 412.

<sup>4</sup> Solms-Laubach ('84), p. 165.

<sup>6</sup> Miss Ford ('04), p. 598.

thickenings; no parenchymatous elements or sclerenchymatous fibres are here found among the tracheae.

Surrounding this xylem-mass is a zone of thin-walled tissue, in which are scattered more xylem-elements (Fig. 2). These latter are in the form of an irregular broken ring, and are undoubtedly of the same nature as the secondary tracheae discovered and described by Boodle<sup>1</sup> in *Psilotum triquetrum*, and which have also been recorded as occurring in that species by Miss Ford. Their radial arrangement in *P. flaccidum* is in some cases quite as obvious as in the other species, though perhaps in none of my preparations were they so numerous as in those figured by Boodle. As this author found in *P. triquetrum*, so in *P. flaccidum* there is no sign of a definite cambium.

Surrounding the zone of secondary xylem-elements is a band of thin-walled elements presumably of the nature of phloem, but this tissue is very difficult to recognize, especially in transverse sections. There is no well-defined pericycle.

The cells of the inner cortex have their walls much thickened by a brown substance (Fig. 3) which is laid down apparently more or less irregularly.<sup>2</sup> The cell-walls decrease in thickness as the exterior of the stem is approached. The epidermis is one cell wide and has a thick cuticle. In the subterranean part of the stem stomata were not found.

As one passes up the stem it becomes wider in diameter, as does also the central stele, which also becomes more completely circular in transverse section. The two protoxylem-groups at each end of the band become more distinct, while a third protoxylem arises between them on the outside of the metaxylem, and eventually the stele becomes triangular in outline (Fig. 3). An exactly similar state of affairs is found in *P. triquetrum*.<sup>3</sup> In this part of the stem there is a tendency in places to a slight mesarch structure,<sup>4</sup> the protoxylem-elements being found not quite at the ends of the xylem arms. The secondary tracheides here begin to diminish in amount, so that when a point is reached where the stem is triquetrous they have almost completely disappeared. When the stem becomes triangular three more protoxylem-groups make their appearance between the three already present, so that with a triquetrous stem is associated a hexarch stele (Fig. 4). Mesarch structure is here sometimes very marked, and in some cases is certainly due to the insertion of a leaf-trace (Fig. 5), though at other times no leaf-trace is given off. The question of mesarchy will be discussed subsequently.

<sup>1</sup> Boodle, L. A. ('04), p. 505.

<sup>2</sup> Mr. Brooks has pointed out to me that in *P. triquetrum* this substance is often laid down spirally on the longitudinal walls. In all cases examined in *P. flaccidum* the thickening due to this substance was reticulate. Sometimes, however, it seemed probable that the first layers of the substance were laid down spirally.

<sup>3</sup> Boodle, L. A. ('04), p. 514.

<sup>4</sup> Cf. *P. triquetrum*, Boodle, L. A., l. c.

At about the same level as that at which the stele begins to be hexarch, fibres similar to those found in *P. triquetrum* appear in the middle of the xylem. The simple pits in the walls of the fibres are easily seen. Occasionally parenchymatous cells are found among the tracheides (Fig. 4), and sometimes gaps may occur in the xylem-ring. The endodermis also becomes much clearer, and the thickenings on the radial walls are shown up very distinctly in sections stained with methyl violet and Bismarck brown.

The cortex becomes more or less differentiated into an inner zone with fairly thin cell-walls, a middle zone of parenchymatous cells with somewhat thicker walls, and an outer assimilating layer. The epidermis here has numerous stomata.

In this region of the stem the structure is therefore very similar to that of *P. triquetrum*.

Sooner or later the number of protoxylems of the stele increase in preparation for the first bifurcation of the stem, where in all my specimens the fibres in the middle of the stem disappear. A leaf was constantly associated with this bifurcation, and in all cases examined received a vascular supply, such that practically one-half of the divided stem-stele was in the axil of the leaf-trace. Sometimes this leaf was carried up on to the fork of the stem above the bifurcation, but when this was the case its vascular supply originated in connexion with the bifurcation of the stem-stele.

In the branches of a higher order central fibres were not observed, while in the flattened branches the number of protoxylem groups was constantly four, two of the six xylem arms of the triquetrous stems gradually dying out. Thus in *P. flaccidum*, except in the region of a bifurcation, and in the transitional region between the triangular and flattened stems, the number of xylem arms would appear to be double the number of ridges on the stem.<sup>1</sup> This is apparently a different state of affairs to that found in *P. triquetrum*, where any close connexion between the number of protoxylem-groups and the number of ridges on the stem is not evident. The relationship in *P. flaccidum* is, moreover, often masked owing to the early preparation for the bifurcation of the stele.

The xylem in the flattened branches occupies the centre of the cylindrical stele, and is cruciform as seen in transverse section, the four protoxylem-groups occurring at the end of each arm near the endodermis, so that the stele is here quite exarch (Fig. 6). The tetrarch arrangement was obvious in the smaller sporangiferous branches.

The protoxylem consists of a few tracheidal elements at the ends of the xylem arms with spiral thickenings on their walls. The metaxylem-

<sup>1</sup> The transitional region between the hexarch and tetrarch stele is, however, generally of much greater extent than that between the triquetrous and flattened stem.



elements have scalariform thickenings, and gradually increase in size from the protoxylem inwards. Transitions between spiral and scalariform thickenings occur. As in the lowest part of the aerial stem, parenchyma and fibrous elements are usually not present in the xylem except in the very young stems below the growing point, where the elements that will form the metaxylem are as yet undifferentiated.

Extending round the xylem is a mass of thin-walled tissue, presumably of the nature of phloem. No lignification of the elements, such as occurs in *Tmesipteris*,<sup>1</sup> could be noticed, but in some cases, though by no means in all, the phloem cells were lignified slightly at the corners, as in *P. triquetrum*.<sup>2</sup>

This phloem tissue appears to be composed of two kinds of elements. In longitudinal section all the cells appear elongated, but some are much longer than the others and contain abundant contents, among the latter being numerous highly refractive globules, no doubt similar to those occurring in the sieve-tubes of Ferns described by Poirault<sup>3</sup> (Fig. 7). They are quite obvious in unstained sections, and show up very distinctly in sections stained with ruthenium red. The elements containing these globules would presumably correspond to sieve-tubes, but not the slightest sign of callus could be found on staining with London blue or corallin soda, though this apparent absence might be due to the use of spirit material. The cross walls of these elements were sometimes very oblique, and sometimes practically at right angles to the longitudinal walls; in some cases the highly refringent globules were observed to be clustered against the wall, but in no case were sieve-plates observed. Nuclei were generally, but not always, absent from these cells, and in one case a disorganizing nucleus was observed.

Associated with these cells, and as a rule outnumbering them, are other elements of about the same diameter, but not so long. In these a large nucleus is always present, which often, but not always, presents a more or less elongated shape. From this account it will be seen that on the whole the phloem of *P. flaccidum* is very similar to that of *P. triquetrum*.<sup>4</sup> In the phloem of *Tmesipteris*, however, Miss Sykes<sup>5</sup> records sieve-tubes only, and these differ from those of *Psilotum* in having lignified walls and numerous lateral sieve-plates on them.

Surrounding the stele is the endodermis, which, as in the triquetrous region of the stem, forms a well-defined layer; its radial walls show their lignification plainly when stained with methyl violet and Bismarck brown.

Immediately outside the bundle, and completely surrounding it, is a cylindrical band of rather large cells, which often have thick walls. The

<sup>1</sup> Sykes, M. G. ('08), p. 70.

<sup>3</sup> Poirault, G. ('93), p. 139.

<sup>5</sup> Sykes, M. G., l.c.

<sup>2</sup> Ford, S. O. ('04), p. 593.

<sup>4</sup> Ford, S. O., l. c.

central part of the walls of these cells appears to be much more lignified than the part bordering on the lumen, which in some cases would appear not to be lignified at all. The cells are elongated longitudinally, and have pointed ends, the latter being much more lignified than the rest of the wall. Simple pits are very numerous on the walls of these cells, and are easily observed.

On the narrow diameter of the stem the thickened layer abuts directly on the chlorophyllous assimilating layer (Fig. 8). This extends completely round the stem as a layer of from two to four cells wide just inside the epidermis, and filling up all the space between the epidermis at the ends of the longer diameter of the latter. In longitudinal section these cells show the peculiar shape already noted for those of this tissue in *P. triquetrum*.<sup>1</sup> The walls are thin and wavy in contour, touching the adjacent cells in three or four places only, so that intercellular spaces between the cells are large and numerous.

Between the chlorophyll-containing tissue in the wings and the fibrous layer round the bundle are parenchymatous cells, which pass gradually into the latter.

The epidermis is a single layer of regular cells with a very thick outer wall; as in *P. triquetrum*<sup>2</sup> the innermost layer of the outer wall is not cuticularized, while the outer layers become more cuticularized the further they are from the inner one. Under high magnifications the layering of the outer wall is very distinct. The epidermal cells are somewhat elongated longitudinally.

Stomata are numerous, as would be expected in an assimilating stem; they are sunk somewhat below the outer level of the epidermis. They are somewhat peculiar in shape, there being only a single ridge on each guard-cell, a state of affairs which is somewhat rare.<sup>3</sup> In *P. triquetrum*, according to De Bary, both ridge of entrance and ridge of exit are absent.

2. *Leaf-trace*. When a leaf-trace is given off from the stele the xylem arm on the side of the leaf becomes elongated, and ultimately two or three tracheides, or sometimes more, are detached, which pass up to the leaf, making an angle of about 30° with the main stele (Fig. 9). These tracheae are small, and have spiral or scalariform thickenings on their walls. As a rule the number of elements is too small to enable one to determine the position of the protoxylem. Surrounding the tracheae are some elongated thin-walled cells continuous with those of the phloem of the stem-stele. In some cases a distinct endodermis was observed.

3. *The Leaf*. The leaves are small lanceolate structures about one millimetre long, and similar in shape to those of *P. triquetrum* (Fig. 10). There is an epidermis with a very thick cuticle, this layer being continuous

<sup>1</sup> Ford, S. O. ('04), p. 592.

<sup>2</sup> De Bary, A. ('84), p. 77.

<sup>3</sup> De Bary, A. ('84), p. 35.

with that of the stem. Stomata were not observed, and Miss Ford states that they are absent from the leaf of *P. triquetrum*. The interior of the leaf is composed of parenchymatous cells continuous with the assimilatory tissue of the stem. The cells in the leaf, however, do not possess to any marked degree the intercellular spaces characteristic of the outer cortical cells of the stem, and in this respect the leaf differs from that of *P. triquetrum*. They contain, however, conspicuous nuclei and many chloroplasts.

The leaf-trace, the xylem of which, when it exists, terminates at the level of the leaf insertion, is continued into the leaf by some narrow elongated cells. These appear to be completely absent from the leaves of *P. triquetrum*.

4. *The Sporophyll.* The forked sporophyll has almost exactly the same structure as two scale leaves connate at the base. Transitions between ordinary leaves and sporophylls are found in which the leaf is divided for a part only of its length.

Between the two prongs of the forked sporophyll arises the sporangiophore, which consists of a short axis bearing, and completely fused with, a synangium of three confluent sporangia. In all cases examined a vascular bundle supplied the spore-producing member. As a rule about two or three tracheides are given off from the ends of one of the xylem arms of the stem-stele in exactly the same way as the leaf-trace originates. The sporophyll-trace (Fig. 11) passes up through the cortex in much the same way as the leaf-trace, and elongated parenchymatous cells pass into the two forks of the sporophyll as into the leaf. The xylem does not, however, terminate at the level of insertion of the sporophyll, but passes up into the axis of the sporangiophore. Here the number of xylem-elements increases, so that a section passing transversely through the three loculi also passes through a vascular bundle in the axis between them, composed of five to eight tracheae. These, however, terminate below the middle of the synangium.

Bertrand<sup>1</sup> states that in *P. triquetrum* the axis of the synangium is completely without vascular tissue, although elongated cells are present here.

The vascular structure of the sporangiophore found in *P. flaccidum* is interesting in comparison with *Tmesipteris*. In *Tmesipteris* Miss Sykes<sup>2</sup> found the sporophyll bundle divided into three, the two lateral strands passing one into each lobe of the forked sporophyll, the central strand passing into the axis of the sporangiophore. This is similar to what takes place in *P. flaccidum*, only here the sporophyll bundles, like the leaf bundles, are represented by a few elongated parenchymatous cells.

The bundle in the axis of the sporangiophore of *Tmesipteris* then divides into three at the base of the bilocular synangium; the two lateral

<sup>1</sup> Bertrand, C. E. ('83), p. 213.

<sup>2</sup> Sykes, M. G. ('08), p. 74.

ones pass round the outside of the septum, the middle one passes a short way into the septum. In *P. flaccidum* the central bundle, which passes into the middle of the tissue between the three loculi, corresponds to the bundle in *Tmesipteris* that passes into the septum, while no representative of the two lateral branches is to be found.

The structure of the synangium seems otherwise identical with that of *P. triquetrum*, and therefore need not be further discussed here.

#### GENERAL CONSIDERATIONS.

From the present investigation it will be seen that *Psilotum flaccidum* closely resembles *P. triquetrum* in internal structure. In the occasional occurrence of very distinct mesarch xylem, in the more general presence of leaf-traces, and in the presence of a vascular bundle in the axis of the sporangiophore, *P. flaccidum* approaches *Tmesipteris* more nearly than does the other species, and in these respects serves as a connecting link.

It is a difficult question to decide which of these three species approaches most nearly the ancestral type from which they all sprang. The most general opinion is that they are all reduced forms in accordance with their epiphytic habit, yet Bower,<sup>1</sup> though he does not dispute this reduction theory, on the other hand does not seem inclined to accept it, and leaves it an open question, while Lignier<sup>2</sup> regards the Psilotaceae as very primitive.

So far as regards the structure of the Psilotaceae, there is little evidence obtainable in support of either view. The feeble development of secondary wood in the base of the aerial stem in both *P. flaccidum* and *P. triquetrum* might be used as an argument in favour of reduction, but it might also be regarded as having originated independently in the genus, especially as Miss Sykes,<sup>3</sup> in spite of careful search, failed to find any trace of it in *Tmesipteris*. The rootless condition of the plant may also be due to reduction, and this seems the more probable when its peculiar epiphytic habit is considered.

The same question of primitiveness or reduction arises when the microphyllly of *Psilotum* is considered. Jeffrey<sup>4</sup> and Lignier<sup>5</sup> regard the Psilotaceae as primitively microphyllous forms connected with the Lycopodiales, the first-named writer also associating the Equisetales with them. The general opinion is, however, that the leaves are reduced structures. In this connexion the occasional occurrence of mesarch xylem in both *P. triquetrum* and *P. flaccidum* may be of importance. Boodle<sup>6</sup> thinks that 'the most natural conclusion is that the aerial stem of *Psilotum* has

<sup>1</sup> Bower, F. O. ('08), p. 413.

<sup>3</sup> Sykes, M. G. ('08), p. 78.

<sup>5</sup> Lignier, O. ('03), l. c.

<sup>2</sup> Lignier, O. ('03), p. 95.

<sup>4</sup> Jeffrey, E. C. ('02), p. 144.

<sup>6</sup> Boodle, L. A. ('04), p. 514.



been reduced from the mesarch to the exarch type in connexion with the disappearance of the leaf-traces'. Miss Sykes<sup>1</sup> holds a similar view, for she thinks mesarch structure in *Tmesipteris* and exarch structure in *Psilotum* are to be explained as due to the different size of the leaves in the two genera.

The occurrence of very marked mesarchy in connexion with the insertion of a large leaf-trace in *P. flaccidum* appears to me to lend support to this view.

Boodle also considers that the secondary thickening in *Psilotum* is reduced from the more normal type, and is to be correlated with the reduction of the leaves to scales, thus causing a diminution of transpiration.<sup>2</sup> This writer's views appear to me to offer the most likely explanation of the secondary thickening and occasional mesarchy in *Psilotum*, but it should not be forgotten that these features can be explained as primitive structures, and not as due to reduction.

There remains to be considered the question of the spore-producing member. Scott<sup>3</sup> and Bower<sup>4</sup> regard the sporophyll as foliar, the former writer considering the sporangiophore in *Psilotum* as part of the sporophyll of which it is an adaxial outgrowth, and which thus occupies a similar position to the sporangiophores of the Sphenophyllales. Bower limits the term sporophyll to the forked bract, the sporangiophore borne on it being non-foliar in nature; it is here associated with the subtending sporophyll, but in other groups may be quite independent of it.

Bower considers the sporangiophore as an organ *sui generis*. The sporophyll he regards as a modified foliage leaf.

Miss Sykes,<sup>5</sup> adopting the view of Strasburger, Goebel, and Bertrand, regards the sporangiophore as a branch of the main axis bearing two leaves (the forked 'sporophyll') and terminating, in the case of *Psilotum*, in three confluent sporangia. Her chief argument in favour of this view is based on the fact that in *Tmesipteris* the bundle in the axis of the sporangiophore, after giving off a strand on either side into the lobes of the sporophyll, is continued into the septum, a branch being again given off on each side which runs round the periphery of the septum. The central strand, Miss Sykes thinks, may terminate the axis of the sporangial branch.

I find myself unable to accept this view. It has been shown that a bundle corresponding to the central one in *Tmesipteris* is also present in *Psilotum flaccidum*, and here it seems simply to serve as a vascular supply to the synangium. Miss Sykes herself seems to regard the lateral bundles in *Tmesipteris* as serving the purpose of supplying the large masses of developing spores with plentiful supplies of food and water,<sup>6</sup> and there

<sup>1</sup> Sykes, M. G. ('08), p. 77.

<sup>3</sup> Scott, D. H. ('07).

<sup>5</sup> Sykes, M. G. ('08).

<sup>2</sup> Boode, L. A. ('04), p. 511.

<sup>4</sup> Bower, F. O. ('08), p. 426.

<sup>6</sup> Sykes, M. G. ('08), p. 80.

seems no reason against supposing that these lateral branches have gradually replaced an original single central one. Moreover, in *Psilotum* ordinary leaves and sporophylls occur in exactly similar positions with regard to the axis, and sporophylls may take the place of leaves on the lower parts of the shoot, and leaves may replace sporophylls on the upper part. Again, the vascular supply of leaves and sporophylls in *P. flaccidum* arises in exactly the same way from the stele of the main axis. These facts all point to the homology of the sporophyll with the ordinary leaf.

As to the morphological value of the sporangiophore of the Psilotales, I share the feeling expressed by H. H. Thomas<sup>1</sup> with regard to this organ in the Equisetales, namely, that the evidence derived from the group under consideration is not conclusive in favour of any of the theories advanced to explain its nature.

#### SYSTEMATIC POSITION OF THE PSILOACEAE.

Recent views on the affinities of the Psilotaceae have lately been brought together so thoroughly by Lady Isabel Browne,<sup>2</sup> that it will be unnecessary to restate them all in detail, though it may perhaps be as well to emphasize some points in this connexion.

For long a relation between the Lycopodiales and Psilotaceae was recognized, so that the latter order was included in the former class. This resemblance is closest between *Lycopodium* and *Psilotum*, both genera having exarch protosteles, while lately an occasional occurrence of mesarchy has been observed in *Lycopodium*<sup>3</sup> as well as in *Psilotum*. The mode of branching is considered as dichotomous in both genera, both are microphyllous, and in the Psilotaceae the relation of the sporangiophore to the sporophyll is similar to that of the sporangium to the sporophyll in the Lycopodiales. If Lang's prothallus referred provisionally to *Psilotum*<sup>4</sup> actually belongs to that plant, it furnishes additional evidence of this relationship, for it is very similar to the prothallus of *Lycopodium clavatum*.

In 1897 Scott<sup>5</sup> pointed out the close resemblance existing between the sporangiophores of the Psilotaceae and those of the Sphenophyllales. This resemblance is shared by anatomical characters, for the stele of *Sphenophyllum* consists generally of a triquetrous exarch protostele with three protoxylem-groups, though sometimes each protoxylem-group is replaced by two. The normally exarch stele of *Psilotum* recalls that of *Sphenophyllum*, while the repetition of the triquetrous condition in the base of the aerial stem of *P. triquetrum*,<sup>6</sup> *P. flaccidum*, and *Tmesipteris*,<sup>7</sup> and in

<sup>1</sup> Thomas, H. H. ('09), p. 258.

<sup>3</sup> Sinnott, E. W. ('09), p. 138.

<sup>5</sup> Scott, D. H. ('97), p. 27.

<sup>7</sup> Dangeard, P. A. ('91), Pl. XII, Fig. 10.

<sup>2</sup> Browne, Lady Isabel ('09), p. 114.

<sup>4</sup> Lang, W. H. ('04), p. 571.

<sup>6</sup> Boodle, L. A. ('04), p. 514.



the smaller branches of the first species is at any rate interesting. The feeble secondary thickening in both species of *Psilotum* also recalls the normal secondary growth of wood in *Sphenophyllum*. As regards stem anatomy the comparison of *Tmesipteris* has been rather with *Cheirostrobos*, where mesarch xylem sometimes occurred. In this respect *Psilotum* probably approaches *Cheirostrobos* more nearly than *Tmesipteris*.

Scott's view has been adopted by A. P. W. Thomas<sup>1</sup> and Bower,<sup>2</sup> who go further than Scott and place the Psilotaceae as an order of the Sphenophyllales. The chief arguments against the view are to be found in the phyllotaxis and in the mode of branching. In the Sphenophyllales the leaves are borne in whorls, and from the constancy of the character throughout the group, Scott<sup>3</sup> is inclined to attach much importance to it. In the Sphenophyllales the branching was axillary, while in the Psilotaceae it is dichotomous. But as Bower<sup>4</sup> points out, branching is axillary in the Equisetales, yet dichotomy may occasionally occur, and it is easy to imagine that a group where both modes of branching were possible might give rise to one line of descendants where axillary branching prevailed, and to another where the branching was dichotomous.

Moreover, it seems to be not impossible that the branching in *Psilotum* is either now axillary, as Prantl supposed, or has been derived from an axillary mode of branching. The constant relation of a leaf to the stem bifurcation seems to me to support this opinion. This would remove one of the chief differences between the Psilotaceae and the Sphenophyllales, and in any case the fairly near relationship of the two groups seems to be almost proved. On the other hand, the resemblances between the Psilotaceae and the Lycopodiales seem too great to warrant the present tendency to separate the two phyla widely, and to me the most logical position appears to be that of Scott in 1907,<sup>5</sup> in which he regards the Psilotales as having most in common with the Sphenophyllales, but as possessing also some of the Lycopodineous affinities that have been previously attributed to them.

Lignier,<sup>6</sup> however, holds a very different view. To him the Psilotaceae are primitive types allied to the Lycopodiales, while the Sphenophyllales are related to the primitive Fern stock through *Archaeopteris*. Further back still the Lycopod phylum and Fern phylum are connected, the common ancestor consisting of a dichotomizing axis bearing small appendages or phylloids. These are represented to-day in the leaves of the Psilotaceae and Lycopodiales. In the Fern phylum branches of the axis were specialized for assimilatory purposes and became leaves.<sup>7</sup> Thus, on Lignier's view, the leaves of the Psilotaceae and Sphenophyllales are not homologous, and

<sup>1</sup> Thomas, A. P. W. ('02), p. 350.

<sup>3</sup> Scott, D. H. ('07), p. 166; ('09), p. 626.

<sup>5</sup> Scott, D. H. ('07), p. 166.

<sup>7</sup> Cf. Tansley, A. G. ('08), p. 6.

<sup>2</sup> Bower, F. O. ('08), p. 423.

<sup>4</sup> Bower, F. O. ('08), p. 424.

<sup>6</sup> Lignier, O. ('03), p. 95; ('08), p. 278.

the two groups are connected so far back that the gap between them is about as great a one as exists between any two groups of the Pteridophyta. The arguments against Lignier's view have been put forward very clearly by Lady Isabel Browne,<sup>1</sup> and it is unnecessary to repeat them here. The suggestion of an axillary mode of branching in the Psilotaceae might, however, add another objection to the list, and the resemblance between the Psilotaceae and Sphenophyllales appear too pronounced to warrant their distant separation.

It seems to me quite possible that the Psilotaceae may connect the Sphenophyllales and Lycopodiales, while at the same time *Archaeopteris* may be a connecting link between the Sphenophyllales and the Ferns. It should, however, be noted that Kidston<sup>2</sup> regards the Archaeopterideae as Pteridosperms and not Ferns at all. Moreover, it must be admitted that our knowledge of *Archaeopteris* is so slight that this form can at present be of little use in phylogenetic considerations.

In any case, whether the Psilotaceae are reduced or primitive, the evidence at present available seems to indicate a relationship between these forms and the Sphenophyllales, and also, though less marked, with the Lycopodiales.

#### SUMMARY.

1. In external appearance the aerial stems of *Psilotum flaccidum* differ from those of *P. triquetrum* in being rounded below, triquetrous above this, and ultimately flattened. In the triquetrous region branching probably takes place in planes successively at right angles; in the flattened part branching is all in one plane. A leaf seems to be constantly associated with stem bifurcation.

2. The stem-stele is band-shaped in the lowest part of the aerial shoot, but becomes triquetrous. When the stem is triquetrous the xylem-mass is typically hexarch; in the flattened parts the stele changes from a hexarch to a tetrarch condition.

3. The leaves often receive a vascular supply, and when this is the case with a leaf below the stem fork, the leaf-trace is given off so that one branch of the stele is practically in the axil of the leaf-trace.

4. Secondary thickening similar to that found in *P. triquetrum* is also found in *P. flaccidum*.

5. Mesarch structure occurs occasionally in the lower part of the aerial stem, sometimes in connexion with the leaf-traces.

6. The sporangiophore-trace is given off in the same manner as a leaf-trace. It is continued into the sporangiophore, and terminates in the central tissue between the three confluent sporangia. It is thus similar in position to the median bundle in the synangium of *Tmesipteris*.

<sup>1</sup> Browne, Lady Isabel ('09), p. 115.

<sup>2</sup> Kidston, R. ('06), p. 434.

7. The sporophyll is probably homologous with a foliage leaf. The evidence is insufficient to decide whether the sporangiophore is foliar in nature or is an organ *sui generis*.

8. The Psilotales are probably allied to the Sphenophyllales and Lycopodiales, but show greater resemblances to the former.

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## EXPLANATION OF PLATE XXV.

Illustrating Mr. Stiles's Paper on *Psilotum flaccidum*.

Fig. 1 is from a photograph by W. Tams of Cambridge.

Fig. 1. Part of aerial stem of *Psilotum flaccidum*. Natural size.

Fig. 2. Transverse section of the base of the aerial stem of *P. flaccidum*, showing secondary xylem elements.  $\times 117$ .

Fig. 3. Transverse section of the stem, showing triquetrous stele.

Fig. 4. Transverse section of triquetrous stem with hexarch xylem. In the middle of the xylem are some fibres, while a few parenchymatous cells also occur in the xylem.

Fig. 5. Transverse section through the end of a xylem arm of the stele, showing very distinct mesarch structure.  $\times 176$ .

Fig. 6. Transverse section through a flattened branch with tetrarch stele.

Fig. 7. Phloem in longitudinal section.

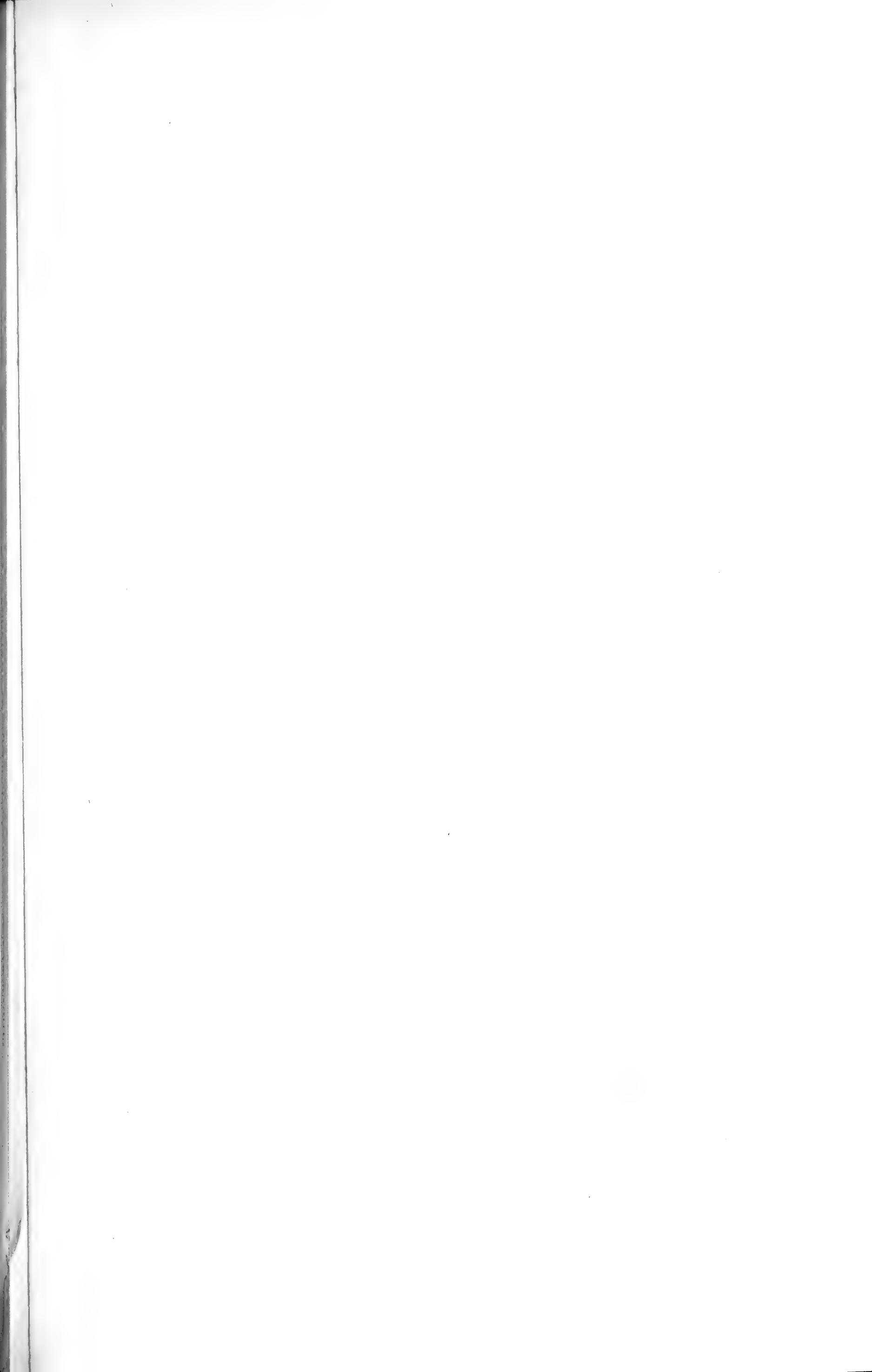
Fig. 8. Epidermis and assimilating layer of stem in transverse section.  $\times 160$ .

Fig. 9. Transverse section showing bifurcation of tetrarch stele with leaf-trace on one side about to be detached.  $\times 117$ .

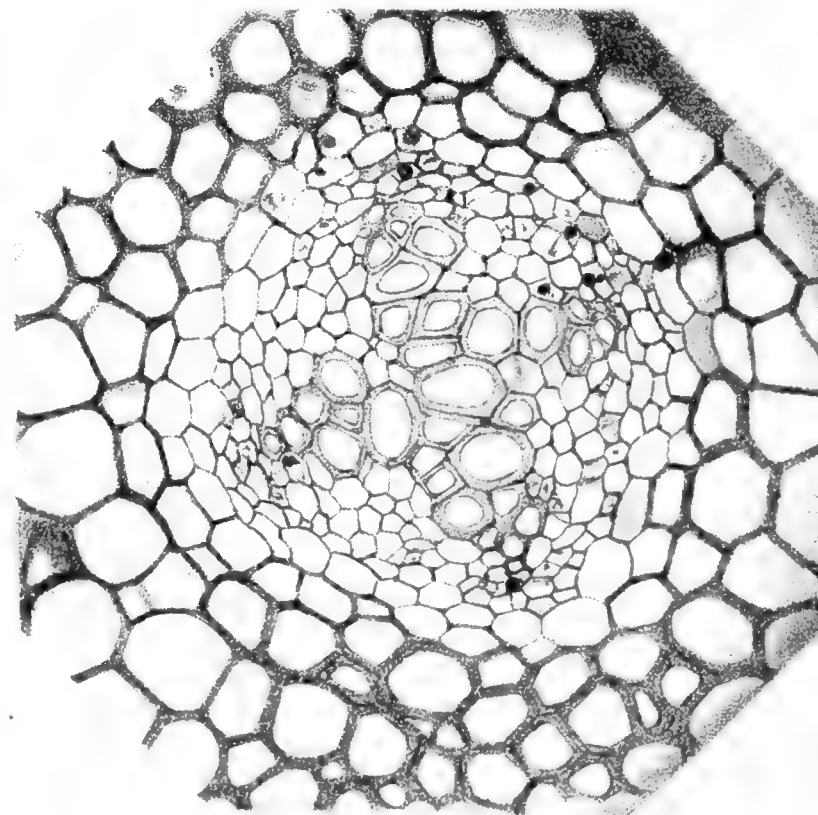
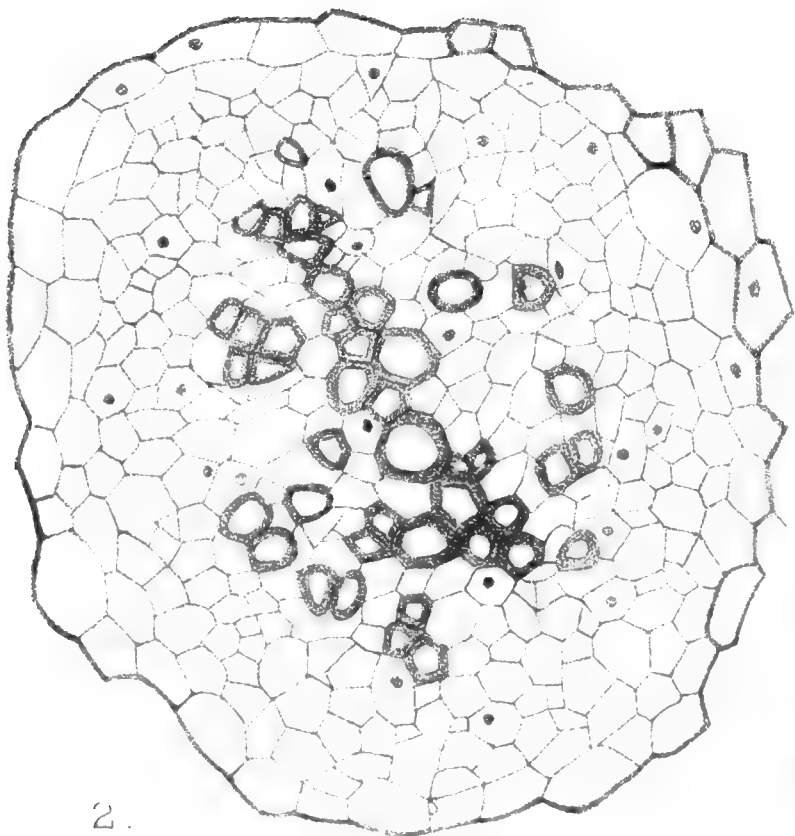
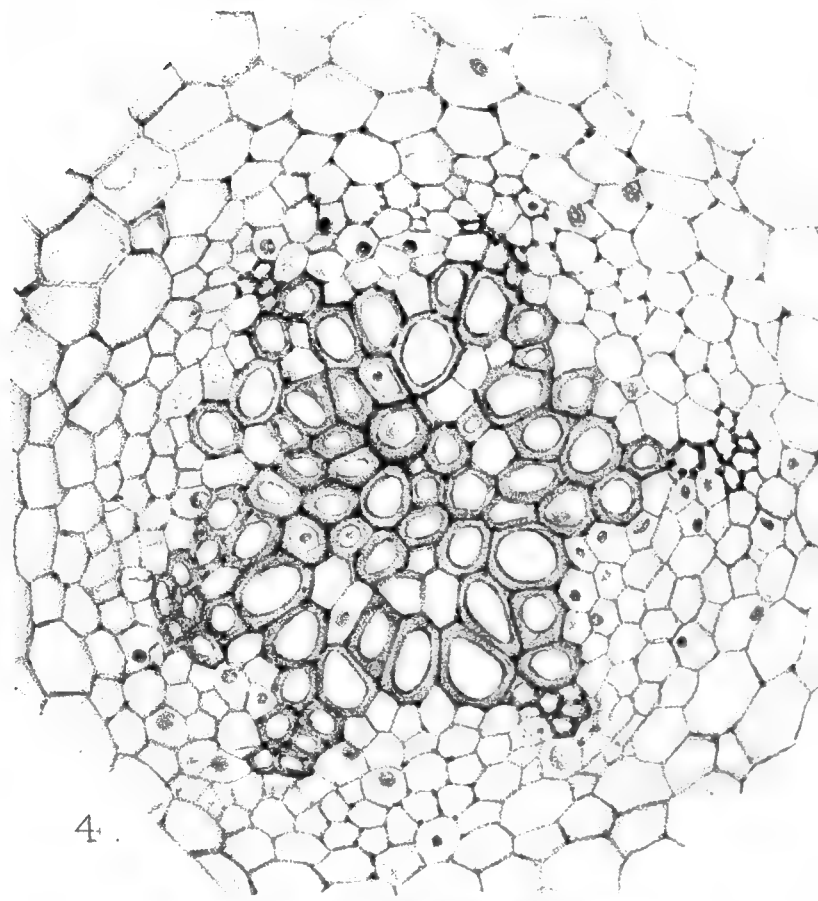
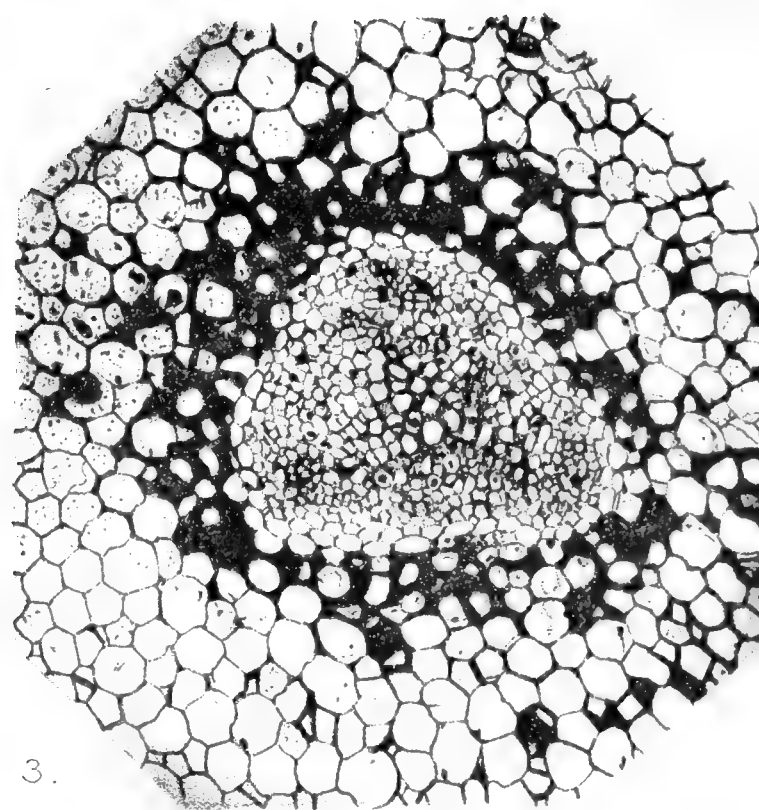
Fig. 10. Longitudinal section of the leaf.  $\times 57$ .

Fig. 11. Sporophyll-trace in transverse section.  $\times 176$ .



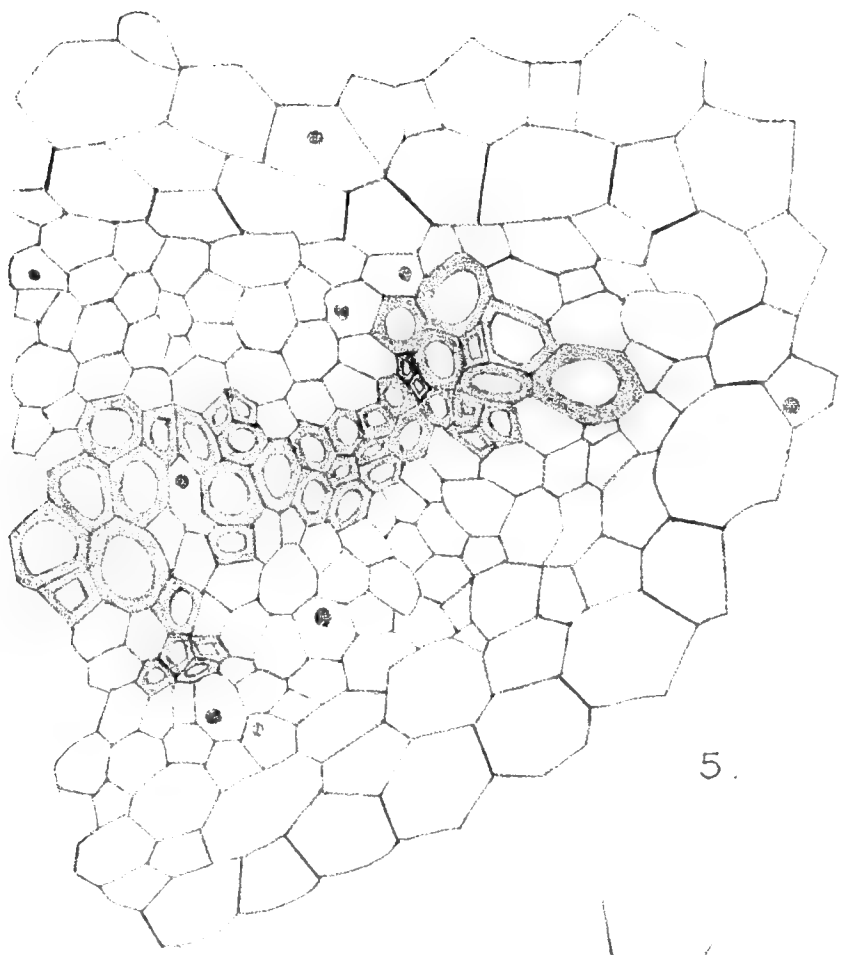




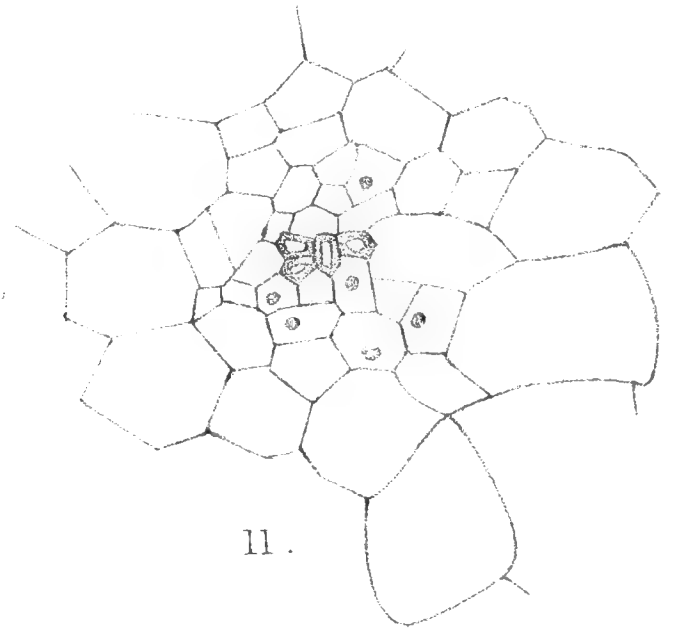




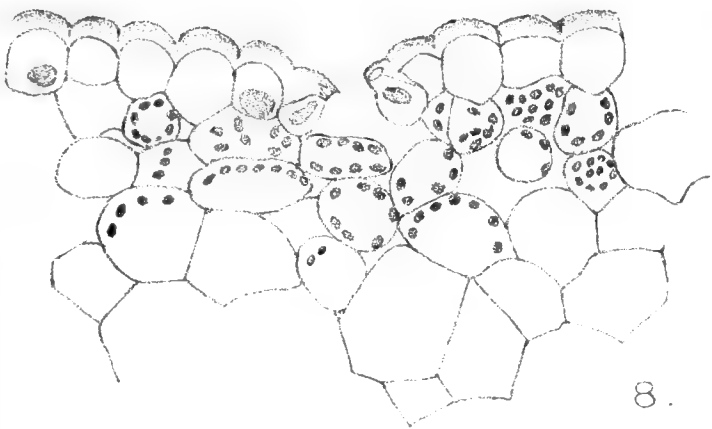
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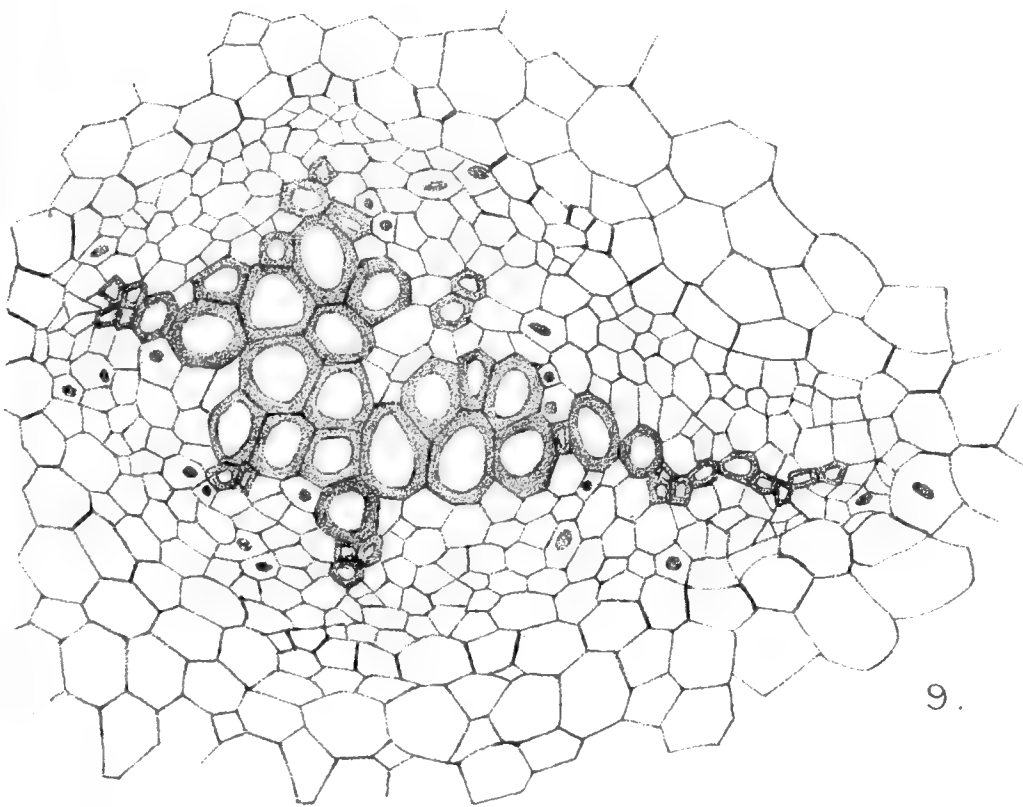
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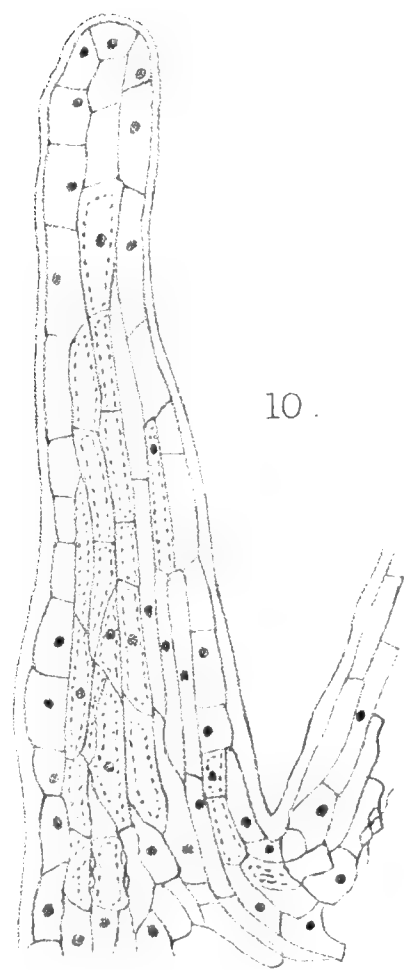
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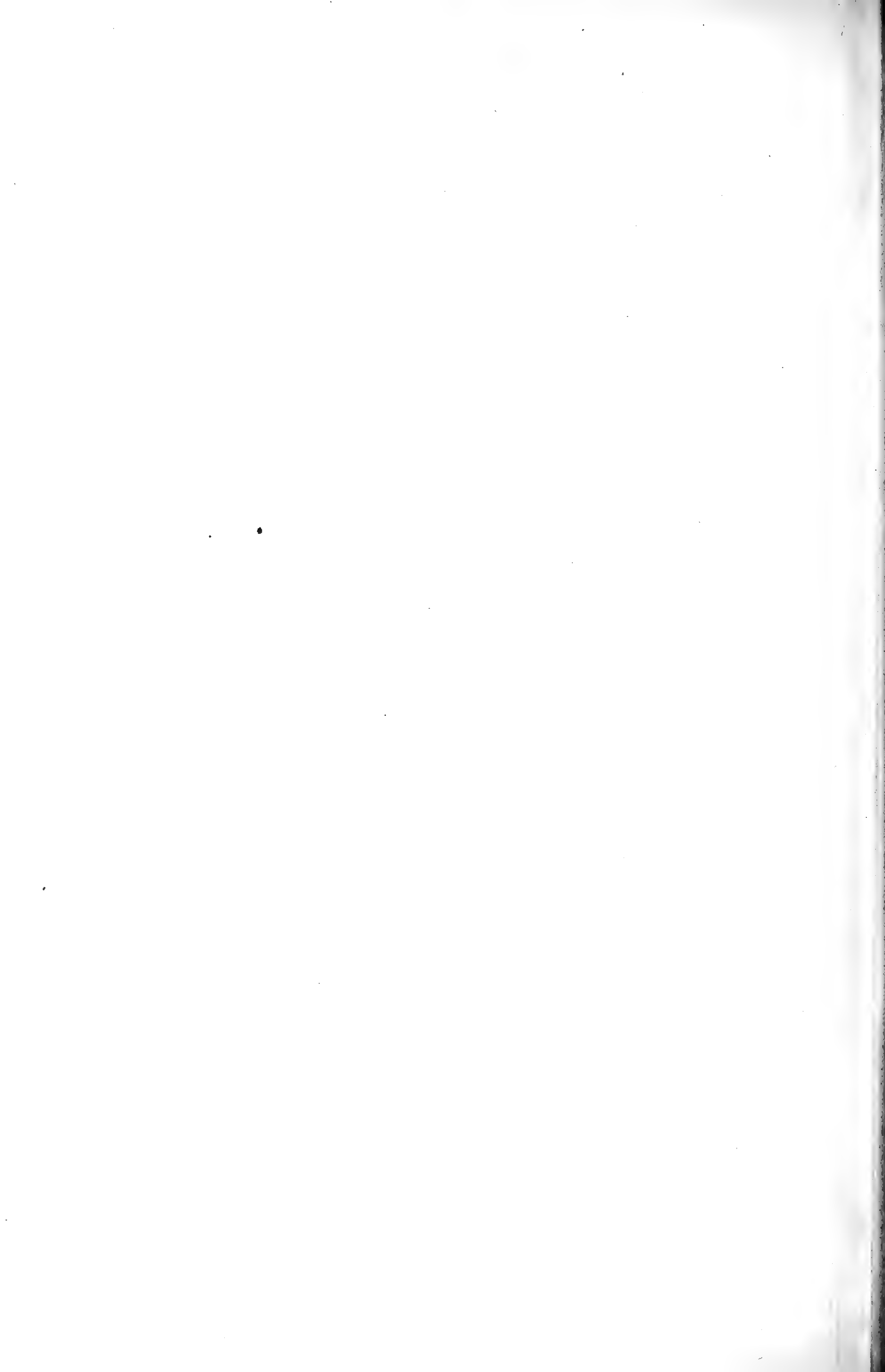
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10.



# The Internal Anatomy of 'Nilssonia orientalis'.

BY

MARIE C. STOPES, D.Sc., Ph.D., F.L.S.

*Lecturer in Palaeobotany at Manchester University.*

With Plate XXVI and a Figure in the Text.

AMONG the desiderata of Botany is information regarding the anatomy of the numerous plants in the Mesozoic rocks which are known only from impressions. The uncertain value of external impressions for phylogenetic and systematic work has often been emphasized in recent years.

Even among the Palaeozoic plants, so many of which are known by their internal anatomy, it is a rare and fortunate chance, or series of chances, which gives both internal anatomy and external impression of the same specimen. Such a fortunate chance has materialized in a nodule of Cretaceous age in the case of the plant hitherto known only as a leaf impression by the name *Nilssonia orientalis*, Heer.

Foliage impressions of this species have been described several times from Mesozoic rocks from the Orient. Nathorst<sup>1</sup> figures impressions of the plant in his 'Beiträge zur Mesozoischen Flora Japans', Yabe<sup>2</sup> figures fragments from Korea, and Yokoyama<sup>3</sup> records the same species for Japan. So that we know that the species was well distributed in the Orient in the Mesozoic times.

Among my specimens of Cretaceous, plant-containing nodules from Japan was one which revealed, when the matrix was broken, a fragment of a fairly sharp external impression of foliage. The stone split so as to expose part of the leaf in surface view, and to retain the bulk of it embedded in its matrix. The exposed portion (see Fig. 1, Pl. XXVI) was enough to identify the leaf as that known as *Nilssonia orientalis*, Heer, and it was found possible to cut a series of sections through the remainder of the leaf. These sections, though not so perfect as could have been desired, yet show anatomical petrifications of the tissues, from which it is possible to reconstruct

<sup>1</sup> Nathorst, A. G. : Beiträge z. Mesozoischen Flora Japans. Kais. Akad. d. Wiss. Wien, 1890, pp. 2-20, Pl. I-VI.

<sup>2</sup> Yabe, H. : Mesozoic Plants from Korea. Journ. Coll. Sci. Tokio, xx, 1905, pp. 1-59, Pl. I-IV.

<sup>3</sup> Yokoyama, M. : Jurassic Plants from Kaga, Hida, and Echizen. Journ. Coll. Sci. Tokio, iii, 1889.

the anatomy of the leaf. There are also several other fragments of the same foliage in the matrix. From all these sections the following anatomical description is compiled.

#### DESCRIPTION OF LEAF.

*External appearance.* As is seen in the small portion of the leaf shown in Fig. 1, Pl. XXVI, the blade is about 4 cm. across, with a midrib from which laterally running veins pass out straight to the margin. These veins are about 0.5 mm. apart. The character of the venation is better shown in the drawing, Fig. 4, Pl. XXVI, where the veins are seen to run undivided from midrib to margin, as is characteristic for the species in most cases, though they may branch a little.

*Internal anatomy.* The leaf shows no marked differentiation of an upper and lower surface. As there is no palisade tissue, the bundle alone exhibits a distinct indication as to which side is uppermost. The sections are cut across the lamina, at right angles to the laterally running veins, and therefore parallel to the midrib.

*The epidermis.* In the well-preserved portions of the leaf the cells of the upper and lower epidermes are alike in character, though in the less favourably petrified regions those of the lower epidermis are much the more obliterated of the two. The individual cells are squarish, about 0.02 mm. in diameter, and are not markedly different in size from the mesophyll beside them. See *e.*<sup>1</sup> and *e.*<sup>2</sup>, Text-figure, and *e*, Figs. 2 and 3, Pl. XXVI.

The cuticle does not seem to have been noticeably thickened, and there is no sign of hairs or protuberances.

*Stomata* are not recognizable on the upper surface, which appears to have been clothed by an unbroken epidermis. In several sections they are to be seen in the lower epidermis, lying in the portion of the leaf between the bundles (Text-fig., *st.*). They are not quite perfectly petrified, but the guard-cells seem to have been placed at the oblique angle usual in Gymnosperms.

*The mesophyll.* The *ground tissue* of the mesophyll shows no special differentiation; the cells are roughly roundish or oblong in shape (see Text-fig.), and are arranged so as to leave but little space between them. There seems to be none of the differentiated transfusion tissue common in modern Cycad leaves.

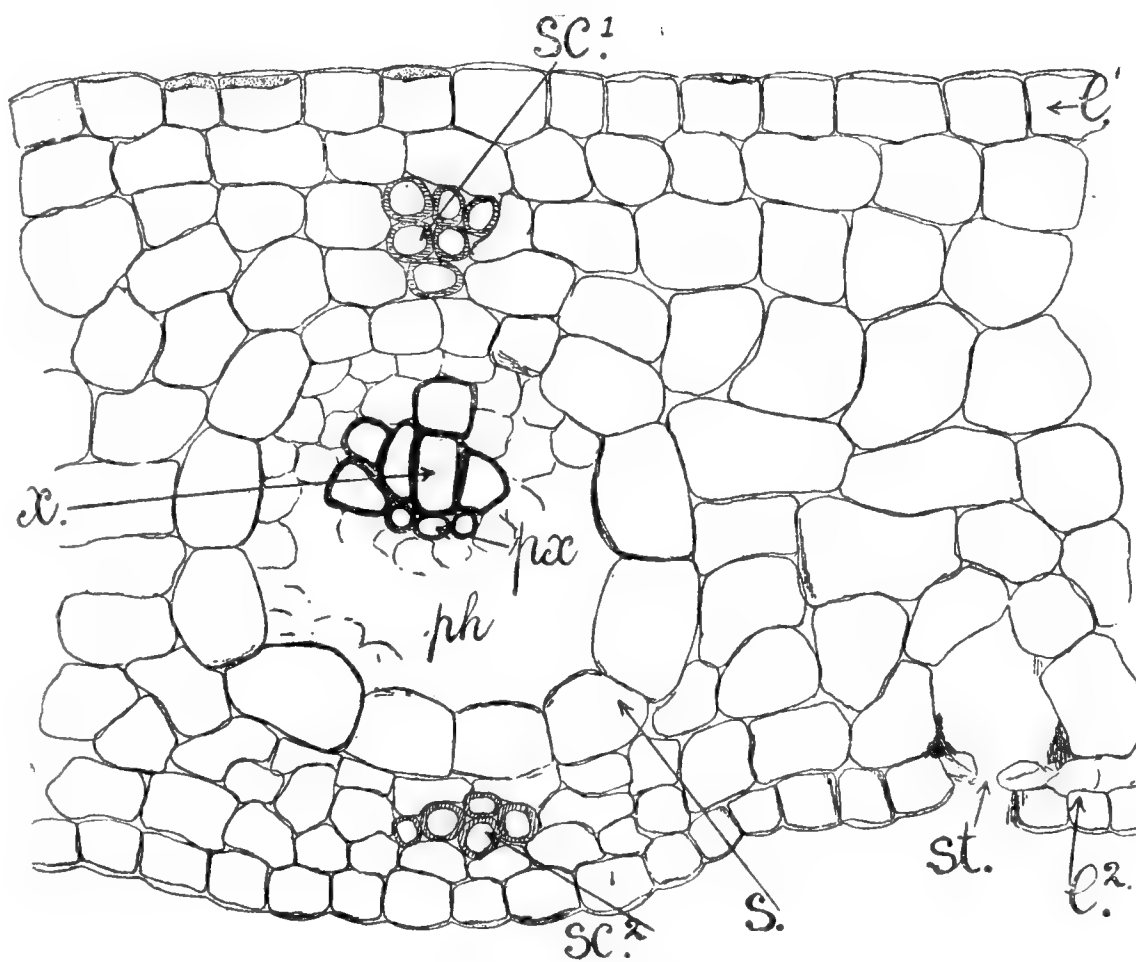
*Sclerenchyma* appears to be developed only in small groups of three or four cells above and below the vascular bundles; see *sc.*<sup>1</sup> and *sc.*<sup>2</sup>, Text-figure. The cells are not very much thickened.

*Resin-canals.* I cannot speak dogmatically about the presence or absence of these structures. They are certainly not present in the way they are in living Cycads, namely, in direct relation to the vascular bundles, either between each pair as in *Encephalartos* or above each as in *Dioon*.



From most of the sections of the fossil one might conclude that resin-canals were absent, as they are in many Cycad genera.

Two sections, however, clearly show canals which have every appearance of being resin-ducts. This is seen in Figs. 2 and 3, Pl. XXVI, *r*. In both the leaf-sections which show the phenomenon, there is only one canal in the tissues. It lies near to the edge of the leaf, as is seen in Fig. 2, Pl. XXVI. The appearance of the structure is exactly that of a resin-canal, with its epithelial lining partly preserved (see Fig. 3, Pl. XXVI). From this, it seems very reasonable to conclude that the canal is really a resin-duct. It lies between two bundles, and is large compared to them.



TEXT-FIGURE. Slightly diagrammatic drawing to show the anatomy of the leaf of *Nilssonia orientalis*, Heer. *e*.<sup>1</sup>, upper, and *e*.<sup>2</sup>, lower epidermis. *st.*, stoma, only on the lower side. *sc*.<sup>1</sup> and *sc*.<sup>2</sup>, groups of sclerenchyma above and below the bundle. *s.*, bundle-sheath. *px*, protoxylem. *x.*, xylem, which appears to be entirely centripetal. *ph*, space, in which are fragments of badly petrified phloem.

As is seen in Fig. 2, Pl. XXVI, the canal lies near the edge of the leaf, which must have been towards the apex in this section, and it is not impossible that an early type, such as the plant we are considering, may have had a large resin-canal near the border of the leaf, without having others regularly between each pair of bundles.

*The vascular bundles.* The bundles lie approximately equidistantly in the leaf, about 0.5 mm. apart, and are simply collateral in structure.

The *bundle-sheath*, though not highly specialized, is clearly recognizable round several of the better-preserved bundles. It consists of large, roundish cells (*s.*, Text-fig.), on which I have not been able to observe any pitting.



The *xylem* of the bundle is apparently entirely *centripetal*, for the small elements which appear to be protoxylem lie on the side towards the middle of the bundle (see *px*, Text-fig. and Fig. 4, Pl. XXVI). There are two or three of the small protoxylem-elements and about half a dozen cells of metaxylem averaging 0.01 mm. in diameter (*x.*, Text-fig.).

The *phloem* is hardly preserved, the only indication of its nature being the fragments of walls in the space below the xylem.

Between the xylem and the bundle-sheath a few cells of soft parenchyma are preserved.

To sum up. The leaf has not a particularly differentiated epidermis or mesophyll. The bundles have each a fairly distinct bundle-sheath, and there are small strands of sclerenchyma above and below. The wood is entirely centripetal. The resin-canals appear to be very few in number, normal in structure, but very large, and running near the edge of the leaf.

The leaf has a structure which is reminiscent of Cycads, and which might be looked upon either as primitive, or as lacking specialization owing to its habitat. What the habitat was we do not know. Although in living Cycads the xylem is usually mesarch, the bulk of the wood is centripetal and the centrifugal elements are apt to degenerate towards the apex, and in some species there are always very few of them. Those species of living Cycads which have resin-canals at all have them in numerical proportion to the bundles, usually one to each. But as some of the living Cycads have no resin-canals at all, it is not surprising to find a fossil in which so few are developed.

The fact that all the wood in the bundle is centripetal might, not unreasonably, suggest a comparison with the several species of *Cordaites* in the Palaeozoic petrifications which had only centripetal wood. I do not wish, however, to enter here into such a theoretical discussion about structures of the real phylogenetic value of which we know so little. *Cordaites* has been pressed into service rather frequently of late.

For the present it suffices to describe the leaf as one which has a distinctly Cycad-like structure, but is simpler in general organization and in its vascular bundle than the living Cycad leaves. The fact that all its wood is centripetal might reasonably be considered as a primitive feature.

A word must be said as regards the position of the species *orientalis* in the genus *Nilssonia*. Most writers place the Nilssonias in the Cycadophyta, a classification which is confirmed by Nathorst's last monograph.<sup>1</sup> *Nilssonia orientalis*, Heer, resembles *N. tenuinervis*, Nath., and differs

<sup>1</sup> Nathorst, A. G.: Über die Gatt. *Nilssonia* Brongn. Kongl. Svenska Vetenskapsakad, Bd. xliii, No. 12, 1909.

somewhat from the rest of the species, coming closer to *Taeniopteris* in general appearance. Seward<sup>1</sup> says (p. 123), 'Heer's figures of *Nilssonia orientalis*, Heer, probably represent a *Taeniopteris*.' And Nathorst (l. c., p. 29) also considers *N. tenuinervis* as doubtfully a *Nilssonia*, and that its affinity is more rightly indicated by the name *Nilssoniopteris*.

Judged merely from the external features, this fine-veined, entire form of *Nilssonia* does seem to come nearer the genus *Taeniopteris*. But as that genus is most probably an artificial one, like many of the Palaeozoic foliage genera, the definition of its limits is not really important at present. Whether the plant I am describing belongs to the one group or the other, it has been described and is well known under the name *Nilssonia orientalis*. The internal anatomy of its foliage is now discovered, and has the features described above, which are clearly Gymnospermic rather than fern-like. They are, moreover, quite of the type that one might hold to be primitively Cycadean.

<sup>1</sup> Seward, A. C.: Catalogue of the Mesozoic Plants. The Wealden Flora, Pt. I. British Museum, London, 1894.

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## DESCRIPTION OF PLATE XXVI.

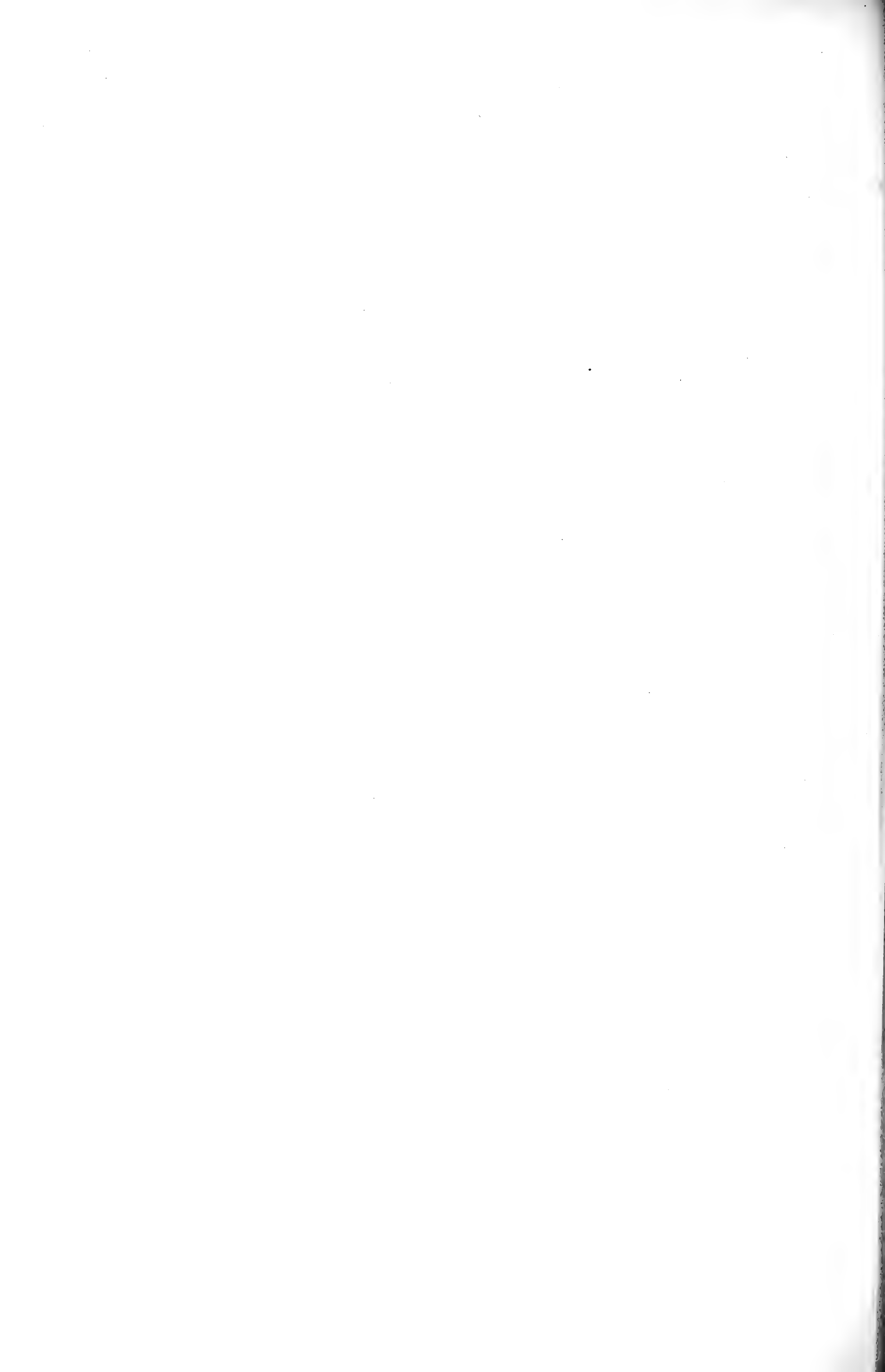
Illustrating Dr. Marie C. Stopes's Paper on *Nilssonia orientalis*.

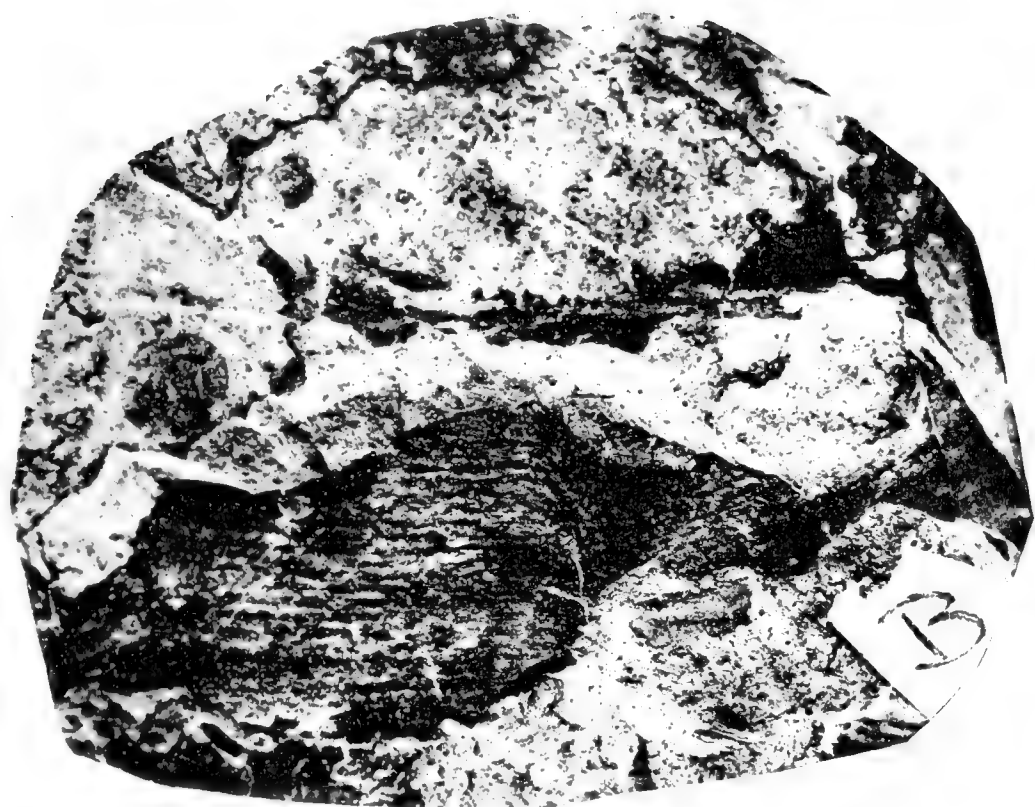
Fig. 1. Photograph of part of the broken nodule showing a portion of the leaf of *Nilssonia orientalis*, Heer, as an impression.  $\times 2$ .

Fig. 2. Photograph of part of the leaf in the matrix, showing the petrification of its internal anatomy. *ed.*, the edge of the leaf. *r.*, resin-canal and *v.*, the vascular bundle near it. *e.*, the upper epidermis.

Fig. 3. Enlarged photograph of the resin-canal and the bundle beside it. *e.*, upper epidermis. *px.*, protoxylem of the bundle. *r.*, resin-canal, with some of its epithelial lining, *ep.*

Fig. 4. Drawing of part of leaf of *Nilssonia orientalis*, natural size.



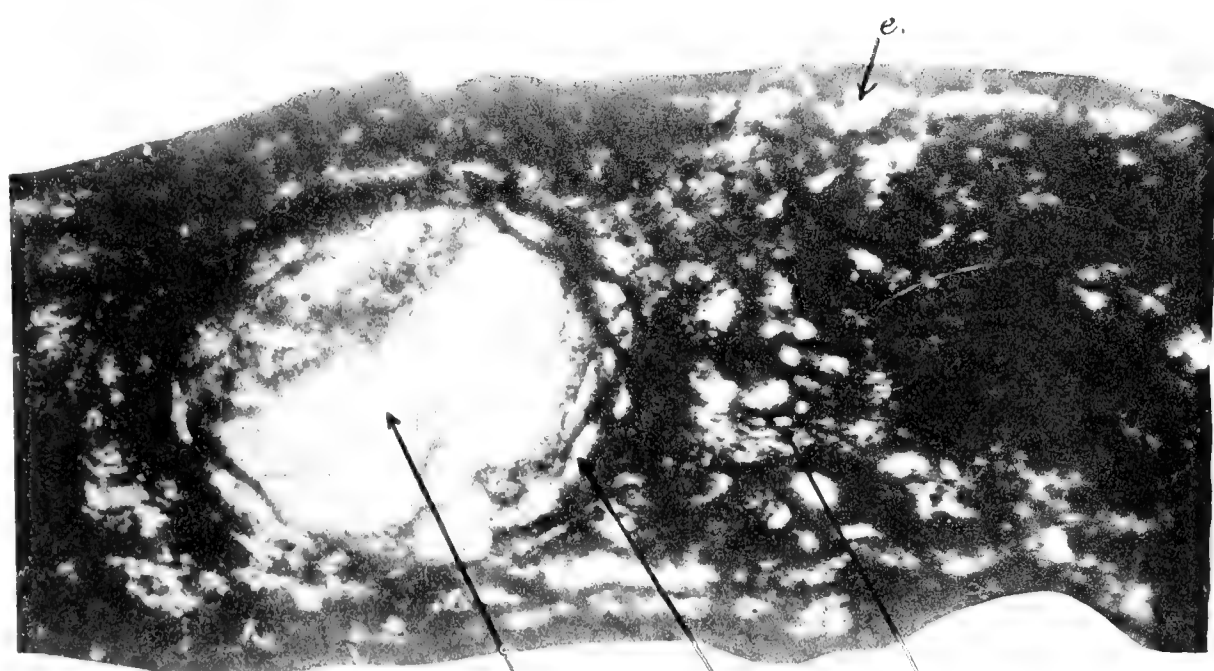


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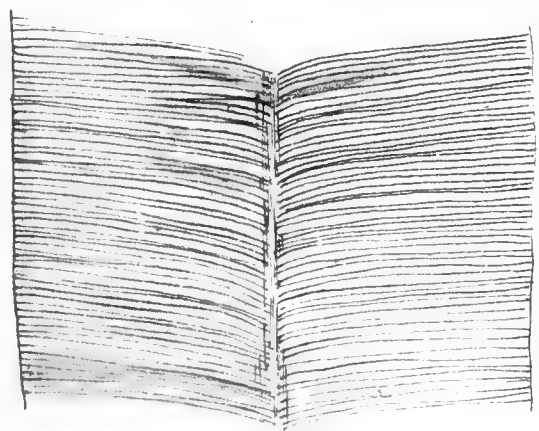


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Hirth, coll.



3.



4.

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# The Anatomy of Cretaceous Pine Leaves.

BY

MARIE C. STOPES, D.Sc., Ph.D., F.L.S.

AND

E. M. KERSHAW, M.Sc.

With Plates **XXVII** and **XXVIII**.

*OBJECT of the paper.* To describe and illustrate the anatomy of two new species of Pine leaves from the Cretaceous of Japan, and to consider them in relation to those described from the Cretaceous of America and living forms.

*Introductory remarks.* The Coniferae are the only large group of plants of which it can be said that we have any considerable knowledge of the representatives in the Cretaceous period.

The interesting monograph on American Cretaceous Coniferae by Hollick and Jeffrey ('09), which brings together the numerous facts obtained and published already by these two authors, gives to the botanical world a solid basis for the construction of a conception of the characters of the group during Cretaceous times; but although a foundation is thus already laid, much remains to be done to complete our knowledge of the group, because the only source of anatomical information has hitherto been these American deposits. It seems to be worth while consequently to describe two new species of leaves of this affinity, which come from the Upper Cretaceous of another continent.

The specimens were obtained by one of us (M. C. S.) in Japan, in those mineral nodules which have been discovered recently, and open out the possibility of obtaining data of the anatomy of a mixed flora of Cretaceous age (see Stopes and Fujii, '10). The Gymnosperm leaves now to be described are both represented by isolated leaves, lying in the granular matrix of the nodule among other plant petrifications, some of which were Gymnosperms, others Angiosperms and Ferns. But although the species are thus only known from isolated leaves, the petrification of their anatomy is so excellent that their specific characters can be determined satisfactorily.



## PREPINUS JAPONICUS, spec. nov.

*Description of leaf.* In the specimen we have a length of about 1.2 cm. of the leaf, in the course of which it does not appreciably vary in shape or diameter. The photograph on Pl. XXVII (Phot. 1) represents the shape of the leaf in cross section. It appears to have been slightly crushed and contracted, as the assimilating tissue between the sclerenchyma and the transfusion tissue shows, but this crushing has altered the shape of the leaf very slightly, if at all. The greatest diameter of the leaf is 1.5 mm., and it has five sides.

In its shape the leaf closely resembles that of *Prepinus statenensis* as described by Jeffrey ('08). He notes (p. 208) that in its polygonal outline his leaf differs from any species of living *Pinus*, and he correlates this form with the fact that the leaves are in fascicles in numbers together, and not as in modern Pines in clusters of two, three, or five.

The *epidermis* is composed of small, squarish cells with very thick outer walls. The stomates are distributed on all sides of the leaf, and average about ten on the upper surface (the longest edge), six or seven on the two lower edges, and two or three on the lateral ones. They are of the normal Gymnosperm type, with sunken guard-cells placed at an oblique angle. In the intercellular space beneath them, fungal hyphae are to be seen in a good many cases in the specimen. Photograph 2, Pl. XXVII, shows an excellently preserved pair of guard-cells between the sclerenchyma patches on either side.

The *hypodermal sclerenchyma* is much broken up owing to the numerous stomates, and forms triangular groups with the point of the wedge towards the centre of the group (see Pl. XXVII, Photos. 1 and 2, and Pl. XXVIII, Fig. 1, *scl.*). The individual cells are often extremely thick-walled, and their pit canals are often visible.

The *assimilating tissue* lies in small patches between the sclerenchyma and in a narrow zone outside the large central mass of transfusion tissue. In the sections this tissue is generally poorly preserved and crushed, but here and there its cells are visible, and show that the tissue was only five or six cells deep. In a few cells are indications that the walls were infolded as in the living *Pinus*, but this is not very clear.

The *resin-ducts* are two in number, laterally placed, slightly towards the upper side of the lateral edges of the leaf. They lie between the sclerenchyma and the transfusion tissue, almost filling the distance between them (see Pl. XXVIII, Fig. 1, *g*). The duct is 0.07 mm. in diameter, and is surrounded by thirteen to fourteen epithelial cells.

The *vascular tissue*, if the transfusion zone surrounding the bundle proper be included, bulks largely in the leaf. In this it closely resembles the American *Prepinus*.

The actual *bundle is double*, and is surrounded entirely by a sclerized zone or sheath, which forms a tongue of hard tissue between the two strands (see Pl. XXVIII, Fig. 1, *v.sc.*). These sclerized cells are small, and have not such thick walls as the hypodermic sclerenchyma. They are comparable to the sheath cells noted by Jeffrey ('08, Pl. XIV, Fig. 17) in his Cretaceous Hard Pines. They are not represented in his *Prepinus* as splitting the bundle, but otherwise seem much like the inner thick-walled sheath he describes. Whether it is right to compare this sheath with that described by Stopes ('03) for *Cordaites*, is a point about which we wish to reserve our opinion for the present.

The *xylem* in the two strands in our leaf is in small quantities, and the elements very small, 0.01 mm. in diameter. The position of the phloem is apparent, but its cell-structure is not sufficiently well petrified for description. There is no indication of centripetal xylem or other unusual structure in the bundle.

The *transfusion sheath* is a broad zone of closely packed, fairly uniform cells (see *t*, Phot. 1, Pl. XXVII, and Fig. 1, Pl. XXVIII) about 0.05–0.06 mm. in diameter. These fit closely together, have fairly thick walls, and appear to have been pitted, though only in a few cases are the pits still to be seen on their walls. There seems to have been no admixture of parenchyma cells with them. The outer zones of these cells may possibly be looked on as something of the nature of an endodermis: in the outermost row of cells the radial walls show an appearance suggestive of a slightly crushed endodermis, but the sheath is not a distinctive one.

Jeffrey ('08, p. 211) notes the absence of an endodermis in his *Prepinus*, but his photographs do not absolutely determine this point, and the sections of the American *Prepinus* which Prof. F. W. Oliver of London University kindly lent us do not determine it either.

*The reasons for placing our leaf in the genus Prepinus* are as follows. Neither Jeffrey ('08) nor Hollick and Jeffrey ('09) give an actual diagnosis of their genus. When we pick out the more essential features from Jeffrey's description, however, they are: (1) the contour of the leaf, with five approximately plane surfaces, which differs from any known *Pinus*; (2) the number of leaves in a fascicle; (3) the two resin-canals, placed laterally; (4) the broad transfusion zone; (5) the sheath of thick cells round the bundle; (6) the single bundle; (7) the large amount of centripetal wood in the bundle; (8) the apparent absence of an endodermis; (9) the presence of strong hypodermal isles beneath the epidermis.

Jeffrey places much stress on the shape of the leaf and the correlation of this with the number of leaves in a fascicle, in which this fossil genus differs from any type of *Pinus*. In his view regarding this feature we entirely concur, and think that for diagnostic features it is the most important.

Our new species, from the description given above, will be seen to agree with *Prepinus* of Jeffrey in all of the nine points except as regards the absence of centripetal xylem and the splitting of the bundle by a tongue of the inner thick-walled sheath. These two features, though of great *specific* importance, do not appear to us to weigh sufficiently against all the other points of agreement to exclude our species from the genus *Prepinus*. Further, only leaves are known in both cases, and among fossils, until the plants are known fairly completely, it is always a pity to multiply genera. For the present then we include our species in the genus *Prepinus* of Jeffrey, and as he has given no diagnosis of the genus, we offer one now.

PREPINUS, Jeffrey, 1908.

Gymnospermic foliage resembling *Pinus*, but with many leaves in fascicle. In section the leaf has five straight sides; two lateral resin-ducts; a large zone of transfusion tissue round the bundle which may or may not be divided; endodermis apparently absent; hypodermal sclerenchyma strands strongly marked.

Only species:—*Prepinus statenensis*, Jeffrey, described *Annals of Botany*, 1908.

Our species separates itself from Jeffrey's through the absence of centripetal xylem, and the splitting of the bundle by the tongue of small thick-walled cells of the sheath.

*Prepinus japonicus*, spec. nov.

Leaf 1.5 mm. in diameter, central bundle split into two strands by the inner thick-walled sheath, no centripetal xylem recognizable.

Locality:—Upper Cretaceous, Japan. Collected by M. C. Stopes.

Type:—the figured slide has been presented to the British Museum, Department of Geology.

*Discussion.* The characteristic shape of our leaf, and its similarity to those leaves of *Prepinus* for which the feature was discussed by Jeffrey ('08, p. 208), seem sufficient ground for presuming that the leaves were in a multifoliar fascicle as they were in *Prepinus statenensis*. Our leaf is so characteristic and so remarkably like Jeffrey's in all important particulars save the absence of centripetal wood, that it seems to us to leave no doubt that it is truly of the same genus. It is only necessary to think of the living Cycads, in some species of which centripetal wood is found in the axis though it is absent from others, to realize that the point is hardly one of generic distinction. Our leaf comes from a geological horizon slightly more recent than the one which yielded the American specimens, and one might be tempted to present the view that the loss of the centripetal xylem was correlated with this. One might also point to the fact that the younger

species approximated also to the modern type of *Pinus* in having a bundle, not separated actually into two as is frequent in the modern species, but split by the ingrowth of the inner thick-walled sheath.

For the moment, however, we find facts more attractive than theories, and will describe another species of leaf from this horizon.

PINUS YEZOENSIS, sp. nov.

This species resembles much more closely the living *Pinus* than do any of those described by Jeffrey. As is seen in Phot. 3, Pl. XXVII, it is oval in section, with no straight side, and has a very well marked endodermis round the bundle and comparatively small transfusion zone. The leaf is about 1.1 mm. in diameter.

The *epidermis* consists of small, rather roundish cells, about 0.01 mm. in diameter. The stomates are of the usual Gymnosperm type and are few in number.

The *hypodermal sclerenchyma* consists of a zone, two or three cells deep, of small cells with but slightly thickened walls.

The *assimilating tissue* of the leaf is well preserved, and consists of large cells (0.08 mm. in diam.) entirely like those of modern Pines, with curved walls folded into ridges projecting into the cell cavity (*a* in Phot. 4, Pl. XXVII, and Fig. 2, Pl. XXVIII).

The *resin-canals* are two in number, placed laterally and towards the upper side of the leaf. They lie immediately under the hypoderm; see *g*, Phot. 5, Pl. XXVII. Each canal is about 0.035 in diameter, and is surrounded by a dozen epithelial cells which are roundish in outline, and very well petrified; see *ep*, Phot. 5, Pl. XXVII.

The *vascular tissue* is surrounded by a large, well defined *endodermis*. This is seen clearly in Photos. 3 and 4, Pl. XXVII, and Fig. 2, Pl. XXVIII, *en*, and is entirely similar to the endodermis in the living Pines.

The individual cells are large and oval, about 0.06 in diameter, and their radial walls fit together and appear to have been slightly thickened.

In view of Jeffrey's ('08, p. 216) statements about the American Cretaceous Pines, the exceedingly clear endodermis and the infolded walls of the mesophyll are both noteworthy features of this species.

A few *sclerized cells* occur within the endodermis, and these may possibly represent the remains of an inner thick-walled sheath such as was noted in *Prepinus*. There are one or two cells at the sides of the bundle which may have been transfusion cells, but the bulk of the tissue within the endodermis consists of the vascular tissues proper.

The *xylem* is arranged in radial series, a dozen or more rows of tracheides separated very widely by large medullary ray cells (see Phot. 4, Pl. XXVII, and Fig. 2, Pl. XXVIII, *m* and *x*). The xylem appears to have been entirely centrifugal.



The phloem is exceptionally well preserved for a fossil (see *ph*, Fig. 2, Pl. XXVIII, and Phot. 4, Pl. XXVII), and is relatively large in amount.

This leaf comes much nearer the living type of *Pinus* than do any described by Jeffrey or Hollick and Jeffrey. Hence, as they use the generic name of the living forms for their species, there appears no excuse for our creating a new genus to include our species, though on the whole we are not in favour of using the same generic name for living forms and fossil fragments. Only where the fossil plant is approximately known in its entirety is it wise to include it in a living group.

Our leaf, however, is so remarkably like the specialized and well-characterized *Pinus* leaf that the use of the modern generic name may be allowed.

*Pinus yezoensis*, sp. nov.

Leaf oval in outline, diameter about 1 mm. Central vascular strand large; radial wood strands separated by large rays; endodermal sheath very well marked; mesophyll with infolded walls; hypoderm slightly developed; resin-canals two, laterally placed.

Horizon.—Upper Cretaceous, Hokkaido (old Japanese name *Yezo*). Collected by M. C. Stopes.

Type:—the figured slide has been presented to the British Museum, Department of Geology.

*Discussion.*—The interest of this Cretaceous leaf lies in its complete likeness to the living types. Hollick and Jeffrey ('09, p. 13), in speaking of their Cretaceous species of *Pinus*, remark that 'these remains, when sufficiently well preserved, possess one feature which, in general, serves to distinguish all of the Cretaceous Pines thus far examined by us from those now living, and that is the very wide zone of transfusion tissue surrounding the leaf bundles. . . . The endodermal sheath separating the transfusion tissue from the mesophyll is also less clearly marked than in living Pines, or may be entirely absent.' In Jeffrey's ('08) fuller description of the leaves (p. 216) he enumerates four points in which the leaves of true Pines of the Staten Island deposits differ from those now living. They are: (1) 'In the better development of the transfusion elements round the bundle.' Our new species, from the Japanese Cretaceous, has less rather than more transfusion tissue than is common in living Pines. (2) 'In the differentiation of the transfusion elements into an inner sheath composed of elongated tracheidal elements, and an outer, much broader zone made up of more nearly isodiametric elements with thinner walls.' The Japanese species does not show such differentiation. The few slightly sclerized cells above and below the bundle might possibly be considered as remnants of the inner sheath, but this theoretical view entails its application to living *Pinus*, which the new fossil species resembles in this feature.

(3) 'In the probable absence of an endodermis.' The Japanese fossil is seen to have a particularly well developed endodermis. (4) 'In the absence of infolding of the walls of the mesophyll.' The Japanese species has excellently preserved cells showing deep infolding of the cell-walls of the mesophyll.

It is clear, therefore, that the new species approximates extremely closely to the living type, and judging from the foliage, can be no more than specifically distinct from any living species. In the shape of the leaf, which is oval, with no straight edge such as is found in the Pines usually, we see a suggestion that there may have been only one leaf in the fascicle. The living *P. monophylla* is more circular in outline, however, and we would do no more than point out the suggestion offered by the shape of the leaf in *P. yezoensis* and note that it is also supported by the fact that there is a *single* bundle in *P. yezoensis*, as there is in *P. monophylla*, while there is usually a double one in the modern Pines with two or three needles in a fascicle.

These two new species from the Cretaceous show that the modern type of leaf in Pines was evolved by that time, and that at the same time and in the same place were living trees with the older type of *Prepinus* foliage.

We wish to tender our thanks to the Director of the Royal Gardens, Kew, for the supply of living species of Pine leaves which he kindly provided for comparison with the fossils.

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- JEFFREY, E. C. ('08): On the Structure of the Leaf in Cretaceous Pines. *Ann. Bot.*, vol. xxii, no. lxxxvi, pp. 207-20, Pl. XIII and XIV, Oxford, 1908.
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## DESCRIPTION OF PLATES XXVII AND XXVIII.

Illustrating the Paper by Dr. Stopes and Miss Kershaw on the Anatomy of Cretaceous Pine Leaves.

### PLATE XXVII.

Phot. 1. *Prepinus japonicus*. Photograph of transverse section of the leaf, showing its five sides and general anatomy. *scl.*, hypodermal sclerenchyma. *g.*, resin-canals, two in number. *t.*, transfusion tissue round the bundle.

Phot. 2. *Prepinus japonicus*. High power photograph of part of the leaf to show (*st.*) stomata lying between the groups of sclerenchyma (*scl.*). *t.*, transfusion tissue.

Phot. 3. *Pinus yezoensis*. Photograph of a transverse section of the leaf, showing its oval outline and general anatomy.

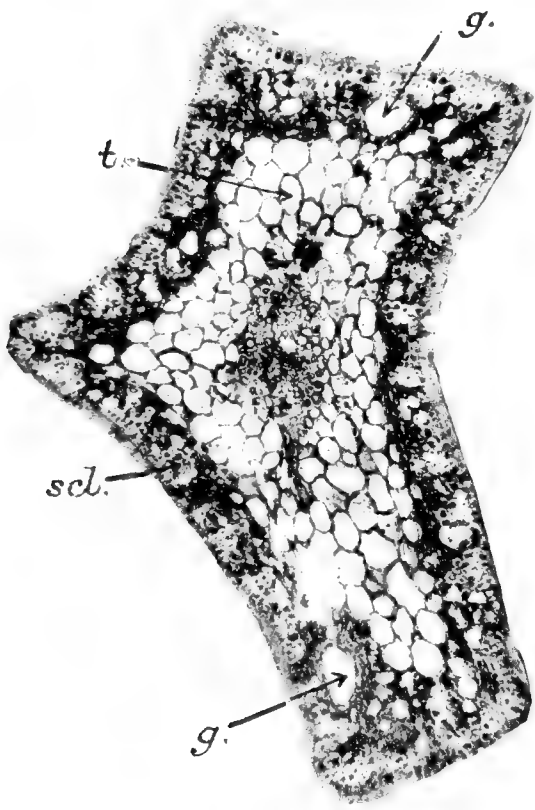
Phot. 4. *Pinus yezoensis*. Enlarged view of part of the leaf. *h.*, hypodermic sclerenchyma. *a.*, assimilating cells, with infolded cells. *en.*, endodermis. *ph.*, phloem. *x.*, xylem; note the large ray cells between the rows of tracheides.

Phot. 5. Part of the edge of the leaf enlarged to show the resin-canal (*g*) lying at the edge below the hypoderm cells (*h.*). *ep.*, epithelial cells of resin-canal. *a.*, assimilating cells with infolded edges.

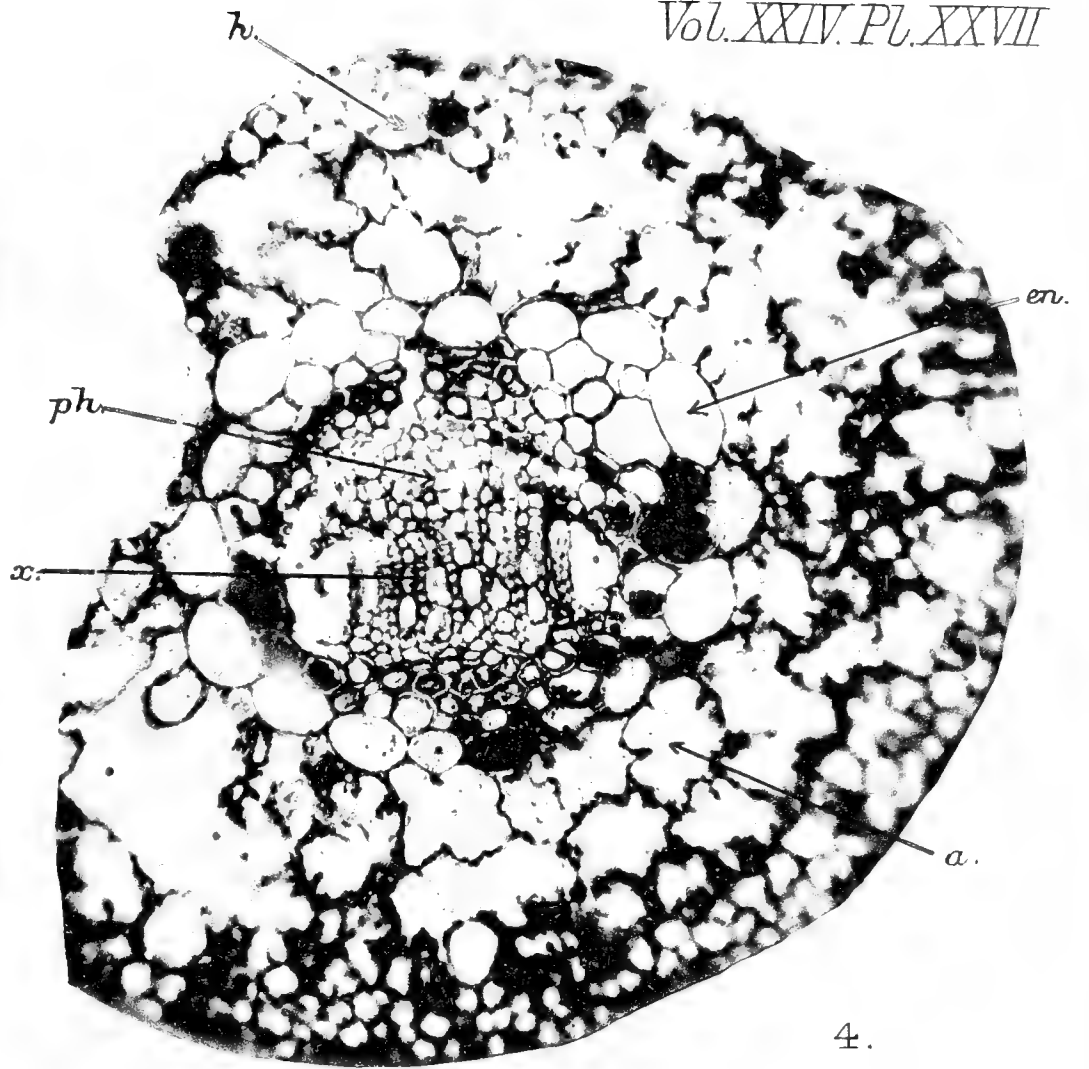
### PLATE XXVIII.

Fig. 1. *Prepinus japonicus*. Drawing of detail of leaf. *scl.*, sclerenchyma patches. *st.*, stomates. *g*, resin-canal. *c.a.*, crushed assimilating tissue. *v.sc.*, sclerized sheath of vascular strand. *t.*, transfusion zone.

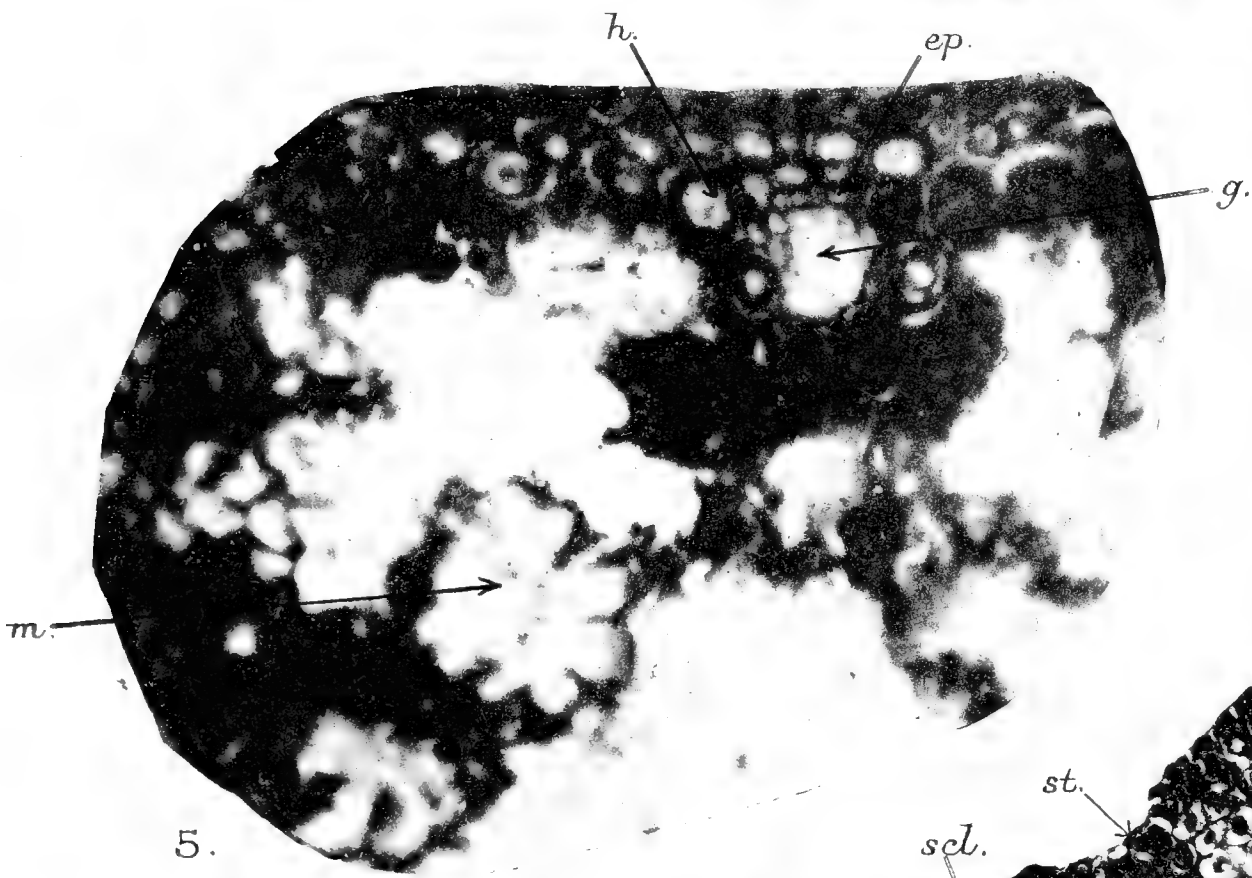
Fig. 2. *Pinus yezoensis*. Drawing of detail of leaf. *a.*, assimilating cells. *en.*, endodermis. *x.*, xylem. *m.*, medullary ray cells. *ph.*, phloem.



1.



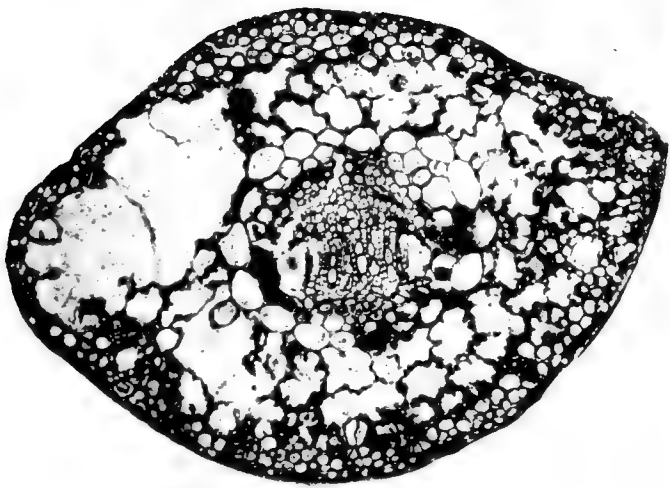
4.



5.



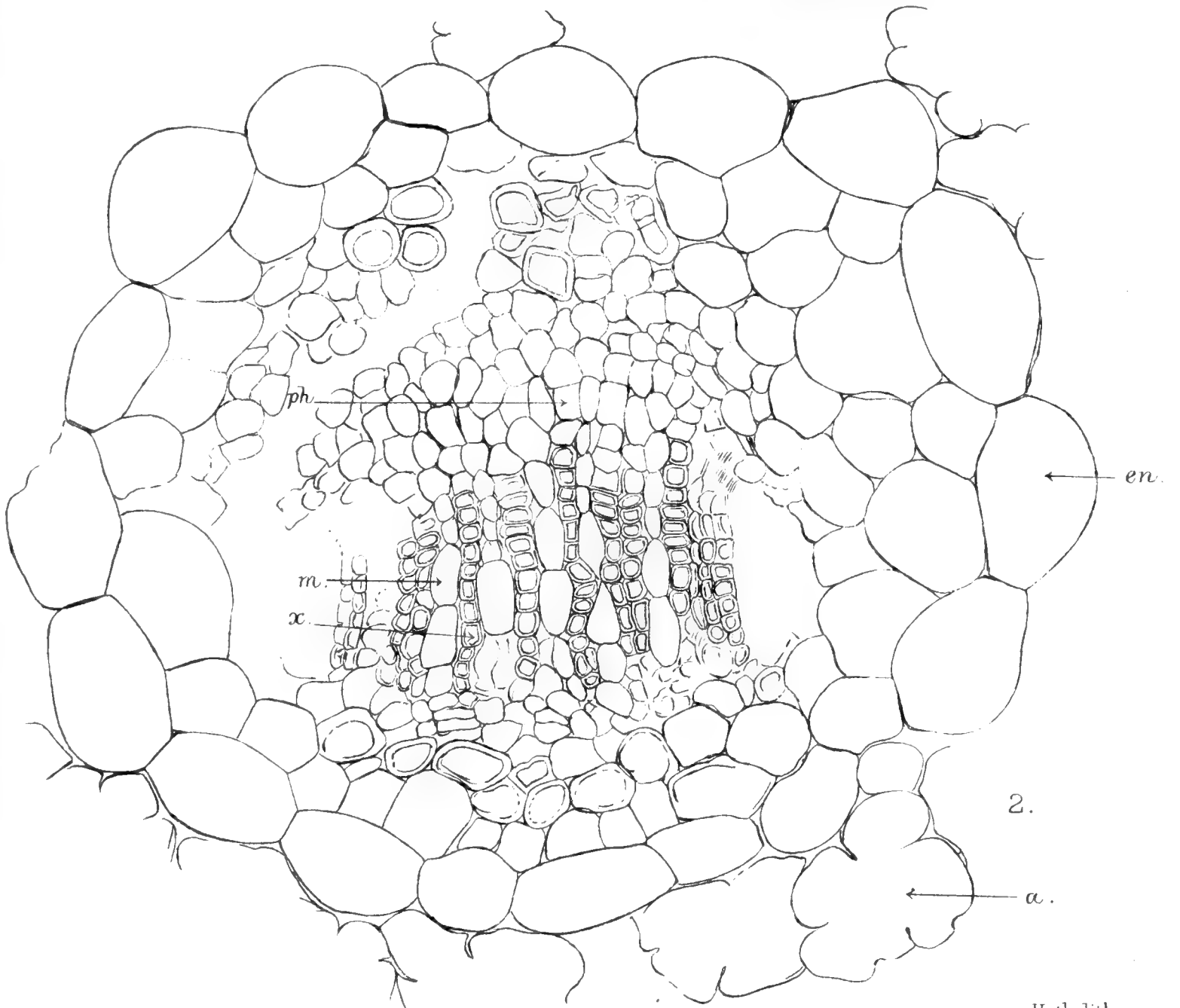
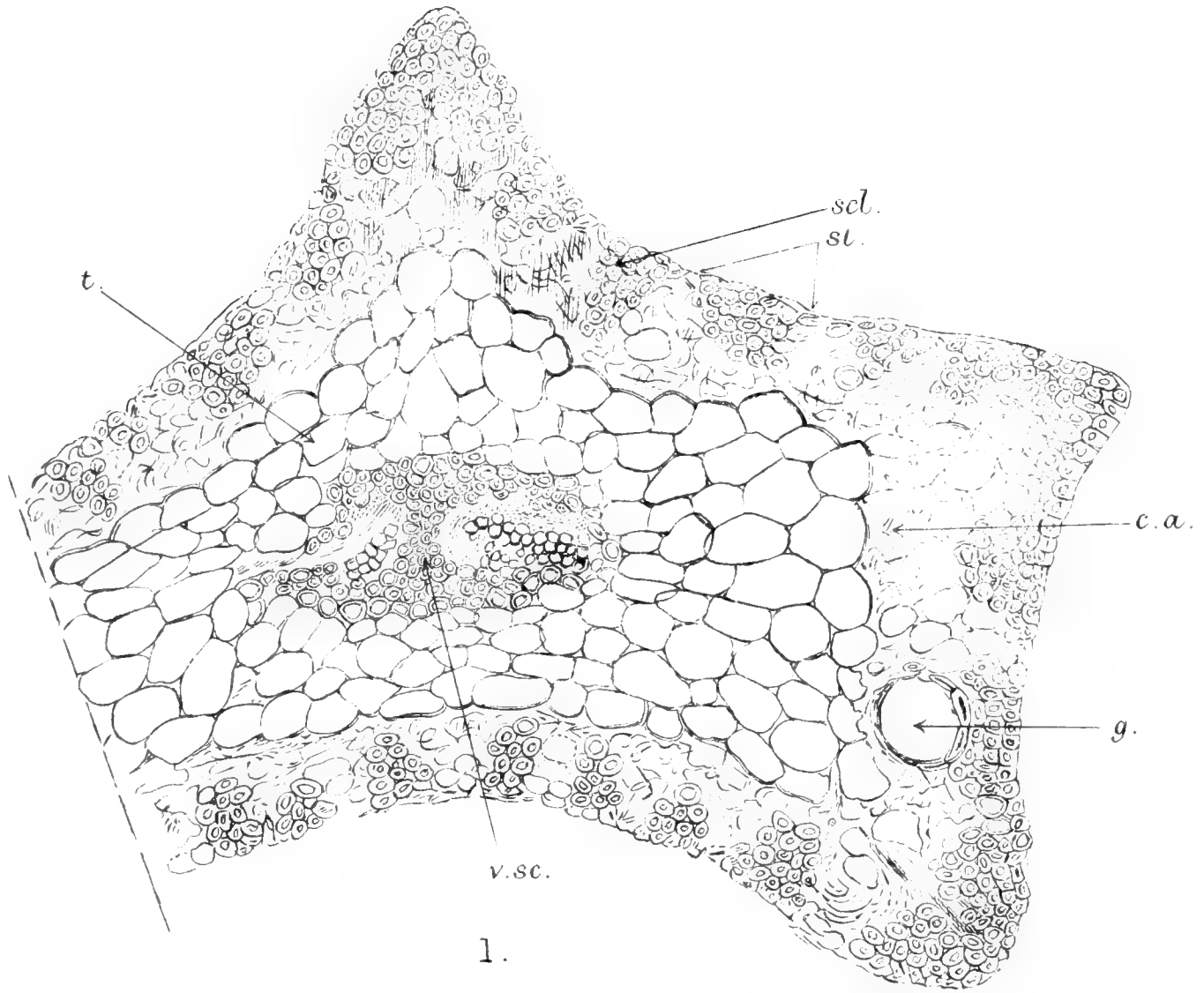
2.



3.

Huth, coll.





Huth. lith.

STOPES & KERSHAW.—CRETACEOUS PINE LEAVES.



# The Gametophytes and Embryo of *Sciadopitys verticillata*.

BY

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With Plates XXIX-XXXI.

OUR knowledge of the gametophytes and embryo of *Sciadopitys* is very meagre, consisting essentially of a brief and fragmentary account of the mature archegonium and embryo published by Professor W. Arnoldi in 1901, and Thomson's description of the megaspore membrane. I have thought it worth while to prepare a fuller account of the developmental stages in connexion with the gametophytic structures, fertilization, and embryo of this interesting genus, in the hope of filling a gap in our knowledge of the Coniferales as a whole.

All of the material for this investigation was obtained from young trees growing in the Pinetum of the Royal Gardens, Kew. For facilities afforded in collecting material and also preparing it in the Jodrell Laboratory, I am much indebted to the Director, Lieut.-Col. Prain, F.R.S. I am also very much indebted to Mr. L. A. Boodle, F.L.S., for many kindnesses. Circumstances did not permit of my remaining at Kew sufficiently long to obtain all of the necessary material, but Mr. Boodle was kind enough to send me living cones from time to time; without this help many of the interesting stages would, of necessity, have been omitted.

## THE MALE GAMETOPHYTE.

From the material collected at Kew Gardens it appears that the microspores mature early in April, and pollination occurs between two and three weeks later. At maturity the microspores have a very thick, hard, resistant exine or exospore, and a well-developed but thin endospore. The thick, hard, outer membrane offered such resistance to the knife in cutting that it was impossible to obtain very thin sections of the microspores. Sections, however, were obtained sufficiently thin to make out all of the essential details. As shown in Pl. XXIX, Figs. 1, 2, 3, and 4, the endosporium appears to be very thick, but this appearance is due to the



fact that the sections here represented are not median. The inner membrane, seen in transverse section, is in reality quite thin. The microspores themselves are not exactly spherical in form, they are slightly elongated or egg-shaped. At one end of the long axis the exospore becomes sharply attenuated, and at the end of the spore it is very thin. This peculiarity of the spore-wall is indicated in Figs. 2, 3, and 4.

The contents of the mature spore consist of a large centrally placed nucleus suspended in a rather dense granular cytoplasm, as shown in Fig. 1. The stage here figured is one just preceding the germination of the spore, for its nucleus is obviously preparing for mitosis. In Fig. 2 is represented the first spindle in the germinating microspore, the chromosomes being at the equatorial plate. Fig. 3 represents a later stage with the chromosomes at the poles of the spindle. It will be observed from Figs. 2 and 3 that the long axis of the spindle is parallel with the long axis of the spore, and consequently one of the cells resulting from this division will find itself placed directly behind the weak spot in the spore-wall. The result of this first division of the germinating spore is represented in Fig. 4. The daughter-nuclei are seen to be separated from one another by a delicate membrane, so that there are now two cells organized.

This division of the microspore takes place within the microsporangium, and it is the only division which occurs before pollination. In fact no other divisions occur until the pollen-tube is formed. Unlike the Abietineae (Coulter and Chamberlain, '01; Ferguson, '04; Lawson, '09) and *Podocarpus* (Coker, '02), there is not a trace of vestigial prothallial cells, and therefore the two cells that are formed are to be regarded as the generative cell and tube-cell respectively. At the time of pollination the pollen-grain is in the condition represented in Fig. 4. The grain is slightly oval in form, with the tube-cell in touch with the thin part of the exospore.

The young ovules, among the Coniferales, show a considerable variety of special adaptations in the form of devices for receiving the wind-conveyed pollen. In many of the Cupressineae which I have observed, the micropyle, at the time of pollination, exudes a small globule of transparent liquid. The purpose of this is no doubt to catch the pollen more effectively, and as the liquid evaporates the pollen-grains descend into the micropyle and find themselves lodged upon the apex of the nucellus. I have observed practically the same thing in *Cephalotaxus* and *Taxus*. In *Pinus* the integument of the young ovule extends outwards in the form of two free limbs which, upon receiving the pollen, curve towards one another, and the grains are thus led into the micropylar canal. In *Pseudotsuga* (Lawson, '09), on the other hand, the pollen-grains are caught in the stigmatic surface of hair-like structures at the lip of the micropyle, and here they germinate, sending down the pollen-tubes through the micropylar canal. The grains themselves in this case never reach the nucellus. In

*Sciadopitys* there is a specialized pollen-receiving device which is quite unlike any of these. At the time of pollination the integument extends but very slightly above the surface of the nucellus, in such a fashion as to form a wide gaping but shallow micropyle. Shortly before the pollen is shed, the upper part of the nucellus becomes sharply differentiated into an organ which, for convenience, I will call the pollen-cushion. This cushion consists of comparatively large cells with intercellular spaces, and with thin cell-walls forming a loose soft tissue which stands out in sharp contrast against the small-celled firmer tissue of the nucellus below. These cushion-cells secrete at the surface a transparent liquid substance, which, from an examination of the fresh ovules, appeared to be more refractive and more dense than water. This pollen-cushion, therefore, not only serves as a stigmatic surface for receiving the pollen, but also furnishes a soft loose tissue into which the young pollen-tubes may penetrate with very little resistance.

Now, while such special adaptations as these pollen-receiving devices may not be of direct phylogenetic importance, they are nevertheless very interesting. They show a series of modifications of structure which probably followed close upon the adoption of the pollen-tube as a means of conveying the sperm-nuclei into the egg.

In *Sciadopitys* the pollen-cushion is ready to receive the pollen early in April, and, as stated above, the integument at this time projects very slightly above the surface, forming a wide micropyle into which the pollen is blown by the wind. During the latter half of April numbers of pollen-grains were found in this position. In some ovules but one or two grains were found, while in others the micropyle was crowded full of them. A few of them are shown in Fig. 6.

The next step in the germination of the microspore was its rapid enlargement and the casting off of the exospore. This takes place immediately after the pollen-cushion has been reached. These changes are shown in Fig. 5. Meantime, the integument continues its growth forward, as may be seen in Fig. 6, and the micropyle is gradually closed. The complete closure of the micropyle occupies about three weeks. During this period the pollen-tubes have made their appearance, and have penetrated for some considerable distance into the tissue of the pollen-cushion.

Although the pollen-tube has attained a considerable length, no nuclear activities occur until the middle of June. About this time the generative nucleus divides, giving rise to a large, more or less, spherical cell with a distinct cell membrane and a free nucleus. These structures, by their later behaviour, were readily identified as the body-cell and the stalk-nucleus. Their position and relative size may be easily made out in Fig. 8, which represents a longitudinal section of the pollen-tube immediately after the division of the generative cell. It will also be seen from this figure that these two structures remain for some time in the spore end of

the tube, while the tube-nucleus advances with the growing tip. Early in July, however, the stalk-nucleus follows in the wake of the tube-nucleus down the tube, leaving the body-cell in its original position in the spore end. This shifting of the free nuclei in the tube is shown in Figs. 9 and 10. As the tubes grow almost in a straight line down through the soft tissue of the pollen-cushion, no difficulty was met with in obtaining complete longitudinal sections showing all the essential changes during their development. In Fig. 7 is represented the general course of the tube as it appeared three months after pollination. As shown in Figs. 9 and 10, there are large numbers of starch grains in the cytoplasm of the tube, and the amount increases as development advances.

Up till this time the male gametophyte has undergone an uninterrupted development, so that about the middle of July we find the body-cell and the stalk-nucleus fully organized, and the pollen-tube lying embedded in the soft tissue of the pollen-cushion. And in this condition it remains until the following spring. Occasionally it was observed that the tube may penetrate for a short distance into the firmer tissue of the nucellus below the cushion, but this was quite exceptional. No further nuclear changes take place in the tube until eleven months later. In this regard the male gametophyte of *Sciadopitys* shows a marked resemblance to *Pinus* (Coulter and Chamberlain, '01; Ferguson, '04), but is quite unlike *Cephalotaxus* (Lawson, '07), which also takes two seasons before fertilization is accomplished, but where the pollen remains practically dormant for a year after pollination.

Early in June of the following year—that is, about fourteen months after pollination—the body-cell in *Sciadopitys* gradually descends into the tube, and finally occupies a position near the tip a little distance behind the stalk- and tube-nuclei. But during this migration of the body-cell the tube has not only penetrated the entire nucellar tissue, but has reached one of the archegonial chambers. In number and position those chambers resemble those of *Pinus*, but are very much deeper. In Fig. 48 the body-cell is represented in the region just entering the archegonial chamber, while the tip of the tube has reached the neck-cells. Fig. 11 shows, with the details more highly magnified, a portion of the pollen-tube containing the body-cell. In its descent in the tube the body-cell loses its spherical form and appears considerably elongated. It retains, however, its distinct membrane which separates it from the cytoplasm of the tube. The nucleus is very large and perfectly spherical, and as it approaches the tip of the tube it shows evidences of preparing for mitosis. The spindle of this division was not found, but several tubes showed the stages immediately following mitosis. The division of the body-cell evidently takes place in the tip of the tube immediately over the neck-cells of the archegonium, and it results, not in the formation of two cells as in *Sequoia* and the

Cupressineae, but in two free nuclei. From the large number of cases examined showing these stages, it became quite evident that there was no cell membrane separating these two male nuclei from one another. The two structures were found lying quite freely in the cytoplasm of the body-cell, as shown in Fig. 12, and one of them is slightly but distinctly larger than the other. Both of them are carried through the neck-cells and into the archegonium by the tip of the tube. In two cases they were found lying in the egg cytoplasm just below the neck-cells. One of these is represented in Fig. 49.

#### THE FEMALE GAMETOPHYTE.

The number of ovules produced on each ovuliferous scale is quite large. They varied from five to fifteen or more according to the position of the scale on the cone, the lower scales producing the larger number, and decreasing as the scales became smaller towards the apex of the cone. They first appear as minute papillae-like structures growing out at right angles to the dorsal surface near the base of the scale, and arranged in two or three rows more or less crowded together. The nucellus or sporangium proper is the first to develop, but this is very soon followed by the integument, which, in the earlier stages, appears as a ring of tissue surrounding the base of each papilla-like nucellus. This condition was found early in March, and from this time on a complete series of stages was obtained by collecting material every two or three days. The ovules take about four weeks in their development before they are in a condition to receive the pollen. During this period the integument grows a little more rapidly than the sporangium, so that at the time of pollination it projects a little beyond the level of the apex of the latter.

The sporogenous tissue does not become differentiated until after the pollen has been received in the micropyle. Fig. 6 represents a longitudinal section of an ovule about three weeks after pollination. Although, as here shown, the pollen-cushion has been differentiated and the pollen received, and the micropyle nearly closed, there is as yet no trace of sporogenous tissue in the megasporangium. Except for the highly differentiated character of the pollen-cushion all of the cells of the nucellus resemble one another. During the last week in May, however, certain cells, deep in the centre of the nucellus at a point level with the insertion of the integument, become enlarged and take on a sporogenous character. These cells are few in number, and in one or two cases appeared singly. Whether they could always be traced back to a single cell I am unable to state, but it seems probable. These cells are not only large and with deeply staining nuclei, they are also actively merismatic. They divide rapidly and frequently, and soon give rise to a large and sharply differentiated sporogenous group



which I interpret to be the archesporium. In the early stages of the group, that is when it consists of four or eight cells, the individual cells appear to be exactly alike, being quite large with dense granular cytoplasm and deeply staining nuclei, and stand out in sharp contrast to the surrounding smaller sterile cells. About this time, however, the most centrally situated cell of the young archesporial group enlarges and becomes differentiated into a functional megaspore-mother-cell. This cell not only differs from the other archesporial cells by its large size, but also by the presence of numerous starch grains in its cytoplasm which were not found in any of its neighbours. These facts are well brought out in the longitudinal section of the sporangium represented in Fig. 15. Here it will be seen that the mother-cell lies in the centre of a large group of sporogenous cells located deep in the tissue of the nucellus.

It should be noted that the form of the sporangium is unusual. The ovules being quite numerous, they become crowded and closely packed together. The nucellus consequently takes on a flattened form, its breadth in transverse section being just about twice its width, as may be seen in Fig. 14. Another interesting point is brought out in this figure, namely, that the insertion of the integument is not at the same level around the base of the nucellus. This insertion is much higher up, at the narrower side of the flattened nucellus. This arrangement obviously gives strength to that region of the nucellus in which the sporogenous tissue is developing. I have not observed this in any other coniferous ovule.

As above stated, there is but a single functional megaspore-mother-cell differentiated out of the sporogenous group. There was no evidence to show that its origin was any different from that of the other archesporial cells which surround it. And although the latter continue their merismatic activity for some time, there is no reason to regard them as other than sporogenous. They eventually develop into a large group of nourishing tapetal cells which completely surrounds the megaspore. This group of cells is, therefore, as in *Pseudotsuga* (Lawson, '09), tapetal in function and archesporial in origin. It persists for about a year, but is finally absorbed at the time of the formation of the permanent prothallial tissue.

I was fortunate enough to obtain a fairly complete series of stages showing all the essential points in connexion with the reduction division of the megaspore-mother-cell. In the first place, this cell is easily detected by its large size, by the presence of starch grains in its cytoplasm, and by its relatively large and deeply staining nucleus. The latter body, even in the resting stage, is much larger than any of the tapetal nuclei. The chromatin in the resting condition consists of delicate threads which interlace with one another, giving the appearance of a reticulum. There is always present at least one large nucleolus. As division approaches, the chromatin threads become more sharply defined, and what appeared to be

a reticulum is really the effect of the separate threads interlacing and crossing with one another. This condition is shown in Fig. 17.

During these changes in the chromatin there is a gradual enlargement of the nuclear cavity, presumably resulting from the increase in the amount of nuclear sap. As shown in Fig. 18, with the enlargement of the nuclear cavity the reticulated nature of the chromatin becomes completely lost, and the individual threads of which it is composed become sharply visible. A somewhat similar stage is shown in Fig. 19, but here the threads are isolating themselves from one another, and are spreading out into the enlarged nuclear cavity. It may be seen also from this figure that the chromatin threads are finely granular. The separation of the threads from one another continues until they are quite evenly distributed through the nuclear sap, and, as shown in Fig. 20, the threads become shorter and thicker, and their granular nature becomes more pronounced. This shortening and thickening of the threads proceeds rapidly until we finally have the appearance represented in Figs. 21 and 22. A large number of preparations were made at this time showing all intermediate stages as well as those here figured. In none of them was I able to find any evidence that the chromatin consisted of a continuous thread. In fact, all of the evidence seemed to indicate the opposite, namely, that the number of chromatin threads in the early stages correspond with the number of chromosomes into which they later develop. And I am strongly of the opinion that this is also true for the resting stage of the nucleus.

Up to the stage represented in Fig. 22, there was no evidence of the reduction in the number of threads having taken place. In fact, in Fig. 23, which is undoubtedly a later stage than those represented in Figs. 21 and 22, we still find the diploid number. But at the stage shown in Fig. 23, there was some evidence of the actual fusion and consequent reduction of the chromosomes beginning to take place; and while the actual fusion was not followed with certainty, the reduced number always appeared after this stage and never before. In Fig. 24 is represented a slightly later stage, which I interpret to be immediately after or during the actual fusion of the chromosomes in pairs.

This stage is almost immediately followed by the disappearance of the nuclear membrane, the separation of the paired chromosomes from one another, and the formation of the reduction spindle. The spindle fibrils originate out of the cytoplasm after the manner which prevails throughout the flowering plants. An early stage of their development is shown in Fig. 25, with the reduced number of heterotype chromosomes lying freely in the cytoplasm and attached to the growing spindle fibrils. The spindle in the early stages is multipolar, but very soon becomes bipolar. The poles are at first quite broad, but later become sharply pointed (Figs. 26 and 27). After repeated counting the reduced number of chromosomes appeared to



be eight, being just half the sporophyte number, which was easily and much more frequently estimated to be sixteen. Fig. 28 represents a transverse section of the mother-cell in a plane through the equatorial plate, showing the eight heterotype chromosomes as they appear in polar view. The daughter chromosomes now pass to the poles in the usual way, as shown in Fig. 29, and after being more or less crowded together, where it is difficult to identify the individual chromosomes from one another, they become very much vacuolated by the accumulation of nuclear sap. The membranes of the daughter-nuclei are formed as a result of the nuclear sap coming in contact with the cytoplasm in the manner described for Angiosperms (Lawson, '04).

It is a curious fact to note that no cell-plate formation follows this division. This is shown in Figs. 29 and 30. The two daughter-nuclei lie freely in the cytoplasm of the mother-cell, one at each end. The interest of this point is its similarity to what occurs in the microsporangium. It will be remembered that in all Gymnosperms no cell-plate is formed immediately after the first division of the microspore-mother-cell in the development of the tetrads, and that cell-plates are only formed after the simultaneous division of the two daughter-nuclei. This is also true for the great majority of the Dicotyledons. The process of tetrad formation here found in *Sciadopitys* clears up much of the doubt that has existed in regard to the organization and number of cells concerned in the axial row of megaspores among the Coniferales.

The daughter-nuclei resulting from the heterotype division are no sooner organized than preparations for the second division set in. This division is simultaneous, and several preparations showed the twin spindles lying one behind the other in the mother-cell. In regard to their position, these differ from the corresponding spindles in the microsporangium. In the latter case the spindles lie side by side, not one behind the other. This difference has a direct bearing on the position of the cell-plates which are formed after this division. The twin spindles are shown in Fig. 31 with the reduced chromosomes at the equator, which indicates how exactly simultaneous are the divisions. What follows results in a very curious arrangement, for two cell-plates are now laid down midway between the newly formed daughter-nuclei. Now, as the two spindles lie one behind the other in the mother-cell, with their long axes practically contiguous with one another, we have two pairs of daughter-nuclei in a single row and practically in the same plane. By the formation of the cell-plates the end cell of each pair becomes separated from its neighbour. No plate, however, is formed between the two middle nuclei, and so it comes about that the axial row or tetrads are represented by three cells, the middle one containing two free nuclei. This curious arrangement is clearly demonstrated in Fig. 32 and the four following figures. That this is the regular and normal

process in the formation of the megaspore tetrads in *Sciadopitys* I have no doubt, for it was repeatedly demonstrated by a large number of preparations showing every step in the process.

It was stated above that the presence of starch granules was a characteristic of the megaspore-mother-cell, and that previous to the reduction division the starch was rather uniformly distributed throughout the cytoplasm. Just before the second division in the formation of the tetrads, however, the bulk of the starch grains settle in the base of the mother-cell. The result of this is that when the tetrads are formed, the basal cell of the axial row contains much more starch than the other cells (Figs. 32, 33, 34, and 35). This is an interesting point because this basal cell becomes the only functional megaspore of the axial row. Fig. 33 represents the tetrads of the axial row fully organized, with the basal cell containing an abundance of starch, the middle cell with its two free nuclei, and the top cell of the row with its single nucleus. Fig. 34 shows the axial row where the two free nuclei of the middle cell have shifted their position, demonstrating quite conclusively that no cell membrane separates them. This figure also shows that the nucleus of the top cell is already betraying signs of disorganization.

The basal megaspore of the axial row now enlarges, but its growth is quite slow. Between the stages represented in Figs. 35 and 36 fully four weeks have elapsed. But during this period the two nuclei of the middle cell and the nucleus of the top cell show every evidence of degeneration, and they eventually become quite abortive. The first noticeable change in the functional megaspore is its great elongation down through the tapetal cells, as shown in Fig. 36. This is now followed by the formation of a large central vacuole which causes a growth in all directions. Fig. 37 represents a longitudinal section of the functional megaspore and the three abortive megaspores as they appear six weeks after the organization of the axial row. It will be seen that the functional megaspore now consists essentially of a huge vacuole with but a thin film of cytoplasm at the periphery, and a single nucleus at the base. A more highly magnified representation of the last vestiges of the abortive megaspores is shown in Fig. 37.

During all of these stages in the development of the megaspores the number of tapetal cells has increased considerably, so that eventually there are several layers of them surrounding the axial row.

The outline drawing in Fig. 13 is intended to represent the relative position and condition of the two gametophytes in the ovule as they appear at the end of the first year's growth, which closes about the middle of July. It will be seen that the pollen-tube has not penetrated beyond the tissue of the pollen-cushion, and that the large functional megaspore lies embedded in the centre of a large group of nutritive tapetal cells. No further changes take place in either gametophyte until the following spring.

This postponement of the development of the gametophytes, and consequent delay of fertilization for twelve months, occurs in a number of other Coniferales, but is evidently not peculiar to any particular family. We have it occurring, for instance, in *Cephalotaxus* (Lawson, '07; Coker, '07), *Torreya* (Coulter and Land, '05), *Pinus* (Coulter and Chamberlain, '01; Ferguson, '04), and in *Sciadopitys*. Why this delay should occur in these few genera is hard to explain. The habit may be a special adaptation to meet special conditions of nutrition; on the other hand, it may possibly represent the vestige of a habit of a resting period of the spores at a very early period in the phylogeny of the group when the spores were free. In support of this view, however, there is at present no evidence.

In *Sciadopitys* no further nuclear changes occur in either the pollen-tube or megaspore until the following spring. Early in March of the second year, however, the nucleus of the megaspore divides, and this is immediately followed by a large number of free nuclear divisions. The formation of prothallial tissue now proceeds in the usual way. The free nuclei are parietally placed, and after the last simultaneous division, cell-walls are formed between them. There are thus formed the primary prothallial cells which are open on the inside and exposed to the central vacuole. These structures have been sufficiently described and figured for other Coniferales (Coulter and Chamberlain, '01; Ferguson, '04; Coker, '03; Lawson, '04, '07, '09) since their first discovery by Mlle Sokolowa in 1890.

The primary cells now rapidly elongate and encroach upon the central vacuole after the manner already described for the Abietineae, Cupressineae, and other families. Cross-walls are now formed, and a considerable amount of cellular prothallial tissue is produced before the ingrowing cells meet in the middle and occupy the space of the central vacuole.

During the organization of the prothallial tissue there is developed a distinct and thick megaspore membrane. Its structure is very like that of *Pinus*, but not quite so thick. It has a distinct fibrillar exospore, and is uniformly thick except in the region of the archegonium, where it tapers out and becomes quite thin. A sectional view of a part of the membrane is shown in Fig. 39. (See Thomson, 1904.)

#### THE ARCHEGONIA.

The archegonia originate as superficial cells, and make their appearance early in April at the apex of the prothallium before the permanent tissue of the latter has been completely organized. The original initial cell was not positively identified; probably on account of its similarity to the ordinary prothallial cells. The first stage observed was after the first division of the initial cell, showing a single neck-cell and a somewhat larger cell below, which develops into the central cell. In Fig. 40 is represented

a little later stage, where the first neck-cell has divided into two and the central cell has enlarged to about twice its original size. It seems to be the rule among the Coniferales that the archegonia originate as superficial cells, but there are exceptions, as in *Sequoia* (Lawson, '04) and *Widdringtonia* (Saxton, '09), where some at least of the archegonia originate deep in the prothallial tissue.

The nourishing jacket cells become differentiated quite early. In the very young stages they look very like the young central cell. They were frequently mistaken for archegonial initials. These cells, although they continue to multiply in number until the archegonium is mature, do not show any increase in size. Those surrounding the very young central cell presented the same dimensions and appearance as those associated with the more mature egg-cell.

The number of archegonia developed varied from four to six, four being the number more commonly met with. They were always found situated at the apex of the prothallium, forming a single group, and their position in regard to one another is like that of *Pinus*, *Abies*, *Pseudotsuga*, and *Cephalotaxus*, the individuals being separated by sterile prothallial tissue. In the mature state they are never as wide as the archegonia of the Abietineae, being rather narrow, long, tapering structures more nearly resembling those of *Cephalotaxus*. Each archegonium is surrounded by its own single layer of nourishing jacket cells.

The young central cell enlarges very rapidly and elongates in a direction towards the centre of the prothallium. During the growth of the central cell its nucleus always remains directly under the neck-cells. The cytoplasm is quite coarsely granular and contains a number of small vacuoles. An early stage is shown in Fig. 41. The enlargement and downward growth of the cell continues, and, as shown in Fig. 42, the cytoplasm becomes charged with granules of food substance, evidently taken in through the jacket cells. As growth proceeds the vacuoles become larger and much more numerous, until, as illustrated in Fig. 43, the entire cell cavity takes on a frothy appearance in identically the same manner as it does in *Cephalotaxus*, *Pseudotsuga*, *Picea*, and *Abies* (Miyake, '03; Lawson, '07). As more food substance gathers in the cytoplasm of the cell, the vacuoles appear to flow together, finally forming a single large one some distance behind the nucleus. Here, again, the archegonium differs from that of the Abietineae, for among the latter there is no large vacuole, either in the central cell or the egg-cell.

As shown in Figs. 40, 41, and 42, during all the early stages of the archegonium the neck-cells are superficially placed in regard to the apical surface of the prothallium. This condition, however, is soon changed, for all of the sterile tissue at the apex grows forward for a considerable distance, leaving an open canal leading to the neck of each archegonium. These



canals or archegonial chambers occur in all Coniferales, where the individual archegonia are isolated from one another, but as a rule they are quite shallow. In *Sciadopitys*, however, they are very deep—so deep that the mature archegonia eventually find themselves buried in the prothallial tissue a considerable distance behind the apex, as shown in Figs. 44 and 48. Stages in the development of the archegonial chamber may be seen in Figs. 42, 43, and 44.

Meantime, the neck-cells have divided repeatedly, forming a single tier. At maturity these cells partly separate from one another on the outer side and spread out in a fanlike fashion. Directly under the neck-cells the central cell is quite narrow, but broadens out rather abruptly, as shown in Figs. 43 and 44. There is thus formed a narrow pocket near the neck, and in this pocket the nucleus remains throughout the developmental stages of the central cell.

When the latter reaches its full size, the nucleus undergoes a division which results in the organization of the egg nucleus and the ventral canal nucleus. The spindle is formed directly under the neck, as shown in Fig. 45. A later stage of this mitosis is shown in Fig. 46, where the chromosomes are at the poles. A careful search was made for a ventral canal cell membrane, but although all stages of this mitosis were observed, no such membrane could be found. I am quite convinced that a cell-wall is not formed after this division. A considerable number of archegonia showed the condition represented in Fig. 47, where the ventral canal nucleus was found lying freely in the cytoplasm just above the egg nucleus. It will be remembered that among the Abietineae a definite membrane is formed which separates the ventral canal cell from the egg-cell. In a previous memoir (Lawson, '07 and '09) I have called attention to the probable phylogenetic importance of this. In the present case of *Sciadopitys* the absence of this membrane becomes interesting when we couple it with the fact that the functionless prothallial cells of the pollen are also absent. It would almost seem that there had been a simultaneous elimination of these vestigial structures.

The egg nucleus and ventral canal nucleus are no sooner formed than they both undergo a change. The latter immediately disorganizes and functions no further, while the former enlarges enormously and moves down and takes up its position in the centre of the cell. Mature archegonia ready for fertilization are shown in Figs. 44, 48, and 49.

#### FERTILIZATION.

As in the Abietineae and *Cephalotaxus*, the arrangement and grouping of the archegonia are such that it is possible for the contents of one pollen-tube to fertilize but a single archegonium. After passing through the nucellar tissue, the pollen-tube enters one of the long archegonial chambers,

and continues its growth until the neck-cells are reached. Meantime, the body-cell descends and takes up a position in the tip of the tube, and here undergoes division, giving rise to the two sperm nuclei. The tip of the tube apparently passes between the neck-cells, and its contents are discharged into the upper cytoplasm of the egg. The course of the tube in its passage through the archegonial chamber is shown in Fig. 48.

Before and during the fertilization period the cytoplasm of the egg becomes heavily charged with coarse granules of food substance and numerous large so-called 'proteid vacuoles'. Meantime, the nourishing jacket cells undergo a curious modification to which Arnoldi ('01) has called attention. The inner wall of these cells—that is, the wall lining the egg-cell—becomes strengthened by coarse, irregularly branched, reticulated thickenings. These thickenings project beyond the surface of the cells, and in sections may be seen extending into the cytoplasm of the egg. It would seem that there was a necessity for strengthening these walls without interfering with the transfusion of substances from the jacket cells into the egg. The thickenings were evidently not of cellulose, for they stained black after being treated with Flemming's triple stain. They are probably of a chitinous nature. In surface view they appear as in Fig. 51.

When the egg-cell is ready to be fertilized its nucleus lies in the centre of the cell just above the vacuole, as indicated in Figs. 44 and 48. The relative size of the egg nucleus and sperm nucleus may be observed in Fig. 49, the former being many times the size of the latter. Although the two male nuclei enter the egg, only one of them—presumably the larger one—unites with the female nucleus. The actual fusion of the sex nuclei was not observed, but the immediate result is shown in Fig. 50. Here we see the first spindle of the sporophyte organized within the boundary of the membrane of the egg nucleus.

#### THE EMBRYO.

The position of the first cleavage spindle seemed to vary within the area of the fusion nucleus. The long axis of the spindle was sometimes found parallel to the long axis of the archegonium; in others it was at right angles to this position. In Fig. 50 it is lying obliquely. There seemed to be no definite polarity in this respect.

Before this first mitosis is complete the membrane of the fusion nucleus fades and finally disappears, leaving the two first free nuclei of the pro-embryo in the cytoplasm just about the middle of the egg. A sufficient number of stages of the spindle were found to observe with fair accuracy the number of the chromosomes, which were found to be sixteen. I was, however, unable to distinguish a male and female group which have been described by Miss Ferguson ('04) in the case of *Pinus*.

The first two free nuclei remain in the middle region of the egg until



the second mitosis takes place, which follows closely upon the first. The resulting four free nuclei now pass to the base of the archegonium before the third series of divisions occur (Fig. 52). During their passage to the base all four nuclei enlarge. The next stage observed was that represented in Fig. 53, where another division has taken place just before the base of the archegonium has been reached. The next division results in the formation of cell-walls between the nuclei. As in the *Pinus* and other Abietineae, there are three tiers of cells and one tier of free nuclei constituting the pro-embryo. The middle tier of cells develops into suspensors and the end tier is carried forward in the ordinary way. Before the elongation of the suspensors, however, the cells of the end tier divide repeatedly, forming three or four rows which taper to a point. Fig. 54 represents an older stage where the suspensors are very much elongated and pushing forward a large group of embryo cells at the end. Arnoldi ('01) describes an additional stage where buds and smaller secondary suspensors are given off from these cells. This I was unable to find. But this was probably because I did not collect material later than July 7, and the stage of development at that date is represented in Fig. 54. I hope in another season to confirm this interesting later development of the embryo described by Arnoldi.

On the whole the embryo of *Sciadopitys* is rather unique; it does not bear a close resemblance to either the Abietineae, Cupressineae, or Taxaceae.

#### SUMMARY.

At pollination the microspores have already germinated. Each one has a very thick exine and a thin inner wall which surround two cells, the generative cell and the tube cell.

At this time the integument of the ovule projects very slightly above the level of the nucellus in such a manner as to form a wide but shallow micropyle.

The upper part of the nucellus becomes differentiated into a loose tissue of large thin-walled cells for receiving the pollen. This structure has been called the pollen-cushion.

As soon as the pollen-grains have been received they send out tubes which penetrate the soft tissue of the cushion.

The generative cell now divides, giving rise to a large spherical body-cell and free stalk-nucleus.

At the end of the first season's growth, which closes about the middle of July, the male gametophyte contains three nuclear structures, viz. the tube-nucleus, the stalk-nucleus, and the body-cell. No further nuclear changes take place until the following spring.

In June of the next year the body-cell descends towards the tip of the pollen-tube, which, meantime, has entered an archegonial chamber.

The division of the body-cell nucleus takes place immediately over the neck-cells of an archegonium, and this gives rise to two male nuclei of unequal size. Definite male cells are not formed, but merely male nuclei, as in the Abietineae.

The contents of the tip of the pollen-tube are discharged into a single archegonium.

A single megaspore-mother-cell is organized, whose nucleus undergoes a heterolytic mitosis, and resulting in the reduction in the number of chromosomes.

The sporophyte has sixteen chromosomes and the gametophytes eight.

After the reduction division of the megaspore-mother-cell nucleus no cell-plate is formed. The two daughter-nuclei lie freely in the cytoplasm. The first step in the formation of the megaspore tetrads is the same as that of the microspore tetrads.

The division of the daughter-nuclei occurs simultaneously, but the spindles lie one behind the other, and not side by side as in the microsporangium.

As a result of this second division the axial row of megaspores consists of three cells, the middle one of which contains two free nuclei.

The basal cell of the axial row becomes the functional megaspore, the other two become abortive.

The functional megaspore becomes very large, and contains a huge central vacuole, but no further germination takes place until March of the following year.

A distinct tapetum, consisting of two or three layers of large nutritive cells, is organized, and completely envelops the germinating megaspore. The tapetum is evidently of archesporial origin.

The megaspore remains unicellular until March of the second season's growth.

The nucleus now divides, and this is immediately followed by numerous free nuclear divisions.

The resulting free nuclei become distributed in the parietally-placed cytoplasm, and the first or primary prothallial cells are formed by the development of cell-walls.

The organization of the permanent prothallial tissue is brought about in the manner described for the majority of the Coniferales.

The archegonia originate at the apex of the prothallium. They are four or six in number, and each is enveloped by its own single layer of nourishing jacket cells.

During the fertilization period the inner walls become curiously modified by heavy, reticulated, chitinous-like thickenings.

The archegonia are isolated from one another by sterile tissue, and each is provided with a deep archegonial chamber.

A ventral canal nucleus is formed, but no cell membrane separates it from the egg.

The mature egg nucleus is many times the size of either of the male nuclei.

The union of the sex nuclei occurs in the middle region of the egg, and the first segmentation spindle is immediately formed within the membrane of the fusion nucleus.

The first division of the sporophyte is immediately followed by a second, forming four free nuclei in the pro-embryo.

These now sink to the base of the archegonium, and undergo further divisions.

Eventually the pro-embryo consists of three tiers of cells and one tier of free nuclei. The middle tier of cells become the suspensors, and the embryo proper consist of a large mass of cells, at least sixteen in number.

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## EXPLANATION OF FIGURES IN PLATES XXIX-XXXI.

Illustrating Dr. Lawson's Paper on *Sciadopitys*.

All figures were drawn with the aid of the camera lucida. The following oculars and objectives were used:—

Figs. 1, 2, 3, 4, 5, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, Zeiss oc. 8, obj.  $\frac{1}{12}$  oil imm.  $\times$  about 1600.

Figs. 14, 15, 45, 46, 47, 52, 53, 54, Zeiss oc. 4, obj. C.  $\times$  about 250.

Figs. 40, 41, 42, 43, 44, 48, Zeiss oc. 4, obj. A.  $\times$  100.

Figs. 10, 11, 12, 37, 39, Zeiss oc. 4, obj.  $\frac{1}{12}$  oil imm.  $\times$  about 1000.

Figs. 8, 9, 50, Zeiss oc. 8, obj. C.  $\times$  about 450.

Figs. 13, 49, Zeiss oc. 8, obj. A.  $\times$  about 160.

Fig. 1. A cross section of a microspore some time before pollination, showing the single centrally situated nuclear considerably enlarged and preparing for mitosis. April 22.

Fig. 2. The same at a later stage, showing the spindle of the first division of the microspore nucleus. This and the following two figures shows the characteristic hard thick exine tapering out and becoming very thin at one side. April 22.

Fig. 3. The same, showing the chromosomes at the poles of the spindle. April 22.

Fig. 4. The same, showing the daughter-nuclei fully organized and separated from one another by a very thin cell membrane. April 22.

Fig. 5. A section of a microspore a few days after pollination, as seen at the surface of the pollen-cushion of the nucellus. Here the exine has been completely thrown off, and the spore itself has become elongated and considerably enlarged. The generative and tube-cells are fully developed. April 24.

Fig. 6. A longitudinal section of a young ovule taken about three or four weeks after pollination, showing the integument and nucellus with the pollen-grains collected on the surface of the pollen-cushion. The micropyle has not yet been closed. May 25.

Fig. 7. A longitudinal section of the upper part of the nucellus, showing the nature of the highly differentiated tissue of the pollen-cushion, into which the pollen-tubes are penetrating. July 17.

Fig. 8. A longitudinal section of a pollen-tube after the division of the generative cell, showing the body-cell and the stalk-nucleus still in the spore region of the tube. June 17.

Fig. 9. The same, showing the relative positions of the three essential structures of the tube. The body-cell has remained within the spore region of the tube. The stalk and tube nuclei have descended into the tube for a considerable distance, the latter in advance of the former. June 17.

Fig. 10. The same, more highly magnified to show the structure of the nuclei and the presence of large masses of starch grains in the cytoplasm of the tube. July 17.

Fig. 11. A section of a pollen-tube taken just eleven months later than that shown in Fig. 10. Here the body-cell has descended into the tube and is situated near the tip. Its nucleus is very much enlarged in preparation for mitosis. June 19.

Fig. 12. A longitudinal section of a body-cell, showing the result of the division of the body-nucleus. The two male nuclei appear in sectional view to be of unequal size, but lie freely within the cytoplasm of the body-cell with no membrane separating them from one another. June 15.

Fig. 13. An outline drawing of a longitudinal section of an ovule.

Fig. 14. A transverse section of the megasporangium, showing the functional megaspore-mother-cell embedded in the tapetum. It will also be seen that the sporangium is connected with the integument at opposite sides of the broadest diameter, giving strength and support to the sporangium region. June 4.

Fig. 15. A longitudinal section of a megasporangium, showing the position of the large megaspore-mother-cell completely surrounded by two or three layers of tapetal cells. June 4.

Fig. 16. A longitudinal section to show the relative size of the megaspore-mother-cell to the surrounding tapetal cells. June 3.

Fig. 17. A megaspore-mother-cell with the nucleus enlarged and preparing for the reduction division. May 12.

Fig. 18. The same at a later stage, showing the chromatin in the form of a tangled spireme and the nuclear cavity considerably enlarged. May 12.

Fig. 19. The same at a slightly later stage, with the chromatin threads more slightly differentiated. June 3.

Fig. 20. The same with the chromatin threads thicker, shorter, and more evenly distributed through the nuclear cavity. June 3.

Fig. 21. A slightly later stage of the same, with the chromatin in the form of thick granular threads. June 3.

Fig. 22. The same with the granular chromatin threads still more shortened and thickened. June 3.

Fig. 23. The same with the chromatin threads so shortened and thickened that this granular nature is much less evident. Definite chromosomes are now formed. June 3.

Fig. 24. A later stage of the same, showing the double nature of the chromosomes. June 3.

Fig. 25. A megaspore-mother-cell at the time of the formation of the reduction spindle and showing the form of the heterotype chromosomes. June 4.

Fig. 26. A megaspore-mother-cell, showing the mature reduction spindle with the chromosomes at the equator. June 4.

Fig. 27. A reduction spindle with the heterotype chromosomes facing the equatorial plate. June 4.

Fig. 28. A transverse section of a megaspore-mother-cell with the heterotype chromosomes at the equatorial plate. June 4.

Fig. 29. A megaspore-mother-cell in the later phase of the reduction division, with the chromosomes at the poles of the spindle, and before the organization of the daughter-nuclei. June 4.

Fig. 30. The same a little later, with the daughter-nuclei organized, and the chromosomes very much vacuolated and giving a reticulated appearance. There is no trace of a cell-wall separating the daughter-nuclei from one another. June 4.

Fig. 31. A mother-cell, showing the simultaneous division of the daughter-nuclei. The twin spindles lie one above the other with the chromosomes, which are clearly reduced in number from the equatorial plates. June 4.

Fig. 32. The result of the second division of the mother-cell. Here it will be seen that cell-walls are formed after this division, and, as a result of this, these cells constitute an axial row. But in consequence of there being no cell-wall formed after the first division, and two cell-walls formed after the second division, we find the middle cell of the axial row containing two free nuclei, and the two end cells one each. June 4.

Fig. 33. A somewhat later stage of the same, showing the definite cell-walls separating the two end cells of the axial row from the middle cell, and the latter containing two free nuclei. It will be noted also that starch is much more abundant in the basal cell of the axial row than in the other two. June 4.

Fig. 34. A still later stage of the same, showing a shifting of the two free nuclei of the middle



cell, which proves quite clearly that no membrane separates them from one another. It may be seen also that the nucleus of the top cell of the axial row shows signs of disorganization. June 4.

Fig. 35. The same at a later stage. Here we see the two nuclei of the middle cell and the nucleus of the top cell showing signs of disorganization, and the basal cell considerably enlarged, with its nucleus in a normal active condition. June 4.

Fig. 36. A longitudinal section of the axial row. It will be seen that the basal cell has enormously enlarged and becomes really the functional megaspore. The middle and top cells show no growth whatever, and their nuclei are very much disorganized. June 12.

Fig. 37. A longitudinal view of the very large functional megaspore and the three abortive, more or less disorganized, functionless spores at the top. It will be noted also that the functional megaspore consists for the most part of a large central vacuole, and the relatively small amount of cytoplasm present is parietally placed. June 19.

Fig. 38. A more highly magnified view of the three abortive megaspores. The three nuclei become crowded together and altogether difficult to recognize as nuclei. The two free nuclei of the middle cell become flattened against one another. June 19.

Fig. 39. A part of the megaspore membrane as seen in longitudinal section. June 19.

Fig. 40. A longitudinal section of a very young archegonium, which at this stage consists of a central cell and two neck-cells. April.

Fig. 41. The same, showing the enlargement of the central cell and the differentiation of the jacket cells. May 15.

Fig. 42. A longitudinal section of two young archegonia, showing the great increase in length and breadth of the central cell. May 15.

Fig. 43. The same at a later stage of development, when the cytoplasm of the central cell has become very much vacuolated, and the sterile tissue at the apex of the prothallium has grown forward, leaving the archegonium behind, and forming a single archegonial chamber leading to the neck-cells of each archegonium. May 25.

Fig. 44. Two mature archegonia ready for fertilization, each containing a large central vacuole in the rear of the egg nucleus. June 19.

Fig. 45. The nucleus of the central cell in process of division. The spindle is formed directly under the neck-cells. June 15.

Fig. 46. The ventral canal spindle with the chromosomes at the poles. June 15.

Fig. 47. The neck region of an archegonium, showing the relative size and position of the ventral canal nucleus and egg nucleus soon after they are formed. June 15.

Fig. 48. A longitudinal section of the apex of the nucellus and upper part of the prothallium, to show the penetration of the pollen-tube through the nucellar tissue and archegonial chamber to the neck-cells of the archegonium. June 19.

Fig. 49. A longitudinal section to show the relative size of the two male nuclei as they lie in the upper cytoplasm of the archegonium immediately after their discharge from the pollen-tube through the neck-cells. June 27.

Fig. 50. A longitudinal section of the fusion-nucleus immediately after fertilization, to show the position and form of the first sporophyte-spindle within the confines of the membrane of the egg nucleus. June 29.

Fig. 51. A surface view of the chitinous-like reticulated thickenings on the inner walls of the jacket cells during the period of fertilization. June 29.

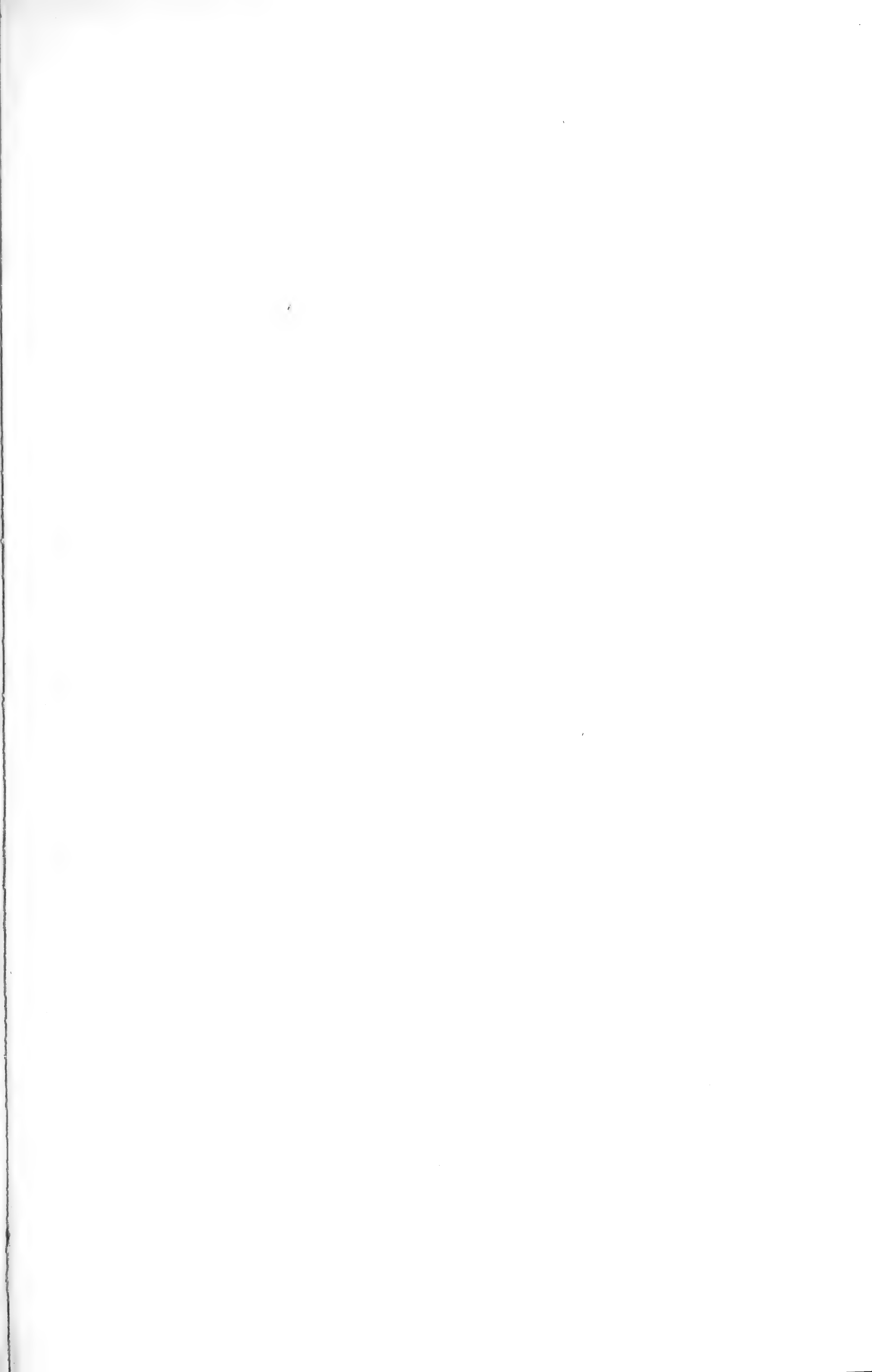
Fig. 52. A longitudinal section of a pro-embryo, showing four free nuclei descending to the base of the archegonium. June 29.

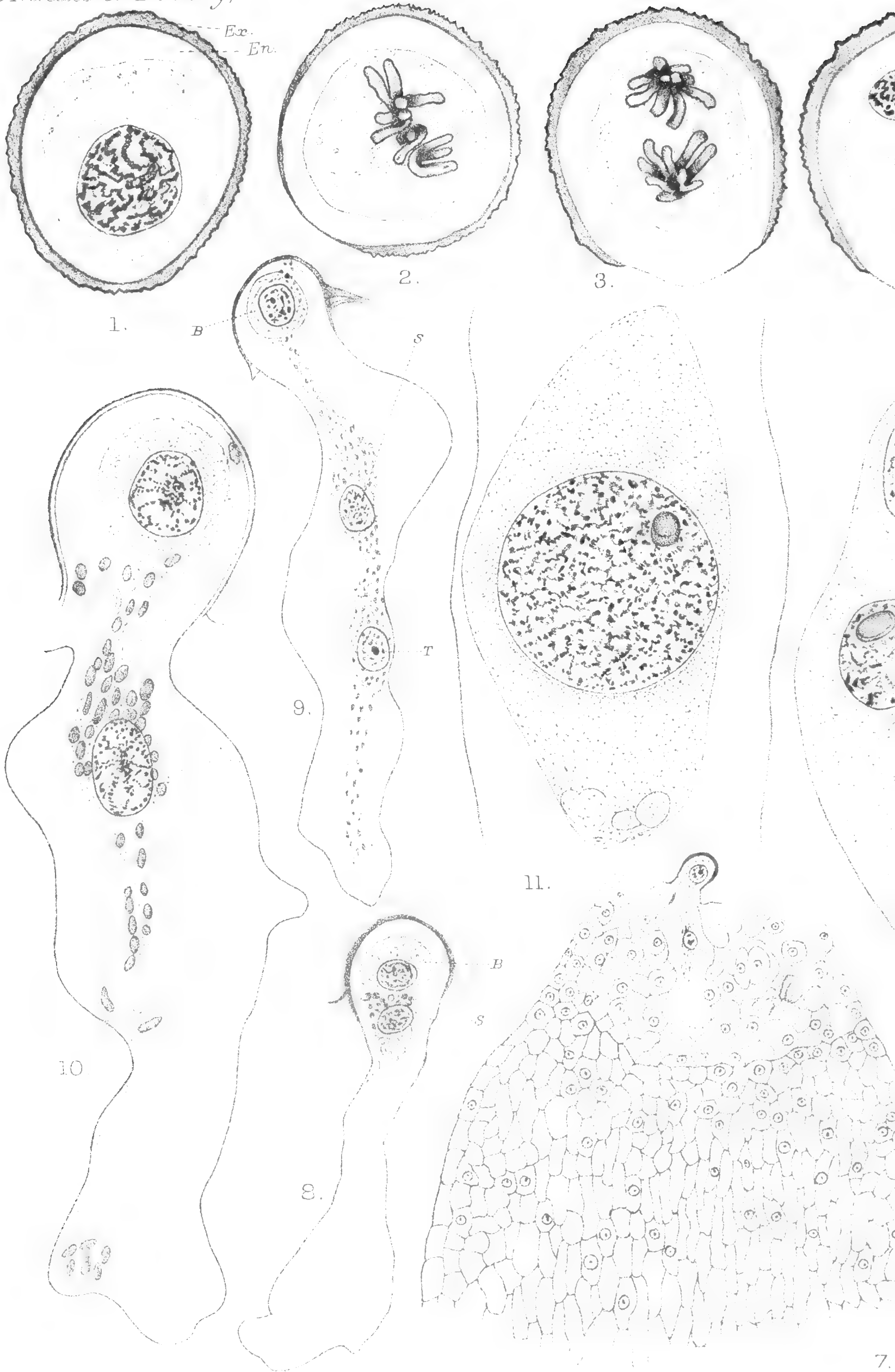
Fig. 53. The same at a later stage, to show the arrangement of the free nuclei in three tiers just previous to the formation of walls between them. July 2.

Fig. 54. A later stage in the development of the embryo after the elongation of the suspensors. The embryo proper at this time consists of several layers of cells which are carried forward into the prothallial tissue by the elongated suspensors. July 7.







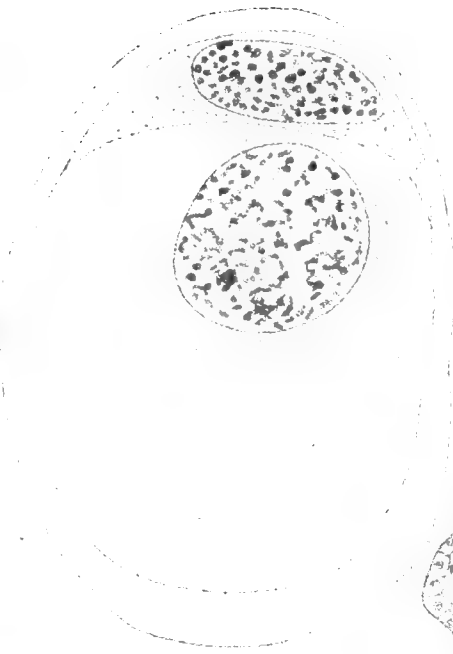


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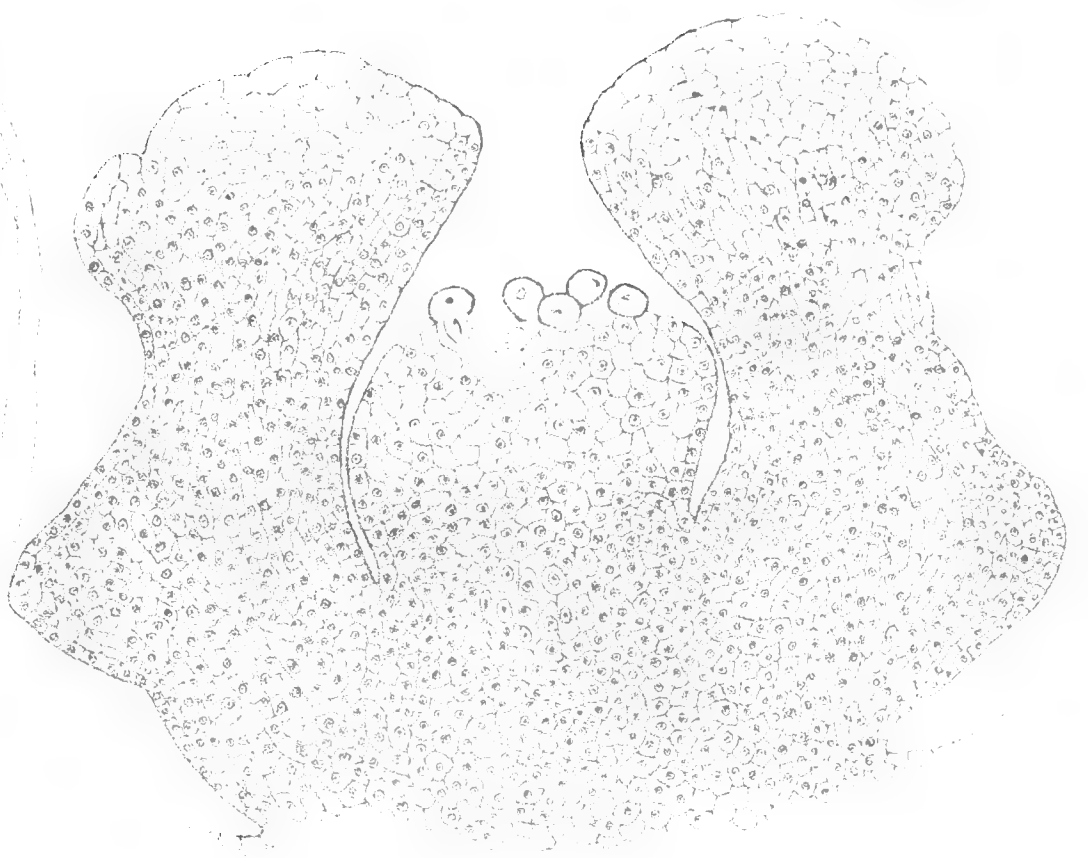
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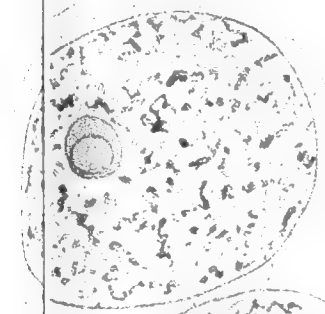
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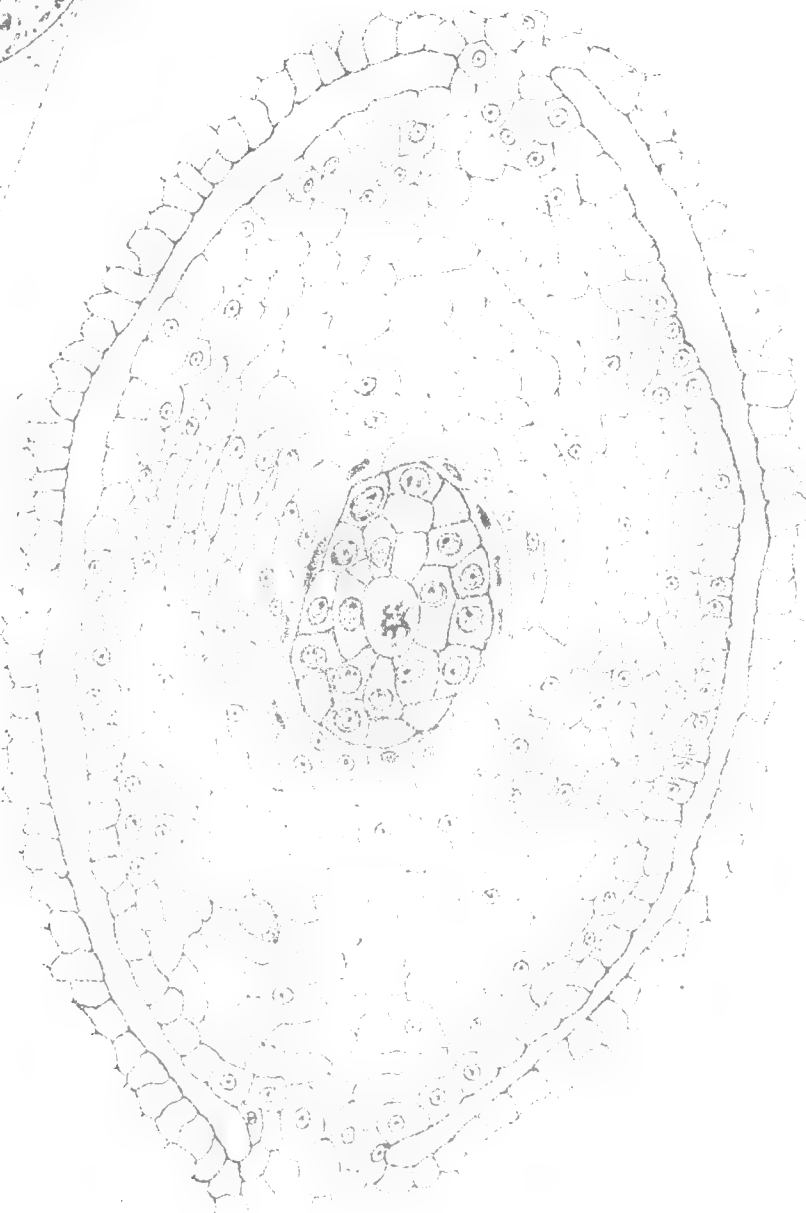
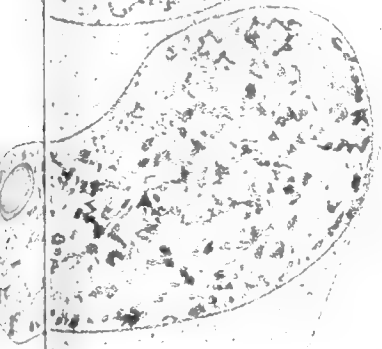
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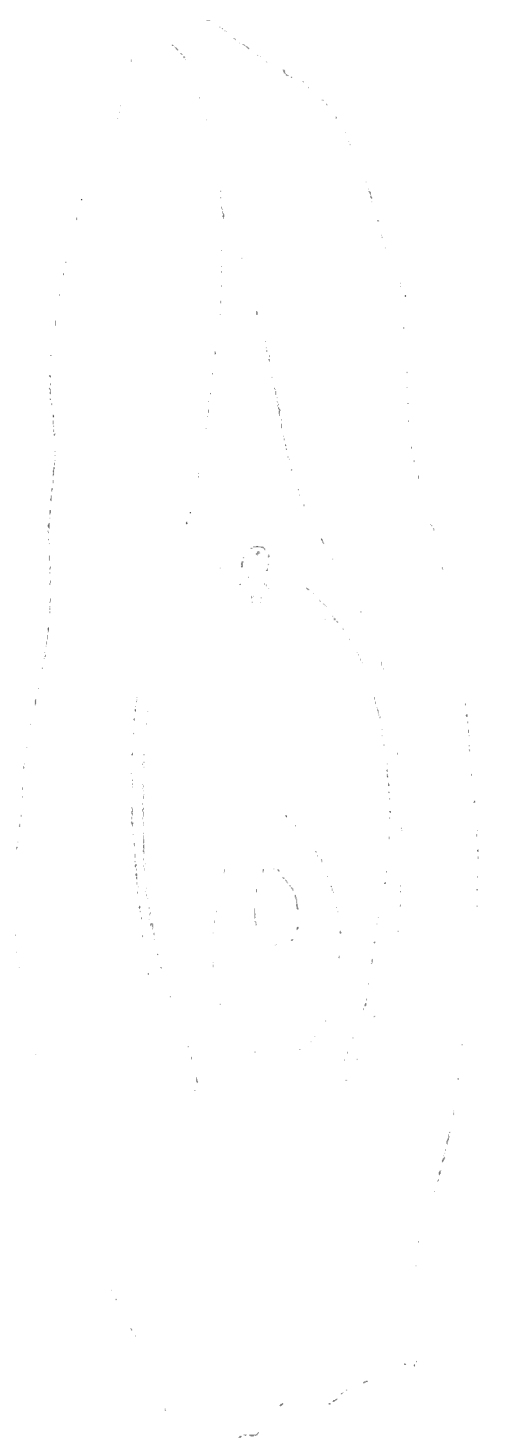
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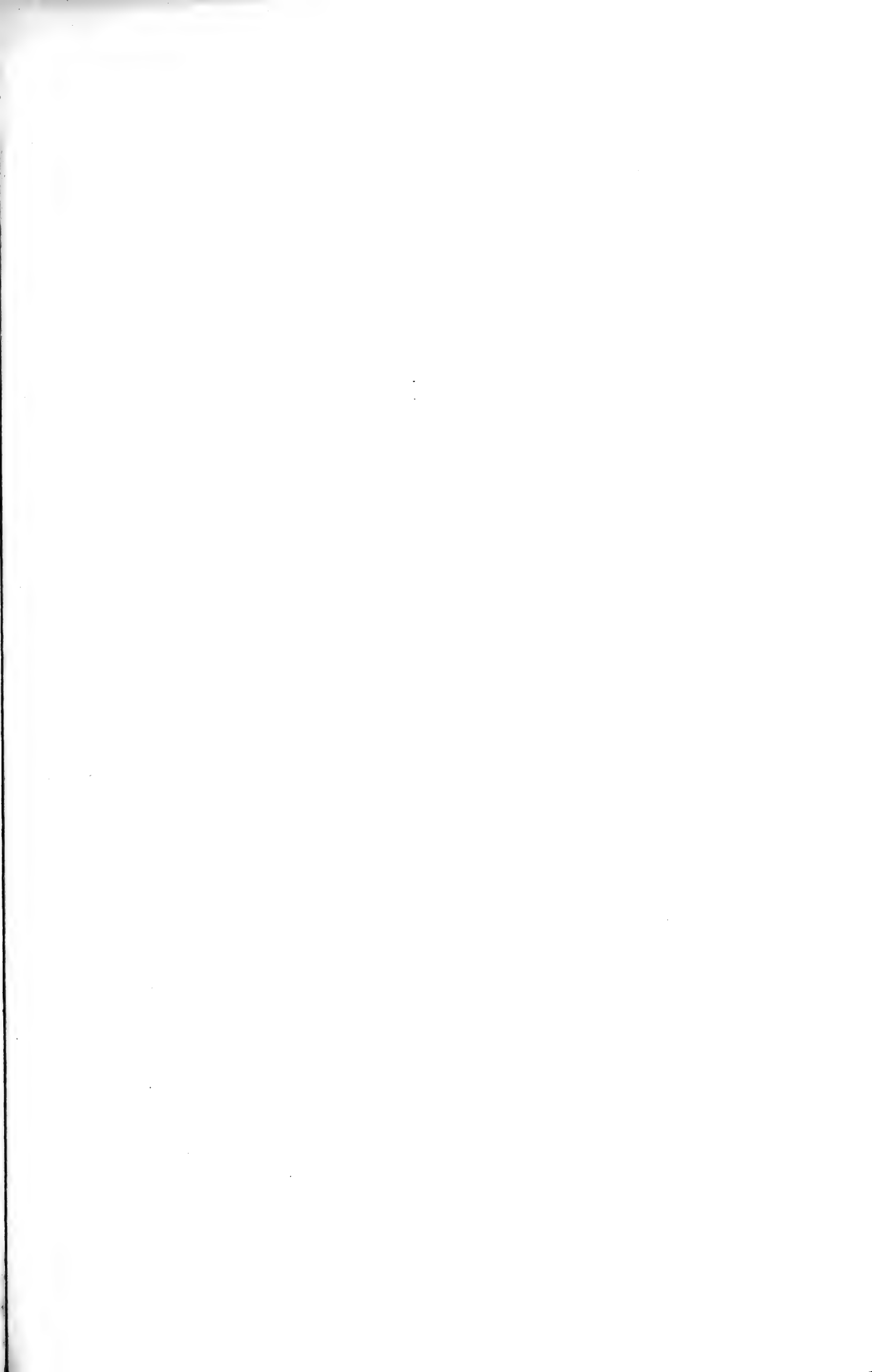


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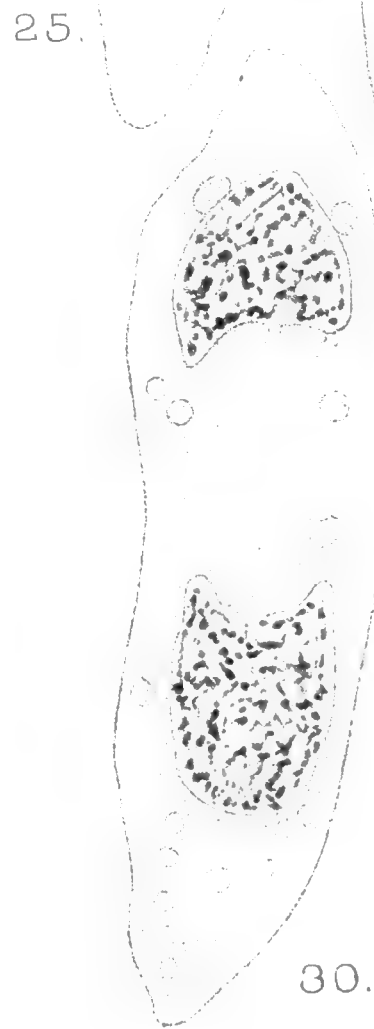
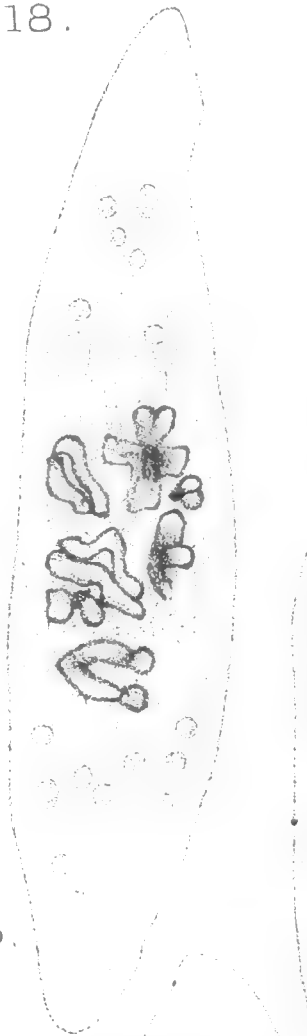
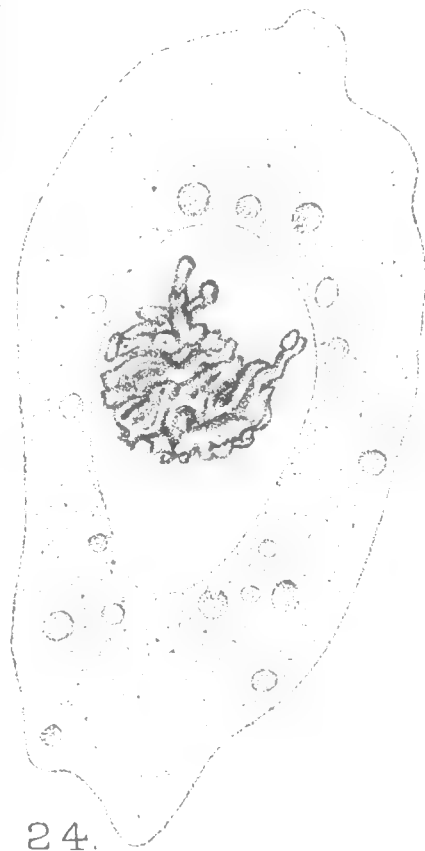
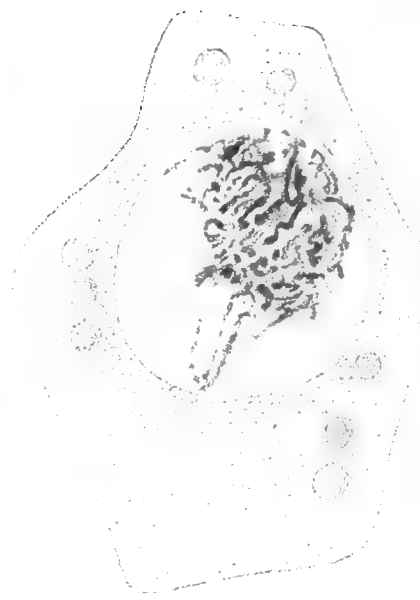
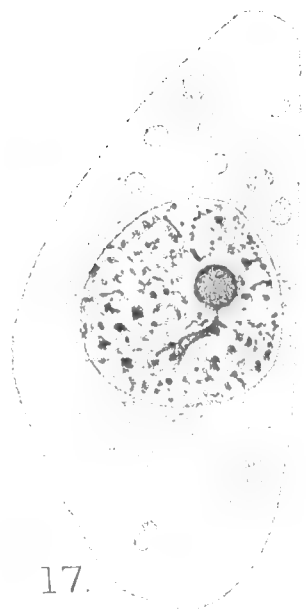
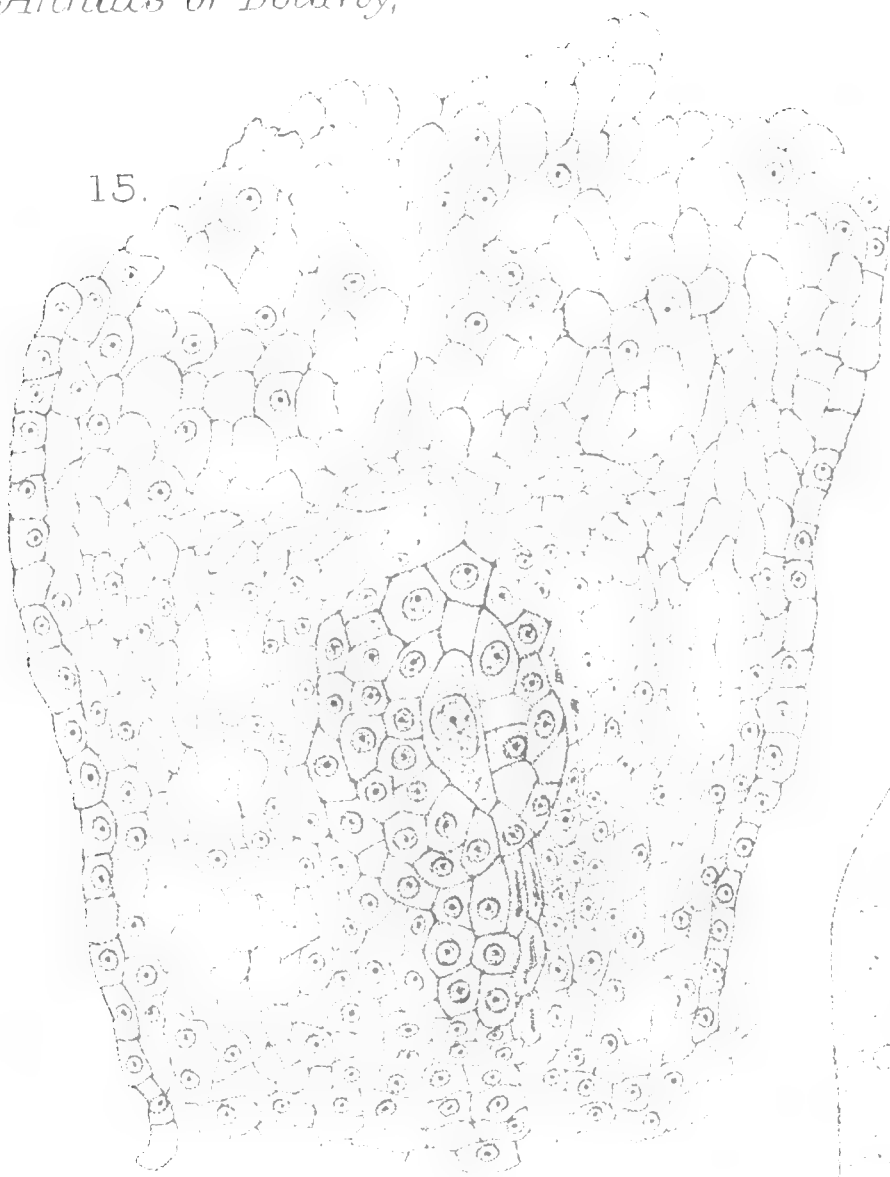


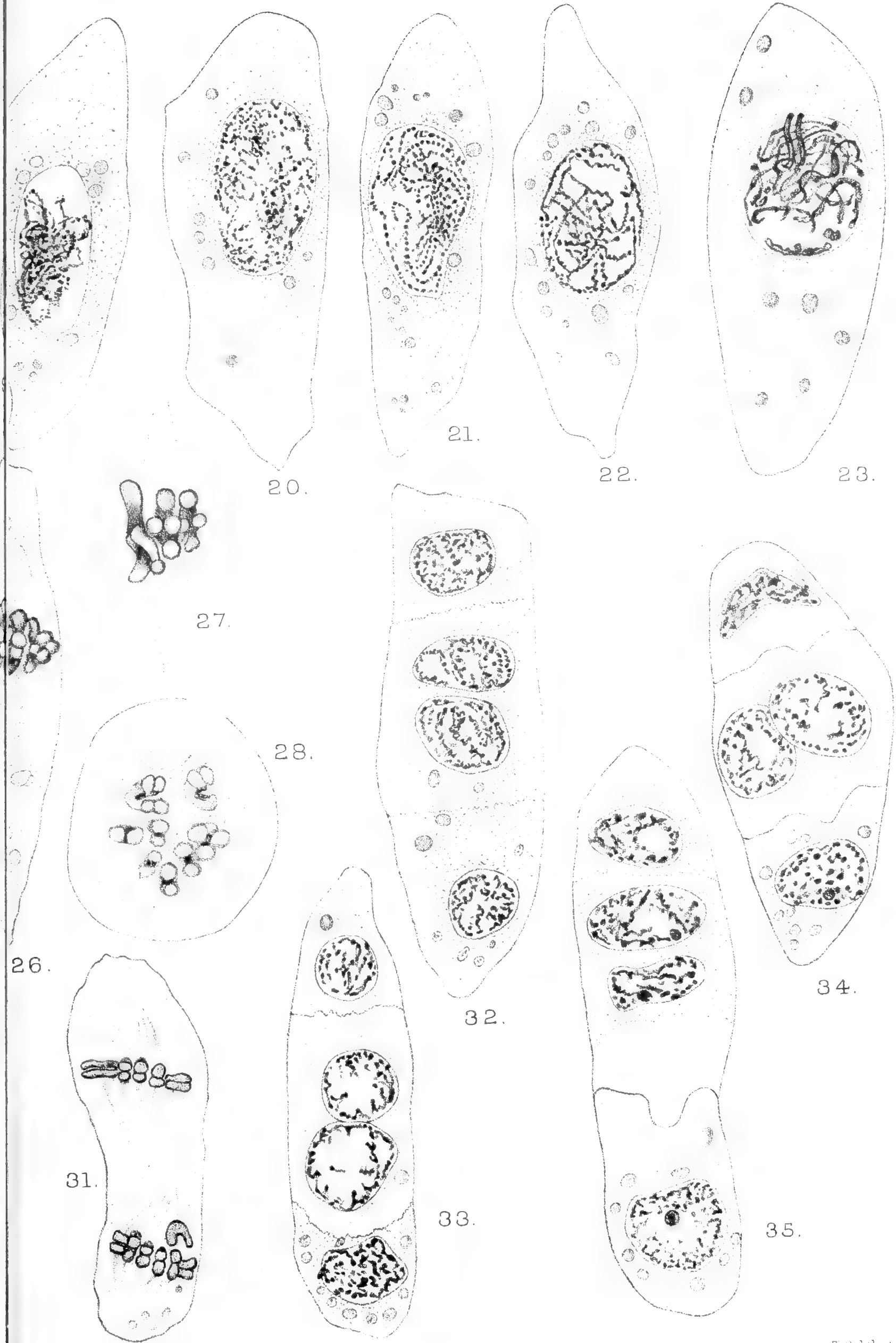
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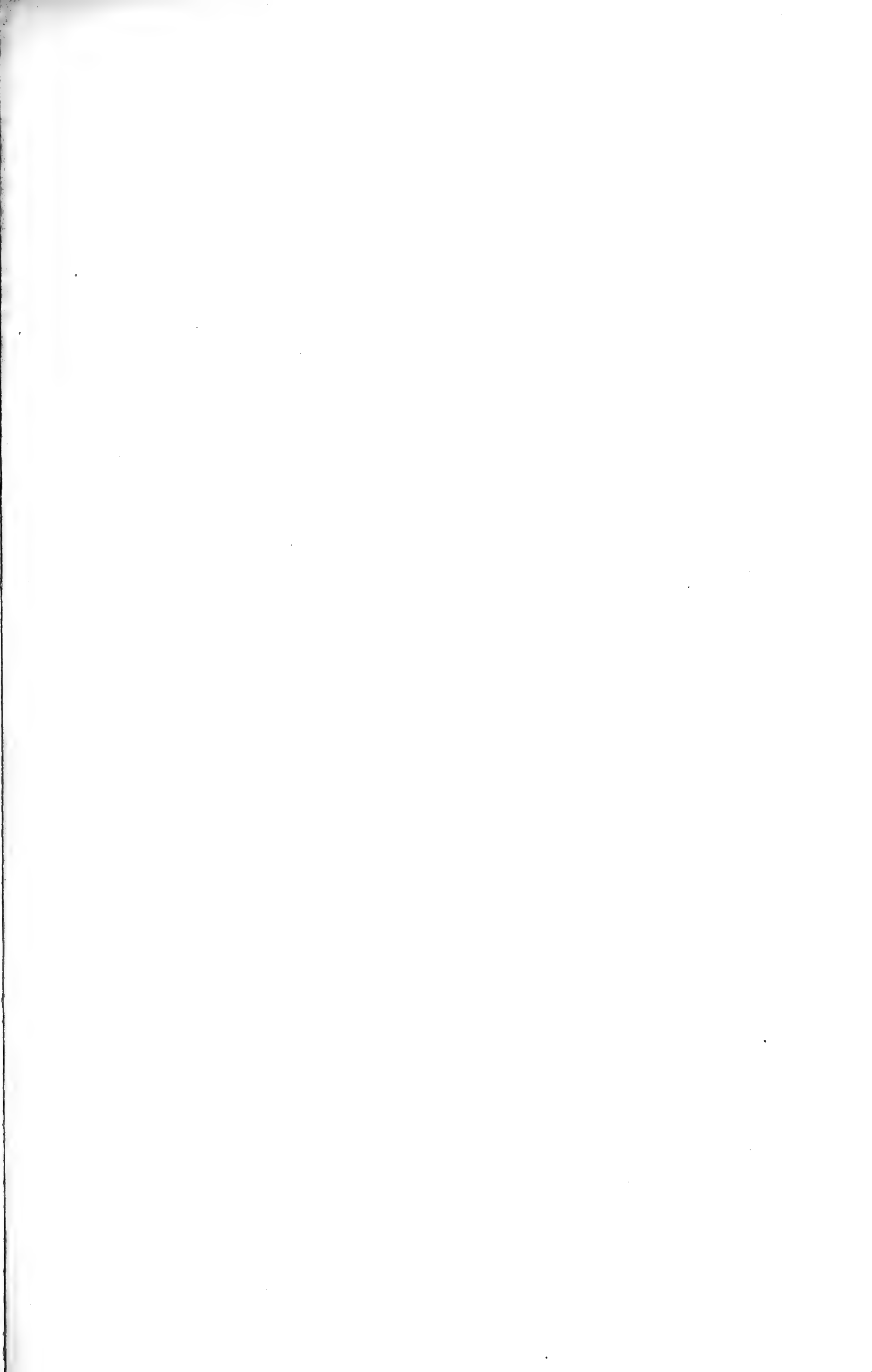






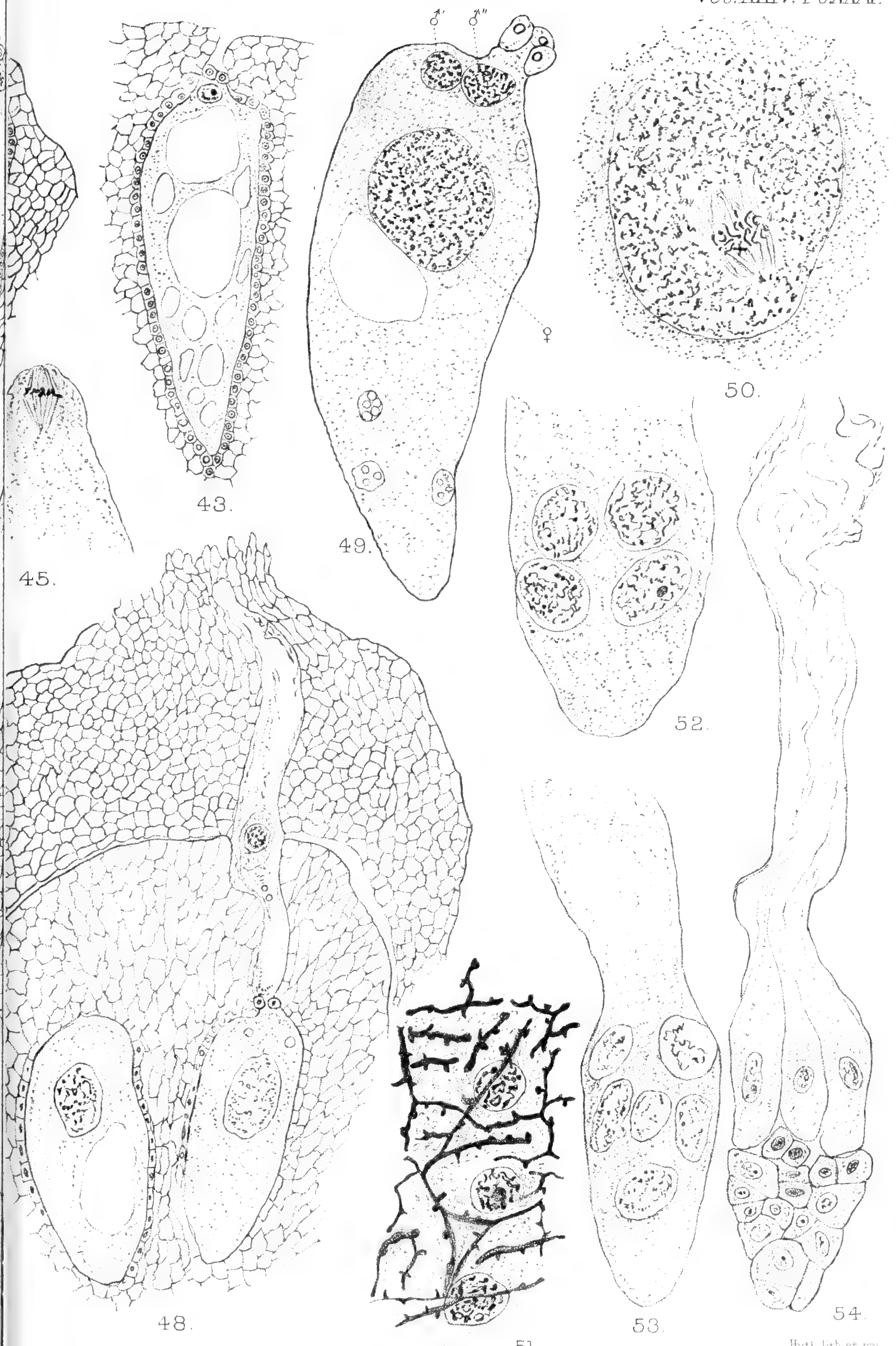








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# Studies in the Phylogeny of the Filicales.

## I. Plagiogyria.

BY

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With Plates XXXII and XXXIII and five Figures in the Text.

FROM very early days the position of the annulus of the sporangium has been held to be a character of the first importance in the comparative treatment of Ferns. In the year 1800 both Bernhardt<sup>1</sup> and Swartz<sup>2</sup> had introduced features of the annulus as defining the larger divisions of the family, but it remained for Bernhardt<sup>3</sup> later to distinguish under the names *Helicogyratae* and *Cathetogyratae* the Ferns respectively with oblique and with vertical annulus. This distinction was adopted by Presl in his 'Tentamen' of 1836 (p. 20), and he made the oblique annulus the character of his first sub-order, while the vertical annulus characterizes the second sub-order of Ferns in his system. Presl's classification was further adopted by Sir William Hooker in his 'Genera Filicum' (1842); it is embodied in the synopsis at the conclusion of that work, with the remark that it is 'the most full and complete that has yet been published'. But, on the other hand, Kunze<sup>4</sup> criticizes Hooker's neglect of exact detail of position of the annulus in some of his figures, and praises Schott ('Genera Filicum', 1834) for his accuracy in delineation of the annulus; he points out the higher degree of organization of the stomium in the *Cathetogyratae* than that usual in the *Helicogyratae*.

It is in the paper of Kunze just quoted that the first description is given of any species of the genus now recognized under the name of *Plagiogyria*; he found in the Ferns described as *Lomaria niponica* and *euphlebia* a condition different from that of all Polypodiaceae, uniting the type of annulus having a highly organized stomium with the oblique annulus

<sup>1</sup> Bernhardt, J. J.: Schrader, Journal für die Botanik, ii, 1800.

<sup>2</sup> Swartz, O.: Schrader, Journal für die Botanik, ii, 1890.

<sup>3</sup> Dritter Versuch einer Anordnung der Farnkräuter. Schrader, Neues Journal für die Botanik, 1806.

<sup>4</sup> Pteridologische Studien. Ueber eine noch unbeobachtete Form der Sporangien (*Sporangia plagiogyrata*). Bot. Zeit., 1849, p. 865.

of the *Helicogyratae*. The stock of these Ferns was not examined, but, on other general grounds, he referred them to the genus *Lomaria*, not seeing in the peculiarity of their sporangia any sufficient reason for removing them from that genus. A more full description with careful drawings was given by him in the following year,<sup>1</sup> but again without the axis having been observed. In leaving these species within the genus *Lomaria*, notwithstanding the divergence of structure of their sporangia, Kunze remarks (l. c., p. 62) that in his view the microscopical features will never lead to a natural arrangement, and they can only be used as indications; the species may, however, be designated as a section of the genus under the name of *Plagiogyria*. Next in point of date came the monograph of Mettenius,<sup>2</sup> in which he separated the sub-genus from *Lomaria* under the generic name of *Plagiogyria*; but Sir William Hooker<sup>3</sup> still retained it as *Lomaria*, where it remained in the 'Synopsis Filicum'.<sup>4</sup> His reasons for this are explicitly stated ('Species Filicum', iii, p. 2); the passage is interesting as illustrating the attitude of a great systematist in the year 1860; he wrote: 'I am not disposed to retain *Plagiogyria* separate from *Lomaria*, although constituted by a botanist by no means addicted to establishing new genera on slight grounds. It has peculiarities in the base of the stipes and in the presence of certain glands called by Mettenius *aerophorae*; but notwithstanding this structure, and even should the capsules in all the species referred to *Plagiogyria* prove to be helicogyrate, yet the habit and sori are so entirely in accordance with true *Lomaria* that, unless the student has the opportunity of examining very perfect specimens, or unless he examines the structure of the annulus of the very minute capsules under the high power of a microscope, the genus cannot be identified. Kunze, who first recognized the physiological differences, only proposed to form a group or section, under the name of *Plagiogyria*, but even that would be inconvenient to retain in a work whose main object is to assist the tyro in the verification of genera or species; and natural habit is often a safer guide than minute microscopic characters.' On this footing convenience of use for purposes of recognition takes precedence over natural affinity in the systematic method.

The monograph of Mettenius above alluded to established *Plagiogyria* as a substantive genus, notwithstanding the divergent opinions of Hooker and of Kunze. Comparisons had been drawn by various writers between the Ferns of the *Plagiogyria* section of *Lomaria*, and other species of *Lomaria*, and of *Stenochlaena* on grounds of external form; but it was pointed out by Mettenius that there were broad differences notwithstanding the superficial similarity; the characters recognized by him as distinguishing

<sup>1</sup> Kunze, G.: Farrnkräuter (Schkuhr's Farrnkräuter, Supplement). Zweiter Band, p. 61, Taf. CXXV, and 91, Taf. CXXXVIII.

<sup>2</sup> Ueber einige Farrngattungen. II. *Plagiogyria*, 1858.

<sup>3</sup> Species Filicum, iii, p. 2, 1860.

<sup>4</sup> l. c., p. 182, 2nd ed., 1883.

*Plagiogyria* were not only the oblique annulus, but also certain peculiarities of the vegetative organs (l. c., p. 7), such as the swelling of the leaf-bases, the spongy tissues (aerophorae) which they bear, and the absence of peltate scales; also he notes that the spores are tetrahedral. This monograph, to which allusion will frequently be made below, gives by far the most complete account yet published of this interesting type of Ferns. Later writers seem to have left the genus severely alone, so far as detailed investigation is concerned. Prantl<sup>1</sup> does not even mention it in his valuable remarks on the systematic importance of the annulus, nor does he place the genus by name in his system of the Polypodiaceae. Of later writers Diels<sup>2</sup> places *Plagiogyria* as a substantive genus under the Pterideae, and in next proximity to *Cryptogramme*. Christ<sup>3</sup> also maintains it as a substantive genus; he places it at the end of the Pterideae (Hook.), and next to *Actiniopteris*, with *Blechnum* as the first genus of the succeeding Blechneae. Raciborski,<sup>4</sup> on the other hand, follows Hooker's 'Synopsis' in still including the species under *Lomaria*.

It is quite plain from the above notes that the Ferns now grouped in the genus *Plagiogyria* occupy an anomalous position in the whole system; moreover, the knowledge of their characters is still very incomplete; the prothallus has never yet been described, and the anatomy is still very imperfectly known. It thus appears desirable to submit them to a fresh examination, with the object of widening the basis of their comparison with other forms. More especially does this seem necessary in view of the fact that *Plagiogyria* alone of described Ferns appears to combine the distinctly oblique annulus of some Simplicies and of the Gradatae with certain general characters of the Mixtae. It will be seen later that this conjunction of features usually distinct gives the genus a special importance in estimating the probable phyletic relations of the Mixtae to other relatively primitive types of Ferns.

As at present described, there are eleven species of the genus *Plagiogyria*; two of these inhabit the Western hemisphere, and one of them, viz. *P. semicordata*, (Pr.) Christ, was collected on Sir John Peak, on my recent visit to Jamaica; living plants and fresh spores were brought to Glasgow, as well as dry specimens and preserved material. The remaining nine species are Eastern, being chiefly represented in China and Japan; the material available was referred to *P. pycnophylla*, (Kze.) Mett., a species native in the north of Hindustan, the Malay Peninsula, and in Java; it was derived partly from Darjeeling, from Mr. Hemsley, and largely from Buitenzorg through the kindness of Dr. M. Treub. From the same source

<sup>1</sup> Das System d. Farne. Arb. aus dem k. bot. Gart. zu Breslau, i, p. 4.

<sup>2</sup> Engler und Prantl: Pflanzenfam., i, 4, p. 281.

<sup>3</sup> Farrnkräuter der Erde, p. 175.

<sup>4</sup> Die Pteridophyten der Flora von Buitenzorg, p. 162.

I also received material of *P. glauca*, (Bl.) Mett. There is also in my hands material apparently of *P. pycnophylla*, collected by Dr. Lang on the Malay Peninsula. It is upon these supplies that the following description is based.

#### EXTERNAL CHARACTERS.

The stock in all of the species examined was upright and relatively massive, with a habit similar to that of the Osmundaceae; it projects above the soil for some inches to a foot in height, and is entirely covered externally by the persistent bases of the leaves of former seasons, which are enlarged, and coated with dark sclerenchyma. Occasionally the axis bifurcates, the branching being clearly dichotomous; this was specially observed in several examples of *P. semicordata*. In *P. pycnophylla* dichotomy was not seen, but here and there stolons are found attached to the stock; these may be attenuated at their base, taking there a horizontal or oblique course, and bearing at first only stunted scale-leaves; but sooner or later (and in some cases without any attenuated basal region at all) the stolon expands into a massive trunk, bearing numerous and more closely disposed foliage leaves. A formation of stolons was also observed in *P. glauca*, in essentially the same way as in *P. pycnophylla*, while dichotomy was not seen in that species. Stolons were not observed in *P. semicordata*. From the specimens examined it would thus appear that the terminal dichotomy of *P. semicordata*, and the lateral formation of stolons, as in *P. pycnophylla* and *glauca*, are complimentary methods of increasing the complexity of the shoot-system; *P. semicordata* has apparently retained the more direct and primitive, and *P. pycnophylla* has adopted a more indirect and probably a more recent method of carrying this out.

The leaves are of the simple pinnate type characteristic of the genus *Blechnum*, but they present several points of special interest, for the most part already noted by Mettenius; these are here detailed afresh in order to fill in the picture of the habit of the genus. The leaves form a dense terminal rosette, being arranged on a spiral plan which increases in complexity as the axis enlarges distally. There is a sharp differentiation of the broader sterile from the narrower and often longer fertile fronds. The foliage leaves are of a rather rigid texture in both species, while in *P. semicordata* there is a marked serration at the distal end of the pinnae (compare Mettenius, l. c., Taf. XV, Fig. 1); this is less marked in the leaves of *P. pycnophylla* (compare Kunze, l. c., Taf. CXXV). The leaf-base in both species is enlarged, widening out laterally, and thickening in a marked degree; on the abaxial side it bears a projecting flange, which is specially prominent in *P. pycnophylla*; on either side of this, but not maintaining any degree of regularity in number or in position, are the well-known 'aerophorae' of Mettenius, which appear in the mature leaf as projecting brownish masses of pulverulent tissue leading through the dense



sclerotic covering to the softer tissues within. Passing upwards the enlarged leaf-base tapers gradually without any definite limit into the petiole, which is more or less clearly four-angled, while dwarf wings project slightly from the lateral faces. In the upper region of the leaf the pinnae are inserted upon these wings; in *P. pycnophylla* the pinnae are some little distance apart from one another, and are very shortly stalked, but in *P. semicordata*, especially towards the tip of the leaf, they run together at their bases, so that the rachis is quite continuously winged; these features are repeated in the fertile leaves, though in less pronounced degree (Pl. XXXII, Figs. 1 and 2). The venation is of the sub-Taeniopterid type (Mettenius, l. c., p. 1), and the forking of the veins is inconstant; a considerable number of them run to the margin unbranched, there traversing the minute serrations which are specially prominent in *P. semicordata*.

In the young circinate condition the leaves are completely covered with a dense felt of curly hairs, which are septate, unbranched, and terminated by a single large cell with mucilaginous contents; in essentials they are similar to those of the leaves of the Osmundaceae. Peltate scales are entirely absent, as noted by Mettenius (l. c., p. 1). In this condition the young leaf is completely protected from risks of evaporation from its surface. The felted covering is more strongly developed in *P. pycnophylla* than in *P. semicordata* or *glauca*.

Probably in intimate relation to this is the development of those 'aerophorae' described by Mettenius, or pneumatophores as they would now be called. Their presence on the leaf-base has already been noted, and drawings of them are given in most illustrated treatises; they are here represented as seen on the bases of young leaves of *P. glauca* in Figs. 3 and 4. In the upper region of the leaf they were also recorded by Mettenius as occurring in *P. pycnophylla*, one at the base of each pinnule; here they are clearly to be seen in the mature leaf (Fig. 1); but it is in the young condition, while the leaf is still circinate, that they are most prominent (Figs. 5 and 6). In *P. semicordata*, though the pneumatophores are present on the leaf-base, they are not recognizable on the distal region of the leaf. This may be put in relation with the fact already noted that the hairy investment is less developed in this species than in *P. pycnophylla*.<sup>1</sup>

<sup>1</sup> Pneumatophores are well known as prominent objects on the leaf-base in the genera *Alsophila*, *Hemitelia*, and *Cyathea*, where they are often of large size; in the mature state of the leaf they appear as roundish or oval pores traversing the hard sclerotic coat, and are filled with friable and dry tissue (see Mettenius, l. c., pp. 3-5, where are also references to other writers). In the upper leaf of these Ferns there are along the marginal wings, and especially close to the bases of the pinnae and pinnules, pale and slightly turgid spots, which show by the presence of stomata and by the spongy subjacent tissue that they are of importance for ventilation. They do not, however, project beyond the surface to any considerable degree. But occasionally at spots corresponding to these the tissues grow out into processes of considerable size, and the 'aerophorae' or pneumatophores in the upper leaf of *P. pycnophylla* are cases in point. Similar processes are seen also in *Dryopteris* (*Polypodium*) *decussata*, (L.) Urban, and in *Dryopteris* (*Polypodium*) *Thomsonii*, (Jenm.) C. Chr.



The fertile leaves resemble the sterile, except that their pinnae are narrower, and their margins sharply recurved so as to protect the sporangia (Figs. 1 and 2). These are borne in large numbers upon the slightly enlarged receptacles which lie above the distal ends of the veins; the sori are thus slightly elongated between midrib and margin. They seem to be always distinct laterally, and the insertion of sporangia on the leaf-surfaces between the veins, as described by Mettenius (l. c., p. 5), has not been observed.

On the same page of his memoir as that just quoted, Mettenius also describes for *P. scandens* (l. c., Fig. 21) how the neighbouring secondary nerves of the pinnae are joined by intramarginal anastomoses. *P. scandens* from the Khasya hills has since been reduced to *P. pycnophylla*; of this species I have material from Darjeeling and from Java; in neither of these was any evidence found of such anastomoses. So far as my observations go the secondary veins are never united distally, and the sori which they bear are always laterally distinct from one another, except in those cases where there has been a basal forking of the vein.

The roots are of the usual Fern type; they are not so numerous as in some other upright growing Ferns. Judging from the appearance in transverse sections, there are on the average more than two to each leaf; but they do not arise in any definite or constant numerical relation to them (Fig. 6). They spring from the central dictyostele, and usually arise from the margins of the foliar gaps.

#### ANATOMY.

The anatomy of the genus *Plagiogyria* has received little attention hitherto. Mettenius (l. c., p. 3) has recorded the fact that in *P. semicordata* a single vascular bundle enters each leaf from the stem; this then divides into three as it passes into the swollen region of the leaf-base, but the

It is to be noted that they are associated in these Ferns with a voluminous and slimy secretion completely covering the young circinate leaf; it arises from numerous branched hairs, the distal cells of which are enlarged as in *Osmunda* and *Plagiogyria*, and produce mucilage internally. It appears probable that there is a relation between the existence of the mucilaginous covering over the young part and the formation of the projecting pneumatophores; the latter are longer in the cases quoted than the thickness of the mucilaginous investment of the young part, and therefore project beyond its surface; thus they would readily serve the purpose of gaseous interchange to the tissues within (compare Fig. 5). Moreover, their most important function appears to be past on the development of the leaf, for then they are apt to shrivel. From these facts the conclusion seems justified that they are provisions for the aeration, not of the mature parts, but of the young parts while covered by mucilage, and thus cut off from ready gaseous interchange with the atmosphere. It is to be noted, however, that they are not always present where even a thick mucilaginous felt exists; this is shown by the case of the Osmundaceae. From a morphological point of view they cannot be held as parts of high importance for purposes of comparison, since their inconstancy, even within a genus, indicates that they are opportunist growths, rather than permanent morphological features in any phylum.

Organs similar to those described above have been noted by Kühn (Flora, 1889, p. 486) in *Nephrodium stipellatum*, Hk., and an abstract of his results appears in Haberlandt's Phys. Pflanzen-anat., 4. Aufl., p. 400, where the same function is attributed to them as suggested above.

branches again unite at the upper limit of the swelling into a single strand, and in this form it passes up through the whole length of the leaf. The only statement I know as to the structure of the stock is by Gwynne-Vaughan;<sup>1</sup> he figures a transverse section of a rather small stock (l. c., Fig. 10), with the remark (l. c., p. 697) that it is radially dictyostelic, but still remains very close to solenostely.

A transverse section of a large stock of *P. pycnophylla* accords entirely with this description (Fig. 6); it appears very irregular in outline as a consequence of the closely aggregated leaves with their decurrent insertion. Their arrangement is on a complex spiral, which varies with the size of the stock. Each is plainly served by a single V-shaped vascular strand, which is accompanied on its way by a broad flanking sheet of dark sclerenchyma on its outer side, and a small patch of the same in the fork of the V. Each leaf-base is subtended on its adaxial side by a deep involution of the surface, of semilunar outline convex inwards, and lined by sclerotic tissue. It will be observed, by comparison of the leaf-traces in the section figured, that internally, opposite each leaf-trace at its first separation from the axial stelar ring, there is a patch of sclerenchyma; as the leaf-trace passes outwards this sclerenchyma expands, taking gradually the semilunar form; it is in the middle of these patches that the involutions make their appearance, widening out (as seen in the transverse section) till the leaf-base which each subtends is completely separated from the stock. A similar involution has been noted in other Ferns, and among the Helicogyrate types which show it are *Anemia* and *Onoclea*.<sup>2</sup>

The middle of the massive axis is occupied by a column of pith, the central region of which is sclerotic, while isolated smaller strands of sclerenchyma about its periphery mark the position where the successive foliar gaps will be formed by passage outwards of the leaf-traces. Surrounding the pith is an almost complete solenostele, interrupted only here and there by the separation of the foliar traces from it: these appear first as outwardly projecting bays opposite to the sclerotic strands already mentioned, and finally they break away from the solenostele by abstriction right and left. The roots, which are seen in large numbers traversing the cortex, spring as a rule from the margins of the gaps thus left by the outgoing traces.

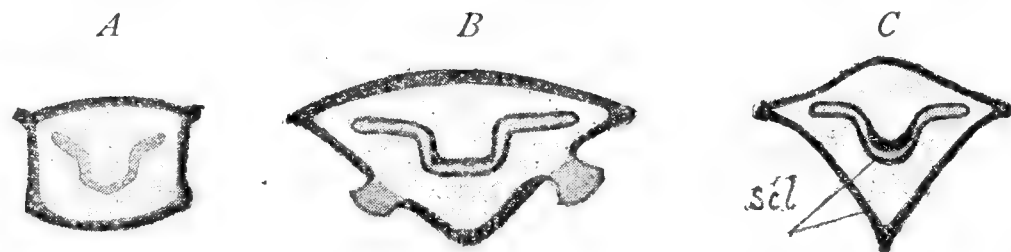
The stelar ring is delimited internally and externally by a brown coloured endodermis, which is continuous through the foliar gaps, and is lined internally by a pericycle of 1-3 layers. The phloem is abundant both

<sup>1</sup> Solenostelic Ferns, II. Ann. Bot., xvii (1903), p. 689. Gwynne-Vaughan names the plant on which he worked *Plagiogyria biserrata* (= *Lomaria semicordata*); but as his material came from the Eastern tropics this cannot be correct; there are no herbarium specimens of the same collection, so it is impossible to check the determination; but all the characters point to the plant having been *P. pycnophylla*.

<sup>2</sup> Compare Gwynne-Vaughan: On the possible existence of a Fern stem having the form of a lattice-work tube. New Phyt., iv, p. 211.

on the peripheral and central sides of the xylem, but thins off considerably at the foliar gaps. The xylem, which is massive, is composed of tracheides with parenchyma scattered through it; the protoxylem is not clearly defined when seen in the mature condition.

The leaf-trace is initiated in the stelar ring, and may be recognized early by an indentation of the inner limit of the xylem opposite one of the sclerotic groups in the pith, which, as mentioned above, subtend the leaf-trace, and accompany it outwards. As this indentation deepens a group of small tracheides (protoxylem) intermingled with parenchyma becomes apparent at its apex, while opposite it the outer limit of the xylem becomes strongly convex, forming the projecting bay already noted. Subsequently, by abstrictions of the stelar ring at some distance right and left of the indentation, with incurving of the sheaths, the leaf-trace is separated, and passes outwards. Entering the leaf-base, which is deeply keeled so as to be triangular in section, the leaf-trace widens out, and loses its acute shape, the apex of the V expanding and rounding off; the sclerenchyma meanwhile



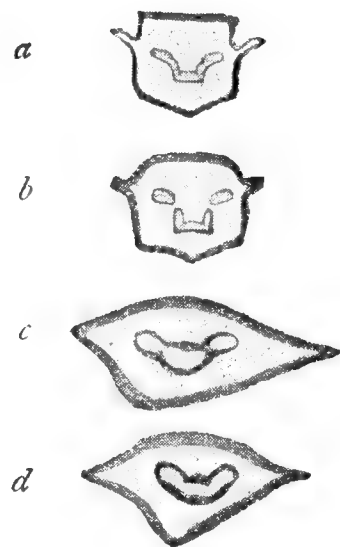
TEXT-FIG. 1. Transverse sections of the petiole of *P. pynophylla*. *A* is taken from a point above the basal swelling; *B*, from a point about the middle of the swelling, and it traverses two of the pneumatophores. *C* is from a point near to the insertion of the leaf upon the axis.

spreads out so as to ensheath the whole strand, the periphery of the section being also covered by a hard sclerotic band (Text-fig. 1, *C*). A section higher up shows the leaf-base further enlarged, and the strand widened out still more, especially in its middle part, while the section may often traverse one or more of the large pneumatophores, which are seated on the oblique abaxial faces (Text-fig. 1, *B*). As the upper limit of the swollen region is reached the vascular strand again contracts, and its sclerenchymatous sheath disappears; the adaxial surface of the strand becomes slightly crenate; examination of these crenations shows that they correspond to the groups of cavity-parenchyma (*Liickenparenchym*), which indicate the position of the protoxylem-groups; the number of these is about nine, three on each of the lateral flanges, while of the other three one is median, and the other two are right and left of it at the angles of the sharp curvature of the leaf-trace (Text-fig. 1, *A*). This structure is maintained upwards to the point where the pinnae are borne. The structure of the leaf-base is found to be the same in *P. glauca*.

A curious difference of vascular structure is, however, seen in the leaf-base of *P. semicordata*. It was noted by Mettenius (l. c., p. 3, and Figs. 12 and 13) that the leaf-trace in this species divided into three strands in the

enlarged leaf-base, but that these unite again at its upper limit, traversing the rest of the petiole as a single strand. The main fact is correct, but there seems to be some indefiniteness in the exact point of reconstruction of the single strand. Transverse sections at the base of the leaf show a single vascular strand of V-shape, but very soon the apex of the V widens out, and in place of the single angle there are two, as in the letter W, with a very flattened middle angle (Text-fig. 2, *d*). At each of the lateral angles of the W is a protoxylem-group, while the metaxylem forms a continuous strap-shaped band with a slightly hooked adaxial curve at either of its two margins; then follows a pericycle of several layers, and the endodermis. Thus the structure in essentials is the same as in *P. pycnophylla*, but there is considerable difference in the proportions, while the number of protoxylem-groups is smaller, and the vascular strap much narrower. A little higher up from the base the xylem becomes constricted at a point some distance from its margin, so that a mass about equal to a quarter of the whole is separated off from the rest; the phloem and sheaths also curve inwards, and a marginal strand is thus constricted off; the same happens (but not necessarily at the same level) at the other margin, so that the condition is reached of a larger median strand, which is placed abaxially and has two protoxylems in its concavity, and two smaller marginal strands slightly nearer the adaxial surface of the leaf (Text-fig. 2, *b*, *c*). This state is maintained for some distance upwards, and in one case which was followed out the strands remained separate for some six inches above the enlarged base, and were reconstituted to a single strand only in the last half-inch of the rachis below the lowest pinnae. The reconstitution of the single strand repeats in converse the steps of disintegration which took place below (Text-fig. 2, *a*). The result is that in the upper region of the leaf the strand of the rachis is similar in all essentials to that of *P. pycnophylla*, but the protoxylem-groups are fewer, the median one being absent, while the more pronounced W-shape of the middle region is a curious specific difference, which seems to go along with the absence of the median protoxylem.

In order to verify the position and relations of the protoxylem in the axis and bases of the leaves of a well-grown stock of *Plagiogyria*, serial sections have been cut through the apical region, so that the actual position of those xylem-elements which are earliest lignified may be directly observed (Fig. 7). It is some time after the stelar tube is already defined in the



TEXT-FIG. 2. Transverse sections of the petiole of *P. semicordata*. *a* is from a point below the lowest pinnae, and it shows a single strand. *b* is from a point lower down, and the strand is seen to have divided into three. *c* is in the swollen region of the leaf-base, and the strands are uniting again. *d* shows the strands fully united as they are seen at the base of the petiole before insertion upon the axis.

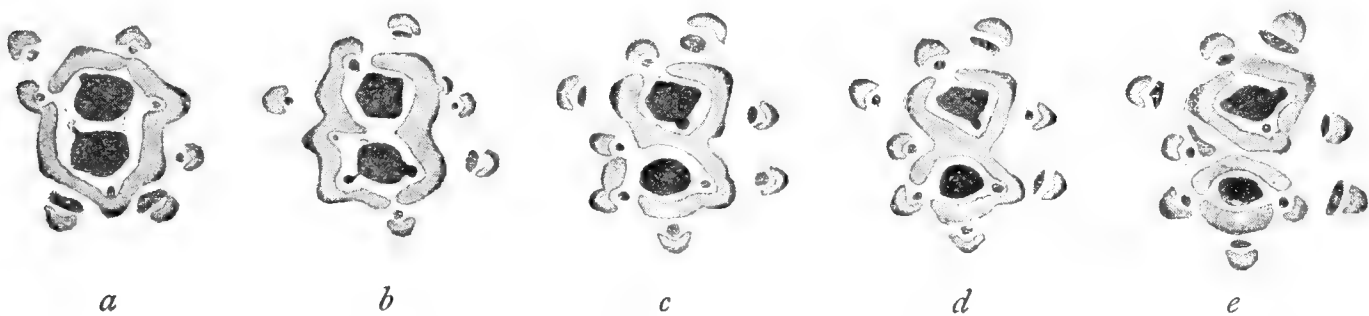


young stem, as seen in transverse section, that the first lignification of the xylem takes place; prior to that event it is easy to make out the outlines of the dictyostele, and to recognize where the initiation of the foliar traces will occur; these are first indicated by the appearance of a group of tracheides in a mesoxylic position. That the position is originally mesoxylic is clearly seen in Figs. 8 and 9, the former of which shows the very first lignification of two tracheides, with the young tracheides of the metaxylem still thin-walled, but easily recognized; the latter represents an older state with metaxylem surrounding the smaller protoxylem-elements. The reason for being quite explicit on this point is that, on passing upwards from the point where the protoxylem is first recognizable, the mesoxylic position is soon lost; the indentation of the margin of the stele appears as above described on the side adjoining the pith, and the metaxylem shifts right and left, with the effect that the protoxylem appears as though it were endoxylic; but that is not its original position: in the first instance it is mesoxylic. Opposite each indentation of the stelar tube the band of sclerenchyma which subtends each leaf-trace may early be recognized, first as a projection from the large central core, and later as a separate band, which passes outwards, taking a position in the angle of the leaf-trace; these points are readily observed in Fig. 7. The central protoxylem of the leaf is usually a single group (*P. pycnophylla*), though cases have been seen in which two groups are present from the first, separated by a median tract not yet lignified (*P. glauca*). About the same time a discontinuous fringe of protophloem makes its appearance on the abaxial side of the stelar tube. Passing upwards along the stelar tube the indentation opposite each leaf-trace deepens, and the protoxylem widens out; sooner or later it divides into two strands, disposed right and left, a division which is usually completed before the margins of the V-shaped leaf-trace are abstricted from the stelar tube. These two protoxylem strands diverge further as the leaf-trace passes out, and run directly into the two abaxial angles of the W-shaped meristele of the petiole, as described above (Text-fig. 1). About the same level as this forking of the first protoxylem-group, or a little later, there appear two other protoxylem-groups at some distance right and left of it; they do not always appear simultaneously, and in point of origin they are quite distinct from the median protoxylems; they pursue their course upwards into the margins of the foliar strand. In the stelar tube itself meanwhile, apart from the protoxylem-groups thus noted, no definite protoxylem is apparent, but the lignification which begins sporadically, and chiefly towards the exterior of the stelar tube, soon becomes general. (Compare Fig. 7 for the position of the protoxylems in the whole section.)

In *P. semicordata* the two protoxylems at the median region of the leaf-trace continue their separate course upwards into the leaf-base, and occupy the angles of the W-shaped strand; the abstriction of the strand

upwards into three, peculiar to this species, takes place so that the median protoxylem-groups remain in the central strand of the three, while the lateral protoxylems continue into the two lateral strands; in fact the abstriction is only a temporary interruption of the metaxylem, and of the tissues and sheaths surrounding it. In *P. pycnophylla* and *glauca*, however, there are usually three protoxylems in the central region of the leaf-strand as it passes into the basal region of the leaf, the third one (and sometimes more) appearing in a median position, but not by branching of the original one. In the lateral arms of the leaf-trace also, as it passes upwards into the leaf, a multiplicity of protoxylem-strands appear on the adaxial face, their position being indicated in the mature state by groups of cavity-parenchyma, as already noted.

In following up the details of dichotomy as seen in the mature stem of *P. semicordata*, by means of serial sections successively from below upwards, the first change is in the division of the central sclerotic core into two by constriction, together with the widening of the dictyostele into an



TEXT-FIG. 3. Five successive sections of the stock of *P. semicordata*, showing the disposition of the vascular tissues at the region of a dichotomy; the lowest section is to the left, and shows the central sclerotic core already divided before the stele itself becomes constricted. ( $\times 2\frac{1}{2}$ .)

elliptical outline, but without any cessation of the usual formation of foliar gaps where the leaf-traces pass off (Text-fig. 3, *a*). When the two sclerotic cores have separated completely from one another, and have passed to the poles of the ellipse, the stele begins to encroach inwards on either side (Text-fig. 3, *b*); the projecting surfaces meet, and the several tissues successively fuse (Text-fig. 3, *c*); the process is accompanied by involution of the outer surface of the stele (Text-fig. 3, *c, d*), and this is continued till the several tissues separate again, the division being now in a plane transverse to the axis of the ellipse; the xylem separates first, then the phloem, and finally the sheaths. The result is the formation by fission of two equivalent steles (Text-fig. 3, *e*). Throughout the whole process leaf-gaps and foliar strands are formed from both of the steles, even obliquely on the sides of them which face one another. The structure shows plainly that it is a case of true dichotomy, resulting in two initially equivalent shanks.

All the material available for the study of the anatomy of *Plagiogyria* has been of old plants, with the dictyostelic structure fully developed, but it has been seen above that *P. pycnophylla* bears stolons, arising in close



conjunction with the leaves. Since Prof. Gwynne-Vaughan has concluded from the study of the lateral shoots of various Ferns that 'the ontogeny of the vascular system of the plant as a whole is very frequently repeated, although more or less imperfectly, in the development of the lateral shoots' (l. c., p. 724-5), this turns attention with special interest to the stolons of *P. pycnophylla*.

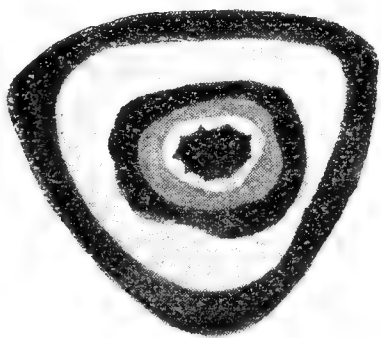
The origin of the stolons appears to be always in connexion with the leaf-base, and from the adaxial side of it; but there is some variety in the proportionate size of the subtending leaf and of the stolon which it bears, and this presents an interesting morphological condition in certain cases. A simple example is seen in Fig. 10, where the subtending leaf has developed to a considerable size, and shows at its apex the circinate curvature; it is in fact normal, but arrested in an early state. From its adaxial side has arisen the stolon, in this case a narrow one, with long internodes. In other cases, however, the stolon is larger in proportion, and the subtending leaf is smaller, being arrested in its growth at an earlier stage; when this is extreme the appearance is as if the leaf-apex were substituted by a stolon, though in face of the former cases this cannot be accepted as the correct interpretation of them. The internal vascular structure accords well with these results of external observation.

The attachment and basal structure of a relatively thin stolon is illustrated by the series of transverse sections in Fig. 11 (I-XIII), while they show also the relation of the stolon to the leaf, the adaxial face of which is constantly turned to the left in these drawings. In (I) the keeled leaf-base is shown traversed by a vascular strand which is, however, more contracted than it is usual for the leaf-trace to be; this becomes crescentic higher up, with the horns directed abaxially (II), and thickens (III); soon a region differentiates in the centre of the xylem (IV), consisting of phloem, pericycle, endodermis, and sclerenchyma in succession inwards from the xylem, which has now opened into a complete ring; except for the slight lateral horns the structure is very similar to that shown in the internode of *Davallia aculeata* by Gwynne-Vaughan (l. c., Pl. XXXIV, Fig. 20); the strand has in fact widened into a solenostele, which still retains, however, the abaxial horns of the original foliar crescent. Presently the ring opens on the abaxial side (VI), and a part of the ring representing its abaxial region between the two horns abstricts first by one margin then by the other, and separating completely (VII, VIII) passes on as the vascular supply of the abortive leaf-apex. The solenostele very shortly closes the gap (IX, X), the point of closure remaining obvious for some time as a thinner region; the ring soon opens again, but now on the adaxial side (XI), to give off the vascular supply of the first scale-leaf of the stolon (XII, XIII).

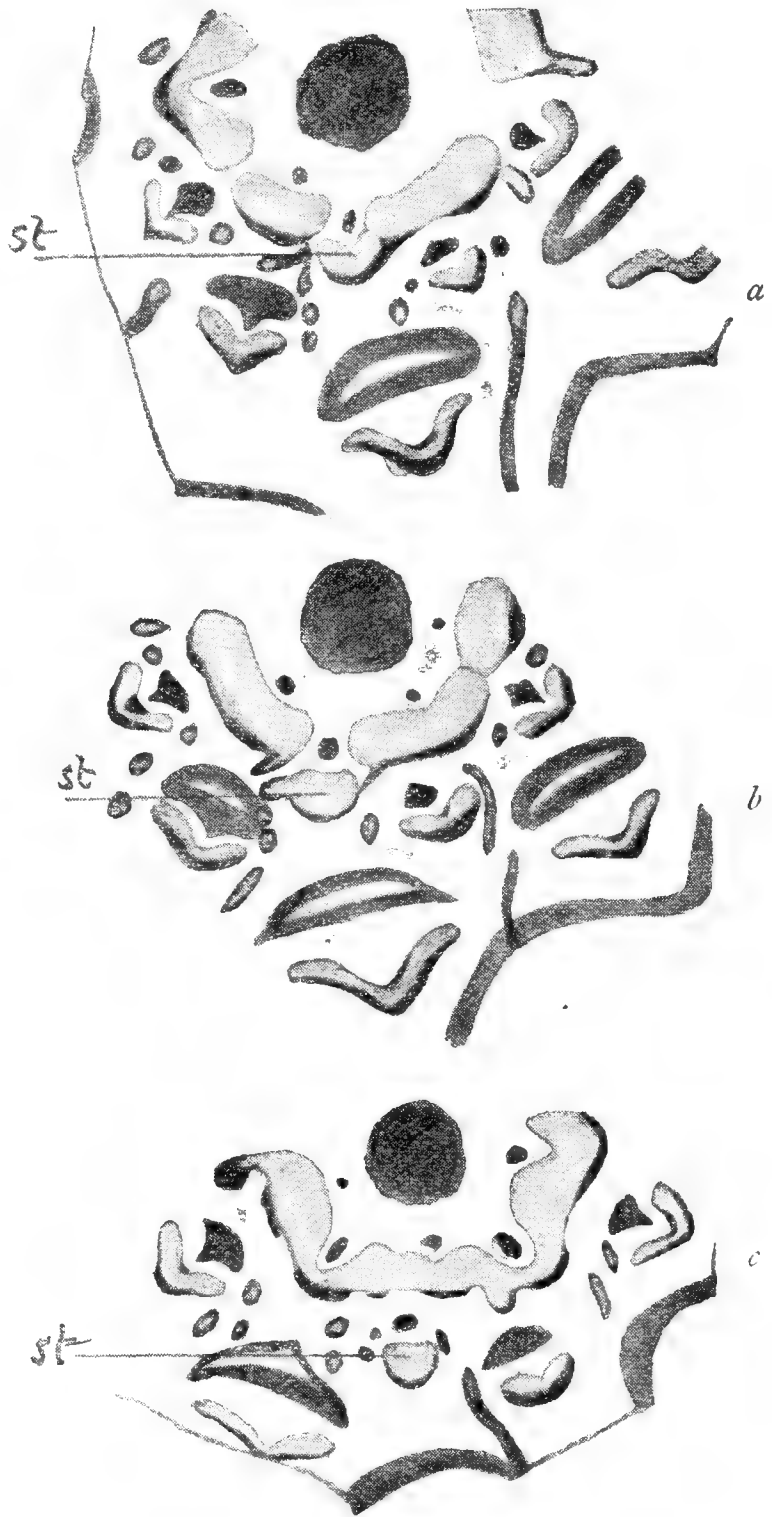
An example of a typically solenostelic structure is seen in Text-fig. 4,

which is taken from a stolon of considerable thickness, but with long internodes; that is in fact the usual structure in such cases.

The details of relation of the stolon to the subtending leaf do not, however, appear to be constant, as is shown by Fig. 12, I–V. These were taken from a thicker stolon with a closer arrangement of the leaves, and since the leaf-gaps overlap the condition is dictyostelic though of a simple type. A comparison of these with Fig. 6, from a larger stock, shows that the former is constructed on the same plan as the latter, but simpler. It will be noticed that the leaf-trace is a single V-shaped strand, and that as it is given off the stele opens, a portion of its vascular tissue separating off first from one then from the other of the margins of the meristeleles which remain: as in the larger axes a strand of sclerenchyma parts from the central sclerotic core of the axis, and passing outwards inaugurates the involution of surface which finally separates the leaf from the axis. The origination of the leaf is marked externally by the projecting keel, which is apparent even below the level of separation of the leaf-trace. In Fig. 12, I,



TEXT-FIG. 4. A typically solenostelic stolon of *P. pycnophylla*; the dark shading indicates the sclerenchyma, the lighter shading shows the stele. ( $\times 4$ .)



TEXT-FIG. 5. Three successive sections transversely through a stock of *P. pycnophylla*, of which *a* is the lowest, showing the origin of a stolon (*st*) in place of a leaf; *b* and *c* show its complete separation from the dictyostele of the axis, with a protostelic structure. ( $\times 2$ .)

which is the lowest of the series, a leaf-trace has passed outwards from the stele, together with its accessory sclerenchyma, which also isolates itself from the central sclerotic core. It may be noted further that roots have arisen right and left from the meristeleles near to the points where the leaf-strand had separated from them. In section II each of the shanks

of the V has put out a process from its inner face, while there is a rounding off of the apex of the V so as to give a circular internal contour; the ends of the divergent shanks of the V then become abstricted (see section III, which, however, shows that to the right still attached); after pursuing a short independent course they both end blindly. Meanwhile, the remainder of the leaf-strand closes into a complete circle, and in fact becomes a solenostele, while the sclerotic strand widens out laterally, and forms a slit centrally (section IV); higher up this widens as in the case of a normal leaf, and thus the secondary stolon passes off from the primary stolon which produced it. Section V of the series shows the parent stolon after the separation is complete.

How nearly a stolon thus produced may in position and in outline correspond to a leaf is shown by a tangential section represented in Fig. 13; here the stolon ranks with the leaves in position and in form; in fact the only feature which distinguishes it is the vascular supply, which has clearly the character of a solenostele. Moreover, if the origin of the 'trace' of such a stolon be examined in a series of sections of the stock, it will be seen that it originates just as a leaf-trace does; but it may be found to differ in form and outline from the very first (Text-fig. 5, *a*), while as it passes outwards it assumes a protostelic structure and circular outline (Text-fig. 5, *b*, *c*).

Clearly this history of origin of a stolon differs from that previously described; here the leaf appears to be completely replaced by the stolon; its vascular supply is mainly taken up in constituting the solenostele, while the remainder terminates in blind endings right and left. In the previous case the leaf has an apex which is traversed by a vascular strand, but early arrested; there is a difference also in the origin of the solenostele of the stolon from it. Without the history of development of the stolon from its first stages, for which the material did not suffice, it is impossible to be sure whether or not the apex of the leaf is ever involved, but certainly in some cases the origin of the stolon is on the adaxial surface at some distance from the apex.<sup>1</sup> Mettenius<sup>2</sup> has drawn attention to the variation in position of the buds in the Hymenophyllaceae, sometimes on the axis, sometimes axillary, and again sometimes upon the leaf-stalk itself, but always subtended by the leaf. The case of *Plagiogyria* appears to be of the same nature, but it plays in a region further removed from the leaf-base. This latter fact may be related to the closer aggregation of the leaves upon the shoot, and Mettenius has already discussed this point (l. c., p. 625) in the case of the Hymenophyllaceae with crowded leaves, in which also he found the position of the bud to be some distance from the leaf-base.

<sup>1</sup> It is not possible to say how far the case of *Plagiogyria* corresponds to any of those examples worked out developmentally by W. Kuppner, *Flora*, 1906, p. 337.

<sup>2</sup> Ueber Seitenknospen bei Farnen, p. 615.

The apparent merging of the leaf into the stolon seen in some of the cases in *Plagiogyria* may be compared with the transitions from leaf to stolon described by Goebel in certain species of *Utricularia* ('Organography,' Engl. ed., vol. ii, p. 240). He states that 'we find all transitions between foliage leaves and stolons, for instance in *Utricularia longifolia*, *U. bryophila*, *U. coerulea*, and others'. The stolons of *Plagiogyria* seem similarly to fluctuate between the two types of structure, but here it is a matter of balance between the leaf-primordium and the stolon-primordium, and the resultant structure seems to take the character of the more prominent partner.

From the description thus given of the vascular structure at the base of the stolon in *P. pycnophylla*, it appears that there may be a short basal region which is technically protostelic; this is followed by a region which is solenostelic with leaf-gaps at intervals, a structure which is characteristic of the lower part with long internodes; it is succeeded by a dictyostelic region, where the leaves are more closely disposed and the leaf-gaps overlap, this being the condition of the mature stock. Similar results were obtained from *P. glauca*; the bases of the stolons were there found to be clearly protostelic, but the stele widened out rapidly to the solenostelic and dictyostelic state, the stolons in the material available being short, and expanding at once into leafy buds. These observations are of interest in showing the primitive vascular condition of the stolons at their base, which has its special bearing where, as in *Plagiogyria*, the adult structure is not far removed from solenostely.

The structure of the root is diarch, and calls for no special remark.

It has been pointed out elsewhere ('Land Flora,' p. 659) that in Eusporangiate types the segmentation of the apex of the stem, root, leaf, and of the wings of the leaf, is more complex than that seen in the Leptosporangiate Ferns, and that this is shared in some degree by the Osmundaceae; in fact that there is some accord between the segmentation of the sporangium and that seen in the embryonic condition of the tissues of the several vegetative parts. It becomes then an interesting question to see what is the state in *Plagiogyria*, a genus in which some analogy has been recognized with the Osmundaceae.

It may be said briefly, and without entering into unnecessary detail, that the segmentation of stem, leaf, and root of *Plagiogyria* has not been found to depart from the usual Leptosporangiate type; the wings of the leaf also show the usual marginal series, with a regular segmentation (Fig. 14), a condition shared by all other Leptosporangiate Ferns, as far as is known, with the exception of the Osmundaceae, which latter occupy a peculiar primitive position.



## SORUS AND SPORANGIUM.

The mature sorus and sporangium of *Plagiogyria* have been described by Mettenius (l. c.), and his figures have been quoted by Diels (Engler and Prantl, i, 4, p. 281). But hitherto no account has been given of the development which would make it possible to enter on an exact comparison with other Ferns. The drawing of Mettenius, quoted by Diels as his Fig. 149, F, gives a very fair idea of the disposition of the sporangia upon the forked veins; this is shown also in our Figs. 1 and 2, respectively for *P. pycnophylla* and *P. semicordata*, while it is in the former species that the development has been traced. Sections of the young pinna show that the veins cause a convex swelling of the surface, and it is upon the receptacle thus formed that the sporangia make their appearance (Fig. 15, *c*). Those first formed are almost simultaneous in their origin, but they are followed later by others which are interspersed amongst them; and thus sporangia of various ages are in juxtaposition, giving the sorus the well-known character of the Mixtae. Fig. 18, *c*, shows the outline of such a sorus in a section traversing it transversely, while the sections shown in Fig. 15, *a*, *b*, represent what is seen when cut in a direction following the course of the vein: these two latter sections were cut from the same pinna, and a comparison of them with Fig. 15, *c*, shows that there is no fixed succession of the sporangia such as is seen in the Gradatae. A comparison of numerous sections has disclosed no regular seriation in time of appearance of the sporangia; in fact the sorus is a mixed one, but the formation of the interpolated sporangia is not long continued.

The sporangium originates from a single cell, which has a square, or more commonly an obliquely wedge-shaped base; it soon projects convexly, and segmentation begins by a wall inclined downwards so as to impinge upon one of the sloping sides of the wedge-like base (Fig. 16, *a*); this is according to the type of segmentation seen in *Alsophila* and *Cyathea* (see 'Land Flora', p. 637, Fig. 349, *c*). This wall is succeeded by others inclined to the first (Fig. 16, *b*, *c*) in the usual manner of Fern-sporangia, followed by a cap-cell (Fig. 16, *d*). A comparison of these drawings, together, and with Fig. 16, *e*, *h*, shows that there is some variation of detail in the basal region of the sporangium, and in the first segmentations; but they all result in a relatively massive stalk, which is a natural consequence of the deep inclination of the first segment-wall. It may be remarked that in the case of the larger sporangium shown in Fig. 16, *d*, the first segment-wall must have impinged directly upon the periclinal basal wall, after the manner characteristic of the Schizaeaceae, *Thyrsopteris*, and *Trichomanes* ('Land Flora,' p. 637, Fig. 349, *d*). In older sporangia the cells of the stalk subdivide so that when mature it is commonly composed of six rows of cells in the distal region just below the sporangial head (Fig. 17, *a*, *b*);

the structure may, however, be simpler in the lower region of the stalk. In the sporangial head the segmentation to form the tapetum follows the usual plan (Fig. 16, *f, g*), and it later divides into two layers (Fig. 16, *h*), and becomes disorganized in the usual way (Fig. 17, *c, d*). Meanwhile, the cell remaining at the centre divides to form the spore-mother-cells, apparently twelve in number, and the further development proceeds in the usual way. The total output of spores per sporangium appears to be forty-eight. This is a feature which *Plagiogyria* shares with many of the *Gradatae* and *Mixtae*, but it is to be noted that a limited spore-number (48–64) is also seen in *Matonia*, while in the *Schizaeaceae* the spore-numbers are not high as compared with what is seen in others of the *Simplices*. The spores themselves are tetrahedral, and when fully ripe numerous highly refractive globules are found attached to their external walls; the attachment is not a very close one, as is shown by the fact that they are easily separated in the mounting, and may be found lying in the mounting medium apart from the spores; they are formed just before the maturity of the spore. Apart from the globules the wall of the spore has no very distinctive markings; in general character it shows some similarity to that of *Lygodium* (Engler and Prantl, i, 4, Fig. 192, J), but the spore-characters cannot be held to be altogether trustworthy for comparison among the *Simplices*, as both in *Schizaeaceae* and *Gleicheniaceae* they are variable within the order. The tetrahedral type is, however, the prevalent one among the early *Filicales*.

The mature sporangium has been depicted by Mettenius (l. c., Figs. 2 and 3), and his drawings have been copied by various later authors; they do not, however, give all the detail for necessary comparison with those of other Ferns, especially when it is remembered how important a part the sporangial structure plays in arriving at conclusions as to phyletic relationships.

The mature sporangium is long-stalked (Fig. 18, *a, b*), in fact quite unusually so among Ferns with oblique annulus, in which the sporangia are as a rule short-stalked, or sessile. A comparison in this respect may be made, on the one hand, with the *Osmundaceae*, and ultimately with some *Botryopterideae*; on the other, with *Gleichenia* § *Mertensia*, and especially with *G. dichotoma* ('Land Flora,' Fig. 310, and text, p. 556); with the latter there is also correspondence in the absence of any central cell or cells in the transverse section of the stalk, which is an indication of the less massive structure of the sporangium. There is a gradual transition from the sporangial stalk to the head, giving the whole body that pear-like form which is so conspicuous in *G. dichotoma*; it appears also in *Loxsonia* ('Land Flora,' Fig. 320, p. 572), and in certain species of *Dicksonia* ('Land Flora,' Fig. 330, p. 593). The head is traversed obliquely by the annulus, which is a complete ring, as recognized by the sequence of the cells, though there is variety in the manner of their induration; there is also some irregularity in their form and number—thirty being about the average; there is also some variability in



their sequence, while occasionally single cells of the sporangial wall quite distinct from the annulus may be indurated. But putting these irregularities aside, the annulus of about thirty cells takes an oblique course round the sporangium, being quite continuous past the insertion of the stalk. The obliquely distal area of the sporangial wall surrounded by the annulus, seen in surface view in Fig. 18, *b*, clearly corresponds to the 'peripheral' face in the Gleicheniaceae type, and is here, as in *Gleichenia*, only slightly convex; while the rest of the thinner part of the wall to which the stalk is attached is strongly convex, and corresponds to the 'central' face of *Gleichenia* (Fig. 18, *a*). A similar comparison may be drawn also with *Dicksonia*. Comparing with the Schizaeaceae, the differences appear at first sight more striking than the resemblances, for in most of them the 'peripheral' face is greatly reduced; but it has been seen that the nearest anatomical correspondence is with *Anemia*, and in this genus the 'peripheral' face is larger than in any other genus of the Schizaeaceae; in fact, notwithstanding its short stalk, its apparently transverse annulus, and its peculiar form, the type of sporangium is essentially the same in *Anemia* as in *Plagiogyria*. It is not desirable to press such comparisons too much into detail but it is worthy of remark that on the side of the annulus nearest to the stalk in the sporangia shown in Fig. 18, *b* and *d*, the series of cells is duplicated; this corresponds exactly in position to that extension of the annulus down the side of the sporangium in *Anemia* which is related to the function of dehiscence (compare Engler and Prantl, i, 4, Fig. 192, *g*). Turning to the mechanism of dehiscence as seen in *Plagiogyria*, this leads to the formation of a lateral slit, as in *Dicksonia* and most other Gradatae; but the structural provision for it shows some variability, which may be held to suggest the sort of changes which would be involved in a shifting of position of the dehiscence. In the sporangia of the Simplicies there is no highly organized 'stomium'; and this applies equally for *Anemia*, with which comparison has above been drawn. In *Plagiogyria* the dehiscence is localized laterally by a well-organized mechanism; but a comparison of various sporangia of this plant shows that there is uniformity neither in the exact position nor in the size or number of the cells which determine it. There is usually a well-defined group of four sister cells, which constitute the actual stomium (Fig. 18, *a, b*); on either side of it (sometimes in immediate juxtaposition with these, or sometimes not) is a group of thin-walled cells, which, however, vary in number; thus on the distal side of the stomium there may be only one of these cells (Fig. 18, *d*), or two (Fig. 18, *c*), or three (Fig. 18, *a, b*); on the proximal side there may be two (Fig. 18, *c*), three (Fig. 18, *a*), or four (Fig. 18, *d*), while they may be in direct juxtaposition with the group of cells of the stomium (Fig. 18, *a*), or removed from them on either side by the intervention of a variable number of indurated cells (Fig. 18, *b, c*). This variability, so different from what

is seen commonly in the sporangia of the Leptosporangiatae, is so marked that it again indicates that *Plagiogyria* occupies a position distinct from other Ferns with a 'mixed' sorus; nor is it apparent that the same variability holds in any of the Gradatae. It suggests that sort of plasticity which might have accompanied the shifting of the point of dehiscence from a median to a lateral position—a change which comparison indicates to have actually taken place in the phyletic transition from the Simplices to the Gradatae and Mixtae.

If we regard the sporangium of *Plagiogyria* from the biological point of view, it will appear that it is not highly specialized in accordance with its requirements. There is no regular sequence of the sporangia in the sorus, consequently the oblique position of the annulus is not a feature of high moment. The induration shows a considerable interruption, the thin-walled region including the stomium; it extends down to, or even sometimes across the insertion of the stalk, so that a condition is arrived at which is not unlike that of the Polypodiaceous type; it has, however, the mechanical disadvantage in the case of *Plagiogyria* as compared with these that the end of the indurated annulus is not directed straight to the stalk itself, but obliquely towards it. The sorus not being a crowded one this point is probably immaterial. The retention of the oblique annulus, as in the Gleicheniaceae for instance, with only the modification of the dehiscence having been swung into a lateral position, is in no way surprising when it is remembered that there is only a slight departure here from the primitive sorus of the Simplices.

There has been some difficulty in obtaining germination of the spores; the cultures of spores of *P. semicordata* from Jamaica came on very slowly, and prothalli are only sporadic, many spores failing to germinate. This may raise some doubt of the identification, though the prothalli observed appear in close relation to undoubted sporangia of *Plagiogyria*, and the form and character of the spores from which the individual prothalli arise are those above described. The difficulty of identification lies in the fact that, in the absence of the highly refractive globules above noted (which were seen not to be firmly attached to the mature spore, and seem to disappear on germination), no marked features remain beyond the tetrahedral form. Final certainty of identification must be awaited when the prothalli have produced young sporophytes.

The character of the prothalli observed are, in general, those usual for Leptosporangiate Ferns, being commonly symmetrical in form, and without any distinctive hairs or glands. The interesting feature is in the antheridia, of which the distinctive modes of dehiscence have been pointed out by Heim ('Flora,' 1896, p. 349); the one type characteristic of the Osmundaceae, Gleicheniaceae, *Lygodium*, Hymenophyllaceae, Cyatheaceae, and Dicksonieae, shows dehiscence by throwing off a lid-like cell, which

type may be held to be the more primitive; the other is generally characteristic of the Polypodiaceae, together with *Anemia* and *Mohria*; here the rupture is by breaking down of a distal cell, with the formation of a rosette-like aperture. The latter is the type seen in the antheridia of the prothalli attributed to *Plagiogyria*; the process has been carefully watched; first an increased convexity of the antheridial wall appears near to the distal end; this gradually protrudes more and more, till rupture of the wall begins at one side of the cell; the convex part of the wall is then turned back as on a hinge, and the spermatozoids come out of the opening; there is no extrusion of a complete cell, but the wall of the distal cell is ruptured, and fragments of it remain attached to the antheridium, forming the star-like outline of the aperture.

#### COMPARISONS.

Comparisons may now be instituted between the characters of *Plagiogyria* as above described, and those of other Ferns, with a view to assigning to it an approximate position in the system.

In the bulky upright stock, with closely aggregated leaves and persistent leaf-bases, and showing at least in *P. semicordata* occasional dichotomy of the axis, characters are seen which are shared by such primitive types as the Osmundaceae; this comparison is accentuated by the densely sclerotic tissues which compose it, and cover it externally. The deep involutions of surface which subtend the leaf-bases are closely similar to those seen in *Anemia* (compare Prantl, 'Schizaeaceen,' Pl. III, Fig. 27, *b*), a comparison which is further supported by the similarity in structure of the leaf-trace. The enlarged base of the leaf reminds us again of that in the Osmundaceae, but the details of its structure do not correspond with any exactitude. The pneumatophores which it bears (which are present also in the upper leaf of the Eastern species) are striking objects, and might be expected to give a valuable basis for comparison; this is, however, in some degree discounted by the fact that they are not constant within the genus itself, being absent from the upper leaf in *P. semicordata*. But, putting this aside as not entirely depriving the comparison of value, the existence of similar organs in the Cyatheaceae is a matter for remark. Closely related causally with them, as already suggested above, are the very numerous glandular hairs which secrete mucilage; such hairs are found plentifully in the Osmundaceae of the present day, and in other relatively primitive Ferns, while somewhat similar hairs were characteristic also of the ancient Botryopterideae. The absence of flattened scales is a feature of importance for comparison; it differentiates *Plagiogyria* from the Cyatheaceae, as well as from the genus *Lomaria*, and when taken together with other primitive characters which the genus shows, indicates a relation with the lower rather than with the higher forms of Ferns.

Passing to the leaf itself, the simply pinnate form gives no distinctive point for comparison, but the serration suggests that it has been condensed from some more complex prototype. The simply forked venation points clearly to a primitive position, though, as is well known, many Ferns of advanced affinities still show it.

The existence of buds and stolons is a widespread feature in Ferns, but the peculiar relation which they bear to the leaf in *Plagiogyria pycnophylla* and *glaucæ* resembles in near degree that seen in the Hymenophyllaceae; in them, as has been seen, the bud is subtended by the leaf, but there is some variation in the position of the bud relatively to the leaf-base, and in some cases it appears to be borne upon the leaf; the condition of *Plagiogyria* would then be only a case of the further removal of the axillary bud from the basal position; as already suggested, this may be a consequence of the close aggregation of the leaves upon the axis, which would necessitate a more distal position for the accessory bud. It is true many other Ferns have buds associated with their leaf-bases, but none of them seem so nearly to correspond to what is seen in *Plagiogyria* as do the Hymenophyllaceae.

Turning to the anatomical features, it has been seen that the stolons show at their base a structure that is solenostelic, or even protostelic; in the absence of young plants this may be taken as evidence of a primary structure of the axis, such as is seen in various primitive types of Ferns. The mature axis shows a condition of dictyostely, though this is not of a high grade; the foliar gaps overlap, so that there is technically a dictyostelic state, but, as Professor Gwynne-Vaughan remarked, it is not far removed from solenostely, and it corresponds in fact to that seen in *Anemia*. The leaf-trace itself consists of a single strap-shaped strand, which again is a primitive feature, though shared by some Ferns which have progressed to higher differentiation; it is characteristic especially of the Simplices and Gradatae, and among them it is a feature of those types which are regarded as being anatomically the more primitive. In actual detail of structure the leaf-trace of *Plagiogyria* corresponds very closely with that of *Anemia* (compare Prantl's Figs. 30, 32, Taf. III of his 'Schizaeaceen'), while the whole vascular system of the axis corresponds also in its main features to that shown by him for the same genus (compare Prantl's Fig. 27, *a*, *b*, Taf. III, l. c.); there is, however, in *Anemia* only a single central protoxylem-group in the leaf-trace, while in *Plagiogyria* there are two or sometimes more; but, as has been seen, there is only one at the extreme base, so that the correspondence with *Anemia* is the closest at this point, where the leaf-trace originates (compare Boodle, 'Ann. of Bot.,' vol. xv, p. 382). The mesoxylic origin of the protoxylem of the leaf is a character shared with many Ferns; its quick fading out as it enters the axial system is matched by what is seen in *Anemia* (Boodle, l. c., p. 384). The general character of the vascular



system of *Plagiogyria* is thus relatively primitive, but with distinct advance beyond what is seen in the earliest types; the closest correspondence being with *Anemia*, which, though it belongs to the primitive Schizaeaceae, is one of the most advanced members of that anatomically variable family.

Turning to the fertile leaf and sorus as seen in *Plagiogyria*, the general habit, with narrow pinnae having their margins reflexed like an indusium over the superficial sori, at once suggests the old comparison with *Lomaria*, or stated more generally, with the Pterideae as a whole. But it is to be remembered that the same holds, though with less closeness of the parallel, for the fertile leaves of *Gleichenia* & *Mertensia*; for though here the margins are not thinned off in the membranous fashion of the Pterideae, the venation corresponds in point of the absence of fusions with that of *Plagiogyria*. On the other hand, though *Lomaria* and *Pteris* habitually show fusions of the venation, many of the simpler Pterideae show a complete absence of vein-fusions; this is conspicuously the case with *Cryptogramme* and *Llavea*, genera with which *Plagiogyria* has been specially related by Diels (Engler and Prantl, i, 4, p. 281).

The superficial sorus of *Plagiogyria*, so far as the observations here recorded are concerned, is restricted to the vein, but extended along it, borne on a slightly enlarged receptacle; it is not unlike what would result from an elongation of a sorus of the type of *Gleichenia pectinata* or *dichotoma*, or, on the other hand, it may be directly compared with that of *Cryptogramme* or *Llavea*, to which it corresponds very closely. As regards the composition of the sorus, it has been noted that most of its sporangia arise simultaneously, as is the case in the Simplices; but that subsequently others are interpolated between those first formed, giving the sorus the character of the Mixtae. This character is also shown by *Cryptogramme* and *Llavea*, but it is worthy of note that it is only arrived at as a late and subsequent condition in *Plagiogyria*.

It is, however, the character of the sporangium itself which has always been held as distinctive for *Plagiogyria* among those Ferns with which on ground of habit it has hitherto been classed. The oblique annulus, continuously indurated as it passes the insertion of the sporangial stalk, is a feature unknown elsewhere among Ferns having a mixed sorus, with the solitary exception of *Dipteris conjugata*.<sup>1</sup> The usual comparison of *Plagiogyria* on this point has been with those prominent families of the Gradatae, the Cyatheaceae, and Dicksoniaceae, which share with *Plagiogyria* the features of lateral dehiscence and small spore-output. But it ought to be borne in mind that there is in *Plagiogyria* no basipetal

<sup>1</sup> The peculiar case of *Dipteris conjugata*, of which a phyletic explanation was offered by the discoverer of the fact of its having a mixed sorus, may be held as an example of the adoption of a mixed condition of the sorus by a single species, in a genus having the soral character of the Simplices. (See Miss Armour, *New Phyt.*, vol. vi, p. 238.)

succession of sporangia in the sorus; also that the obliquity of the annulus is equally shared with *Gleichenia*, while in § *Mertensia* the form and size of the sporangium is not unlike that of *Plagiogyria*; it differs, however, in the important features of the much larger spore-output, and the median dehiscence shown by *Gleichenia*. The comparison has also been instituted above with the sporangium of *Anemia*, to which the sporangium of *Plagiogyria* shows similarity in the essentials of construction, and in the indefiniteness of the stomium; but it differs in the important point of number of the spores produced; in these respects there is perhaps a closer comparison with the condition seen in the Matonineae, where also there is an oblique annulus and lateral dehiscence, together with a small spore-output.

These comparisons, all of which place *Plagiogyria* in relation with relatively primitive Ferns as regards the characters of the mature sporangium, raise also the comparison on the basis of segmentation of the young sporangium, as well as of the apices of the various parts (compare 'Land Flora,' p. 650). It has been seen above that the details of segmentation of the young sporangium conform to that type which appears in the more primitive Leptosporangiatae, such as the Cyatheaceae and Dicksonieae: also that the segmentation of the apices is according to that common to Leptosporangiatae at large, while in the segmentation of the wings of the leaf there is nothing to strengthen the comparison with the Osmundaceae, which therefore rests rather on external features than upon internal structure or details of development. This is in fact the position which might have been anticipated.

In this place it is only right to express the regret that the prothalli available for study have been open to some doubt as to identity. Pending the clearing up of this uncertainty it may be remarked that the antheridia observed showed dehiscence of the type characteristic of the more advanced Leptosporangiate Ferns; but in view of the uncertainty that exists it is unavoidable that our comparisons shall at present be confined to the sporophyte generation, and any conclusions must in so far be held to be provisional, pending the facts relating to the sexual generation.

But, subject to this proviso, the facts relating to the sporophyte are distinctive, and they indicate clearly that the affinities of the genus are relatively primitive; this follows from (1) the entire absence of flattened scales, the young parts being protected by filamentous hairs only; (2) the occasional dichotomy of the shoot; (3) the simple venation of the leaves, with absence of connecting commissures; (4) the stelar structure, not far removed from solenostely; (5) the undivided leaf-trace; (6) the absence of a 'true' indusium, the sori being covered by the recurved leaf-margin; (7) the type of sorus with initially simultaneous sporangia, among which others are intercalated later; (8) the structure of the sporangium with oblique annulus and indeterminate stomium; (9) its mode of initial segmentation



leading to a relatively thick stalk; (10) the tetrahedral form of the spores. All these characters indicate for the genus a relatively primitive position, and the strength of that conclusion lies in the number of the facts upon which it is based.

From the comparisons detailed in the above pages it will be apparent that *Plagiogyria* does not naturally fall into any of the families of Ferns which have an oblique annulus; it is a type of which the cross characters indicate some intermediate position, with affinities in various directions; in fact it is a 'synthetic' type. But though the bulk of the indications are clearly towards the Simplicies, they do not relate the genus specially with any family of them; thus the habit suggests the Osmundaceae, the anatomy the Schizaeaceae, and the sorus the Gleicheniaceae or Matonineae, while the venation would compare reasonably with any of these. But it is plainly a primitive type which has broken away from its original characters, as is seen in the dictyostelic structure of the mature stem following on solenostely and even protostely, and in the mixed character of the sorus. It would seem probable that the mixed character of the sorus was acquired directly from the simple state; from the entire absence of any indication of basipetal sequence of the sporangia it is probable that this took place without the intervention of any gradate condition, as in the case of the *Dennstaedtia-Davallia* series;<sup>1</sup> it has here been achieved by the simple interpolation of new sporangia between those formed in the first instance. There is a precedent for this already recognized within the genus *Dipteris*; for while in *Dipteris Lobbiana* the sori show simultaneous origin of the sporangia, *D. conjugata*, which on other grounds may be held to be a more advanced type, has a mixed character of the sorus. It was concluded in that case that a direct progression had been effected from the simple to the mixed condition of the sorus within the limits of a very natural genus; and the conclusion appears probable that a like direct progression has produced what is seen in the sorus of *Plagiogyria*; this conclusion eliminates any near relationship with the Gradatae.

In the dictyostelic structure of the axis, as well as in the mixed sorus, *Plagiogyria* compares with the Mixtae, and especially, as indicated by the characters of position and protection of the sorus, with that relatively primitive group of them, the Pterideae; but it differs from them in its oblique annulus; the question will therefore be whether in any of the Pterideae there is indication of a like feature. Without entering here into details, which must be reserved for a future paper of this series, it may be stated that there is evidence among the Pterideae of the existence of an oblique annulus, and that it is found in the genus *Cryptogramme*, which has already been placed by Diels in near systematic relation to *Plagiogyria*.<sup>2</sup>

<sup>1</sup> See Studies in the Morphology of Spore-producing Members. IV. Leptosporangiate Ferns. Phil. Trans., B., vol. 192, p. 91.

<sup>2</sup> Engler and Prantl, i, 4, pp. 279-81.

But the position of the annulus in *Cryptogramme* is more nearly vertical, and its induration is interrupted opposite the insertion of the stalk; these are steps which lead towards the condition of the annulus common in Ferns with a mixed sorus, and seen in other Pterideae. Such facts indicate the correctness of the conclusion that *Plagiogyria*, though independent of near relation to any of the families of the Simplices, is a form which has broken away from the primitive state in certain features, and that, as a synthetic type, it serves as an indication of the probable origin of those features which characterize a certain group of the Mixtae, viz. the Pterideae, to which it has already been held by systematists to be akin.

The attempt must now be made to locate this synthetic type, *Plagiogyria*, in the system of the Filicales. In the first place the generic separation of it from *Lomaria* cannot be for a moment in doubt; *Plagiogyria* must certainly be held to be a substantive genus; the question will be whether a higher degree of systematic separation is not required for a form which combines such distinct features. It might at first sight seem desirable to found a new order of Ferns to accommodate it; and my first impulse was to establish it as the only genus of a new order, the Plagiogyriaceae. But reflection upon the facts which point to its natural alliance with the simpler Pterideae, and especially the fact that traces of an oblique annulus exist in *Cryptogramme*, indicates that a separation is not desirable. A natural order should be as nearly as possible a phyletic unity. Our conclusion from the comparison of the facts must be that *Plagiogyria* gives the key to certain changes which have accrued in the descent of the Pterideae, such as the progression anatomically towards the dictyostelic state though maintaining the united leaf-trace, and the progression from the simple to the mixed sorus with the swinging of the oblique annulus to the vertical position, together with its interruption at the insertion of the stalk. If, within the Pterideae, there were no forms which shared in any degree the peculiar characters of *Plagiogyria*, then a separate systematic position might be desirable for the genus. But as it now appears that anatomically, as well as in the characters of the annulus, it does not stand entirely apart, the conclusion seems justified that its natural place is within the Pterideae. But of these it must be ranked as the most primitive form, while its affinity in character with the various groups of the Simplices, but not especially with any one of them, indicates a probability that the Pterideae sprang directly from such a source, though not from any one of the families of them at present known. Accordingly no change from the definition of the sub-families or of genera of the Pterideae as arranged by Diels<sup>1</sup> is proposed as a consequence of the new facts; but as regards phyletic grouping, which will be worked into further detail in subsequent papers of this series, *Plagiogyria* will be placed in the lowest

<sup>1</sup> l. c., p. 255.

position, as their oldest type, while *Cryptogramme* will retain the position in near relation to it, as has been correctly recognized by Diels.

As regards the arrangement of the species within the genus, though there are no quite distinctive features of priority, it appears that *P. semicordata* is in certain characters more primitive than *P. pycnophylla* and its closely allied species (or forms), *P. scandens* and *P. glauca*. This is indicated by the prevalence of dichotomy of the axis in the former, while it is, as far as observed, absent in the latter, its place as a method of amplification of the shoot being taken by stolons in the Eastern species. The more marked serration of the leaves in *P. semicordata*, while in the Eastern species they are more nearly entire, may be held to be a nearer condition to a more richly branched ancestry, while the leaf-form of the Eastern species is more completely condensed; the Eastern species show a fuller development of the pneumatophores, which are a special adaptation extended here to the upper leaf, while they are present only on the leaf-base in *P. semicordata*; and this goes along with the more highly developed covering of mucilaginous hairs in the Eastern and Western species. These are only indications it is true, but they all point to *P. semicordata* as being the more primitive species. On the other hand, the division of the leaf-trace in *P. semicordata* into three strands in the basal region of the leaf must be borne in mind, though the bearing of the fact is obscured by their subsequent junction below the lowest pinnae into a single strand again. There is also the fact of the absence of the median protoxylem-strand, which is present in *P. pycnophylla* and *glauca*, but again it is not clear what interpretation is to be put upon this fact.

Lastly, the observations on *Plagiogyria* have their bearing on the relations of those three grades of soral condition characteristic of the *Simplices*, *Gradatae*, and *Mixtae*. When these types were first recognized by me, it was clearly laid down that the three divisions illustrate steps in the evolution of the Filicales, but that they were arrived at not by progression along a single line of descent, but by similar adaptations making their appearance in more than one evolutionary sequence, and the results were grouped according to their common adaptation ('Studies,' v, p. 123). It was specially insisted on that, though at that time no evidence was available to show that the 'mixed' condition of the sorus had been acquired by modification of the 'gradate' condition, 'it is possible that a mixed condition of the sorus may have arisen also by interpolation of successive sporangia without order in a typical simultaneous sorus, such as that of *Angiopteris* or *Gleichenia*; but evidence of this has not come to hand' (l. c., p. 124). Since this was written in 1899 such evidence has been acquired in the case of the genus *Dipteris*, already quoted; here in the broad-leaved *D. conjugata* sporangia of different ages are found side by side in the same sorus, though in other species of the genus, having the more primitive

narrow type of leaf, the sporangia of the same sorus are simultaneous in their origin; there has in fact been a progression from the simple to the mixed type of sorus within the genus. A parallel progression is now suggested as having resulted in the mixed condition seen in the sorus of *Plagiogyria*; from a superficial sorus such as that of *Gleichenia dichotoma* or *pectinata* it would be produced by a slight elongation along the vein, together with an irregular interpolation of younger sporangia between those first formed, but with the characters of the individual sporangium for the most part maintained. Whether or not the *Gleichenia* type was the actual source, it seems probable that there has been here another direct progression from a simple sorus to one of a mixed character. The natural affinity already recognized by Diels and discussed above, between *Plagiogyria* and the simpler genera of the Pterideae, indicates that this direct progression has probably given rise to one of the greatest phyla of Leptosporangiate Ferns. The further discussion of this, and the marshalling of the evidence to support it, must be deferred for the present, but the facts already in hand are sufficient to give the main conclusion a reasonably degree of probability.

The general effect of such a conclusion will be to condense the brush of phyletic lines of the Leptosporangiate Ferns, for, in the case of a very considerable sequence of forms with a 'mixed' sorus, it removes any necessity for the idea that they were arrived at through a stage such as is seen in the Gradatae, and it refers them in origin directly back to the more primitive Simplices.

#### CONCLUSIONS.

1. The genus *Plagiogyria*, merged by Sir W. Hooker in *Lomaria*, is a substantive genus, quite distinct from any other.

2. It shows its relatively primitive character in the stelar structure, the undivided leaf-trace, the simple forked venation, occasional dichotomy of the axis, the absence of flattened scales, absence of a 'true' indusium, the sorus initially simple but later showing mixed character, the segmentation of sporangium, its thick stalk, oblique annulus, and indeterminate stomium, and the tetrahedral spores.

3. It shows resemblances, more or less marked, to all the great series of the Simplices, but not to any one of them so clearly as to point to close affinity.

4. On the other hand its characters indicate that it is rightly allied with the Pterideae, of which it may be held to be the most primitive type.

5. Its 'mixed' character of sorus, without any indication of a gradate sequence of sporangia, combined with its primitive characters and its probable affinity to the Pterideae, shows that a great phylum of Mixtae has probably been derived directly from the Simplices.



## DESCRIPTION OF PLATES XXXII AND XXXIII.

Illustrating Professor Bower's Paper on *Plagiogyria*.

Fig. 1. *Plagiogyria pycnophylla*. Parts of two pinnae seen from below, with a pneumatophore at the base of each; on one side of the right-hand pinna the indusial margin and the sporangia have been removed, so as to show the venation and the absence of distal connexions of the veins. ( $\times 4$ .)

Fig. 2. *Plagiogyria semicordata*. Parts of two pinnae seen from below, from near to the apex of the leaf; on the right-hand side the indusial margin and the sporangia have been removed, so as to show the venation; note the winged character of the rachis, which is specially developed at the apical region of the leaf. ( $\times 4$ .)

Fig. 3. Apical bud of *Plagiogyria glauca*, showing leaves still circinate and bearing the pneumatophores projecting from the enlarged leaf-bases. ( $\times 2$ .)

Fig. 4. *P. glauca*. The persistent base of an old leaf, showing a row of pneumatophores on either side of the midrib. (Enlarged.)

Fig. 5. Circinate apex of leaf of *P. pycnophylla*, showing the pneumatophores projecting through the covering of mucilaginous hairs. ( $\times 2$ .)

Fig. 6. Transverse section through a well-developed stock of *P. pycnophylla*, showing the central sclerotic core (*sc*), the stele (*st*), the leaf-traces (*lt*), and their subtending sclerotic patches (*sp*), which as they pass outwards become hollowed by surface involutions (*inv*). To the left is a solenostelic stolon in place of a normal leaf (*sol*). ( $\times 2$ .)

Fig. 7. Transverse section of the stem of a well-grown plant of *P. pycnophylla* at a point close below the apex, to show the position of the protoxylem-groups, which are indicated as dark dots in the meristemes; note that the first indication of the formation of a leaf-trace is by an outgrowth of the central sclerotic core, and that a mesoxylic protoxylem-group appears opposite it in the meristeme of the axis. ( $\times 6$ .)

Fig. 8. Part of a meristeme of *P. glauca*, showing the first protoxylem-elements already lignified, lying in a distinctly mesoxylic position. ( $\times 100$ .)

Fig. 9. Part of a meristeme of *P. pycnophylla*, showing mesoxylic protoxylem. ( $\times 250$ .)

Fig. 10. A leaf of *P. pycnophylla*, itself arrested in its growth, and bearing on its adaxial face a stolon (*st*); *pn* indicates the pneumatophores.

Fig. 11. A succession of transverse sections through a leaf bearing a stolon, the lowest being placed first; see text. ( $\times 4$ .)

Fig. 12. I-V. Successive sections through a stolon of *P. pycnophylla*, showing a structure not far removed from solenostely; on the right-hand side of the drawings a stolon, which replaces a leaf, is originating, and in IV it shows a stolon structure (*st*). (Natural size.)

Fig. 13. Tangential section through the leaf-bases of *P. pycnophylla*, showing how one of them is structurally replaced by a stolon (*st*) having a stele which is almost protostelic. (Natural size.)

Fig. 14. Transverse section through a young pinna of *P. pycnophylla*, showing marginal segmentation of the usual Leptosporangiate type. ( $\times 300$ .)

Fig. 15 (*bis*). Sections through the sorus of *P. pycnophylla*, in an advanced state, but not mature; *a* and *b* show the 'mixed' character of the sorus, which is covered by the attenuated margin of the pinna, these sections being transverse through the pinna, and following the course of the veins; *c* is at right angles to these, and cuts the vein transversely. ( $\times 30$ .)

Fig. 16, *a-h*. Sections through sporangia of *P. pycnophylla* in early stages of development; for details see text. ( $\times 225$ .)

Fig. 17. Older stages of the same; *a, b*, transverse sections through the sporangial stalk; *c* and *d*, sections longitudinally and transversely through the sporangial head. ( $\times 225$ .)

Fig. 18, *a-e*. Mature sporangia of *P. semicordata*; for details see text. ( $\times 80$ .)

# Note on the Petiole of *Zygopteris Grayi*, Will.

BY

R. KIDSTON, LL.D., F.R.S.

With Plate XXXIV.

UNTIL the publication of Dr. Paul Bertrand's 'Études sur la fronde des Zygoptéridées' last year, in which he unites *Zygopteris bibractensis* var. *Westphalica*, P. Bertrand,<sup>1</sup> with *Zygopteris Grayi*, Will. sp., as its petiole,<sup>2</sup> I was under the impression that it was common knowledge that *Zygopteris di-epsilon*, Will., was the petiole of that stem.

On communicating with Dr. Scott on this subject, I find I have been mistaken in believing that this relationship between *Zygopteris Grayi* and *Z. di-epsilon* was generally known, and on his suggestion the present note is written.

Although *Zygopteris Grayi* has been more or less fully described by several botanists,<sup>3</sup> there are certain points in connexion with the so-called 'axillary shoot' which appear still to await description, and as this is intimately connected with the leaf-trace, even at the risk of repetition it seems desirable to describe the relation of the *petiole* to the so-called 'axillary shoot' and to follow the former until it is given off and free in the cortex.

As the term 'axillary shoot' seems to me very misleading, I must anticipate, for reasons which will be subsequently stated, and substitute the term 'branch' for this structure in the following description.

The stem-stele of *Zygopteris Grayi* is more or less distinctly stellate in transverse section, but the length of the five arms varies in prominence,

<sup>1</sup> The *Zygopteris bibractensis* of Williamson, but possibly not the *Zygopteris bibractensis* of Renault. Ann. des Sci. Nat., 5<sup>e</sup> sér., Bot. vol. xii, p. 171, Pl. IX, Figs. 17 and 18.

<sup>2</sup> P. Bertrand, Études sur la fronde des Zygoptéridées, pp. 106, 107, 222.

<sup>3</sup> 1889. *Rachiopteris Grayi*, Will., Mem. xv, Phil. Trans., vol. clxxx (1889), B., p. 156, Pl. I, Figs. 1, 2, 3, 4 (? Fig. 5); Pl. II, Fig. 5 A.

1900. *Zygopteris Grayi*, Scott, Studies in Fossil Bot., 1st ed., p. 278, Figs. 97 and 98; 2nd ed., vol. i, p. 306, Figs. 115 and 116, 1908.

1908. *Zygopteris Grayi*, Tansley, Evolution of the Filicinean Vascular System, p. 16, Fig. 6.

1909. *Ankyropteris Grayi*, P. Bertrand, Études sur la fronde des Zygoptéridées, p. 107, Pl. XI, Fig. 78.

1880. *Rachiopteris di-epsilon*, Mem. x, Phil. Trans., pp. 537-8, Pl. XXI, Figs. 90 and 91.

1909. *Etapteris di-epsilon*, P. Bertrand, l. c., p. 148, Pl. I, Fig. 6; Pl. XVI, Fig. 110.



according to the state at which they have arrived in giving off branches; that which has just given off a branch being shortest, while that from which one is just about to be given off is longest. If one of the stele arms in the latter condition be examined, it will be seen that its extremity is subtriangular in form, with the distal margin always straight, and terminating in two lateral horn-like points (Fig. 1). Fig. 2 shows a branch just given off. The central area is occupied by a mixed pith, composed of parenchyma and small tracheae, and is a continuation of the mixed pith of the stem.<sup>1</sup> This is surrounded by a zone of tracheae, the adaxial portion being formed of one to three rows of moderately large tracheae, while the abaxial portion contains generally one—rarely two rows. The extremity of the lateral horns usually consists of a few small tracheae, which hold a special relation to the roots. In all essentials the ‘branch’ at this stage resembles the stem-stele with the exception of the five prominent angles.

As the ‘branch’ travels outwards and upwards through the cortex it assumes a slightly more oval form, and the ‘horns’ of small tracheae disappear, though it still contains a mixed pith, and possesses all the essential characters of its parent stem (Fig. 3). At this stage, the xylem of the abaxial side of the branch still usually possesses only a single row of tracheae.

The beginning of the formation of the petiole trace is seen at Fig. 4. This stage immediately follows that described and seen at Fig. 3. The abaxial side of the branch shows a slight increase in the thickness of the xylem, and from each of the abaxial rounded angles tracheae, mostly of a size similar to those forming the branch-stele, begin to appear (Fig. 4, *a'*, *a'*).

Traced higher up these two groups unite and form a bar placed tangentially to the axis of the branch as seen in Fig. 5, *a*, where it is still attached to the branch. Here the pith seems entirely to have disappeared from the branch-stele. The specimen shows a very small clear space in the centre of the axis, but this has much more the appearance of a break in the xylem than a space left by the decay of parenchyma. The specimen is, however, too imperfectly preserved for a definite conclusion on this point.

I have not been able to observe on the specimens at my disposal the actual union of the two groups of tracheae which form the bar or the very early stages of the arm development, but from the examples given at Figs. 4 and 5, there can be little doubt as to the intermediate stages of development which succeed each other.

At Fig. 6 the petiole trace has become quite free from the branch, and advanced some distance outwards as a free organ. The rows of tracheae on the abaxial side of Figs. 2, 3, and 4 have much increased in thickness, though the branch still shows an opening in the central area, but the manner in which fragments of walls of the surrounding tracheae project inwards

<sup>1</sup> See Kidston and Gwynne-Vaughan, *Trans. Roy. Soc., Edin.*, vol. xlvii, p. 468, 1910.

towards the opening rather confirms the opinion already stated as to the branch possessing a solid protostelic structure, though here again the preservation of the specimen leaves the matter in doubt.

The petiole trace seen at Fig. 6 possesses all the essential characters of *Zygopteris di-epsilon*, Williamson. It is of course much smaller than the trace in the fully developed petiole; that seen at Fig. 6 only measuring 1.7 mm. in a direction parallel with the cross-bar, while the measurement of the same portion of the petiole trace in the type specimen of *Z. di-epsilon* is 4.75 mm. It is, however, an ascertained fact that the petiole trace increases in size in its passage outwards, and does not seem to attain its full dimensions even in the immediate base of the free petiole.

That the petiole shown at Fig. 6 and still contained within the cortex of *Zygopteris Grayi* cannot be an immature condition of the petiole which Williamson identified as *Zygopteris bibractensis*, Renault, and which has now been distinguished as *Z. bibractensis* var. *Westphalica* by Dr. Paul Bertrand,<sup>1</sup> is seen from its narrow straight bar and the long thin arms, scarcely incurved, and without any indication of the external band of small tracheae on the outside of the arms, which is so distinctive a feature in the *Z. bibractensis* type of petiole. The very much incurved arms and curved cross-bar of this latter species are also absent from the petiole trace of our specimens. On the other hand, all the characters of the petiole trace found in the cortex of *Z. Grayi* are identical with those of *Z. di-epsilon*.

It might be pointed out that the petiole which Williamson figures on Pl. I, Fig. 4 of his Memoir xv, is referable to *Zygopteris di-epsilon*, and this I think was his opinion, for he refers to it as 'a similar Zygopteroid petiolar bundle' to that he has described in the cortex of *Z. Grayi*.<sup>2</sup>

The morphology of the so-called 'axillary shoot' which I regard simply as a branch must shortly be considered.

Stenzel was the first to observe this so-called 'axillary shoot',<sup>3</sup> but later it was also recognized by Williamson, who described it as a 'circular aberrant organ' which he regarded as a branch, and this is the view that has been generally accepted, and the leaf-trace has been considered to arise from a division of the 'axillary shoot' into two unequal parts. It is thus described by Dr. Scott: 'As we follow the leaf-trace outwards through the cortex, we find that it divides into two strands, of very different form, both lying on the same radius. The outer of the two strands is the foliar bundle, which is continuous with the external side of the original triangular strand, while the inner strand is destined for the axillary shoot.'<sup>4</sup>

In regard to the 'axillary shoot' in the Hymenophyllaceae, Tansley says: 'The stem-like structure of the strand below the junction of the leaf

<sup>1</sup> P. Bertrand, l. c., p. 73.

<sup>2</sup> Williamson, l. c., p. 157.

<sup>3</sup> Stenzel, Die Gattung *Tubicaulis Cotta*, pp. 35-6, 1889.

<sup>4</sup> Studies in Fossil Botany, 2nd ed., vol. i, p. 311, 1908.

and branch traces might of course be explained by the hypothesis that this basal portion is really a stem-structure on which the leaf is inserted, but this consideration, even if valid, will not apply to the structure of the petiole strand *above* its junction with the branch-strand. The whole of the phenomena, taken in conjunction with those of *T. reniforme*, appear to lend decided support to the interpretation of leaf-strand and stem-stele as primitive identical structures;<sup>1</sup> and again further: 'We do actually find, in the Botryopterideae and Hymenophyllaceae, the base of the leaf-trace exhibiting a structure which may be interpreted in this way.'<sup>2</sup> In talking of *Zygopteris* it is stated: 'The leaf-trace departs as an isodiametric strand which divides tangentially as it passes through the cortex, the inner half becoming the stele of the axillary shoot, while the outer becomes the petiolar strand. The latter has at first the form of a tangentially extended band. . . .'<sup>3</sup> It is evident then that Tansley regards the petiole and the remainder of the 'axillary shoot' as two parts of a single organ, and this he believes to be cauline.

Dr. Scott, however, from the study of the stem of *Zygopteris corrugata* where the leaf-traces are given off direct from the stem, without the occurrence of any 'axillary shoot', speaks of them as 'rather of the nature of a dichotomy', and gives the note of warning that 'this fact raises the question whether, as has been suggested, the apparent axillary branching of other species (of *Zygopteris*) and of recent Hymenophyllaceae may not be a modified dichotomy, in which case the "undivided leaf-trace" would really be the stele of the small branch, and the "subtending" leaf would belong to this branch and not to the main axis. The data are insufficient to settle the question, and for the present it seems better to keep up the distinction between the two kinds of branching.'<sup>4</sup> That the explanation of the nature of the 'axillary shoot' given here by Dr. Scott is the correct one is fully borne out by the development of the leaf-trace of *Zygopteris Grayi, Will.*

In this species, no division of the so-called 'axillary shoot' takes place in the departure of the leaf-trace, but it arises from the periphery of that structure as an independent organ, and at no time partakes of the structure of the organ from which it arises. The so-called 'axillary shoot', till after the departure of the leaf-trace, retains a structure comparable with that possessed by the stem, and the only change which takes place in its structure is the possible loss of the pith, but this does not seem to occur until after the departure of the petiole, when it appears to be wholly formed of xylem, and assumes the condition of a solid protostele. As far as I have been able to observe, the petiole carries nothing of the branch with it, except it be a few tracheae from the two abaxial angles of the so-called

<sup>1</sup> Tansley, *Evolution of Filicinean Vascular Structure*, p. 33.

<sup>2</sup> Tansley, *l. c.*, p. 115.

<sup>3</sup> *Ibid.*, p. 17.

<sup>4</sup> Scott, *Studies*, vol. i, 2nd ed. p. 318.

'axillary shoot', which I believe is morphologically a *branch* which gives rise to, but which does not of its own tissue contribute to, the formation of the leaf-trace.

It may be further pointed out that *Zygopteris Grayi* is by no means the most primitive type of *Zygopteris*. In *Zygopteris corrugata*, according to Dr. Scott, there is no 'axillary shoot' in connexion with the leaf departure, and in the older *Zygopteris (Diplolabis) Roemeri*, Solms, from the culm, of which the stem was recently described by Mr. W. T. Gordon before the Royal Society of Edinburgh,<sup>1</sup> the petioles arise directly from the solid protosteles, and their steles assume the characteristic Zygopteroid form in their passage through the cortex.

The presence of a small stem from which the petioles arise, and the occurrence of a mixed pith in *Zygopteris Grayi*, would indicate that it has travelled some considerable distance from the primitive type of Zygopteroid structure.<sup>2</sup>

<sup>1</sup> Dec. 20, 1909.

<sup>2</sup> Note.—*Zygopteris (Ankyropteris) scandens*, Stenzel (Die Gattung *Tubicaulis Cotta*, p. 31, Pl. VI, Figs. 50-5, Pl. VII, Figs. 56-65, 1889), has been considered by some botanists as synonymous with *Z. Grayi*, Will., but is essentially distinct from that plant and is easily distinguished by the form of its petiole trace. The cross-bar of *Z. scandens* is formed of two rows of much larger tracheae than those of *Z. di-epsilon* (= *Z. Grayi*), where the cross-bar is seven or eight rows wide. The arms of *Z. scandens* are slightly incurved and do not possess the outward spreading form of those of *Z. di-epsilon*, but above all, the abaxial arms of *Z. scandens* bend inwards towards each other and are shorter than the adaxial arms. The bar is also slightly curved, which gives the leaf-trace an inequilateral form; or, to put it in other words, the petiole trace of *Z. scandens* has only one plane of symmetry—the vertical, while that of *Z. Grayi* has two planes of symmetry—the vertical and horizontal. These two species cannot therefore be united. (See Stenzel, l. c., Pl. VII, Fig. 63.)

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## EXPLANATION OF PLATE XXXIV.

Illustrating Dr. Kidston's paper on *Zygopteris Grayi*, Williamson.

[All figures are enlarged about 17 times.]

The \* indicates the position of the axis of the stem.

Fig. 1. From specimen contained in the Collection of the Manchester Museum, No. R. 443. *Loc.* Oldham. *Hor.* Halifax Hard Bed.

Fig. 2. From specimen contained in the Author's Collection, No. 308. *Loc.* Oldham.

Fig. 3. From specimen contained in the Collection of the Manchester Museum, No. R. 443. *Loc.* Oldham.

Fig. 4. From specimen contained in the Author's Collection, No. 308. *a'*, *a''*, Bar of petiole. *Loc.* Oldham.

Fig. 5. From specimen contained in the collection of the Manchester Museum, No. R. 441. *a*, Bar of petiole; *b*, Branch. *Loc.* Sunfield, Oldham.

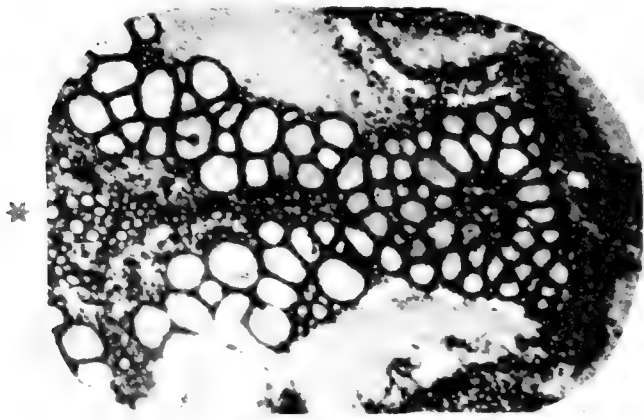
Fig. 6. From specimen contained in the Author's Collection, No. 305. *b*, Branch. *Loc.* Sunfield, Oldham.

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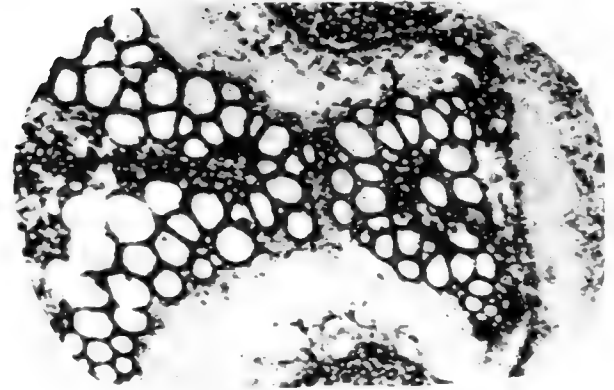
My thanks are due to Professor Weiss, for kind permission to figure and describe the specimens contained in the Collection of the Manchester Museum.



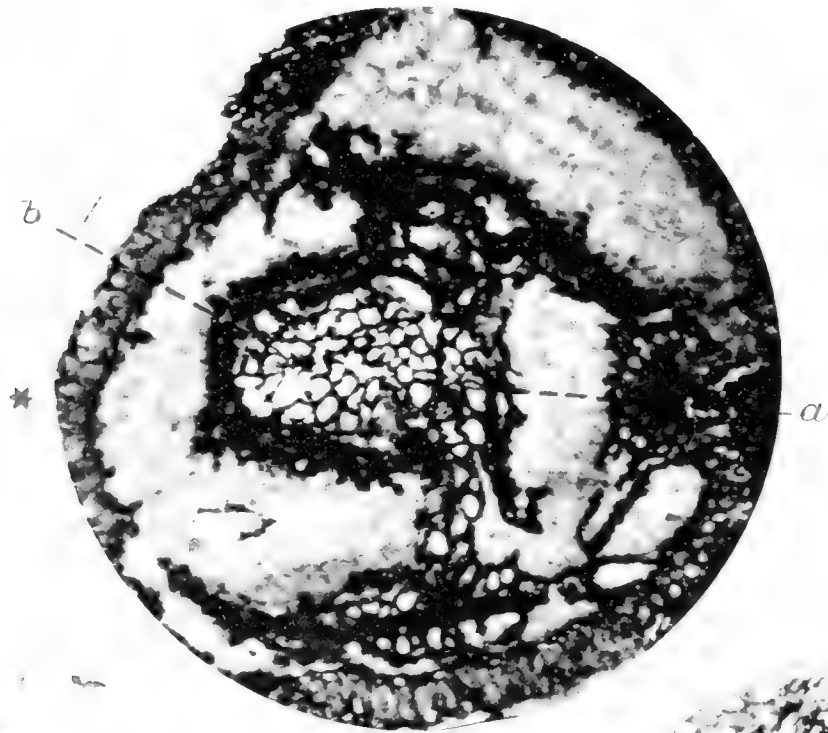




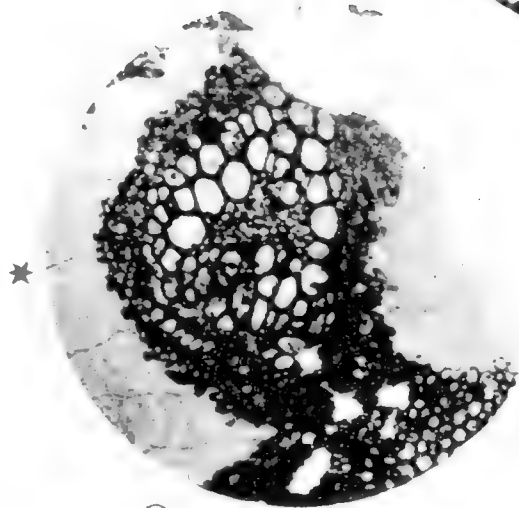
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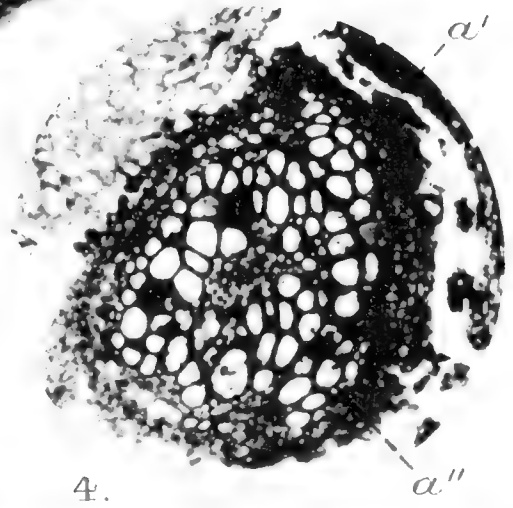
2.



5.



3.



4.

6.

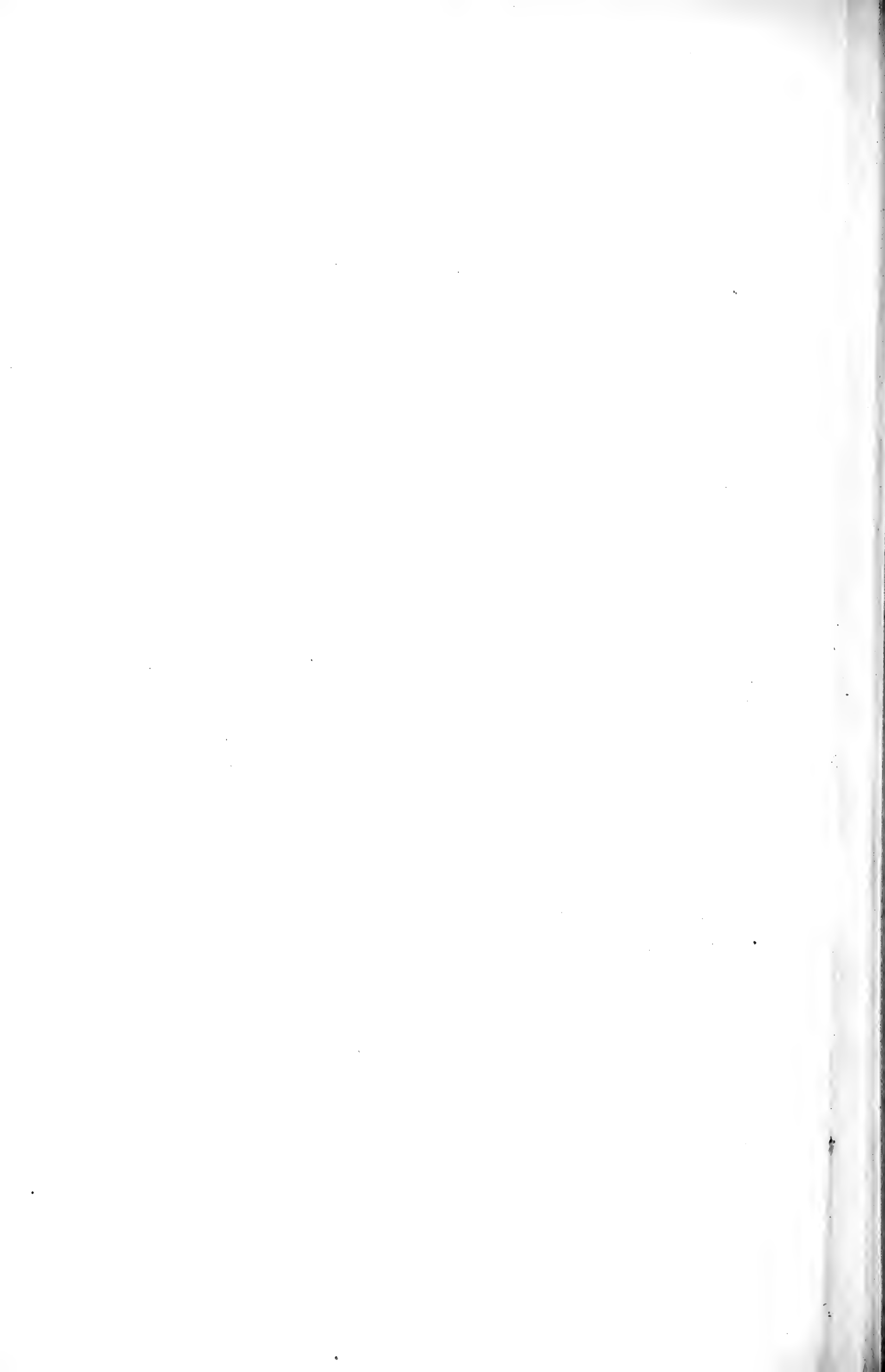


R. Kidston, photo

5. (1) coll.

KIDSTON—ZYGOPTERIS GRAYI Williamson.





# Symbiosis of Ants and Plants.

BY

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With Plates XXXV and XXXVI.

A GREAT deal has been written and published in various works concerning the adaptations of certain plants in the tropics apparently for the combined benefit of ants and the plants themselves, each deriving some advantage from the symbiosis. The subject, however, has by no means been exhausted, as in many cases the observations made by the writers have been too few, or derived from too short an acquaintance with the plants in their natural habitat. The most important general contribution, at least to the study of the myrmecophilous plants of the Eastern Tropics, has been made by O. Beccari, in Malesia ('*Piante ospitatrici*'), but the story of some of the species he has described in this paper is still incomplete, and more remains to be done. Myrmecophilous plants, using the word in its widest extent, are, it would seem, commoner in the Eastern Tropics than in those of the New World, and we possess in the Malay Peninsula a considerable number of plants which have, or are believed to have, close relations with ants, many of which plants occur wild or introduced in the Botanic Gardens in Singapore. These are the following:—

*Pachycentria,*

*Myrmecodia,*

*Hydnophytum,*

*Clerodendron myrmecophilum,*

*Dischidia Rafflesiana,*

*Ficus inaequalis,*

*Macaranga,* 3 species,

*Korthalsia,* 2 species,

*Pleopeltis sinuosa,*

*Lecanopteris carnososa,*

besides a number of epiphytes in the life-history of which ants play a more or less important part. Among these and so-called myrmecophilous plants generally there are many degrees of symbiosis. In some species, and these are not many, we have distinct modifications, which cannot apparently be of any use at all except to induce ants to take up their abode in or about the plant, and in which observation shows that the absence of the ants pro-

duces disastrous results ; such, as I hope to show, are the Macarangas. In these the symbiosis may be said to be complete. In others we find that though the plant possesses modifications utilized often by ants, such modifications have another function of importance to the plant, and there are no modifications which can only serve to attract the ants. Furthermore, in some of these cases the ants have apparently no function to perform that is of any use to the plant. In these cases there is no true symbiosis, even though ants commonly occupy a position on the plant.

Then again, we have a series of plants—epiphytes—which, though undoubtedly deriving advantages from the ants, have no special modification to induce them to occupy the plant, the modification by which the plants obtain the services of the ants being obviously intended originally for a different purpose.

Ants are extraordinarily numerous and varied in habits in the tropics, and are very quick in finding out the most suitable locality to put their nests. Any kind of hole, e. g. in a stick if large enough, is adopted. A cool place under or between leaves, a deserted termites' nest, anywhere in fact where they can put the eggs and larvae away from the action of sun and rain, is speedily utilized, and even if they commonly adopt a particular part of a plant, such as the swollen sheath of *Korthalsia* or the spathes of *Daemonorops*, or even a hollow stem, it does not necessarily follow that this is a case of true symbiosis. When Professor Schimper was stopping in Singapore, as he and I were looking into various oecological questions, we found on a tree of *Ficus inaequalis*, a native of Celebes cultivated in the Botanic Gardens, a number of branches which were swollen just below the nodes, and partly split and hollowed out. These were tenanted by ants. A branch of this fig-tree is figured in the *Pflanzengeographie*, Pl. 83, as an example of myrmecophily. I have since constantly examined this tree and others in the Gardens, and find that these swellings and the ants have quite disappeared. The branches were, I believe, swollen by some disease or abnormality of growth, and in course of development split and the holes produced were occupied by ants. The appearance of the twigs closely resembled that of *Humboldtia laurifolia* (figured on the same plate), a more truly myrmecophilous plant, but in the case of the *Ficus* the swelling and presence of the ants appear to have been abnormal and accidental.

A somewhat similar case occurs in *Pachycentria tuberculata*, an epiphytic plant of the order Melastomaceae, in which the roots, embedded in a mass of vegetable débris, ants' nests, &c., are frequently swollen at intervals into fusiform dilatations. In most of these I find no opening or hollow space, the tubers being quite solid ; occasionally there is, however, an opening and a hollow space within (sometimes after the style of the tuberous stem of *Myrmecodia*), and in this hollow ants have placed their nest. Beccari describes and figures this plant and allied species, but says

that except that he noted that ants frequented this plant he had made no observations on their action on the plant.

The Bull's-horn Thorn, *Acacia cornigera*, a native of South America, has long been cultivated in the Botanic Gardens, Singapore, from a plant sent from England and propagated by cuttings. Naturally the ants which normally occupy the hollow swollen stipules did not accompany the plant, but a descendant of the original importation was for a considerable time occupied by a local species of ant (I believe it was a species I have also found in *Korthalsia scaphigera*). This ant bored into the stipules and tenanted them in much the same way as the South American ant does in the native haunts of the *Acacia*. Unfortunately they have since abandoned the tree, so I am unable to make further observations. But the fact serves to show that ants are always ready to adopt a place suitable for their nesting, even if it is not the class of nesting-place they are accustomed to.

#### KORTHALSIA.

The genus *Korthalsia*, to which I pass on next, comprises two sections of rattans, in one of which the ligule is developed into a close tight-fitting sheath, while in the other the ligule is dilated into a rounded or oblong sheath, or ochrea, of a stiff papery texture, and so dilated that it forms an ideal place for an ants' nest when the ants have perforated it so as to make an entrance, and this ochrea is commonly so perforated and occupied by ants.

There are two species of this section in the Singapore Botanic Gardens, viz. *Korthalsia scaphigera*, Mart., and *K. echinometra*, Becc.; both are wild in Singapore also.

*K. scaphigera*, Mart., when growing in thick jungle is more slender and has narrower leaves and longer and narrower ochreae than the form planted or growing naturally in open exposed spots. In the jungle form growing in the Gardens forest, I find that the ochreae are tenanted by a small species of *Camponotus*, but by no means all the ochreae are occupied. If one on a stem is occupied, usually all the rest, excluding the top one which is still covering the base of the bud, are occupied, but only a comparatively few stems are inhabited by the ants. Where the plant is planted out in the open ground, I find a large proportion of the ochreae are not occupied, and the ant at present occupying the ochreae is quite a different one from the one in the forest. It is a much larger and broader black ant, with powerful mandibles and a remarkably prominent process on the pedicel. Both species, however, belong, I believe, to the genus *Camponotus*.

In the *Korthalsia echinometra*, Becc., cultivated in the Gardens, there are no ants. Beccari mentions the occurrence of two species of *Camponotus* and one *Tridomyrmex* in *Korthalsia scaphigera*, and another *Camponotus* in *K. echinometra*. It appears to me very probable that no special kind of

ant haunts these *Korthalsias*, but that any ant whose habits induce it to adopt this class of dwelling may do so.

The primary use of the ochrea is undoubtedly to throw off rain from the base of the young bud which it at first covers, and thus it is not in any way a modification for the benefit of the ants. The only possible advantage that the ants could bring to the plant would be to defend it against the attacks of caterpillars or other insects. Now neither in the forests nor in the open part of the Gardens are the ochreae always occupied by ants; neither are the unprotected plants of *K. scaphigera* and *K. echinometra* more liable to insect attacks than the protected plants; and, again, the species, like *K. flagellifera*, which do not possess swollen ochreae are equally exempt from insect attacks.

There are, indeed, but few insects which attack the foliage of palms even under cultivation, and even less in a wild state. The commonest caterpillar which attacks any palm is *Erionota Thrax*, one of the skipper butterflies. The caterpillar, which is of large size, is of a light sea-green colour and powdered all over with a white floury substance. It rolls up the leaves of palms and ensconces itself therein, devouring the leaf from within. The white powdery dust (wax) protects it from the action of water, but also from the attacks of ants. I have unrolled a leaf-tube containing one of these caterpillars in the neighbourhood of a nest of the tree ant *Oecophylla smaragdina*, which is a great destroyer of caterpillars. The ants ran all over the caterpillar and attempted to attack it, but were deterred by the white powder, and soon left the caterpillar unhurt.

The ants which frequent *Korthalsia* are smaller and much less ferocious than the *Oecophylla*, and are much less likely to attack so large a caterpillar.

Furthermore, plants of which the adult leaves are attacked by caterpillars are in this country seldom seriously or permanently injured, at least in a wild state, where they grow isolated from other plants of the same species, as these plants commonly do in the forests. The loss of a few adult leaves is quite unimportant to the plant. The matter is different in cases where the young leaves when just opening from the bud are attacked persistently, as will be seen in the case of the *Macarangas*.

Taking the whole facts of the myrmecophily of the *Korthalsia* together, it will be seen that they do not fill the requirements for a true symbiosis. The ants are not always present, yet the plant unprotected does not suffer from their absence; more than one species of ant frequents the ochrea of the rattan; there is no special modification which can only be accounted for as of use to ants; there is no clear benefit to the plant from the presence of ants at all.

## DAEMONOROPS.

In several species of the rattans of the genus *Daemonorops* ants habitually make nests in the large flower spathes. The inflorescence in these palms is enclosed in four or five stiff sheaths which in the *Cymbiformia* section quite cover the dense flower panicles. The two outer spathes are the largest and usually quite enwrap the others. They only open a little when the flowers are ripe, and do not fall off usually till the fruit is quite ripe. The flowers, which are very numerous, brown, and stiffly cartilaginous, are wind-fertilized, the pollen being produced in great abundance and blown away in clouds from between the half-open spathes. In the spathes of *Daemonorops Jenkinsianus* I find nests of a species of *Campotonus*, apparently a form of *C. mitis*. The nest is very simple, consisting of a few walls of soil between the inflorescence and spathe and a comparatively small number of larvae or cocoons, together with some coccids guarded by these ants.

## GONIOTHALAMUS.

There are a few species of the Anonaceous genus *Goniothalamus*, notably *G. Ridleyi*, King, which produce their flowers in masses at the base of the trunk of the tree. The flowers are of large size and dull reddish in colour. They are almost invariably covered by a nest of very small black ants, which pile up powdery soil all over them, so that they are often quite concealed. It would, I think, be difficult for a bee or other insect to get to the honey of these flowers through the nest, yet I think no species of the genus fruits so regularly or heavily as does *Goniothalamus Ridleyi*. That the ants are distinctly attracted by the flowers, is clear from the fact that the flowers from the trunk which are too high up for the ants to cover with the nest are generally densely covered by a swarm of the insects. Owing, however, to the minuteness of the ants and the difficulty of making observations in such a mass of them, I have been unable to definitely decide whether the ants do actually fertilize the flowers by conveying the pollen from one to the other, but I cannot see any other way in which the fertilization can be effected. The ants generally throw up the mounds over the flowers before the buds open, as if in anticipation of the honey within the flowers.

In most species of the genus the flowers are borne on the branches or upper part of the stem, and are brighter in colour, white or orange, and these are not haunted by ants, but doubtless fertilized by Hymenopterous or Dipterous insects. If the flowers of *G. Ridleyi* are, as I believe, fertilized by ants, their position at the base of the stem may be taken as a modification to that end.



This, however, could not be classed as symbiosis, but rather as a modification for fertilization, as the main nest of the ants is apparently always underground near the tree.

#### DISCHIDIA RAFFLESIANA, Wall.

This remarkable plant, so common in the southern part of the Malay Peninsula, has been a subject of a very considerable amount of literature, and has been classed by Beccari and others as a myrmecophilous plant.

It is plentiful in the Botanic Gardens at Singapore, and there is an old Durian tree preserved for its cultivation which is almost covered with this plant. This tree also bears *Pleopeltis sinuosa* and *Drymoglossum piloselloides* in abundance.

This *Dischidia* is one of the Asclepiadeae, a climbing epiphyte, which bears two forms of leaves, one round and fleshy, and one cone-shaped, with an opening at the base (the broadest part), and an inverted rim inside the opening. Through this opening the roots project into the hollow cone. The cone-shaped or rather pitcher-shaped leaves are green, or more usually yellow outside and purple within, and are generally arranged in pairs or fours in an irregular false whorl or cluster.

The sole advantage to the plant obtained by the presence of ants in the pitchers could be but the supplying of food in the form of the nest-débris to the roots enclosed in the pitcher; much after the same way as *Platynerium* is supplied with food by the ants. No insects, so far as we know, attack the plant or are likely to, so that the *Dischidia* would gain nothing by inducing the ants to come as guards. The advantage mentioned is undoubtedly a valuable one to *D. Rafflesiana*, but it must be pointed out that the tenancy of the pitchers by ants is not constant, nor even very common, and there does not seem to be any material superiority of plants tenanted over those which are not tenanted. In the case where there is an ants' nest in the pitcher, the roots seem to be somewhat more extensively developed, and they are soon found in contact with the detritus. All the pitched *Dischidia* that I have met with, viz. *D. Rafflesiana* and *D. complex*, grow in very hot exposed positions on thinly foliated trees, positions where the roots are exposed to an excessively hot sun. The plants scramble over and twine round the branches, and the roots could not be protected from drought as they are in *D. collyris*, Wall., in which the convex leaves are closely appressed to a tree trunk, with their margins touching and so covering completely the roots. The only position in such an exposed place as that usually inhabited by *D. Rafflesiana* where the roots could develop sufficiently for absorptive purposes, is in the hollow of the pitcher where the water-vapour can be absorbed. From the inverted saucer-shaped leaves of *D. collyris* to the pitchers of *D. Rafflesiana* is a comparatively simple development. Beneath the leaves of *D. collyris* and the somewhat similar ones of

*D. coccinea*, ants' nests are almost invariably found, but one would have little doubt that the peculiar shape of the leaves in these plants is primarily adapted for the protection of the roots from drought, and the adoption of them as a suitable nesting-place by ants is a secondary function.

Their relationship with ants is in fact very similar to that in the case of *Platycerium*, and only differs from that of *Korthalsia* in the actual advantage derived from the presence of ants in the two former plants.

The habit of *Dischidia Rafflesiana* is somewhat peculiar. Indeed I do not know an epiphyte which at all resembles it. As Groom says, it is specially fond of decaying trees—perhaps it would be clearer to say dying trees. Its long slender stems creep along the branches, but also pass from branch to branch, and very frequently hang down for some distance. The trees it prefers are undoubtedly half-dead trees with few leaves, so that the plant is most fully exposed to the hot sun. In such a habitat it makes rapid growth, forming a kind of network over the tree, and the pitchers are yellow, or soon become so, the same colouring exactly as *D. collyris* which grows on tree trunks in full sun. If, as sometimes happens, the *Dischidia* seed alights on a shady tree, it grows and develops the long stem and pitchers, but in this case the stem and pitchers are green. It does not, however, make nearly as good a growth in a shady tree, and one never sees such a tree filled with it as one does in an open exposed tree.

The plant is common especially on our sea-coasts in hot exposed places, but disappears entirely inland, and is quite absent from the forests of the plains and hills. I have, however, found it at an altitude of about 3,000 feet in mossy spots on Mount Ophir, but on no other mountain. The pitchers here were green. Mount Ophir, however, contains a number of plants more commonly associated with seashores and not met with in the intervening country, and I think there is reason to believe that at no very distant date, geologically, this mountain was a separate island, and that the remains of its seashore flora have survived on its xerophytic upper slopes.

The object of the pitcher-shaped leaves has constantly been discussed, and it is not my intention here to consider their physiological functions, except so far as relates to their connexion with ants, in fact to their supposed myrmecophily.

I may, however, refer to the various functions that have been attributed to them. Delpino thought that the plant was carnivorous, that the pitchers caught insects which were drowned in the water contained in them after the manner of *Nepenthes*. As only a very few of the pitchers ever contain more than a drop or two of water, and insects do not appear to enter those that do, this theory may be at once dismissed. A theory that they are actual water-vessels containing water derived from rain, and that the roots obtain their water in this way, is somewhat negatived by the fact that a large proportion of the pitchers do not contain water. On examining a con-

siderable number on September 29, after a heavy rain shower in the morning, I found the greater number quite dry inside, three or four with a drop or two of water, and one or two full or nearly so. Groom showed by experiments made in the Botanic Gardens, Singapore, that the roots inside the pitchers absolutely did absorb the water,<sup>1</sup> and further that a considerable amount of earth and humus was found in the pitchers; and though part of this was no doubt débris of bark from the upper part of the tree washed in by storms of rain, there was a quantity of material which could only have been brought by ants. In the plants which he and I examined, we found clay similar to that of the soil at the foot of the tree. Without doubt the ants had carried this soil into the pitchers and made nests therein. Unfortunately we did not investigate what the species of ant was that was utilizing the pitchers as nests, and the tree and *Dischidia* on which these observations were made has long gone.

On examining the pitchers of the *Dischidia* on the Durian tree above mentioned, I found that in most branches of pitchers a touch produced an exodus from the mouth of the pitcher of numerous little vicious ants, which appear to be *Technomyrmex brunneus*, but on opening the pitcher from which they issued, no humus or any trace of a nest was found and no larvae or pupae. The ants appeared to be clustered round the upper end of the cup, behind the incurved lips. Nests of this ant were, however, found under the bark of the tree where loose and among the masses of rhizomes of the *Drymoglossum* and other such spots. They were made of thin brown walls, apparently of vegetable débris, with supporting partitions. The whole tree swarmed with myriads of these ants, which run about on all the branches, and were to be seen busily engaged with coccids on the leaves of the Durian.

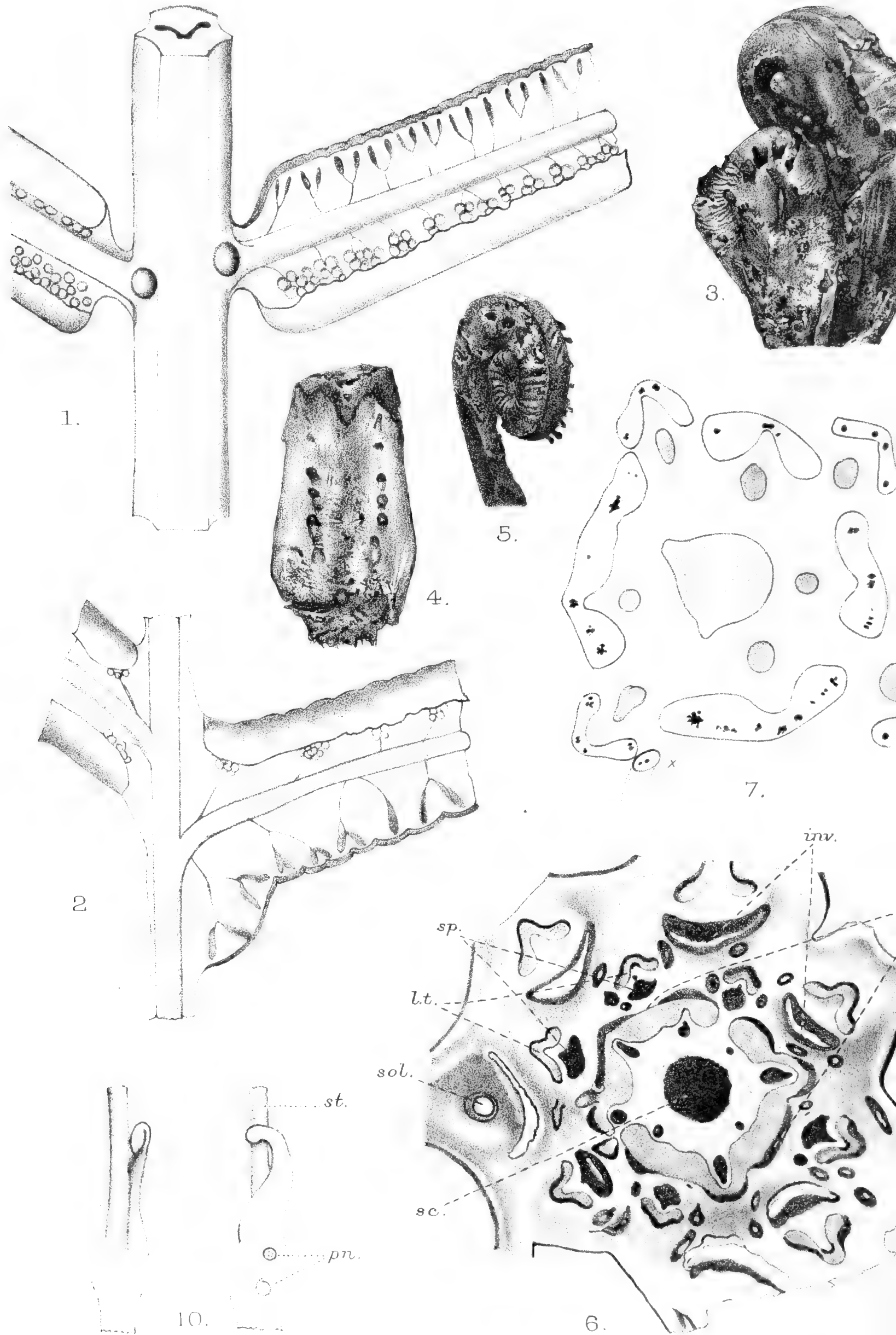
In a good many cases, especially where the slender twining stem hung down below the bough it was climbing on, there were no ants in the pitchers, and in these were often small cockroaches and other insects.

It is clear that this species of ant does not care to make its nest in the pitchers; indeed, many of the nests on the tree were much too large to be included in a pitcher, and as the ants were occupying the tree in immense numbers, and very vicious, no other species of ant would be able to make a lodgement in the pitchers.

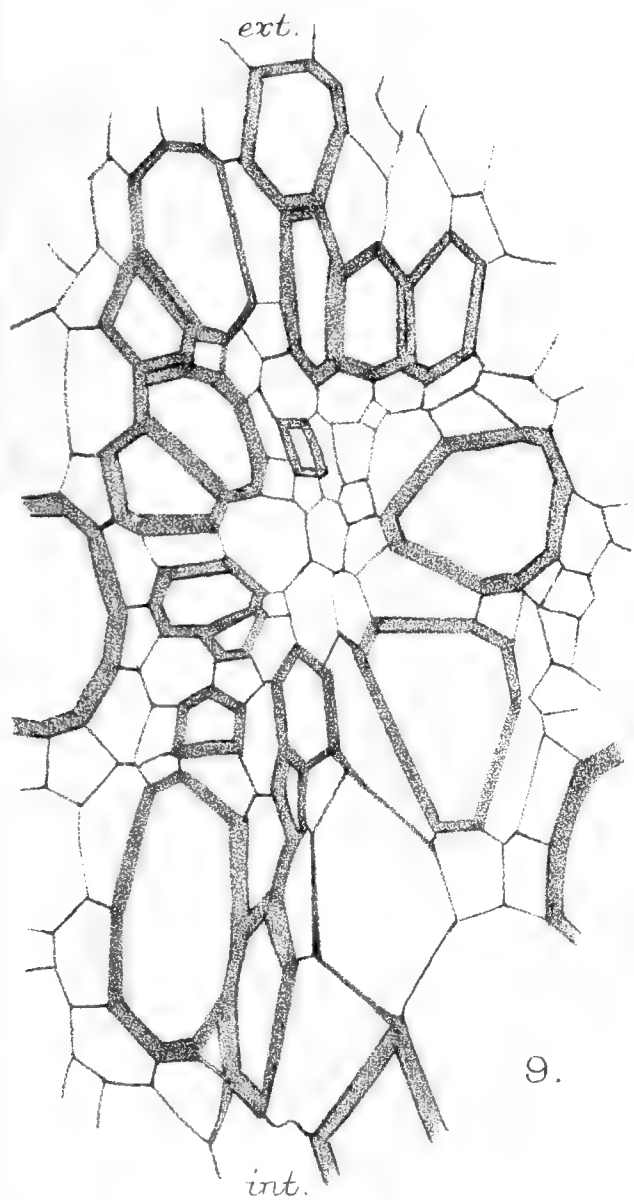
What was the reason, then, of their visiting the interior of the pitchers? They were absent from the pitchers containing water, even in small quantity. The inner surface of the pitcher is lined with wax, and it seems probable that the ants were engaged in collecting this. One may compare this with the recurved 'bud-bract' of *Macaranga triloba* and the wax deposits on the back of the young leaf of *M. hypoleuca*, both of which are visited by species of ants, apparently collecting or eating the wax only.

<sup>1</sup> Annals of Botany. vol. vii, p. 227.

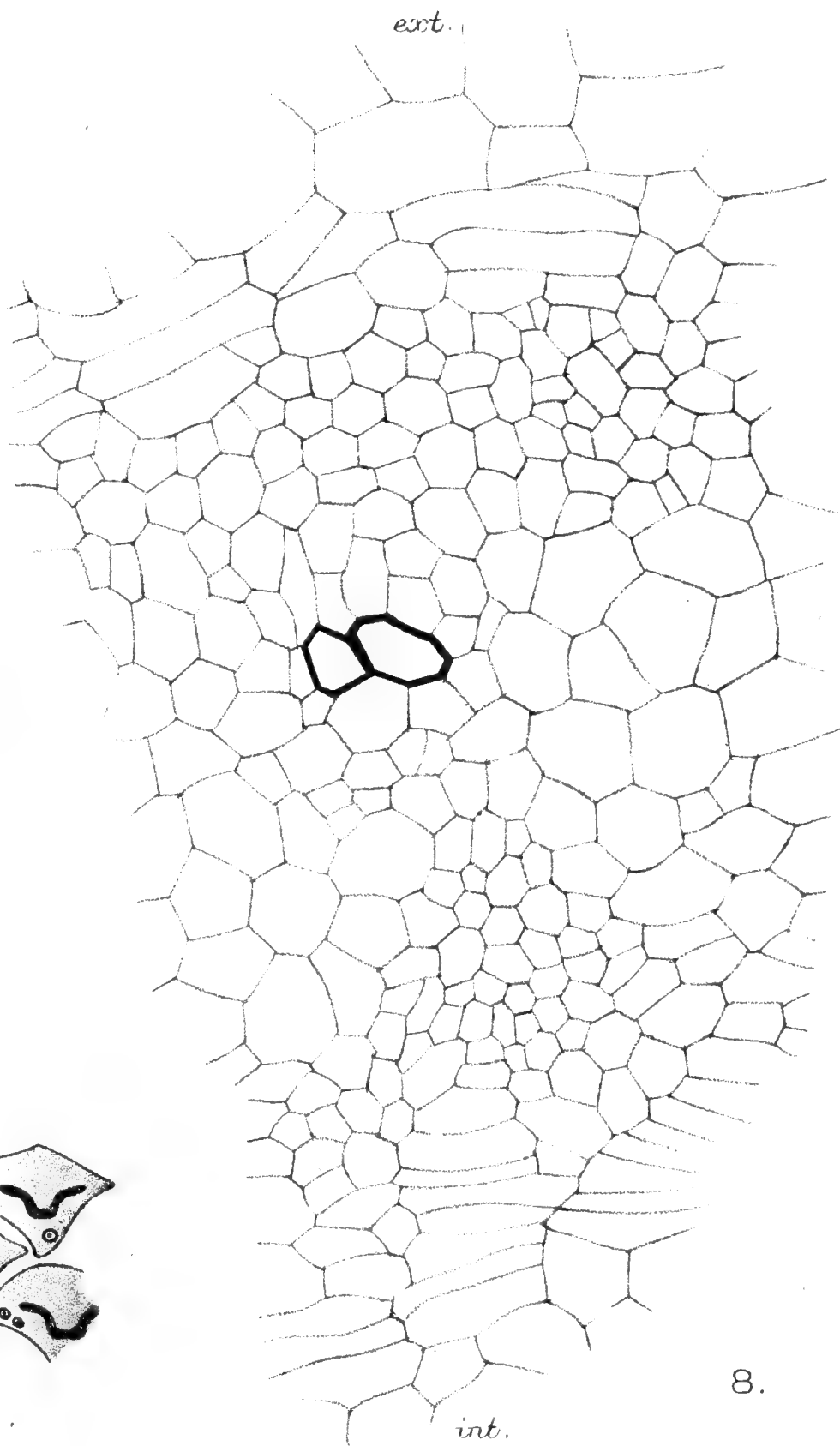




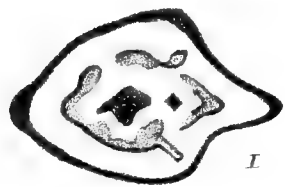




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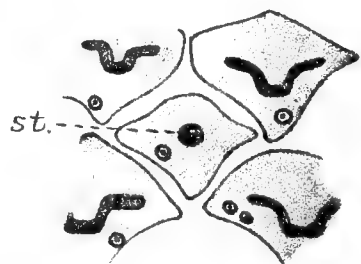
8.



I



II



13.



III

12.



IV



V



I



II



III



IV



V



VI



VII



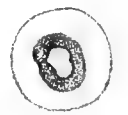
VIII.



IX



X



XI



XII

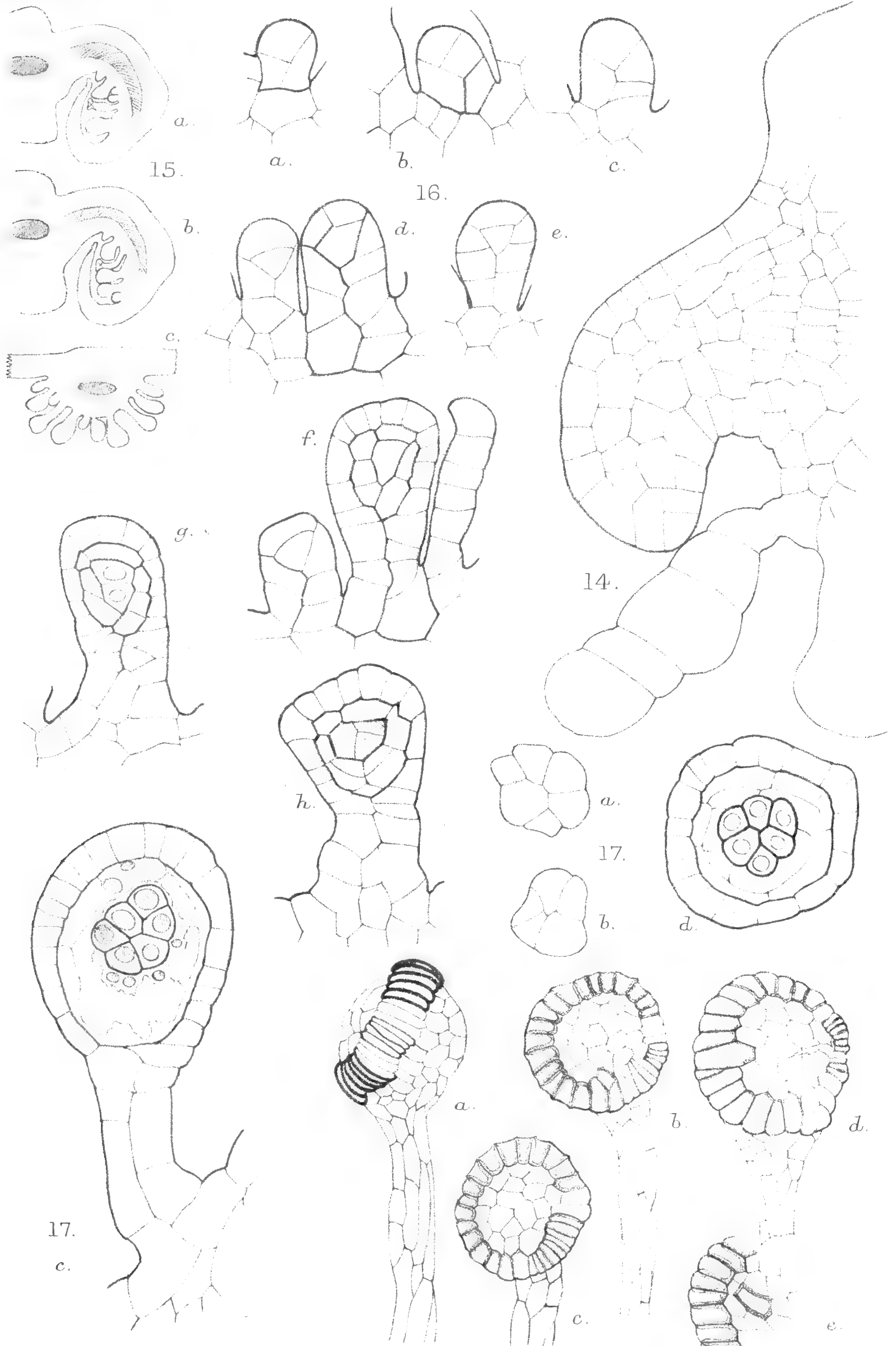


XIII

11.







Hutch, lith et imp.

BOWER — PLAGIOGYRIA.



I moved some plants from the Durian tree to a shady tree near my house, and they were invaded by a species of ant, apparently the same one which frequents the roots of epiphytic orchids. They piled up earth on the basal ends of the deflexed pitchers among the roots of the *Dischidia*, and on opening one of the pitchers I found that it contained numerous workers, with a queen and a number of eggs, some of which had hatched out. There was no soil or other material in the pitcher.

I conclude from these observations that *Dischidia Rafflesiana* cannot be considered as truly myrmecophilous.

#### POLYPODIUM SINUOSUM.

The morphology of this plant is described by Yapp in *Annals of Botany*, vol. xvi, p. 185, Pls. X, XI, XII. The plant is common in Singapore. It is an epiphytic fern with a thick rhizome, tunnelled for almost its whole length and inhabited by the ant *Technomyrmex albipes*, Sm., of which Bingham says, in the Formicidae of the Fauna of British India, 'Occurs throughout our limits and spread over the tropics of the old world.' This species is frequently imported into Europe with tropical plants; I have seen many specimens from Kew Gardens, so that it does not appear to be peculiar to the *Polypodium*, which has not so wide a distribution. As Yapp explains, a tissue consisting of large cells with thin walls and no intercellular spaces is formed in certain definite areas near the apex of the stem; this tissue breaks down at an early period, its place being taken by ant galleries. The communication with the outer air is effected by short passages excavated by the ants themselves.

The position of this tissue seems, he says, to indicate that this was developed originally as a water reservoir, but as it soon disappears it may perhaps have to some degree changed its function, and the galleries may have some other important function to fulfil. He suggests that they may serve as organs for the aeration of the stem, and to a slight extent for the absorption of water.

The fern generally grows in at least partially shaded though not heavily shaded spots, on the boughs of trees, at about 15 to 20 feet from the ground. Like the other myrmecophilous fern described in the same paper, *Lecanopteris carnosula*, a nearly allied plant, it seems almost impossible to cultivate it away from its ordinary habitat; even if a bough carrying it is carefully cut off and transferred to a shady spot, it very speedily perishes, so much so that the native gardeners affirmed it would never grow but on a living tree.

No epiphytic fern native here appears to suffer so much from drought. A few weeks of dry weather on one occasion in the Gardens almost exterminated the plant altogether, though it was very abundant on the branches of a shady *Inga saman*, as well as elsewhere in the Gardens.

After the drought I was hardly able to find a single living plant anywhere. The rhizomes completely dried up and hundreds of plants must have perished. Meanwhile, *Davallia solida*, *Polypodium angustifolium*, and other such plants seemed to be little the worse, though as much or even more exposed. *Vittaria lineata* feels the effect of drought severely, and its thin wiry rhizomes seem to dry up very much, while the fronds get wilted and dry, but I do not think that even it perishes so readily as *Polypodium sinuosum*. As a water reservoir, therefore, the rhizome (at least in its present state, with the ant galleries replacing the original large-celled tissue) must be considered a failure.

But whether the galleries are intended for the absorption of water or for aeration of the stem, the question arises, What benefit, if any, does the plant derive from the presence of ants in the galleries? Their use as defenders against the attacks of caterpillars may, I think, be laid aside. I have never seen any of the epiphytic ferns attacked by caterpillars or any other injurious insects. In fact it is comparatively rare to find any ferns here attacked by insects at all. *Angiopteris evecta* injured by a caterpillar which rolls up the ends of its fronds into a ball, and *Aspidium cicutarium* attacked by a small species of bag-worm, are all the ferns that I can recall having seen damaged by insects.

*Lecanopteris carnososa*, Bl., of which Yapp gives an account in the same paper in which he deals with *Polypodium sinuosum*, is another of our ant-inhabited plants apparently specially modified for their benefit. He is doubtless right in classing it as a *Polypodium* near *P. sinuosum*. It is usually met with in very damp forests at a considerable altitude, where it grows on wet mossy branches of trees, often at some height. Plants, however, occur also on more dry and exposed positions, as it still grows on the branches of lofty Shoreas on the top of Bukit-Timah, a hill of 500 feet elevation, in Singapore. Here it is certainly in a comparatively dry exposed habitat, but from the occurrence in the same spot of other plants more usually found at a considerable altitude, I am inclined to think that this hill was originally of a much greater altitude than it is at present, and was then covered with high wet forest.

The rhizome of this fern is rounded and of very considerable size. It is very fleshy and tunnelled with hollow spaces similar to those in *Myrmecodia*, and tenanted by ants described by Forel as *Crematogaster Yappii*.

#### RELATION OF ANTS TO EPIPHYTES.

Ants play a considerable part in many cases in the growth of epiphytes, and especially in orchids.

While not intending here to go into the question of the epiphytic life of plants in the Eastern Tropics, I would, however, record some observa-

tions on the epiphytic flora and its growth. All trees do not bear epiphytes. On some of those with smooth bark or with longitudinally grooved bark epiphytes are seldom if ever to be found. Whether a tree does or does not bear epiphytes depends on the flow of rain down the branches and stem. Where in smooth-barked trees like *Macaranga* the rain flows quickly off, vegetable débris and spores cannot rest, and no epiphytes are borne. A notch in the bark of one of these may, however, retain a little soil, and epiphytes then usually appear. Lichens are abundant on these smooth-barked trees, and usually absent from rough-barked ones. *Ficus Benjamina* is a smooth-barked tree, but liable to cracks or other injuries, so that it carries epiphytes readily in parts. The boughs are covered with lichens, and where they are more or less vertical, with a strong slope, nothing more grows on the upper side where the great rush of rain-water takes place. On the sides where water more slowly trickles off, mosses and Algae starting from a crack in the bark commence growing. As they increase in growth they retain more and more of the débris, and may cover a considerable patch. In so doing they kill out the lichens.

When the patch is large enough ferns or phanerogamous plants appear. The roots of the orchids and creeping rhizomes of the ferns retain the vegetable débris washed down and blown by wind, and the plants increase until the bough may be covered with epiphytes.

As soon as the orchids commence to grow, or even before, the ants begin to use the spot as a suitable one for their nests. The Pigeon-orchid (*Dendrobium crumenatum*) is one very attractive to ants. It emits slender white roots so as to form a cage at the stem base, which is quickly occupied by a species of ant, a *Dolichoderus*.

This ant brings a quantity of soil and piles it up, and fills in the spaces between the roots beneath which it makes the nest. The earth thus brought up supplies food to the plant and also serves to keep the roots cool and moist.

The old nests as time goes on accumulate on the tree boughs till quite a quantity of soil is supplied to the roots of the various epiphytes.

I do not find that all the great number of epiphytic plants on our trees are supplied in this way with nutriment, but undoubtedly it is one of the factors of the great development of epiphytes in the Eastern Tropics. Occasionally termites add a supply of soil to the epiphyte garden. In *Arenga saccharifera* and other palms of which the leaf-bases remain on the trunk, the termites tunnel out the leaf-bases and replace the destroyed tissue with mud, which remains in after the termites have left. Through this mud such plants as *Davallia solida* push their rhizomes and utilize the soil brought up. Ants too carry this soil further up the trees to form their nests beneath the orchids, &c.

There are, however, no special modifications to induce the ants to nest



among and around the roots of the orchids and other such plants, unless the emission of the roots from the base of the stems in *Dendrobiums* in such a form that it is convenient for the ants can be said to be one. But, especially from observing young plants, I am of opinion that the nesting of the ants among the roots is distinctly advantageous, seedlings not infested by ants being weaker and suffering more from drought. In the case of the two epiphytic ferns, *Platynerium bifforme* and *Thamnopteris nidus-avis*, ants also play a very important part in bringing soil to the roots in the nests of these nest-epiphytes.

#### THAMNOPTERIS.

The Bird's-nest Fern, *Thamnopteris nidus-avis*, L., is very abundant here, and appears as an epiphyte in all kinds of trees, frequently only a foot or two from the ground. Its large nest-like circles of fronds allow of its receiving and retaining fallen leaves, fruits, and other vegetable débris from the tree on which it grows, and through this mass the fronds are pushed up. The roots, dark brown and woolly, push up also through the decomposing leaves, and are especially prominent pushing through the dead fronds at the base of the plant. The decaying leaves and humus retained by the dead fronds not only feed the roots, but keep cool and moist the young growing fronds and roots, so that even on hot days when other epiphytes are suffering, this plant is little the worse. In the mass of dead leaves we find ants nesting. In one plant was a colony of the fierce, mahogany brown ant—*Odontomachus punctulatus*, Fab., apparently—which is so abundant in our forests; known to many from its vicious stinging powers, its long usually wildly expanded jaws, and the extraordinary leaps that it gives when annoyed. It is one of the ants known as fire-ants here. Its sting is quite painful, and the effect lasts for some time.

In another Bird's-nest Fern was the nest of a species of *Phidole* which at first sight resembled *Dolichoderus taprobane*, but did not bite or sting. I am unable to identify the species, which was coloured much as in the *Dolichoderus*, but was a more slender and hairy ant.

These ants, there is little doubt, bring a certain amount of soil from the ground to form their nests among the fronds, and to a certain extent benefit the plant in this way, though this class of plants seems to depend more upon leaves accidentally blown or drifted in from trees near by. The amount of vegetable débris—leaves, fruits, and bits of stick and bark—which accumulates in the crown of this fern is surprisingly large. The fronds spread widely, however, and the circle of fronds has a diameter of from 6 to over 12 feet in large-sized plants; any leaf drifting within this circle is almost certain to slide to the centre.

## PLATYCERIUM BIFORME, Bl.

This fern has three sets of fronds, viz. large and broad erect ones which form the large nest, from below which hang the branched pendant fronds which give the plant its common name, and among which are those which bear the kidney-shaped spore-producing fronds.

The large erect fronds form a kind of basket, which contains a considerable quantity of dead leaves and other detritus drifted therein by wind, and mould formed by the decay of these and the older fronds. It is in this mass that the ants make their nest.

On examining the material used by the ants for their nest in a fern put at some five or more feet from the ground in a tree, I found it consisted to a large extent of sand and minute fragments of laterite. These with fragments of decomposed vegetable matter formed the walls and covering of the nest. This material must have been carried up by the ants themselves. In moving a portion of the nest I found that the ants had a store of some species of coccid on the bark of the tree. This coccid was rounded in outline and light brown in colour. It had doubtless been brought there by the ants, as I saw it nowhere else except in one portion of the nest where it was abundant. This coccid is orbicular, fawn-colour with the edges white, the whole surface dotted over with fine depressions or punctures.

I believe the ant to be *Dolichoderus taprobane*. It appears to be identical with the species which makes its nest beneath the roots of *Dendrobium crumenatum* and other epiphytes. It is a very small species, little over 2 mm. long; the head dark brownish above, abdomen nearly black, and the rest of the body and legs yellowish, all slightly hairy. It forms nests of soil, often in the form of a large sheet irregular and raised, under which an immense number live. Though very small they bite viciously, but the bite, though irritating, is not severe. They certainly attack other insects, and if a termite run in their neighbourhood is broken open so that the termite workers are exposed, the ants attack and carry them off. They will not, however, attack *Termes umbrinus*, a termite which is often to be seen going in long procession to or from a tree or woodwork, where it collects bark to cultivate a species of *Agaricus*, on which to feed the young. The army of *T. umbrinus* is guarded by soldiers flanking it, which repulse enemies by means of a corrosive liquid which they eject. The *Dolichoderus* may often be seen running by the army without attacking any of the termites.

In the *Platynerium* it lives in vast myriads, and must bring up a considerable addition of clay and earth to the roots of the plant. The fronds of this fern do not spread so widely as do those of *Thamnopteris*, and are not nearly as efficient a trap for drifting leaves and sticks as are those of that plant, consequently the amount caught by the *Platynerium* is not

so large. As they wither they curl in and make a large cabbage-like ball in which the vast myriads of ants live. *Platycerium*, when removed from its tree to a plant-house, seldom thrives well unless ants have an access to it from the ground, as during the removal the original inhabitants usually leave the nest. It should, however, be mentioned also that in a plant-house or such place it has not the advantage of the fall of leaves in it, at least to the ordinary extent, and also the water-supply from above is perhaps not what it ordinarily gets. But considering the smaller catch of leaves which this bulkier plant gets and the large amount of soil usually found in the centre, I have little doubt that the main feeding of this plant is effected by the *Dolichoderus*, and that the adaption of the erect fronds, though primarily for the catching of fallen leaves and for covering the roots when they are dead to prevent a loss of water, yet has a secondary function in encouraging the presence of ants, which thus supply an additional source of nutriment without which the plant would not thrive.

#### CLERODENDRON MYRMECOPHILUM, Ridl.

This plant and *Cl. breviflos*, Ridl., are both inhabitants of very wet spots in Singapore, Johore, and other parts of the Peninsula, and both have hollowed stems in which live ants, much in the same way as they do in *Macaranga*. The stems are usually quite simple and unbranched, bare of leaves at the base. There do not appear to be any further modifications to attract ants, and it is difficult to see any advantage their presence can be to the plant. Under cultivation, as the plant is propagated by cuttings, the ants soon disappear and the plant seems none the worse, except that it is very liable to attacks of coccids and aphids which are often to be found beneath the young leaves, which they spoil. Ants, however, protect these insects and often cover them in with walls and masses of sand beneath the leaf. It is quite conceivable that the removal of coccids by ants from the young foliage, which they seriously injure or destroy, to the interior of a hollow stem where it appears from their constant presence in *Macaranga* they are harmless, may be advantageous to the plant, but I have no evidence to offer for this in *Clerodendron*, and the blights I have found on the young leaves are not the kinds transferred to their nests by ants. Beccari, in describing *C. fistulosum* of Borneo, suggests that the tunnelling of the stem by the ants strengthens it. I hardly think this would account for the ants, as the same structure occurs in *Macarangas* even when no ants are present.

#### MACARANGA.

The genus *Macaranga* includes upwards of 100 species of trees and shrubs distributed over the tropical regions of Africa and its islands, Indomalaya, and the Australian and Polynesian regions, but as those defined

include species so very different in habit and structure it would probably be more satisfactory to break it up.

In studying the Malayan species I find that a certain number are myrmecophilous, and as three of these species are abundant in the Botanic Gardens of Singapore, I have for some time been examining these plants, and making notes on them with a view of finding out how far they were actually modified so as to attract and ensure a regular symbiosis, and I think that the following observations will show that the *Macarangas* of this group do fill all requirements for their classification as truly myrmecophilous. I have also examined the herbarium specimens in the herbarium of the Botanic Gardens, Singapore, in order to compare the myrmecophilous species of the genus as at present understood with those which are not tenanted by ants, and which possess no organs modified for myrmecophily. In all the myrmecophilous species the stem in the young plant is hollowed by the disappearance of the pith, and in the hollow stem live the ants, which obtain access to the outside of the plant by holes perforated through the side of the stem at irregular intervals, but one or more appears in each internode. The ants also perforate the septa of the nodes so that a communication exists between all the internodes. In adult trees the ants usually only reside in the ends of the branches, the hollow in the trunk, though long persisting, being abandoned.

In most of the species of the genus, the buds are protected before opening by an opposite pair of triangular or lanceolate sessile leaves or bud-bracts, which have been called stipules in various papers, but which seem to me to bear no relation to the stipules of the Leguminosae nor of the Rubiaceae, but are rather of the nature of bud-scales, and I shall call them bud-bracts throughout this paper. A certain set of the plants included under the genus *Macaranga* in the flora of British India and elsewhere do not appear to possess these bracts at all; such are *M. trichocarpa*, Muell., *M. carolinensis*, Volkeres, *M. cumingi*, Muell., *M. javanica*, Muell. None of these are myrmecophilous. Another series possesses well-developed bracts which are persistent for some time after the bud has expanded, but are not in any way modified for the use of the ants, nor is the stem hollowed or occupied by ants. These trees in fact are not myrmecophilous. Such are:—

<i>M. tanarius</i> , Muell. Arg.	Malay Peninsula.
<i>M. Curtisii</i> , Hook.	” ”
<i>M. populifolia</i> , Muell. Arg.	” ”
<i>M. depressa</i> , Muell. Arg.	Borneo.
<i>M. bicolor</i> , Muell. Arg.	Philippines.
<i>M. megalophylla</i> , Muell. Arg.	Malay Peninsula.
<i>M. riparia</i> , Engl.	New Guinea.

The bracts in these persist for so long that there are often as many as

six pairs in *M. populifolia* on the end of a branch representing the development of as many internodes, often the lowest reflexed from its bud. In *M. megalophylla* these bracts are very large. The myrmecophilous species fall into three groups. In all the bracts are long-persistent as in the last group, and the stem or branches are hollow and inhabited by complete nests of ants belonging to the genus *Crematogaster*. Nearly if not all the species have wax glands on the young leaves, and the young parts of the stem are coated with wax, and on the margins of the young leaves red cylindrical nectaries are borne on the ends of the nerves. These drop off as the leaf attains its full development, leaving no trace. The leaves in all are peltate, entire, or three-lobed.

The three groups into which these fall, or perhaps it would be better to say the three modifications which we find, are as follows:—

(1) *M.* sp. from Sarawak in Borneo, where it was collected both by Mr. Hullet and Dr. Haviland. In this plant the bracts are very large, lanceolate, acuminate, deflexed, coriaceous, not appressed to the stem, but concave, so that they may provide a nidus or feeding ground for ants. It is a very remarkable plant and quite unlike any other known to me, but, unfortunately, I have not seen it alive.

(2) The second group is represented by *M. hypoleuca*, Muell., of the Malay Peninsula and *M. caladifolia*, Becc., of Borneo.

In this group the bracts are lanceolate erect and do not bear food-bodies, but food-bodies are borne on the backs of the young leaves before they are expanded.

(3) The third group is the most elaborately myrmecophilous group of all. It includes *M. triloba*, Muell., *M. Griffithiana*, Muell., *M. Hullettii*, King, and, I believe, also *M. Hosei*, King, but I have not had an opportunity of examining this latter alive, and my herbarium specimens are not very good.

In these the bud-bracts after the expansion of the bud are reflexed and continue to grow, till they come in contact with the stem, to which they are so tightly appressed by their tips that they form a ringlike body almost completely surrounding the stem, and so concave beneath, that the ants, which find their way in at an open corner near the base, can not only hide within, but occasionally bring their larvae there. The under side of these bracts bears many white globular or more or less pear-shaped bodies (the food-bodies), which are at first attached to the lower epidermis of the bract, but later become detached and are conveyed by the ants to the nest in the hollow stem, where the larvae are fed on them. These food-bodies are not, so far as I can make out, commonly, if at all, borne on the under surface of the young foliage leaf as they are in *M. hypoleuca*, although wax glands occur abundantly there. They seem to be continuously produced on the under side of the bract, as they are to be seen of all sizes, and both attached and free. They much resemble the figure of the 'food-bodies' of *Cecropia*



*adenopus* as figured in Schimper's *Pflanzengeographie*, and their origin seems to be of the same nature. I have seen no trace of them anywhere on the *Macarangas* which do not harbour ants in the stems.

The three distinct myrmecophilous species I have been able to study in life are *M. hypoleuca*, *M. triloba*, and *M. Griffithiana*, three very abundant plants in and around the Botanic Gardens; of the last two, indeed, I may say there are hundreds of plants of all stages from seedlings to full-grown trees. I will now proceed to give a fuller account of each of these three species.

#### MACARANGA TRILOBA, Muell. Arg.

This is a tree of no great size (usually attaining a height of about forty feet) which is common in the low country of the Malay Peninsula, occurring in woods usually of secondary growth, or on edges of woods, and usually in dry positions (as compared with *M. Griffithiana* at least, which seems to prefer lower lying, permanently damp spots). In secondary jungle the trees come up abundantly, often close together. It is absent from the original forest, and its presence in any quantity may be taken as a sign that the wood in which it grows is of secondary growth. The stem of the seedling is always green, and is not covered with the glaucous or white wax coating formed on *M. hypoleuca* and *Griffithiana*.

The stem of the plant is hollow and contains almost invariably nests of an ant which has been identified by Col. Bingham as *Crematogaster* near *C. Daisyi* of Forel. I have found stems quite woody and half an inch through still occupied by the ants, and it is quite rare to find a plant which is not or has not been occupied by ants.

The first account of the myrmecophily of this plant was published by Miss Winifred Smith in the *New Phytologist*, ii. 79 (1903), Pls. V and VI. The material on which this account was based was brought to England by Mr. Tansley from the Botanic Gardens of Singapore. It was not, however, very extensive, and many points in the relations of the ants to the plant could not be made by Mr. Tansley for lack of time. The blanks in the history, as given in the *New Phytologist*, I hope to supply in this account of the plant.

The stem of the seedling is at first solid, slender, and woody, containing a small pith, and after it has grown for some inches, the internodes above become more succulent and swollen, dilating in the centre, and gradually narrowed to the nodes. This, however, is less conspicuous in *M. triloba* than in *M. hypoleuca*. The stem is smooth and green outside, not possessing the glaucous or white coating of wax met with on *M. Griffithiana* and *M. hypoleuca*. The terminal internode remains solid for some little time, succulent and green; the swollen internodes are hollow, the pith having disappeared. At first it remains as transverse bars, which later break



down, and in an unoccupied internode small ragged bits only remain. In a plant occupied by ants these are removed, and no trace of the pith is left. The walls of the tunnel are then dark brown from the excreta of the ants. As the stem grows the ants carry their young into the upper internodes by perforating the septa of the nodes, so that only the terminal internode, still green and sappy, is not inhabited, for into this the ants do not attempt to tunnel. In each internode there are from one to three perforations made by the ants to communicate with the exterior. Usually, there is one to each internode, and it is not rare to find an internode not perforated. When more than one is made they are generally in a line close together. They are sometimes made in the upper part, sometimes in the lower part of the internode, and usually in a shallow inconspicuous groove on the side further from the leaf.

It is clear, I think, that their external openings are made from the outside, at least in the case of the first ones on the stem, but the ones made in the upper internodes in some cases, at least after the ants have occupied the lower ones, appear to be made from the inside.

Within the hollows of the internodes are placed the young larvae. The queen ant is usually to be found in the lowest occupied internode.

There is no nest properly speaking, and no material brought from outside. The larvae lie quite loose and motionless within the hollow. They possess on the body a number of very short hairs by which, I imagine, they retain their position on the vertical walls of the tunnel. Around the head of each I have found four or five 'food-bodies' placed near the mouths, presumably for them to eat. The 'food-bodies', which will be described later, are brought into the hollow stem from the bud-bracts through the lateral perforation. I have seen the ants carrying them about in their mouths, and even bearing them off when the stem has been split and they commence to remove the larvae into a safer spot.

Besides the larvae and food-bodies, there is nothing else in the hollow except occasionally some *Coccidae* are brought in. This, however, is more common in *M. Griffithiana* than in *M. triloba*. These coccids are orbicular or elliptic in outline, of a pink colour, and covered with elevated round bosses on the raised back. They are usually put in internodes not occupied by the larvae, and must be brought in quite young, as when adult or nearly so they are too large to pass through the perforations. I have only seen these coccids in the hollowed stems, and have failed to find them on the leaves or outer parts of the plants.

As the tree develops and becomes thicker and more woody, the perforations to the surface disappear, but the hollow tunnel still remains, never entirely closing up, but is now unoccupied by the ants. The ants in fact only remain in the trunk of the tree as long as the external perforations remain. I have seen them still inhabiting the base of a young

tree which was half an inch and more in diameter and had developed a considerable amount of wood. In full-sized trees the ants still remain in the branches.

It is quite rare to find a plant of this species not tenanted by ants, but I have found the shoots from a stool in an isolated position unoccupied, and occasionally a young plant also. This most usually occurs when a seedling has come up at a considerable distance from a parent tree, or from other plants which are inhabited by the ants.

*The bud-bracts.* These are called stipules in the article in the *New Phytologist* and elsewhere, but, as above stated, I prefer to call them bud-bracts.

They first appear as a pair of ovate triangular green bracts enclosing the bud. They have broad bases and an acute point, and often a low keel runs along the back. The under side at the base, as in the leaf, is thickly covered with bladder-glands. As the bud develops the bracts are reflexed, eventually coming into contact with the stem, and continuing to grow, become so elevated in the middle that the two form a hollow ring almost completely surrounding the stem. They are now hard and stiff, and usually of a dark purple colour, occasionally, however, retaining their green colour. On the under surface are innumerable glands, and thickly sprinkled over the surface are the food-bodies, very small, pure, white globular or elliptic bodies attached to the epidermis. Miss Smith, l. c., describes them as golden yellow, but this I think must have been due to the drying of the specimens or the action of the preservative. I have always found them to be milky-white. The ants, which enter the hollow ring by the spaces between their outer corners and the stem, spend much of their time in this enclosed space and seem very unwilling to leave it. They often run about with the food-bodies in their mouths, and convey them to the hollow interior of the stem, where they supply them to the larvae. I have seen larvae actually underneath the bud-bract, evidently brought there by an ant, and lying on the abundant supply of food-bodies, but this is certainly unusual.

Although the ants often remove many of the food-bodies to the nest, they do not clear them all away, though there are usually more to be seen in plants not occupied by ants than in those in which ants are abundant, and as the food-bodies are of different sizes, and apparently are in different stages of development, I conclude that they continue to develop and grow during the life of the bract. They are not to be seen on the bract before it has been reverted and has become appressed to the stem, and has thickened and turned red, when they appear in considerable numbers. They are rather hard externally, and when crushed exude a liquid which does not mix with glycerine and appears to be of an oily nature. The outer coat of the food-body is marked

with cellular markings, and it appears to consist of small irregular cells. There can be little doubt that they are derived from the bun-shaped bladder-glands, which are abundant on all parts of the plant where the food-bodies occur. They are to be seen in all stages of development, and seem to continue developing as long as the bud-bract exists.

*The bladder-glands* are semi-transparent or quite transparent, and appear to be composed of about eight or nine large cells. They occasionally have a golden yellow tint, especially in older leaves. In young leaves they cover the back densely, but are more scattered in adult leaves, in which in some species they last for a long time. They are not confined to myrmecophilous plants, for they are very abundant on the leaves of *M. trichocarpa*, but here they never develop into food-bodies. In shape they are more or less round with the lower side flattened, the upper elevated into the shape of a bun. Miss Smith compares them to the lupuline glands of the Hop.

*The marginal nectaries.* The leaves of *M. triloba* are in seedlings peltate ovate on long petioles, in older plants more or less trilobed green on both surfaces, or especially in young plants dark red. The adult leaf is quite glabrous, but when first emerging from the bud it is hairy, especially on the edge, and thickly covered on the back with bladder-glands. The margins are separated, and at the end of each tooth where a nerve ends is borne a green or reddish 'nectary' or gland. These remain on the leaf till it is adult, when they seem to drop off. They are best developed in *M. hypoleuca*, where they appear first as short cylindrical bodies of a bright crimson with a rounded depression at the top. They increase in length and become more distinctly clubbed, the top bending over slightly so that the depression is on one side.

These nectaries occur in all the myrmecophilous species, but I cannot tell what function they perform, as the ants do not pay any attention to them.

#### MACARANGA GRIFFITHIANA, Muell. Arg.

This tree resembles *M. triloba*, Muell. Arg., in its habit and myrmecophily, though in specific characters it is very distinct. It is a fairly large tree, upwards of 40 feet tall, and has much of the habit of *M. triloba*, but its young branches and leaves are more glaucous from the deposition of wax. The backs of the leaves are, however, less white than those of *M. hypoleuca*. There are also differences in the flowers and fruit which, however, do not affect the question of the myrmecophily. This species seems to prefer the damper spots in and around the Botanic Gardens, even localities which are liable to be flooded from time to time, while *M. triloba* prefers the drier borders of woods.

Like *M. triloba* its stems are hollow and tenanted by ants which also

frequent the deflexed bud-bracts, which somewhat resemble those of the other species. In the swampy ground of the Botanic Gardens, at least, it is by no means so constantly frequented by ants as the allied species. Possibly this is due to some extent to the flooding two or three times a year of the area in which it is abundant, which may affect the abundance of the ants. The nectaries are conic with a depression at the top, and they seem to fall off very young. The young leaf is covered at first with a soft white down, and is densely covered with bladder-glands on the under side, but they are paler in colour, nearly white, and translucent. In the adult leaves they still remain, but are much more scanty. They are absent from the base of the petiole. The bud-bracts are to some extent like those of *M. triloba*, but are green, or more usually, when adult, sienna-brown. The apex is longer acuminate, so that when the bract is appressed to the stem the apex is curved to one side. At the base they are covered in the outer surface in the bud with short brown hairs, which persist after the bract is reflexed. Food-bodies of all sizes are abundant beneath, mixed with the hairs. The glands are smaller and more globular than on the leaf, and usually somewhat irregular in outline.

In other respects these organs much resemble those of *M. triloba*. The *Coccidae* which are preserved in the nest are identical with those in *M. triloba*. It is quite common to find seedlings and adult trees which have never been occupied by ants, a thing which is rare in *M. triloba*.

I notice that, as in the case of *M. hypoleuca*, the plants which are not protected by ants are much more liable to the attacks of caterpillars, the leaves being often gnawed, but I have not found any caterpillars at work. Seedlings which contain no ants may often be met with, which have altogether escaped the attacks of ants, but I have not seen any that have been protected which at the time of protection were attacked. A seedling about four feet high, growing in a spot covered with thick tall herbage, had the lower leaves partially destroyed, apparently by caterpillars at some earlier stage of the plant's life. The upper leaves, however, were quite untouched. On examining the plant I found that the lower half of the stem had no signs of having ever been occupied by ants, there being no entrance holes, while the upper half was colonized and the ants were busily engaged on the young just opened leaf at the top. The leaves had only been gnawed up to the point at which the colonies had commenced; above they were unhurt. This seemed to show that in the early part of the plant's life the ants were absent, and the caterpillars had attacked the young leaves, but that later the plant had been colonized, and the caterpillars had no longer been able to attack the young leaves.

## MACARANGA HYPOLEUCA, Muell. Arg.

This tree is much larger than the other two species, attaining a height of nearly 60 feet, with a stout, grey, smooth stem about 36 to 42 inches round.

It has a much larger spreading head of foliage. The leaves are stiff and coriaceous when adult, and conspicuously white on the back, with a coating of white wax in the form of an easily detached powder, which under a low power of the microscope is seen to consist of small bodies of the shape of curved sausages.

The tree inhabits rather drier spots than *M. Griffithiana*, borders of woods and secondary forests.

The seedling is curiously dilated between the nodes, narrowing to the node at both ends. Though this occurs in the other species it is not so conspicuous in them as in *Macaranga hypoleuca*. The internodes are hollow, and usually tenanted by ants in the same way as the other species, and in the hollows occurs what appears to be the same species of coccid. The young leaves are at first woolly and have their three lobes deflexed; as they expand they become glabrous, and at length develop a white waxy coating. Their margins bear nectaries, as do those of the other two species, but these are larger and more brightly coloured. They first appear as crimson polished cups on the nerve endings. They gradually lengthen as the leaf grows, and become club-shaped with the depression on the one side of the club; when the leaves become fully developed and coriaceous they disappear, as already described. On the under side of the young leaf are abundance of bladder-glands similar to those of the other species, and scattered about between the raised nerves are a number of food-bodies, white and globular. These are most plentiful in plants which are not protected by ants. The ants in plants occupied by them spend much of their time beneath the deflexed blade of the young leaf, and are as unwilling to quit as those that frequent the deflexed bud-bracts of the *M. triloba* are to quit the bud-bracts. I have seen them running about with the food-bodies in their mouths.

Beneath the shelter of the leaf also live acari, which seem to be harmless to the plant and undisturbed by the ants. In nearly adult leaves, before the coating of white wax is fully developed, the whole of the surface of the under side except the nerves is thickly dotted over with bladder-glands, and food-bodies are to be seen here and there on the sides of the raised nerves, especially in the angles formed by the branching of the nerves. The bud-bracts are lanceolate acuminate erect leaves, with a low keel on the back, pale green in colour, with a scanty thin deposit of wax and some brown hairs, but no glands.



They do not bend downwards, but remain erect till they fall off, and do not produce food-bodies.

The glands are very similar to the food-bodies, white and slightly verrucose, but more translucent. The food-bodies are only more borne on the sides of the nerves which carry few glands. It is by no means infrequent to find plants of *M. hypoleuca* unprotected by ants, and this is especially the case in isolated plants. They appear shabby and weak, and almost invariably have the leaves gnawed by caterpillars.

In such seedlings the stems are swollen between the nodes, and hollow, with fragments of pith lying in the hollow, but there are no perforations through the nodal septa, and no ants are to be seen about the plants.

On one of these uninhabited plants I found two caterpillars of different species; one was a Tortricid caterpillar, and with it was a pupa of apparently the same species. This caterpillar was concealed below the deflexed young leaf which it had much injured. The other larva, on a somewhat older leaf, was apparently that of a Bombycid. It was devouring the leaf from the under side. Both the caterpillars and the pupa were just where the ants would have been if the plant had been inhabited.

Another young plant which was unprotected was adjacent to a fair-sized tree, also unprotected. Here again I found the Tortricid larva beneath the youngest leaf. Every leaf on this plant had been injured and the plant was sickly. Another unprotected plant had the same kind of Tortricid caterpillar and a number of larval thrips on the under side of the young leaf, and a dead adult thrips lay on an adjacent leaf. The caterpillar had spun webs containing its excreta beneath the leaf which was injured. As there were many food-bodies beneath this leaf, more than usual, I conclude the caterpillar does not eat them.

Many other unprotected plants in which I did not find any larvae had all, or nearly all, their leaves gnawed, but almost all either had caterpillars beneath the leaves or had obviously been attacked some time previously.

#### SUMMARY OF THE NOTES ON MACARANGA.

The trees of this genus may be divided into those which are inhabited by ants and those which are not.

The latter possess solid stems with a small pithy cavity, which is never hollowed out; the leaves possess no nectaries, nor are they or the stems ever covered with a waxy excretion, but are generally more or less hairy or covered, especially when young, by a woolly or felted coating. The greater number possess bud-bracts, which, however, usually fall off shortly after they are reflexed from the bud, but occasionally persist for some time (*Macaranga populifolia*). Bladder-glands occur on the leaves of some species at least (*M. trichocarpa*), but never develop into food-bodies; they seem always to be absent from the bud-bracts.



Of the myrmecophilous species, we have two series. In both the stem at first is solid and woody, but as it develops the internodes dilate, and the pith, which is proportionately large, disappears and the stem becomes hollow. The bud-bracts are large and more persistent. The leaves bear on their backs numerous bladder-glands, and some of them develop into food-bodies which are used by the ants inhabiting the hollow stem as food for their young.

In most cases the stems and leaves are more or less glaucous or white from the excretion of wax. The young leaves possess nectaries on the edges.

The differences between the two series are as follows:—In one the bud-bracts are lanceolate and leaf-like, and remain sub-erect; they bear neither bladder-glands nor food-bodies. The food-bodies and glands are borne on the under side of the young leaf, which for some time remains with its lobes deflexed. In the other the bud-bracts bear on their outer surface bladder-glands, especially at the base. They are long-persistent, and soon after opening are deflexed, till meeting the stem they form a chamber, in a ring shape, within which food-bodies are developed. The young leaf opens flat and entire, and is not deflexed and bears no food-bodies.

That these modifications are not caused by any action of the ants is shown by the fact that all occur in plants in which ants are absent. It is clear, however, that they cannot be of any use to the plant except for the purpose of attracting ants, and causing them to permanently inhabit the plant. Observations show that plants of these species in which ants are absent suffer seriously from the attacks of caterpillars. Two of these modifications, namely, the hollowing of the stem and the development of some of the bladder-glands into food-bodies, may be said to be complementary of each other, that is to say, neither alone would serve the desired purpose. Food-bodies alone would only attract ants temporarily, as they would take them to their nests as speedily as they could be produced. The additional advantage of the hollow stem induces them to remain as permanent guardians of the plant. Hollow stems which furnish abodes for ants, unaccompanied by any other inducement to them to occupy the plant, do occur in other plants, e. g. *Clerodendron myrmecophilum*, Ridley, but further investigation of these plants is required. In the case of such plants as *Hydnophytum*, the stem is naturally hollowed out with an external opening before the incursion of the ants. But this is not the case in *Macaranga* nor in *Clerodendron*, where the opening to the hollow chamber is made by the ants themselves.

The modifications which bring about the occupation of the plant by ants are for the most part simple developments of organs originally possessing other functions. Bud-bracts occur in most other species of

*Macaranga*, but they usually fall off after they have performed their function of protecting the bud. In *M. triloba* they not only remain attached, but reflex themselves and continue to grow. The bladder-glands occur on other species than the myrmecophilous ones, and perhaps perform an excretory function.

In the myrmecophilous species they continue to develop or rather to increase in size and to produce an oily liquid in their interior, which is attractive to ants. Only a very few of these bladder-glands develop into food-bodies. Indeed their great numbers would make it impossible for all so to develop in so small a space. Why some should so develop and others not is by no means clear.

Hollow stems are not by any means common in plants in this region, and no *Macarangas* which are not otherwise adapted for myrmecophily have any part of the stem hollowed, so far as I have seen. In the case of *Clerodendron fistulosum*, Beccari suggests that the inflated stems producing a strengthening of the plant allow it to grow straight and strong above its competitors, and that the parents of the plant appear to have been slender and herbaceous and badly adapted to sustain the lot of its existence among tropical vegetation, mostly woody. In the view of the relationships of these *Macarangas* to the solid-stemmed non-myrmecophilous species, and the occurrence of many solid-stemmed *Clerodendrons* in our forests, I should doubt the validity of the suggestion.

It is clear, I think, that the advantage which the plant derives from the presence of the ants is protection from the attacks of caterpillars and possibly thrips. It may be taken as certain that the greatest time of danger to the plant from insect attacks is while it is in the seedling stage, while it possesses only one growing-point. The destruction of the terminal bud may cause the death of the whole plant, and even if the plant has already branched, which it does not usually do till it is at least ten feet tall, a caterpillar can and doubtless would destroy the second bud after finishing the first. Buds of plants in the tropics are usually very carefully protected by coverings of bud-bracts, wax, fur, or by other methods. Even in full-grown trees the destruction of the young leaf by insects or fungi does more harm to the tree than an extensive raid of caterpillars on the adult foliage. The damage caused to a tree by the destruction of the adult foliage by caterpillars seldom in a natural state causes any very serious permanent injury to the tree, but the continued damaging of the just expanded leaf is most injurious.

In an adult *Macaranga* the ants only frequent the ends of the boughs, moving on from internode to internode as they develop. The stiffly coriaceous adult leaves of *M. hypoleuca*, and the firm but less coriaceous ones of *M. triloba*, are not attacked by caterpillars, and do not require protection. The defence required is only that of the young leaf during the

early stages of its development, and though the young leaves in an unprotected tree are not entirely destroyed, they are so injured and deformed that the tree has a weak and wretched appearance, and does not develop to such a size or robustness as a guarded tree.

*Conclusion.* Among the large series of plants which have been classed as myrmecophilous, we find that there are all stages of development of a true symbiosis included among them. Thus in the case of *Ficus irregularis* there was seen a commencement of a relationship between the ants and the tree, due to an accidental modification which temporarily affected the tree. Had the swellings on the branches been of any importance to the tree and become a permanent feature of it, the next stage represented by such a state of affairs as occurs in *Pachycentria* might have developed. In this the dilated roots have probably a function of water-storage, and form a feature of the plant, and owing to their peculiar growth they are apt to become hollowed out, and are then, occasionally at least, tenanted by the ants which habitually live among the roots of epiphytes. This stage of development of myrmecophily in which an organ modified for another purpose is adopted as suitable nidus for ants, while, at the same time, there does not appear to be any definite advantage to the plant from their presence, seems to be quite common.

In *Korthalsia* the swollen ochrea seems at first sight to have been evolved for the housing of ants, but as there seems to be no advantage derived from them by this modification, and plants thrive as well without them as with them, and, further, more than one species of ant occupies the ochrea, such a case can hardly be considered a true symbiosis. There are good reasons for the development of the ochreae into their present form for a different purpose, and there is no special modification which is obviously intended to attract the ants or to induce them to remain. To the same case belong, I think, *Dischidia*, though here the plant does derive benefit from the presence of ants, when they inhabit the pitcher-shaped leaves and convey soil to the roots within, and the same advantage is obtained by the nest epiphytes *Thamnopteris* and *Platynerium*. The importance of the ants to such plants as *Clerodendron myrmecophilum*, *Lecanopteris*, *Polypodium sinuosum*, *Myrmecodia*, and *Hydnophytum* is less clear to me, yet all are habitually tenanted by ants, and seem to a greater or less extent to be modified to form a nidus for them. It is possible that the swollen fleshy stems of *Lecanopteris* and the *Myrmecodia*, resting quite exposed on the tree trunks, might be attacked by rats or other enemies were they not defended by the ants; but I have no evidence of this, and more observations are wanted.

In *Macaranga triloba* we have the most perfect development of myrmecophily and a true symbiosis. The hollow stem, the retention of the bud-bracts for some time after their original function of protecting the

bud has ceased to be necessary, their reflection and continued growth, the production of food-bodies, are all modifications which can have no other function than that of attracting the ants and retaining their services as guards. It has been shown too that their guardianship is of the greatest importance to the plant, which almost invariably suffers seriously from the attacks of caterpillars when they are absent.

Very similar to the case of *Macaranga* are those of *Cecropia adenopus*, as described in Schimper's *Pflanzengeographie*, and of *Acacia cornigera*. These seem to be truly myrmecophilous and the symbiosis of the ants with the plants appears to be as complete as possible.

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## EXPLANATION OF PLATES XXXV AND XXXVI.

Illustrating Mr. Ridley's Paper on Symbiosis of Ants and Plants.

### PLATE XXXV.

*Macaranga triloba*, Muell. Arg.

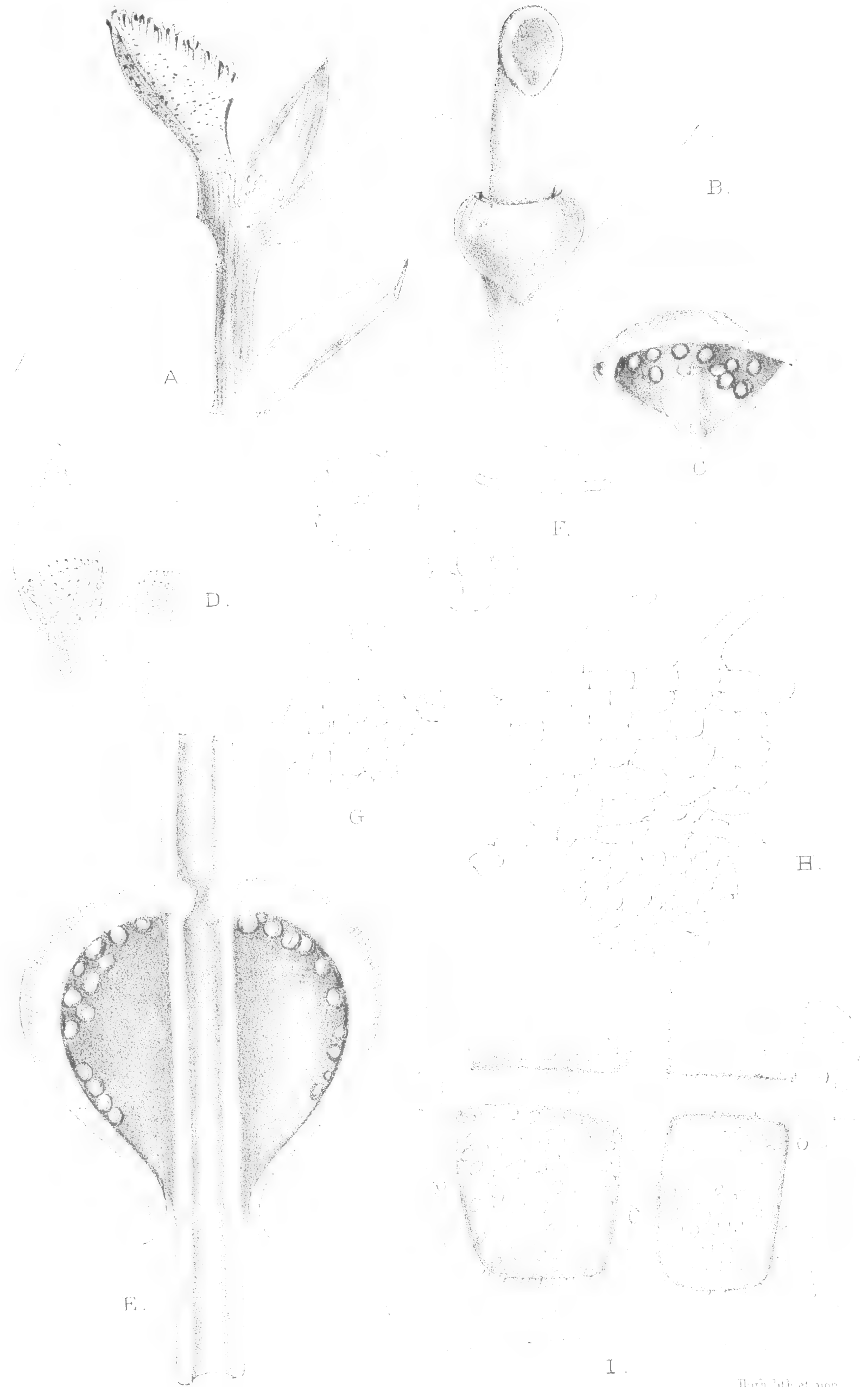
- A. Bud showing bud-bracts spreading.
- B. Portion of stem with bud-bracts reflexed and forming a ring.
- C. Bud-bract from inside, showing food-bodies.
- D. Bud enclosed in its bracts, with bladder-glands.
- E. Section of bud-bract and stem, showing food-bodies.
- F. Bladder-glands.
- G. Food-body.
- H. Section of bud-bract with a food-body.
- I. Under portion of young leaf with bladder-glands.

### PLATE XXXVI.

*Macaranga hypoleuca*, Muell. Arg.

- A. Young stem, showing dilatation of internodes unoccupied.
- B. The same in section, with pith-bars.
- C. Bud with spreading bud-bracts.
- D. Section of stem occupied, with two coccids and the perforation made by the ants.
- E. Young leaf with nectaries and food-bodies.
- F. Portion of young leaf much enlarged with nectaries, and food-bodies.
- G. Nectaries at different stages of growth.



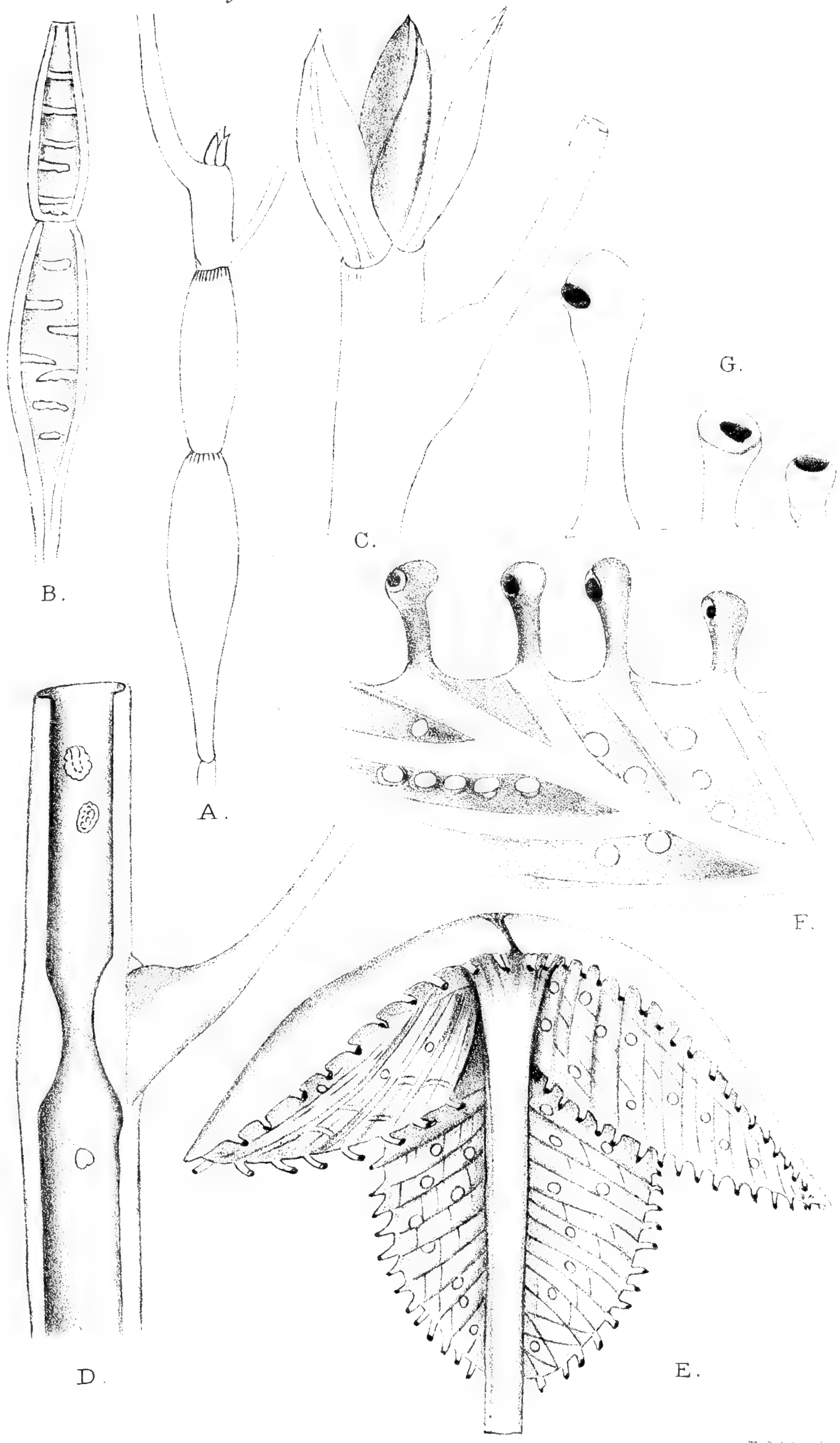


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## NOTES.

**NOTE ON THE MESARCH STRUCTURE OF CERTAIN VASCULAR BUNDLES IN THE COTYLEDONS OF SOME SCITAMINEAE.**—The presence of bundles possessing a distinctly mesarch structure was first observed in this Order by Miss Sargent, in transverse sections of the sucking cotyledon of *Brachytilum Horsfieldii*, and the occurrence of short, broad, spirally-thickened tracheides situated centripetally to the protoxylem was at once confirmed by longitudinal sections of the seed and enclosed cotyledon.

The question arose as to whether this was a primitive character, or whether the presence of this centripetal xylem could be otherwise accounted for. The fact that the bundle was exarch in structure before it entered the cotyledon, then became mesarch for a space, but again became exarch towards the tip of that organ, seemed to indicate that this variation in structure might be due to some local adaptation.

Besides *Brachytilum*, the cotyledons of the Scitamineous seedlings, *Alpinia calcarata*, *Roscoea purpurea*, and *Elettaria Cardamomum*, were examined; those of *Alpinia* and *Roscoea* were found to resemble that of *Brachytilum* closely, not only in the presence of an apparently mesarch bundle, but in other features. In *Elettaria*, however, no such mesarch structure was found.

In the three former species the haustorial part of the cotyledon is provided with two vascular bundles; one of these passes into it directly from the stele, the other travels through the cotyledonary sheath first. Definite mesarch structure is only present in that which comes direct from the stele, but the relations of the constituent bundles of the two strands within the sucking cotyledon can be more easily traced in the other, and thus an explanation of the mesarch appearance of its companion arrived at.

When this trace is on the point of leaving the cortex and entering the cotyledonary sheath, it is joined by two small lateral bundles which form part of a cortical anastomosing system connecting the traces of all the leaves together. Sometimes these two small bundles fuse and join the main strand as one bundle, sometimes there is a short connecting branch between them, but in either case we have as a result a small bundle running close past the protoxylem of the main strand. Thus in Fig. 4 the smaller bundle, B, is formed by the union of the two laterals which have taken the courses indicated by the dotted lines, and it will be seen that one has passed very close to the protoxylem of the main cotyledonary strand,  $c_2$ .

In the second cotyledonary strand which passes direct from the stele to the sucking cotyledon, this crossing of the paths of the three constituent bundles takes place while the two small cortical bundles are associated with the main trace, and

the result is the apparent mesarchy of the whole strand. In Fig. 1 the three bundles are quite distinct, the two small laterals lying one on each side of the main strand. At this point the bundles have just left the hypocotyl. As they pass together up the cotyledon, the xylem from the lateral bundles spreads round the protoxylem of the main bundle, until the whole has a mesarch structure as shown in Fig. 2. In Fig. 3 the whole mass of centripetal wood has moved to one side; its position now may be

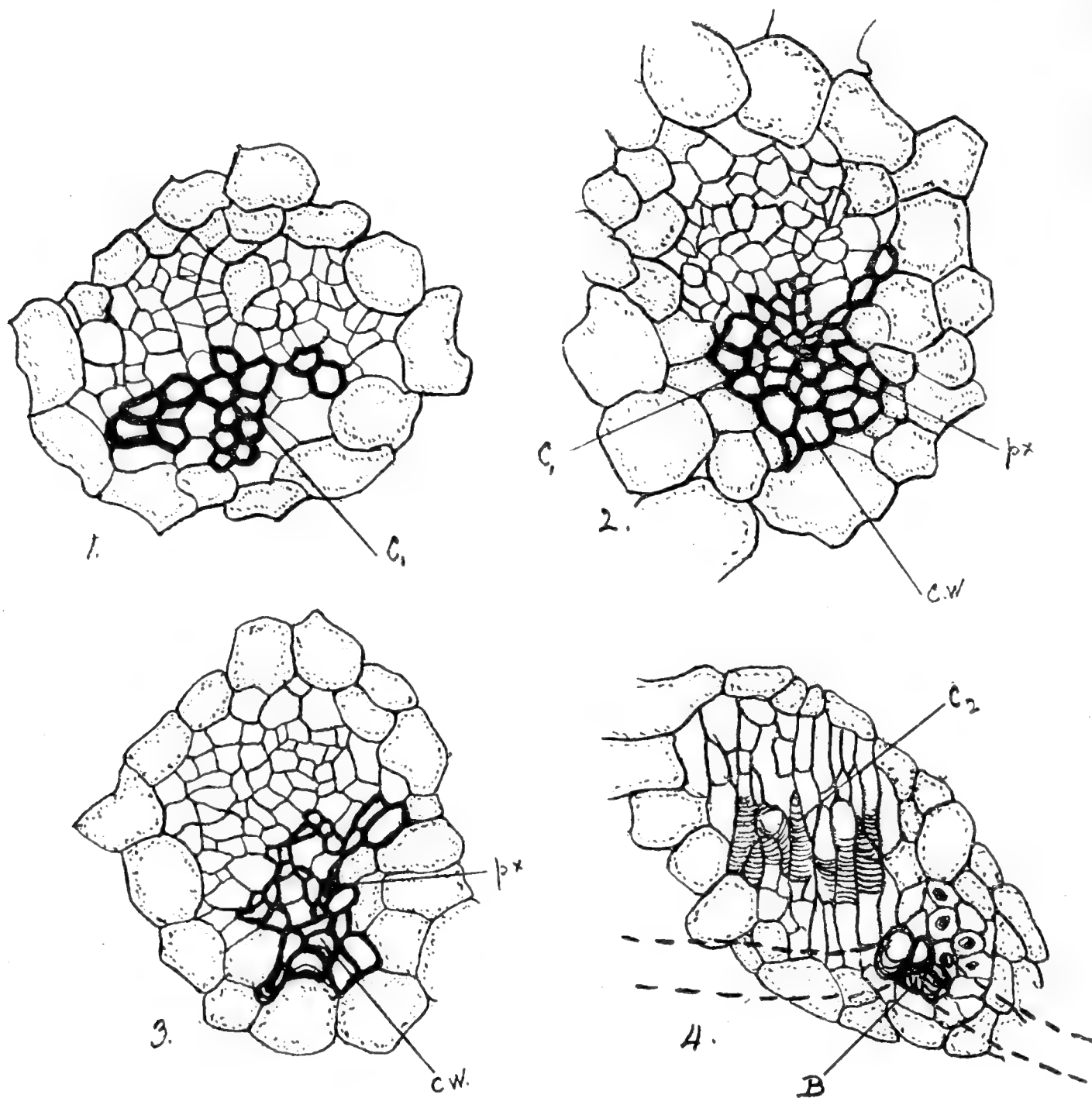


Fig. 1. *Roscoeae purpurea*. Triple strand entering the cotyledon.  $C_1$  = cotyledonary trace.

Fig. 2. *Brachyichilum Horsfieldii*. Vascular strand showing mesarch structure.  $px$  = protoxylem;  $c.w.$  = centripetal wood.

Fig. 3. *Roscoeae purpurea*. Strand becoming exarch again with centripetal wood moving to one side.

Fig. 4. Fused cortical bundles, B, about to join second cotyledonary trace,  $C_2$ .

compared with that of the fused laterals in Fig. 4. Finally in the upper part of the cotyledon the whole strand becomes exarch again.

Figs. 1, 3, and 4 are all taken from sections of seedlings of *Roscoeae purpurea*, but in Fig. 2, a section cut from a seedling of *Brachyichilum* has been substituted for the parallel case in *Roscoeae*, as the mesarch structure was first observed in this set of sections and is very striking. In a neighbouring section a small amount of phloem-like tissue is to be observed accompanying the centripetal wood, and this is also the

case in one of the *Roscoea* seedlings examined. It is, therefore, evident that the presence of centripetal wood in one vascular strand of the cotyledons of *Brachytilum*, *Roscoea*, and *Alpinia*, is due to the fusing of two small cortical bundles after they have become closely associated with the main strand, but before they are wholly merged in it.

It must be added that there is a tendency for large single tracheides to occasionally make their appearance on the side of the protoxylem remote from the metaxylem throughout the whole course of both bundles, but this is to be observed in the cotyledons of many monocotyledonous families. In *Anemarrhena* isolated tracheides occur scattered between the two bundles, and in *Erythronium grandiflorum*, where there are several laterals, their appearance usually precedes the fusion of two of these; in *Iris* the single strand of fused bundles ends in a brush of these spirally thickened elements, while in *Maianthemum* a group of three tracheides was observed in the ground tissue quite apart from any vascular bundle.

In an organ whose most important function is the rapid passage of food substances to the growing plant, the occurrence of such elements is likely to be an adaptive feature rather than a primitive one, and their frequent but by no means constant association with the bundles in a centripetal position does not necessarily indicate their vestigial nature.

*Summary.* The well-marked mesarch structure of one of the cotyledonary strands in the lower part of the sucking cotyledon of certain Scitamineae is evidently due to the relative movements of the constituent bundles after they have become associated together in a single strand, and is therefore of no phylogenetic importance.

E. M. BERRIDGE.

#### PRELIMINARY NOTE ON APOGAMY IN PTERIS DROOGMANTIANA.

—A cytological investigation of the prothallus of this Fern has revealed features of much interest. In the young prothallus, cells each containing two nuclei are common: it appears certain that in this form neither of the paired nuclei has migrated from an adjacent cell, as in every case a nucleus is present in each of the surrounding cells. Our available evidence indicates that the pair of nuclei are formed by the division of the nucleus of an ordinary cell, no cell-wall being laid down between the daughter-nuclei. After division has taken place the two daughter-nuclei remain for some time unfused, but, in most if not all cases, fusion eventually takes place. Stages in the fusion have been observed, and the resultant nuclei are very large and at first often lobed.

We are indebted to Mr. Boodle for kindly supplying us with material for examination.

E. L. STEPHENS.  
M. G. SYKES.



**THE PANAMA DISEASE.—PRELIMINARY NOTICE.**—This fungoid disease on the *Musa sapientum* var. *Gros Michel* was, it seems, first detected in Central America, practically destroying the crops in Costa Rica and seriously threatening to dry up a new source of revenue to Dutch Guiana, where this plant, the banana, was recently introduced. Experts here and in the U.S.A. had not yet succeeded in finding the cause of the disease. I did not hesitate, however, to undertake the research, the result being that I succeeded, after three weeks' assiduous working, in finding a fungus in such a connexion with the diseased tissues as to convince me of its being the cause of the disease.

The general microscopical aspect of these tissues, the ramification of the mycelium, the formation of the spores—chlamydospores and conidia,—the grouping of the spores into clusters, the mode of germination, &c., are all undoubtedly in favour of the conclusion that the *Panama disease* is caused by one of the Ustilagineae, probably in company with a member of the Chytridious order.

I hope to publish the final results of my research at the earliest possible date.

The accompanying figures, drawn from my preparations, will help to corroborate my preliminary conclusions.

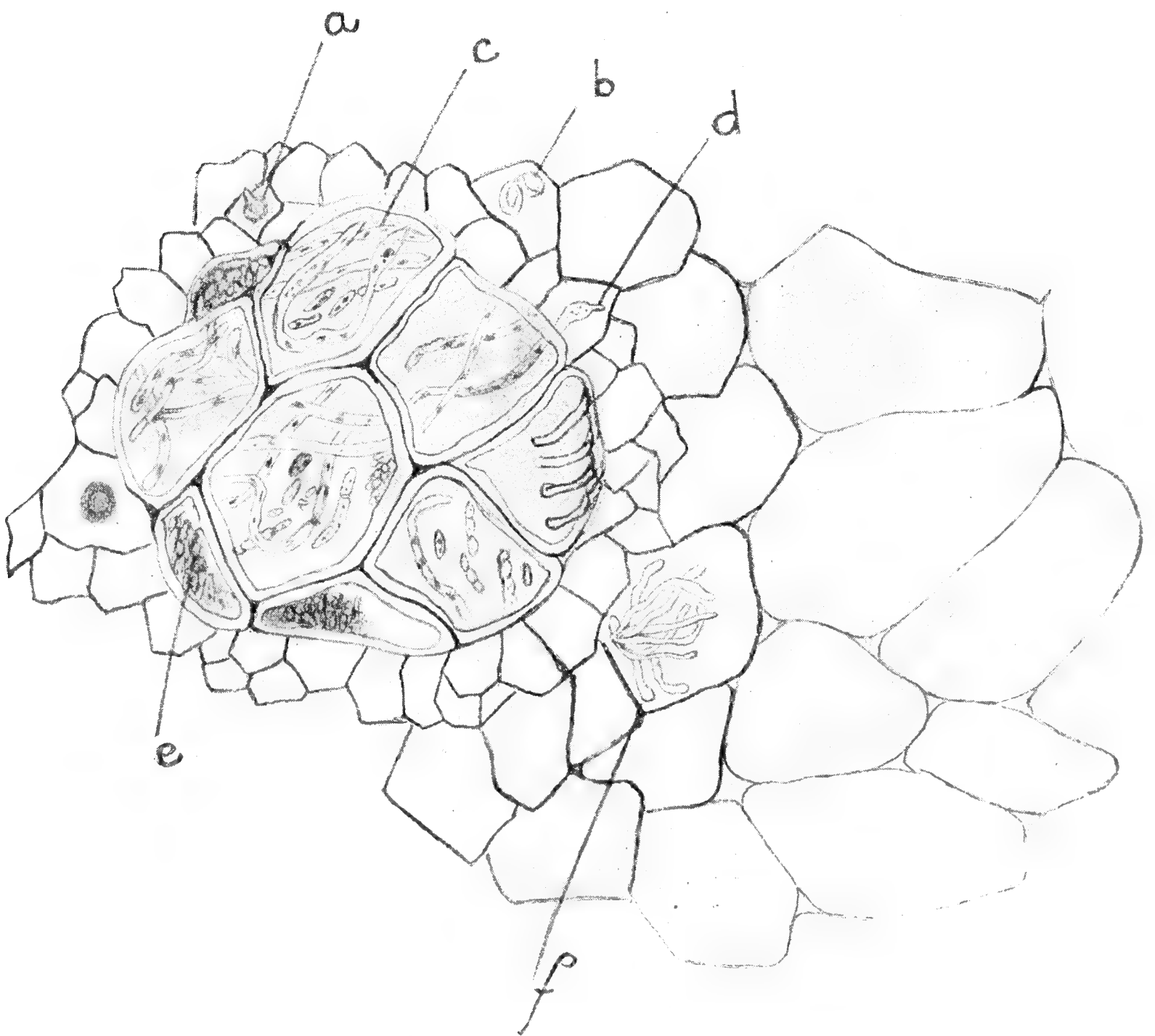


FIG. 1. Transverse section through rhizome.  $\times 255$ . *a*, germinating chlamydospore; *b*, two chlamydospores joined together; *c*, mycelium in wood vessels; *d*, conidium formation; *e*, breaking up of the mycelium into chlamydospores; *f*, haustorial hyphae.

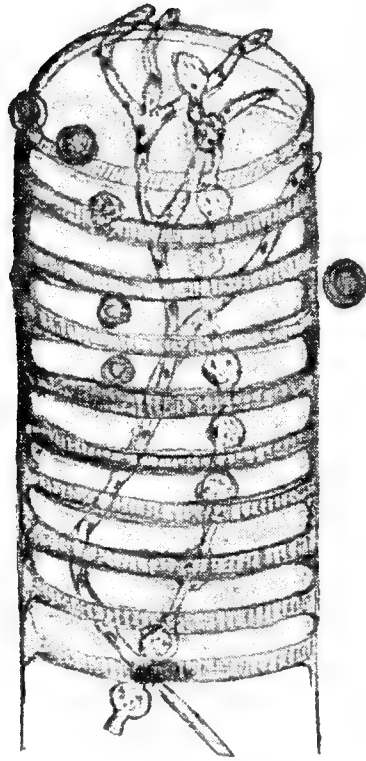


FIG. 2. *A spiral vessel* through which run mycelial threads breaking up into chlamydo-spores.  $\times 255$ .

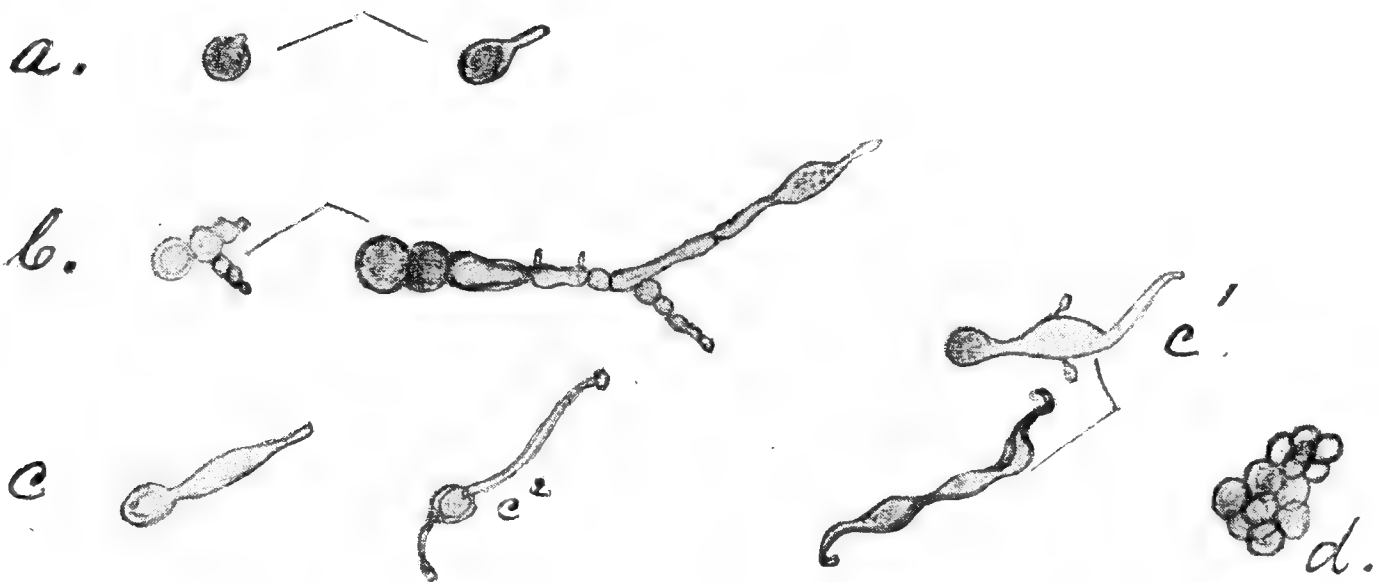


FIG. 3. Germination of spores—different modes.  $\times 255$ .

E. ESSED.

PARAMARIBO,  
March 4, 1910.



# The Journal of Botany

## British and Foreign

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# On the Structure of the Palaeozoic Seed *Mitrospermum compressum* (Will.).

BY

AGNES ARBER, D.Sc., F.L.S.

With Plates XXXVII-XXXIX, and two Figures in the Text.

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## I. INTRODUCTION.

THE numerous fossil seeds occurring in the Palaeozoic rocks have been conveniently grouped by Oliver<sup>1</sup> under the names Platyspermeae and Radiospermeae, these names corresponding to the two series originally distinguished by Brongniart.<sup>2</sup> As the words imply, the Platyspermeae are flattened seeds with a bilateral symmetry, while the Radiospermeae are radially symmetrical. Our knowledge of the internal structure of the Radiosperms has of late years progressed very rapidly. To realize this we need only recall the memoirs on *Lagenostoma Lomaxi*,<sup>3</sup> *Stephanospermum*,<sup>4</sup> *Trigonocarpus*,<sup>5</sup> and *Physostoma*,<sup>6</sup> which all fall within half

<sup>1</sup> Oliver ('03).

<sup>2</sup> Brongniart ('74).

<sup>3</sup> Oliver and Scott ('04).

<sup>4</sup> Oliver ('04).

<sup>5</sup> Scott and Maslen ('07).

<sup>6</sup> Oliver ('09).

MAY 31 1912

a decade. In the case of the Platysperms less progress has been made. A number of these seeds, occurring in the silicified pebbles from the French Upper Palaeozoic rocks, were long ago described by Brongniart,<sup>1</sup> and subsequently figured.<sup>2</sup> Quite recently Bertrand<sup>3</sup> has revised Brongniart's work, in the light of Renault's preparations, and has amended the generic and specific diagnoses. Williamson,<sup>4</sup> working about the same time as Brongniart, contributed a description and figures of a flattened seed from the British Carboniferous rocks, which he named *Cardiocarpon compressum*. His description was necessarily incomplete owing to the fact that he had very few specimens, and that his more important sections were not thin enough to reveal the detailed structure. Since Williamson's paper was published, no further advance seems to have been made in our knowledge of the internal structure of British Platysperms.

Seeds similar to those figured by Williamson under the name of *Cardiocarpon compressum* occasionally occur in sections of the English Lower Coal Measure nodules, or 'coal-balls'. Professor F. W. Oliver, who had formed a collection of such preparations, suggested to me that I should undertake their examination. I am also indebted to Dr. Scott and Professor Weiss, who had both been collecting sections of *Cardiocarpon compressum* with a view to their ultimate description, and who very generously made over to me the whole of their material. Miss Benson and Mr. D. M. S. Watson have also been so good as to lend me sections which have been of great value. The following account is based mainly on the preparations in the University College Collection, supplemented by those from the sources above acknowledged, and from the Williamson Collection in the British Museum. I am indebted to Dr. Smith Woodward for permission to examine the latter. The present investigation, on which a preliminary note has already been published,<sup>5</sup> was begun at University College, London, and I wish to express my gratitude to Professor F. W. Oliver, under whose direction it was undertaken. I have also to thank Dr. Scott for his help and advice in connexion with the study.

In the present paper I propose to use the new generic name *Mitrospermum* in lieu of *Cardiocarpon* for the seed under discussion. The reasons for this change will be deferred until the structure has been considered.<sup>6</sup>

## II. GENERAL CHARACTERS OF THE SEED.

### A. The Sclerotesta.

The seed is a characteristic Platysperm. It is symmetrical about two planes. The first is the plane of flattening, or in other words the plane which traverses the longer axis of the transverse section (Text-Fig. 2, C, *p.p.*).

<sup>1</sup> Brongniart ('74).

<sup>2</sup> Brongniart ('81).

<sup>3</sup> Bertrand ('07 and '08).

<sup>4</sup> Williamson ('77).

<sup>5</sup> Agnes Arber ('10).

<sup>6</sup> See p. 502.

This is called by Bertrand<sup>1</sup> the 'antéro-postérieur' or 'A P' plane, but it is more generally known as the *principal plane*<sup>2</sup> of the seed. The second plane of symmetry is named by Bertrand the 'gauche-droite' or 'G D' plane, but no convenient English term is in use for it. As it is often necessary to refer to it, I propose to call it the *secondary plane*. It lies at right angles to the principal plane, and traverses the shorter axis of the transverse section (Text-Fig. 2 C, *s.p.*).

The flattened sclerotesta or shell is about 5 mm., both in length and breadth, or sometimes more. It is pointed above and broadest near the base. The form is shown in Text-Fig. 1, A, and Pl. XXXVII, Fig. 9, where the seed is represented cut approximately in the principal plane. Median sections in the secondary plane show an outline broadest towards the base and tapering to the micropyle (Text-Fig. 1, B).

There are some indications which suggest that the valves ultimately separated in the principal plane of the seed, but there are no sharply defined dehiscence planes, comparable with those found in such a seed as *Diplotesta*.<sup>3</sup> In the section photographed in Pl. XXXVII, Fig. 4, a stigmarian rootlet has wedged itself into the shell, which has given way at the junction of the valves.

In the centre of the seed-base a small space is left between the valves, forming a basal foramen. Each valve of the shell had a slight, external, angular ridge in the median plane, extending upwards from the base, but dying out in the upper part of the seed (Text-Fig. 2, C, *r*, and Pl. XXXVII, Fig. 10, *r*). The disappearance of the ridge in the upper region of the shell can be traced in a series of five transverse sections<sup>4</sup> cut from one seed by Miss Benson.

At the chalaza, inside the shell, a slightly raised cushion of tissue projects from the floor into the cavity of the seed, occupying the whole width of the seed in the secondary plane, but only part of the width in the principal plane. This projection, which consists mainly of the expanded upper extremity of the main supply bundle,<sup>5</sup> seems to correspond to the structure which Bertrand,<sup>6</sup> in describing other cases, has named a 'crête sous-chalazienne'. As the projection occupies only part of the width of the seed, a slight sinus is left on each side, so that the form of the whole seed cavity approaches a heart-shape. Williamson<sup>7</sup> figures a section across a seed at the level of the sinuses. The somewhat oblique, transverse section shown in Pl. XXXVII, Fig. 10, of the present paper, passes through a sinus on one side, but dips into the basal wall on the other side. The sinuses must have been very shallow, and a section cut precisely in the principal plane would be needed to expose them in profile. The sections represented in Pl. XXXVII,

<sup>1</sup> Bertrand ('07 and '08).

<sup>2</sup> Oliver ('03).

<sup>3</sup> Bertrand ('07<sup>2</sup>).

<sup>4</sup> Royal Holloway College Collection, 351 (7-11).

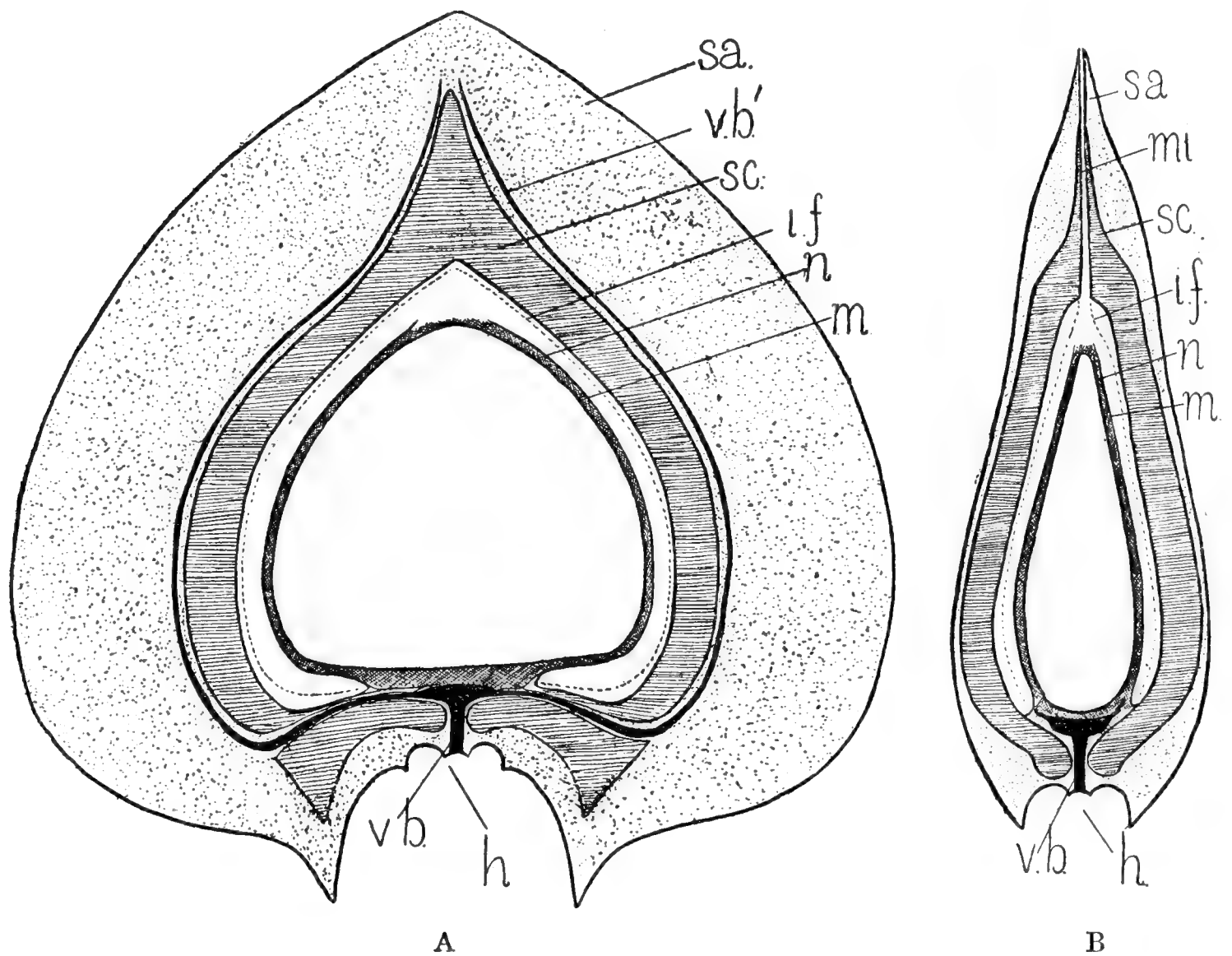
<sup>5</sup> See p. 497.

<sup>6</sup> e. g. Bertrand ('07<sup>2</sup>).

<sup>7</sup> Williamson ('77), Pl. XV, Fig. 124.

Fig. 9, and Pl. XXXIX, Fig. 26, are by no means accurately median, and hence the sinuses can hardly be detected.

The valves of the shell are slightly thickened at the base of the micropylar canal (Text-Fig. 1, B, and Pl. XXXVII, Fig. 2; cf. also Williamson's<sup>1</sup> Fig. 126). The canal is diamond-shaped in section, the longer axis lying in the principal plane (Text-Fig. 2, A). The valves rapidly taper upwards, becoming very thin at the actual orifice (Pl. XXXVII, Figs. 2 and 3).



TEXT-FIGURE I, A and B. Diagrammatic restoration of longitudinal sections of *Mitrospermum compressum* (Will.) ( $\times 8$  or 9). A is cut in the principal plane; B in the secondary plane. (The apex of the nucellus is left incomplete, because no sections have been met with in which the pollen-chamber is clearly exposed. The form of the downward projections of the sclerotesta base in A is slightly uncertain.) *sa.* = sarcotesta; *sc.* = sclerotesta; *mi.* = micropyle; *if.* = inner flesh; *n.* = nucellus; *m.* = megaspore; *v.b.* = main vascular bundle; *v.b'.* = branch vascular bundle; *h.* = hilum.

Figs. 7 and 8, Pl. XXXVII, are photographs of two specimens in the Williamson Collection,<sup>2</sup> which may represent shells, or casts of the shell-cavities, of seeds nearly allied to *Mitrospermum*.

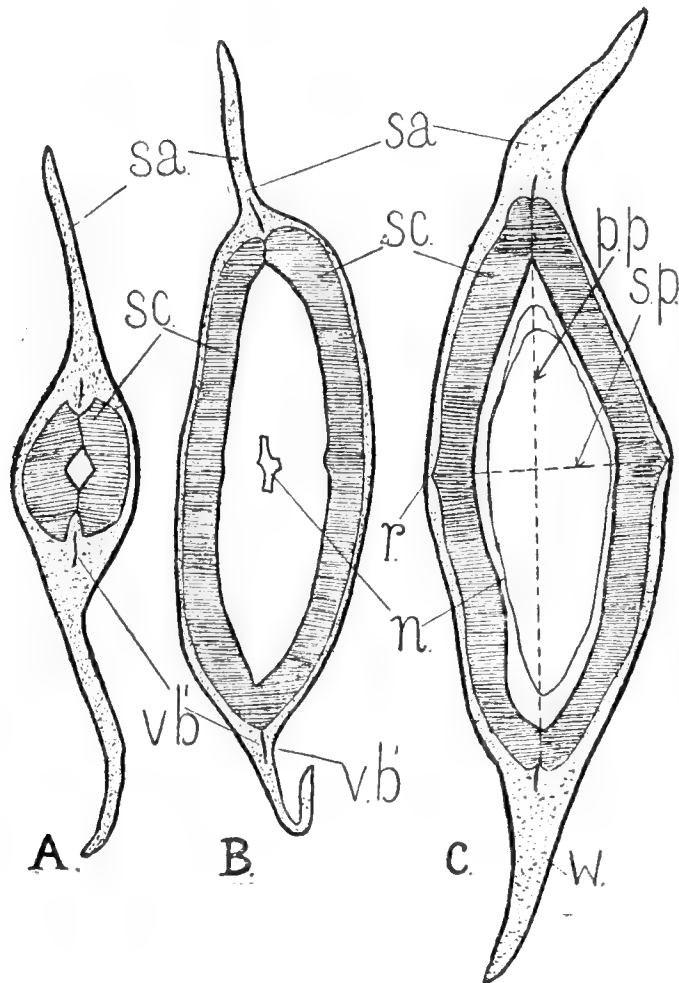
<sup>1</sup> Williamson ('77).

<sup>2</sup> Williamson describes these specimens in his MS. catalogue as follows:—'1427. Possibly a nucleus of some of the Sammaropsid forms of Lesquereux. Little Hutton near Peel, Delph, Lancashire. Mr. Wm. Monkhouse.'



## B. The Sarcotesta.

In the better preserved specimens the shell is enclosed in a delicate sarcotesta. This takes the form of a thin layer of tissue clothing the surfaces of the valves, and at their lateral edges extending beyond them as a wing or flange lying in the principal plane. Sections of the apex of the seed, which pass through both shell and sarcotesta exactly in the plane of flattening, would be needed in order to demonstrate definitely the contour of the wing at the micropylar end. Such sections have not been found, but it is probable that the outline in this plane resembled that diagrammatically shown in Text-



TEXT-FIGURE 2, A, B, and C. Diagrammatic (slightly restored) transverse sections of *Mitrospermum compressum* (Will.) ( $\times 8$  or  $9$ ). A and B are sections from the same seed (slides 1693 and 1694, in Dr. Scott's Collection; from Dulesgate). A is nearer the apex than B. C is a section of another seed nearer the base than either A or B (Slide 1417, in the Williamson Collection, named in Williamson's manuscript catalogue *Cardiocarpon anomalum*). sa. = sarcotesta; sc. = sclerotesta; n. = nucellus; v.b'. = branch vascular bundle; w. = wing; p.p. = principal plane; s.p. = secondary plane; r. = median ridge of sclerotesta.

Fig. 1, A. Transverse sections, which seem from internal evidence to have been cut near the micropyle, show a greater breadth of wing in proportion to the shell than sections taken lower down (cf. Text-Fig. 2, A and C).

A seed with the sarcotesta preserved, cut through the micropyle in a plane at right angles to the plane of flattening (i. e. in the secondary plane), fortunately occurs in the University College Collection (Pl. XXXVII, Fig. 2). It is clear, from this specimen, that the sarcotesta became much thicker at the level at which the shell began to taper off to the micropylar orifice. The micropyle seems to have been of considerable length, and in the upper



part its wall consisted chiefly, if not entirely, of sarcotestal tissue. It is impossible to decide exactly where the shell terminated. Judging from the single section now under discussion, we may say that, roughly, the lower third of the micropylar canal was bounded by the thick but tapering upper region of the shell, the middle third by a prolongation of the shell as a thin stiffening membrane, and the upper third by the sarcotesta alone. The preservation of the section, however, is imperfect, and further specimens cut in this plane are needed before we can arrive at a final interpretation. It is possible that this section may belong to a different, though closely related, species, since both the sarcotesta and 'inner flesh'<sup>1</sup> are developed to an unusual degree.

There is considerable difficulty in ascertaining the form of the sarcotesta at the base of the seed. The reconstruction of this region shown in Text-Fig. 1 is the result of a comparative study of the sections at present available, but it is to be regarded as merely provisional. It appears that the wing formed two downward projections in the principal plane, one on each side of the hilum, so that the seed remotely resembled a mitre in shape. One of the projections is shown cut obliquely in Pl. XXXVII, Fig. 5. Such a form of sarcotesta is unlike that figured by Williamson<sup>2</sup> for *Cardiocarpon compressum*, but closely recalls the *Cardiocarpon anomalum* of Carruthers,<sup>3</sup> and the more exaggerated *Cardiocarpus bicaudatus* of Kidston.<sup>4</sup> The hilum itself consisted of a central papilla, surrounded by a groove, which was bordered by a raised margin (Pl. XXXVII, Figs. 5 and 6, and Text-Fig. 1). It is probable that the whole area included within the raised margin was the hilum.

### C. The Inner Flesh.

In certain sections a thin-walled tissue, which apparently belongs to the integument, is preserved within the shell. It may be called the 'inner flesh', since Scott and Maslen<sup>5</sup> have used this term for a similar tissue occurring in *Trigonocarpus*, on account of its correspondence in position with the inner flesh of a Cycad seed. Fig. 2, Pl. XXXVII, which is a photograph of a longitudinal section cut in the secondary plane, shows a considerable development of the inner flesh. In another section<sup>6</sup> resembling this one, but cut a little to one side of the median plane, we find the inner flesh well developed between the nucellus and the base of the shell. This tissue is also seen in a transverse section<sup>7</sup> in Dr. Scott's Collection. Altogether the manner of occurrence of the 'inner flesh' suggests that it originally formed a continuous lining to each valve, but that it has only been occasionally preserved. The reason for its frequent absence may perhaps be the same

<sup>1</sup> See below.

<sup>3</sup> Carruthers ('72), Fig. 3.

<sup>5</sup> Scott and Maslen ('07), p. 110.

<sup>2</sup> Williamson ('77), Fig. 121.

<sup>4</sup> Kidston ('94), Pl. VI, Fig. 3.

<sup>6</sup> U. C. L., S. 68.

<sup>7</sup> D. H. S. 2275.

as that suggested by Scott and Maslen<sup>1</sup> to explain the disappearance of the inner flesh in *Trigonocarpus*, namely, 'that the cells themselves were of a delicate nature, and also that the hard impermeable coat of the seed prevented the infiltration of the petrifying material before the tissue had undergone disorganization'.

The inner flesh seems to correspond to the 'plaques tylaïres' described by Bertrand<sup>2</sup> as characteristic of the species of *Cardiocarpus* found in the French Carboniferous rocks.

#### D. The Vascular Supply.

A vascular bundle (*v. b.* in Text-Fig. 1) enters the hilum, and passes straight up to the base of the nucellus, through the foramen in the shell to which we have already referred. Below the nucellus, the end of the bundle expands, and gives off two opposite branches lying in the principal plane of the seed. These strands are at first almost horizontal, but they soon bend downwards and outwards, traversing the shell in an oblique direction (Pl. XXXVII, Fig. 9, and Pl. XXXIX, Fig. 26). On emerging into the sarco-testa they curve upwards, and pass towards the micropyle, following the slight groove at the junction of the valves. We may infer that the bundles continued through almost the whole length of the seed, since they can be followed in transverse and longitudinal sections as far as the base of the micropyle, and tracheides have even in one case<sup>3</sup> been recognized close to the upper part of the micropylar canal.

A small number of tracheides are given off from the expanded end of the central bundle, not far above its two main branches, mostly in the plane at right angles to the plane of flattening. Median sections, cut in the secondary plane, expose these tracheides running longitudinally to right and left at the base of the nucellus, and forming a thin, horizontal, tracheal plate. I have not been able definitely to satisfy myself as to whether a nucellar vascular system arises from this tracheal plate. The nucellus as a rule survives only as a carbonized layer showing but little structure, though in certain of the sections in which it is better preserved,<sup>4</sup> elongated elements with indications of tracheal markings can be detected at various levels. It is possible that these elements may prove to be vascular.

#### E. The Nucellus and Embryo-Sac.

The nucellus seems to have been free from the integument from its base upwards, as in *Stephanospermum*<sup>5</sup> and *Trigonocarpus*.<sup>6</sup> The possibility that an appearance of freedom might be due to a contraction and tearing of the nucellar tissues must not be overlooked. There seems, how-

<sup>1</sup> Scott and Maslen ('07), p. 110.

<sup>2</sup> Bertrand ('08<sup>2</sup>).

<sup>3</sup> U. C. L., S. 70. Cf. also D. H. S. 2254 and U. C. L., S. 35.

<sup>4</sup> e.g. U. C. L., S. 36, S. 53, S. 68, S. 69.

<sup>5</sup> Oliver ('04).

<sup>6</sup> Scott and Maslen ('07).

ever, little reason to doubt that in *Mitrospermum compressum* the space between integument and nucellus was natural and not artificial.

In the upper part of the seed the nucellus, as seen in transverse section, is characterized by four salient angles, which lie in the principal plane and the plane perpendicular to it (Pl. XXXVIII, Figs. 17 and 19). It has been already mentioned that near the micropyle the shell cavity becomes diamond-shaped in section. It is probable that this fact explains the four-angled form of the nucellus, since in the living seed it must have fitted the shell cavity fairly closely. The shrinking of the softer tissues, due to fossilization, has, however, caused the angled part of the nucellus to be drawn down to a level below that of the corresponding angled part of the shell-cavity.

There is, unfortunately, no case known in *Mitrospermum compressum* of a typical, well-preserved pollen-chamber at the apex of the nucellus, but a preparation in the University College Collection (Pl. XXXVIII, Fig. 21) recalls one of Scott and Maslen's figures of an oblique longitudinal section of *Trigonocarpus Oliveri*,<sup>1</sup> in which the pollen-chamber 'is apparently cut through'. The marked 'shoulders' of the nucellus in this preparation of *Mitrospermum compressum* also recall certain figures given by Brongniart<sup>2</sup> of *Cardiocarpus sclerotesta* and *C. tenuis*. In Mr. Watson's Collection there is an interesting transverse section, which seems to pass through the pollen-chamber (Pl. XXXVIII, Figs. 17 and 18). The section of the nucellus is very small and almost square. Elongated cells radiate from a tiny space in the centre. If we imagine that the pollen-chamber was of the type found in *Cordaianthus Grand'Euryi*,<sup>3</sup> a section such as the one under consideration might be explained as cutting through the pollen-chamber at the base of the beak. Renault describes the wall of the pollen-canal in this species as formed of cells elongated in the transverse direction, and radiating round the central conduit.

The megaspore and the narrow nucellus are as a rule much shrivelled and carbonized, but the most favourable sections indicate that the megaspore was surrounded by a nucellar tapetum. This is often preserved only as a structureless line. On the outer side of the tapetum, there are a few layers of thin-walled cells, among which, as has already been stated, there are occasional traces of elements which may have been tracheal in nature. This delicate tissue is clothed externally by a carbonized nucellar epidermis (Pl. XXXVIII, Figs. 22 and 23).

A seed of which there are two sections in Dr. Scott's Collection<sup>4</sup> has a tissue within the embryo-sac, which may be a prothallus (Pl. XXXIX, Fig. 24). This consists of irregular roundish cells, and there is no indication of the tubular method of ingrowth characteristic of the modern

<sup>1</sup> Scott and Maslen ('07), Pl. XIII, Fig. 20.

<sup>2</sup> Brongniart ('81), Pl. II, Fig. 1, and Pl. V, Fig. 3.

<sup>3</sup> Renault ('79), Pl. XVII, Fig. 15.

<sup>4</sup> D. H. S., 1802 and 1803.

Gymnosperm prothallus.<sup>1</sup> At one side the valves of the shell (which are not represented in the drawing) are slightly opened, and the appearance suggests that the prothallus is emerging from the seed. It is highly probable, however, that this appearance is purely accidental.

In the case of one seed,<sup>2</sup> in which the nucellus and megaspore are poorly preserved, a number of rather opaque, reticulately-marked bodies are seen scattered in the shell cavity. They are irregularly oval in shape, and measurements of three of the largest showed that the average size was about  $77 \mu$  by  $58 \mu$ . In another section<sup>3</sup> similar bodies are seen embedded in the ill-preserved sarcotestal wing. It is just possible that these objects may be pollen-grains, but it is far more probable that they are foreign bodies.

### III. HISTOLOGICAL DETAILS.

#### A. The Sclerotesta.

There is considerable variation in the thickness of the sclerotesta in different sections. This probably indicates that under the name *Mitrospermum compressum* we are including a group of related species.<sup>4</sup>

The cells making up the sclerotesta are somewhat elongated in the longitudinal direction, and polygonal in transverse section, the radial diameter being the greatest (Pl. XXXVIII, Fig. 11). They have thick walls which in some cases are distinctly pitted (Pl. XXXIX, Fig. 25). The outer cells of the sclerotesta are larger than the inner, which are commonly rounder and more thin-walled. The larger cells, when seen in transverse section, may be about  $100 \mu$  in the radial direction, and less than half this width in the tangential direction.

#### B. The Sarcotesta.

The surface of the sclerotesta is irregular, whether seen in transverse or longitudinal section, and a variable number of small, roundish, thin-walled cells separate it from the large outer cells of the sarcotesta (Pl. XXXVIII, Figs. 13 and 16, *i. c.*). These thin-walled cells constitute the inner layer of the sarcotesta, and form the main part of the wing tissue (Pl. XXXVIII, Fig. 12). When, as occasionally happens, the wing is cut longitudinally in the principal plane, each of the thin-walled cells is seen to be longer than it is broad, and to have a sinuous outline.

The large outer cells of the sarcotesta (Pl. XXXVIII, Figs. 13-16, *m. c.*) usually form a layer one cell thick. Sometimes two or three cells are seen, but probably this is, in most cases, due to the obliqueness of the section.

<sup>1</sup> Sokolowa ('91).

<sup>2</sup> M., R. 953 (5).

<sup>3</sup> U. C. L., S. 42.

<sup>4</sup> Dr. Scott suggests that a seed met with in his slides 2249 and 2250 (two sections from the same seed), and 2259, may represent a distinct type. The sclerotesta is unusually thick, and the distinction between sclerotesta and sarcotesta not very sharp.

The cells may be empty, or filled with carbonized contents. They vary in size; and may, in extreme cases, exceed  $500\ \mu$  in length, but less than half that length is more usual. In transverse sections they measure about  $100\ \mu$  in a direction tangential to the seed surface, and  $50\ \mu$  in the direction at right angles to this. In cases, however, where they are particularly large, they may measure as much as  $200\text{--}280\ \mu$  in the tangential direction.<sup>1</sup> These measurements are taken from the part of the sarcotesta which clothes the shell. The outer large cells of the wing are generally somewhat smaller. The irregularity in size of these cells in different specimens, or even in different parts of the same specimen, is very striking (cf. Pl. XXXVIII, Figs. 14 and 15). In certain cases, in which only a few of these cells are preserved, they are much swollen, and stand up freely from the surface of the shell. Altogether the appearance and behaviour of this layer suggests that the cells may have been *mucilage cells*, analogous to those met with in the surface layers of the testa of many modern seeds, and that the irregularity in size may have been due to the varying amount of water which the mucilage had taken up before fossilization. Mucilage cells are markedly characteristic of the testas of Palaeozoic seeds of the *Lagenostoma* group (*Lagenostoma*, *Conostoma*, and *Physostoma*).<sup>2</sup>

Although the mucilage cells of *Mitrospermum compressum* usually appear to form the outermost layer of the sarcotesta, in more than one case there is a tissue external to them, consisting of small cells radially arranged (Pl. XXXVIII, Fig. 13, *o.c.*). The fact that this layer is usually absent may be accounted for by the nature of the mucilage cells beneath. The swelling of the latter would cause an inelastic outer layer to crack and peel off. This would be somewhat analogous to the case of *Conostoma oblongum*, mentioned by Oliver,<sup>2</sup> in which 'the common outside membrane of the very conspicuous palisade-cells of the testa is sometimes found "blown off", as though a number of these cells had emitted a quantity of mucilage'.

### C. The Seed-Base.

Sections cut in the principal plane do not show such a sharp distinction between the elements of the sclerotesta and sarcotesta in the region below the two branch bundles (Pl. XXXIX, Fig. 26, *b*) as in the rest of the seed.<sup>3</sup> The sclerotesta here tends to become thinner-walled. The histological distinction between the two layers of the integument seems, however, to be variable in this part of the seed, for, though almost lost in certain sections, it is clear in others. Many cells with carbonized contents whose appearance suggests secretory cells are found in the seed-base (Pl. XXXIX, Fig. 30). There are occasional indications of an absciss layer in the hilary region.<sup>4</sup>

<sup>1</sup> Royal Holloway College Collection, 351 (9), and U. C. L., S. 50.

<sup>2</sup> Oliver ('09), p. 110.

<sup>3</sup> Cf. *Trigonocarpus*. Scott and Maslen ('07), p. 101.

<sup>4</sup> e. g. U. C. L., S. 65.



#### D. The Vascular Tissue.

The main supply bundle is usually represented only by tracheides. The expansion, in which it terminates below the base of the nucellus, consists of short reticulate elements (Pl. XXXIX, Fig. 29). In the basal part of the two branch bundles, however, some elongated, thin-walled elements are preserved below the tracheides. This tissue is probably phloem (*ph.* in Pl. XXXIX, Fig. 27). In the same section from which the figure just mentioned was drawn, two small elements with loosely spiral thickenings occur in one place on the lower side of the xylem (Pl. XXXIX, Fig. 28; cf. also Fig. 26). They are about  $6\mu$  wide, and are succeeded by larger elements  $10\mu$  to  $18\mu$  wide, which are reticulate or closely spiral. The phloem is not preserved in the part of the bundle where they occur. The small elements have the characters of protoxylem, and thus the xylem of the branch bundles, at least in the lower part, would seem to have been centripetal in development. I have not met with any case of recognizable phloem in the branch bundles, after they have emerged from the shell, and curved upwards towards the micropyle. The most striking feature of the strands in this part of their course is the extreme flattening which they undergo in the principal plane of the seed, so that the bundle is better described as a tracheal plate. A transverse section in Dr. Scott's Collection<sup>1</sup> shows one of the bundles as a chain of tracheides, twenty elements long in the plane of flattening, but only from one to three elements wide (Pl. XXXVIII, Fig. 20).

#### IV. NOMENCLATURE.

##### A. Specific Name.

In his original description of the seed under discussion, Williamson<sup>2</sup> wrote, 'All the specimens agree in giving to this species much of the general dimensions and contour of the *Cardiocarpum acutum* of Lindley and Hutton, and of the *C. Lindleyi* of Mr. Carruthers, excepting that the latter observer describes his seeds as having a central longitudinal ridge, which my specimens certainly have not. Since these differences exist, it may be well to distinguish my type under the name of *Cardiocarpon compressum*.' It seems, however, that Williamson was mistaken in entirely denying the existence of a 'central longitudinal ridge', since many transverse sections which seem undoubtedly to belong to his type show a slight median ridge on the surface of each valve in the secondary plane (Text-fig. 2, C, and Pl. XXXVII, Fig. 10). In fact one of Williamson's type sections<sup>3</sup> shows a distinct indication of a ridge, though this is rather minimized in his drawing of it.<sup>4</sup>

<sup>1</sup> D. H. S. 2428.

<sup>3</sup> W. 1409.

<sup>2</sup> Williamson ('77), p. 259.

<sup>4</sup> Williamson ('77), Pl. XV, Fig. 124.



The ridge, unlike that in *Cardiocarpum acutum*,<sup>1</sup> only extended a certain distance up the shell and then died out. There is a section<sup>2</sup> from Oldham in the Williamson Collection, which is referred in the MS. catalogue to *Cardiocarpon anomalum*, but which is in reality a most characteristic example of his *C. compressum*. It is a transverse section showing a well-marked median ridge (Text-Fig. 2, C). It was probably this ridge which deterred Williamson from referring the section to *C. compressum*. The attribution to *C. anomalum* is a curious one, since all the other sections in the Collection, referred by Williamson to Carruthers' species *C. anomalum*,<sup>3</sup> have proved to be *Lepidocarpon*s.<sup>4</sup>

A large number of seed impressions of the *Cardiocarpon* type from Devonian and Carboniferous rocks have been figured by various authors,<sup>5</sup> but none of them can with certainty be identified with *Mitrospermum compressum*.

### B. Generic Name.

Brongniart's original description of the genus *Cardiocarpon*<sup>6</sup> dealt only with external characters, and was somewhat vague. It ran as follows:—

'*Cardiocarpon*. Fruits comprimés, lenticulaires, cordiformes ou réniformes, terminés par une pointe peu aiguë.'<sup>7</sup>

Since Brongniart's time various seeds, which he would probably have included in the genus *Cardiocarpon*, have been distributed into several newer genera, e. g. *Samaropsis*, *Cordaicarpus*, *Cyclocarpus*, &c. A critical discussion of these genera, which are distinguished by slight differences in external form, has been published by Kidston,<sup>8</sup> who concludes that it is advisable tentatively to retain Brongniart's generic name *Cardiocarpon* for the whole assemblage of these seeds.

Bertrand<sup>9</sup> has recently published a fresh description of the genus, founded on a re-examination of the silicified seeds previously studied by Brongniart and Renault, in which he discusses internal as well as external characters.

On comparing the structure of *Mitrospermum compressum*, as outlined in the preceding sections of this paper, with the generic characters enumerated by Bertrand for *Cardiocarpus*, we find that they fail to agree in one important point, namely, the type of vascular system. Bertrand restricts the use of the name *Cardiocarpus* to forms in which the hilo-chalazal bundle emits its

<sup>1</sup> Lindley and Hutton ('31), Pl. LXXVI.

<sup>2</sup> W. 1417.

<sup>3</sup> Carruthers ('72).

<sup>4</sup> Scott ('01).

<sup>5</sup> Dawson ('71), Newberry ('73), Andrews ('75), Kidston ('94), &c.

<sup>6</sup> The name has been used by different writers in the forms:—*Cardiocarpon* [Brongniart ('28), Williamson ('77)], *Cardiocarpus* [Brongniart ('81), Bertrand ('08)], and *Cardiocarpum* [Lindley and Hutton ('31)]. Compare Scott and Maslen ('07), footnote to p. 90.

<sup>7</sup> Brongniart ('28).

<sup>8</sup> Kidston ('94), p. 263.

<sup>9</sup> Bertrand ('08<sup>2</sup>).

two branches *before* entering the shell. These two branches remain outside the shell throughout their entire course. He contrasts this type of vascular system with that of *Rhabdocarpus*,<sup>1</sup> in which the main supply bundle passes unbranched through the shell. The two lateral bundles arise from the chalazal vascular plate, and immediately run down again through the shell, forming an acute angle with the main bundle. Bertrand names them 'faisceaux récurrents'. On emerging from the shell, they curve upwards and follow the same course as the bundles of *Cardiocarpus*. Brongniart's *Cardiocarpus nummularis* and *C. tenuis* have the rhabdocarpic type of vascular symmetry, and hence are removed from *Cardiocarpus* and placed in *Cyclocarpus*.<sup>2</sup>

In *Mitrospermum compressum* the vascular system<sup>3</sup> is intermediate between that characteristic of *Rhabdocarpus* and of *Taxospermum*.<sup>4</sup> In the latter the branch bundles, as in *Rhabdocarpus*, arise from the chalazal vascular mass, but they follow the floor of the shell cavity, and on reaching the flanks traverse the shell obliquely from below upwards.

Since the seed described in this paper cannot strictly be referred to *Cardiocarpus* (in Bertrand's sense), or to any of the related genera, it seems advisable to distinguish it by a new generic name. I suggest that *Mitrospermum* might be used in allusion to the form of the seed-base.

*Mitrospermum*, gen. nov.

The characters of the genus are at present those of the only known species:—

*Mitrospermum compressum* (Will.).

1877. *Cardiocarpon compressum*, Will. W. C. Williamson, *On the Organization of the Fossil Plants of the Coal Measures*, Pt. VIII, p. 279.

Bilaterally symmetrical seed, flattened in the plane of symmetry. As usually preserved, the integument consists of a sclerotesta of thick-walled cells, enclosed in a more delicate sarcotesta, which extends into a wing in the principal plane. An outer layer of large mucilage cells is the most conspicuous feature of the sarcotesta. In the best preserved specimens, in addition to the parts of the integument just described, two other layers can be recognized, namely, a thin-walled tissue lining the sclerotesta, and a small-celled layer clothing the mucilage cells of the sarcotesta. The main vascular bundle enters the hilum, and passes through the sclerotesta without branching. Below the base of the nucellus, it gives off two strands in the principal plane, which are at first horizontal, but soon dip slightly downwards and outwards, traversing the sclerotesta in an oblique direction.

<sup>1</sup> Bertrand ('07<sup>4</sup>).

<sup>2</sup> Bertrand ('08<sup>2</sup>).

<sup>3</sup> See p. 497.

<sup>4</sup> Bertrand ('07<sup>1</sup>).

When these branches emerge into the sarcotesta they are separated by almost the whole width of the seed-base. They turn upwards, and pass to the micropyle, keeping close to the sclerotesta throughout their course.

*Localities*:—South Lancashire Coalfield:—Oldham (original locality); Shore, Littleborough (abundant); Dulesgate; Hough Hill, Stalybridge.

*Horizon*: Lower Coal Measures.

## V. THE CONTRIBUTION TO CORDAITES.

The present investigation has thrown no fresh light on the problem—to what plants do seeds of the *Cardiocarpus* type belong? It may, however, be well in conclusion briefly to review the evidence on this point as it stands at present.

In 1872 Carruthers<sup>1</sup> figured two species of *Cardiocarpon*, *C. Lindleyi*, Carr., and *C. anomalum*, Carr., attached to twigs, which he suggested belonged to an extinct Gymnosperm of the *Dadoxylon* type, or, as we should now say, to one of the Cordaitales.

Grand'Eury<sup>2</sup> in 1877 pointed out that a variety of seeds known as *Cardiocarpus* are found associated with *Cordaites* in such a way as to render their identity with this genus very probable. For example, at Chazotte there are rocks filled exclusively with *Cordaites* accompanied by numerous *Cardiocarpus* seeds. Grand'Eury was also fortunately able to adduce more direct evidence. He found catkin-like inflorescences in actual organic continuity with branches bearing Cordaitean leaves, and in some cases these inflorescences contained seeds. The seeds varied in size in different specimens, and those which were best developed were recognizable as belonging to the cardiocarpic type.<sup>3</sup>

The next great advance in our knowledge of the Cordaitean fructification was due to Renault,<sup>4</sup> who in 1879 published an account of the internal structure of the male and female catkins, illustrated by figures which have now become quite familiar.<sup>5</sup> The female catkins were, however, much younger than those whose superficial features had been described by Grand'Eury, and were not sufficiently advanced in development to make it possible to identify the contained seeds with any of the numerous mature seeds, known only as detached specimens.

We may conclude that the work of Carruthers, Grand'Eury, and Renault, has established the fact that *Cordaites* bore seeds of the cardiocarpic type, using the word in a broad sense. Further than this, we find that there is some evidence from association to show that certain species of *Cardiocarpus* are definitely connected with certain species of *Cordaites*.

<sup>1</sup> Carruthers ('72).

<sup>2</sup> Grand'Eury ('77).

<sup>3</sup> e. g. the seeds of *Cordaites nobilis*, see Grand'Eury ('77), Pl. XXVI, Fig. 9.

<sup>4</sup> Renault ('79).

<sup>5</sup> Scott ('08), vol. ii, Figs. 193 and 194.

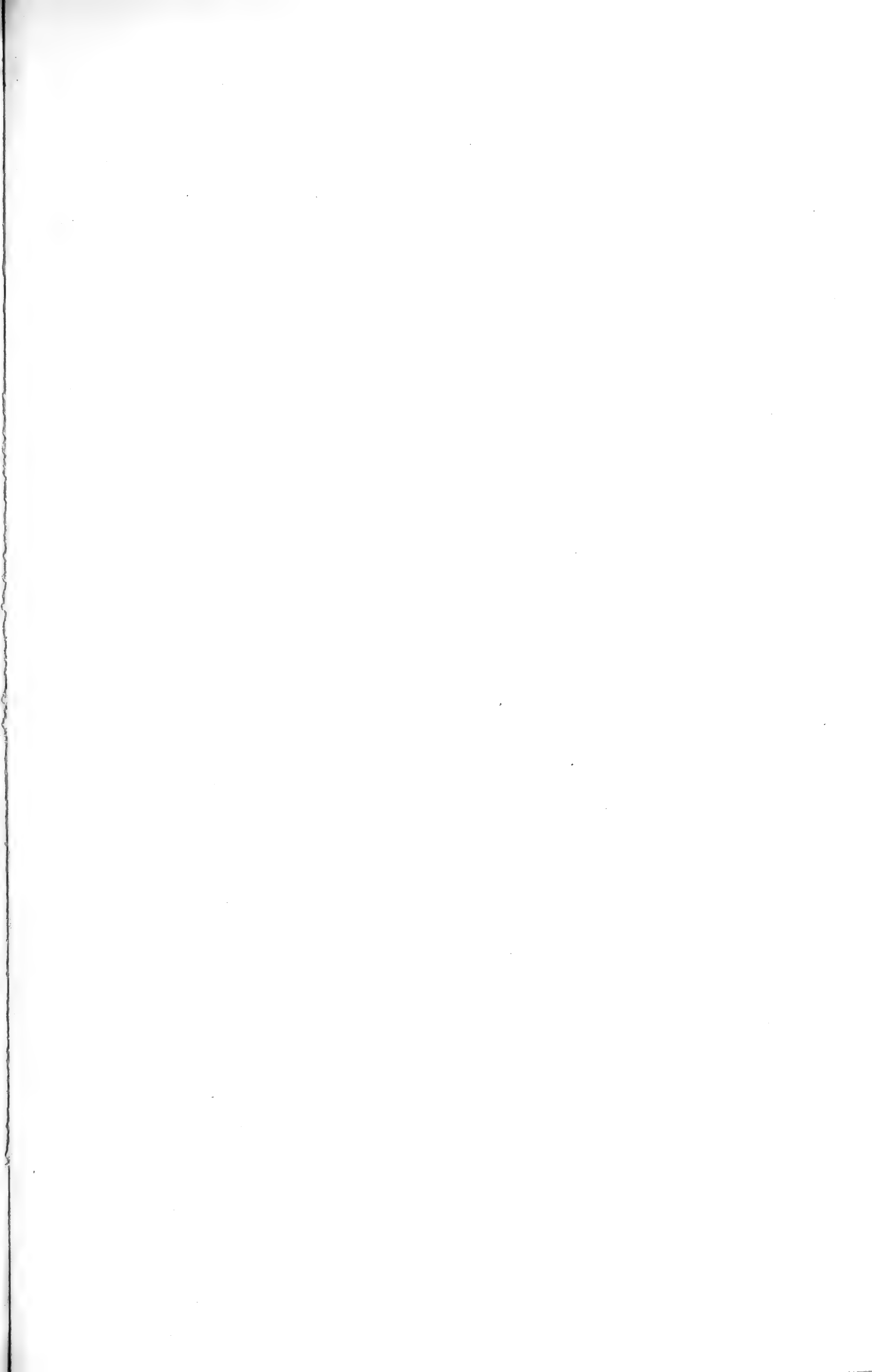
Kidston,<sup>1</sup> for instance, points out that *Cardiocarpon Lindleyi* is almost invariably found associated with *Cordaites principalis*. We are confronted, however, with the curious fact that there seem to be many more species of *Cardiocarpon* than of *Cordaites*. A few years ago Grand'Eury<sup>2</sup> wrote, 'On a institué au moins cinq fois plus d'espèces de graines que de feuilles de *Cordaites*.' As an example he mentions that, associated with leaves identified with, or closely similar to, *Cordaites palmaeformis*, Göpp., five species of seeds are found—in the Franco-Belgian basin, *Cardiocarpus Lindleyi*, Carr., *C. Pitcairniae*, Lind., *C. cornutus*, Daw., and in the Loire basin, *Samaropsis fluitans*, Daw., and *S. forensis*, Gr. Dawson<sup>3</sup> had previously noticed a similar disproportion between the small number of species of Cordaitean leaves, and the numerous species of seeds, occurring in the Canadian rocks with which he was concerned. Oliver<sup>4</sup> has drawn attention to a precisely analogous difficulty in the case of the *Lagenostoma* group of seeds. He writes, 'One petrified seed (*L. Lomaxii*), and at least three impressions, superficially in agreement with the seeds of the *Lagenostoma* group, have been referred to the frond-type Sphenopteris. We are still left with *Physostoma*, *L. ovoïdes*, and the Conostomas, all petrifications from the same group, and—excepting for *Heterangium*—there are no species of *Sphenopteris* yet separated from *Lyginodendron* by anatomical characters to which they could be assigned.' In the case of *Cordaites*, Grand'Eury's explanation is that evolution in this group has proceeded principally in the direction of changes in the reproductive organs, the vegetative structure remaining unaltered owing to uniformity of climate and conditions. It is easy, however, to take an exaggerated view of the uniformity of anatomical structure within these groups. It is likely that in the future a rigorous analysis of the anatomical characters of the Lyginodendreae, and of the Cordaitales, will reveal the existence of many more species, distinguishable on vegetative grounds, than those we know at present. In the case of the Cordaitales this analysis has already begun, and the work of Scott and Maslen<sup>5</sup> on the subject is bringing to light many new anatomical types. On the other hand, it is possible that there may actually be some truth in the idea that there is a disproportion between the number of so-called Cordaitean seeds, and the number of species recognizable by their vegetative characters. Some of these seeds may perhaps prove to belong to other groups. Until 1905 no case was known in which seeds of the Platysperm type could be definitely attributed to any other plant than *Cordaites*. In that year, however, seeds with bilateral symmetry were discovered attached to two Pteridosperms, *Aneimites*<sup>6</sup> and *Pecopteris Pluckenetii*.<sup>7</sup> The notion that every member of the Platyspermeae was necessarily a Cordaitean seed, was thus discredited.

<sup>1</sup> Kidston ('86).<sup>2</sup> Grand'Eury ('05<sup>2</sup>).<sup>3</sup> Dawson ('91).<sup>4</sup> Oliver ('09), p. 111.<sup>5</sup> Scott and Maslen ('10).<sup>6</sup> White ('05).<sup>7</sup> Grand'Eury ('05<sup>1</sup>).

The particular seed with which we have been concerned in this paper, *Mitrospermum compressum*, is always found unattached, and the question of its attribution must be left open until further evidence is forthcoming.

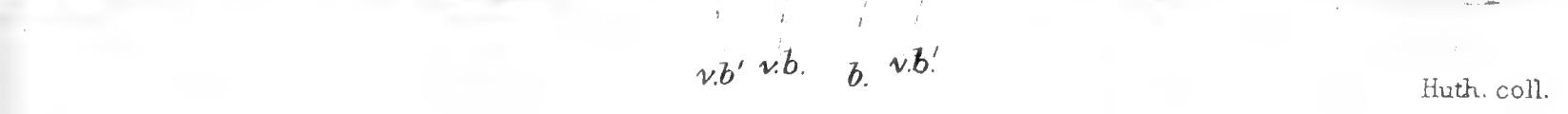
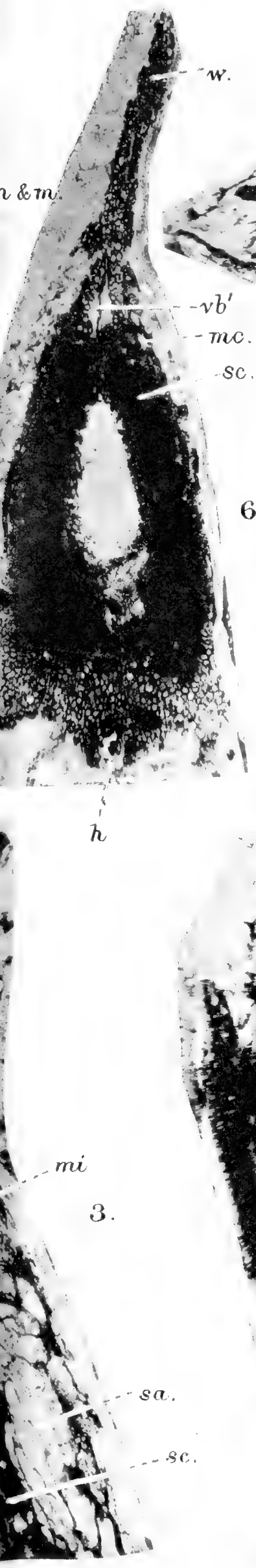
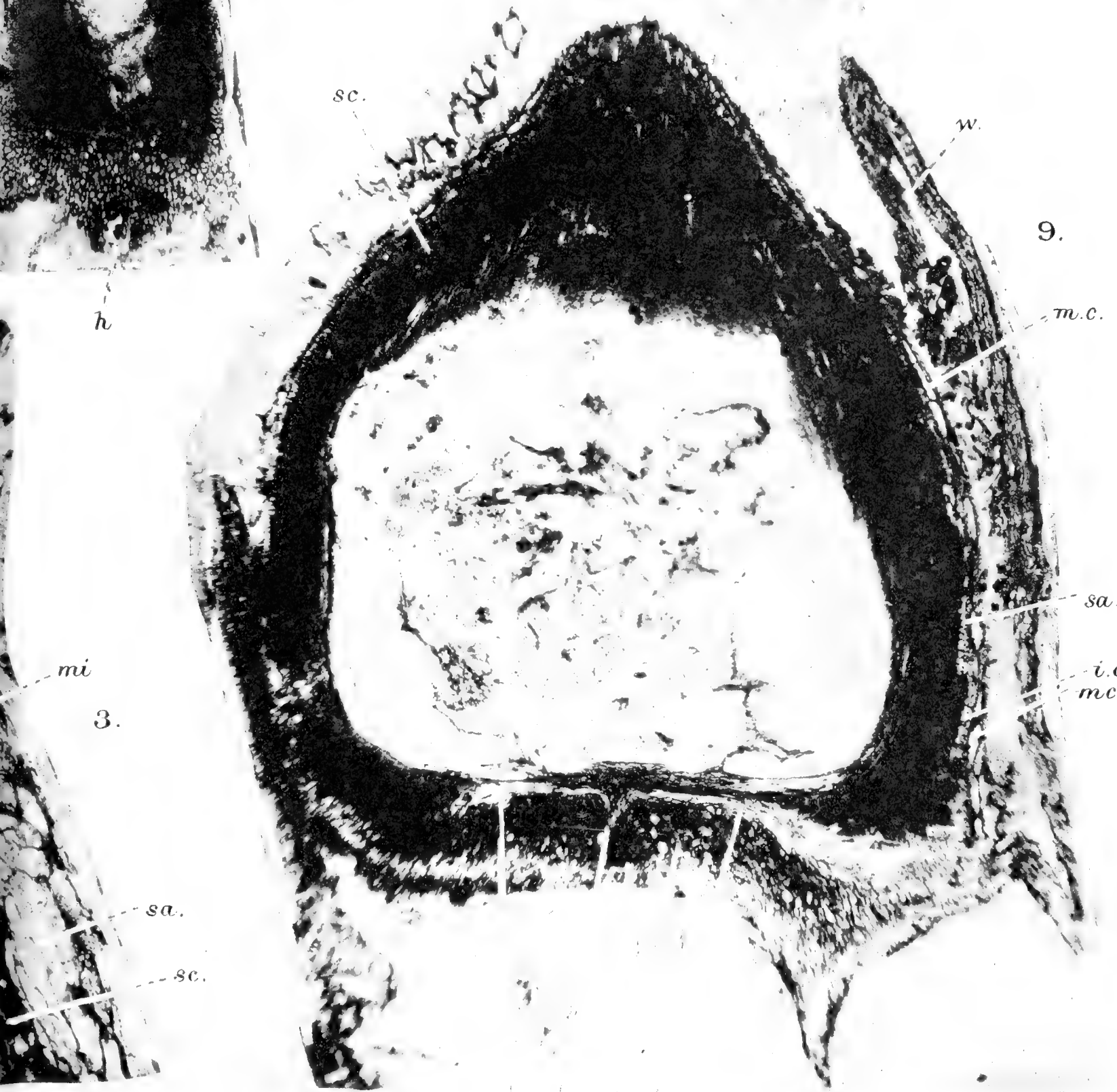
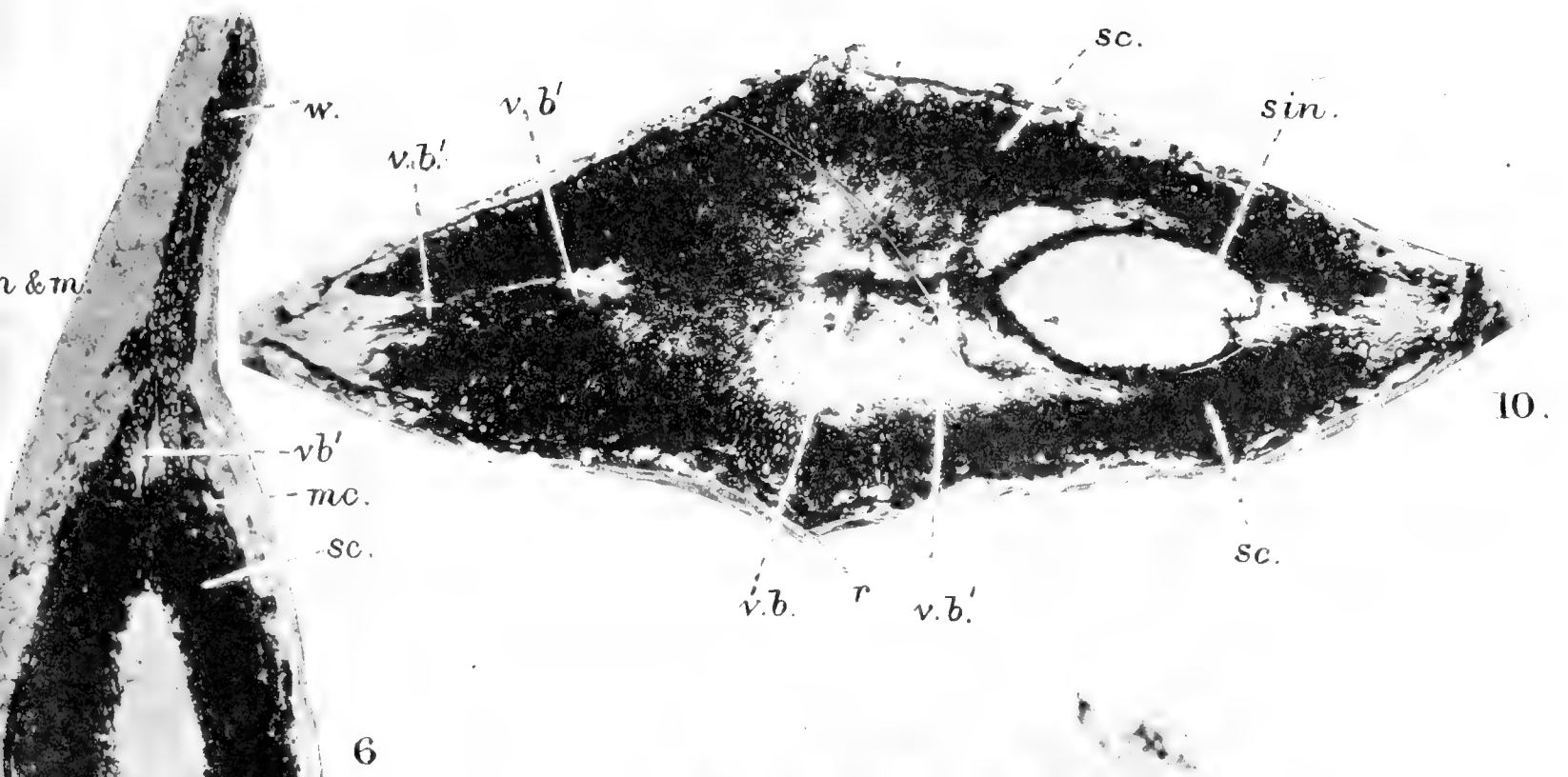
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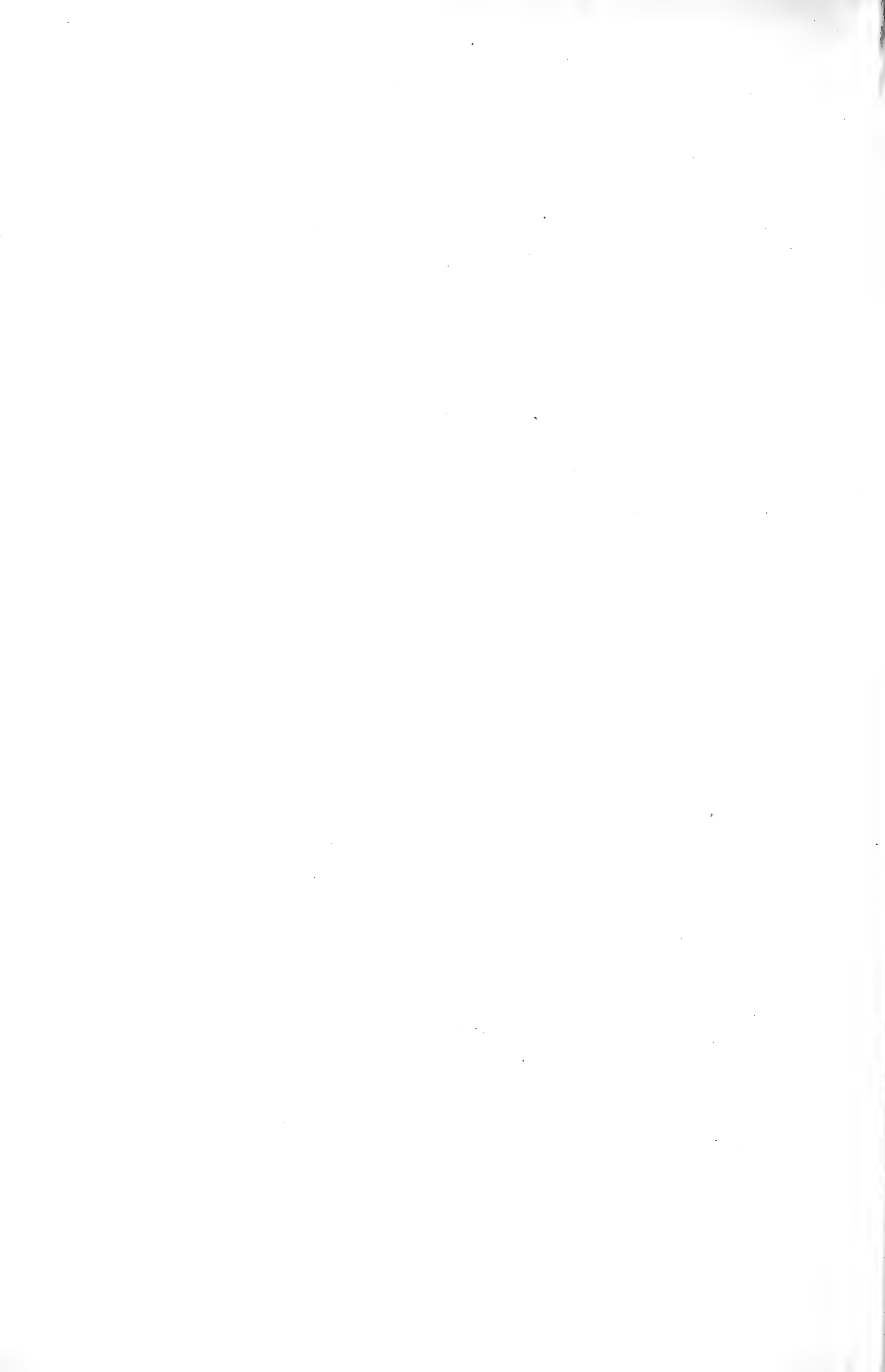
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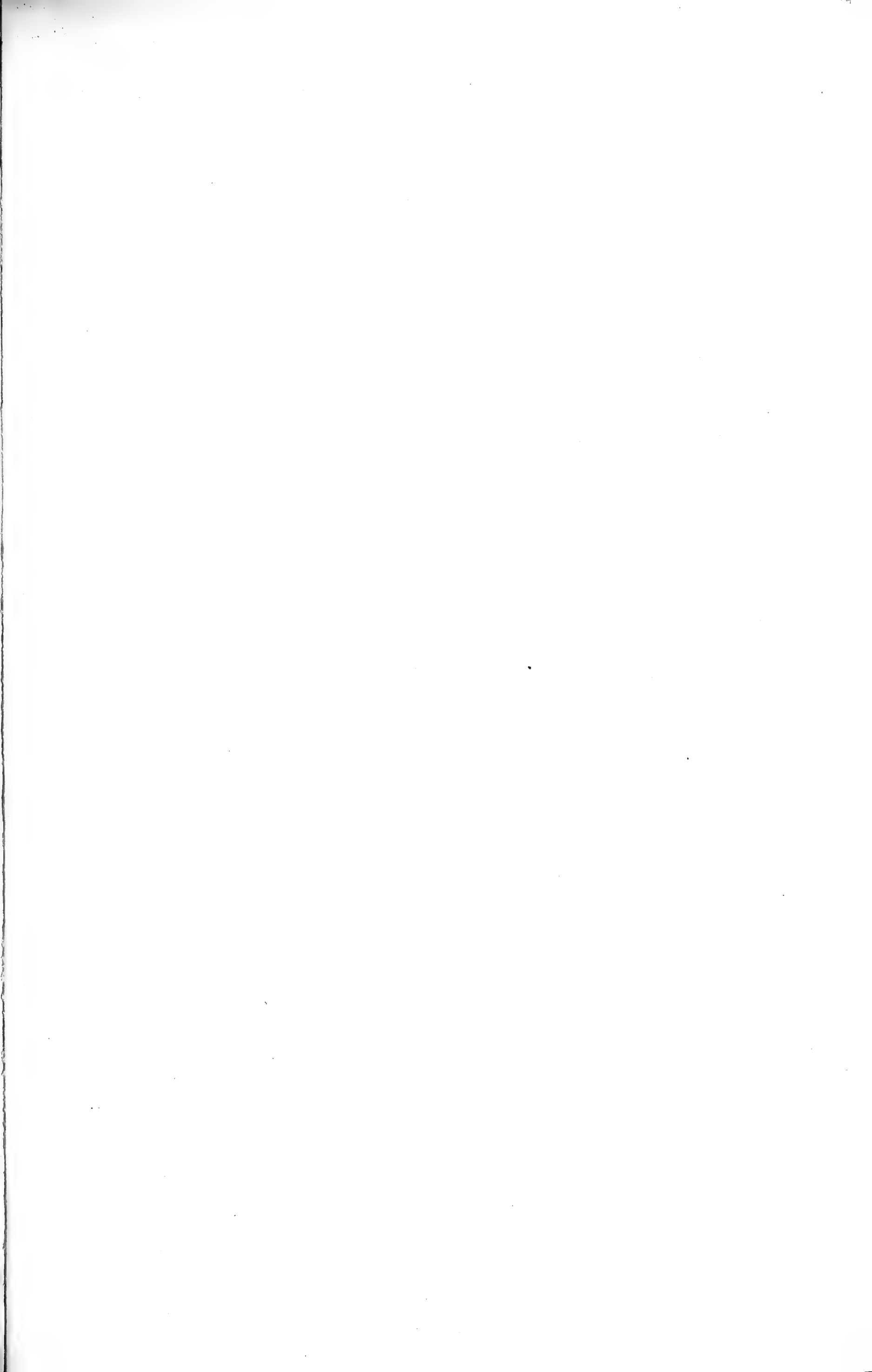


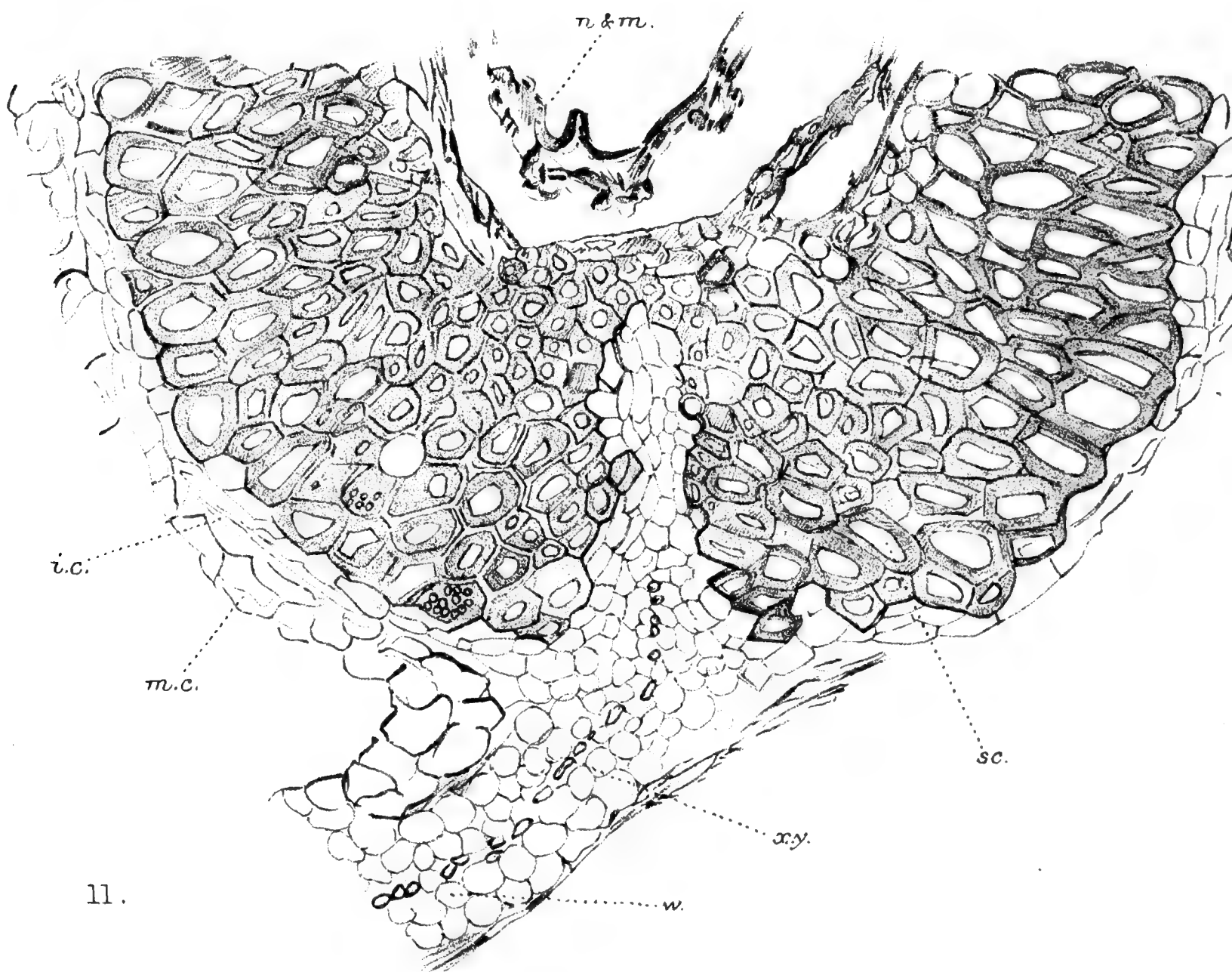
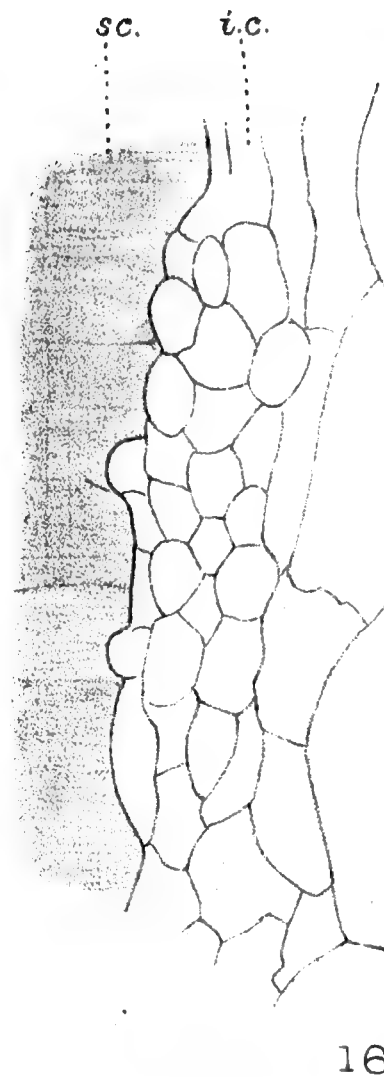
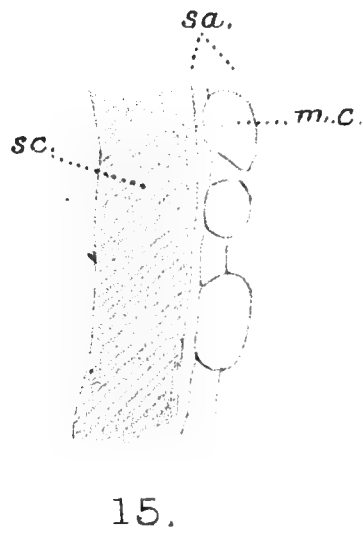
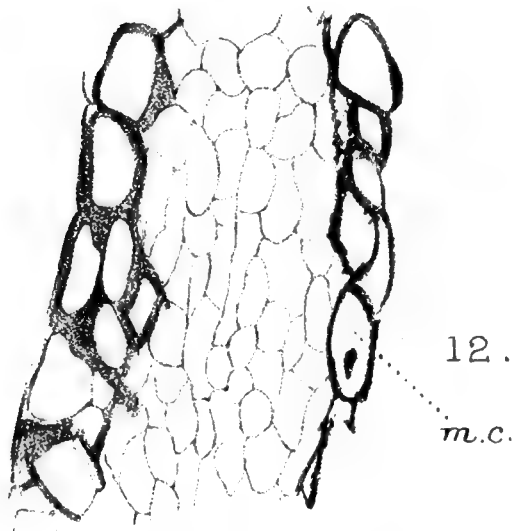
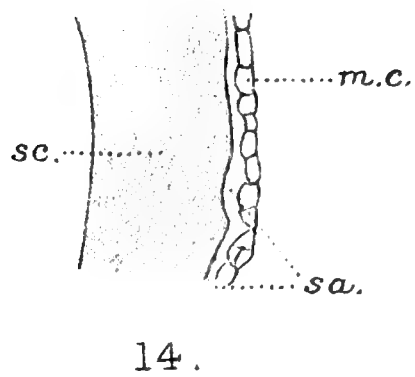
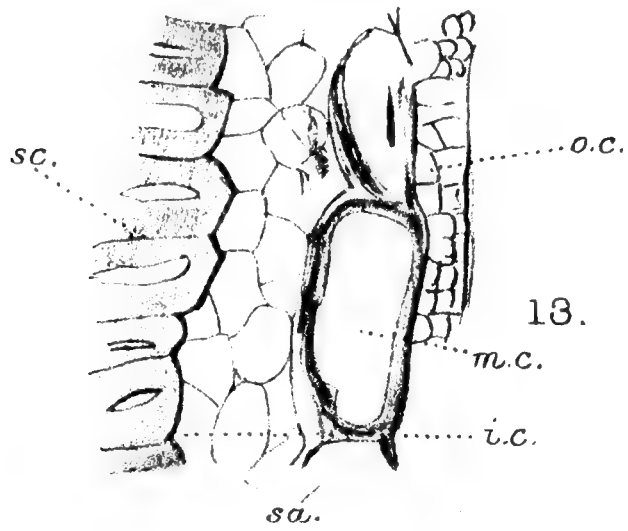




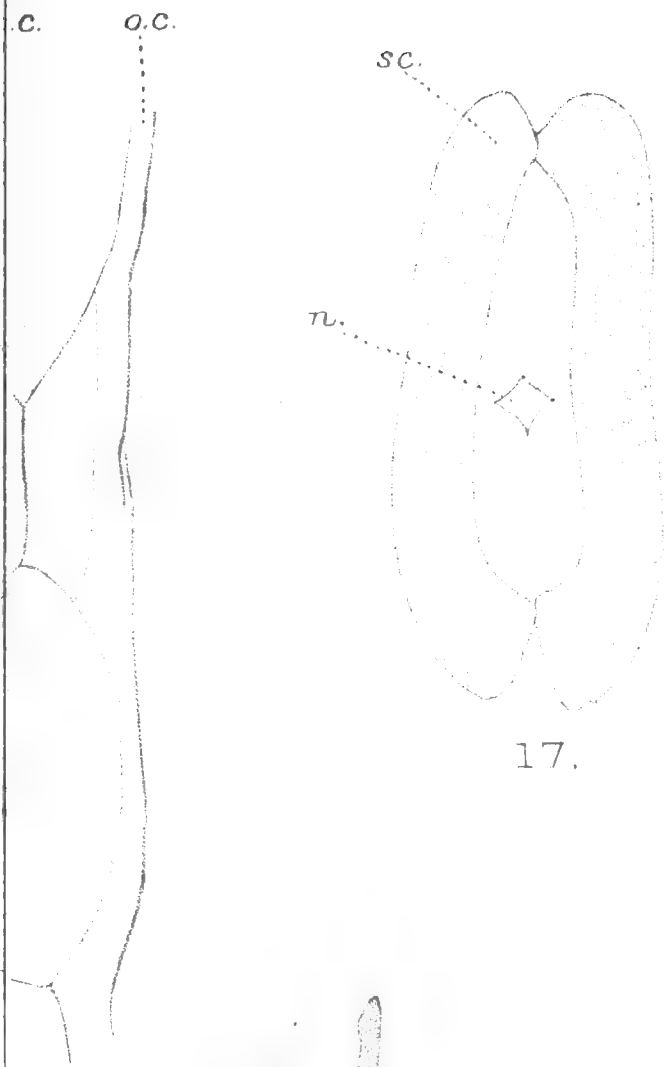








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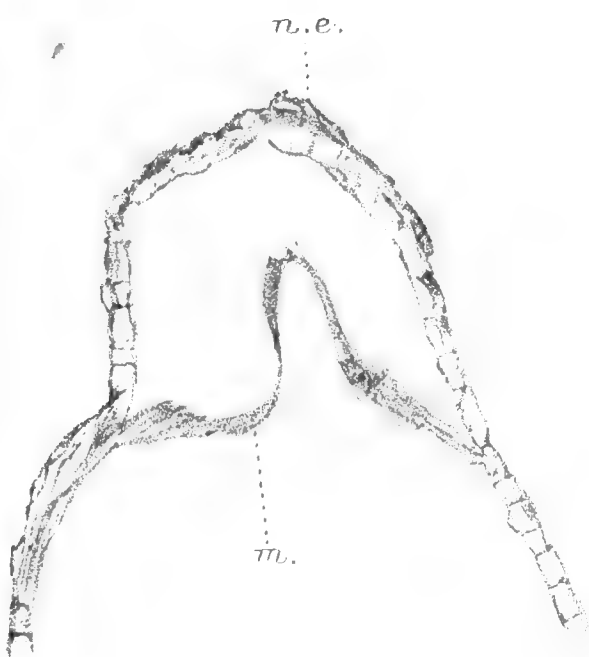
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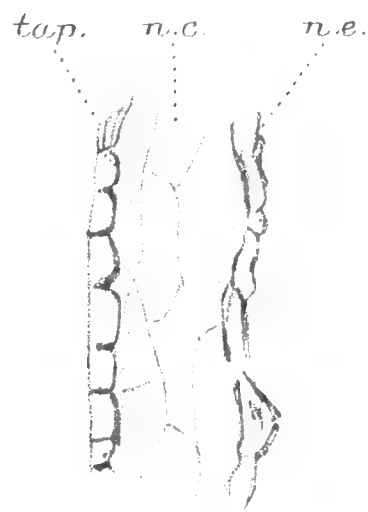
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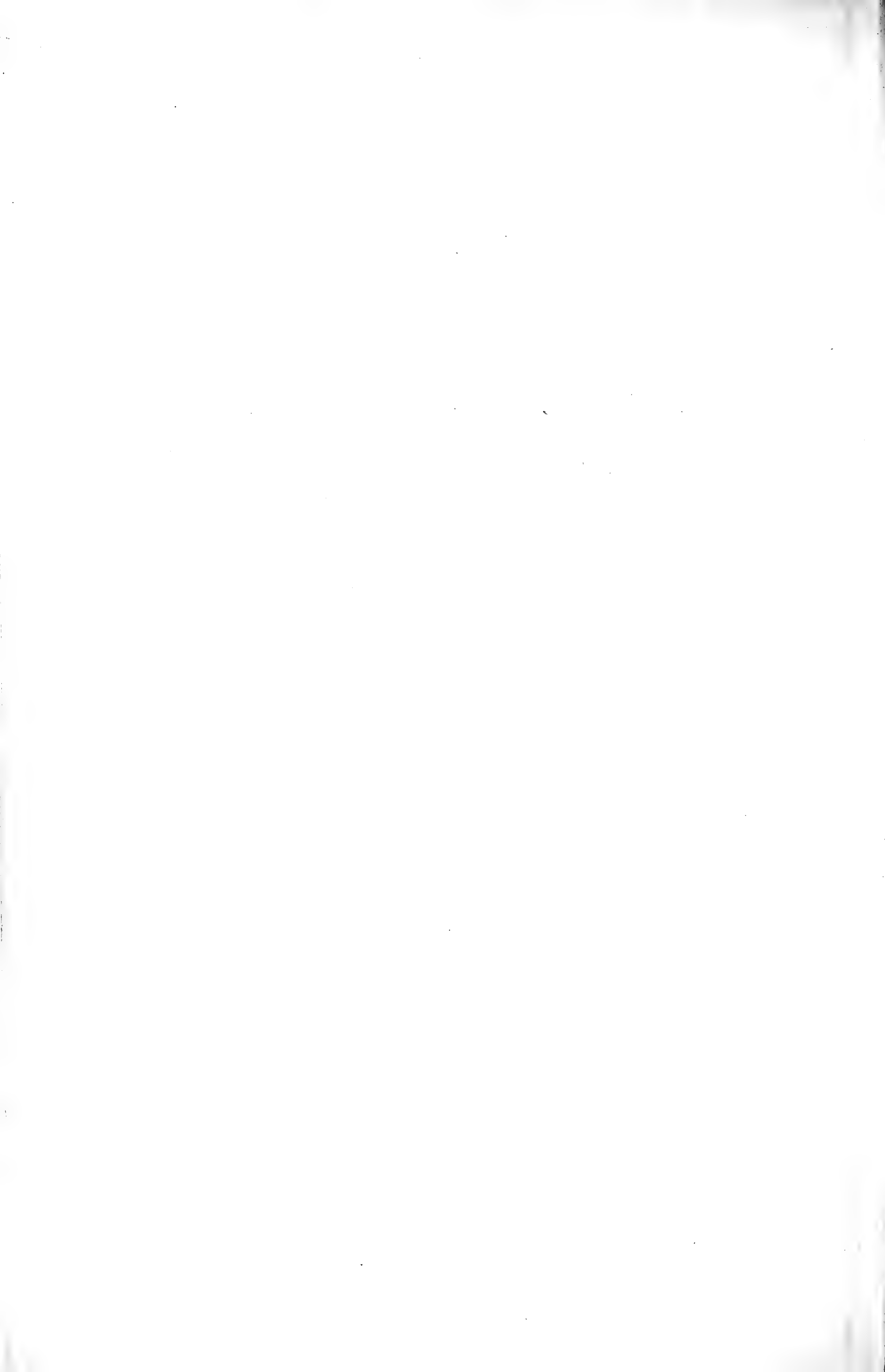


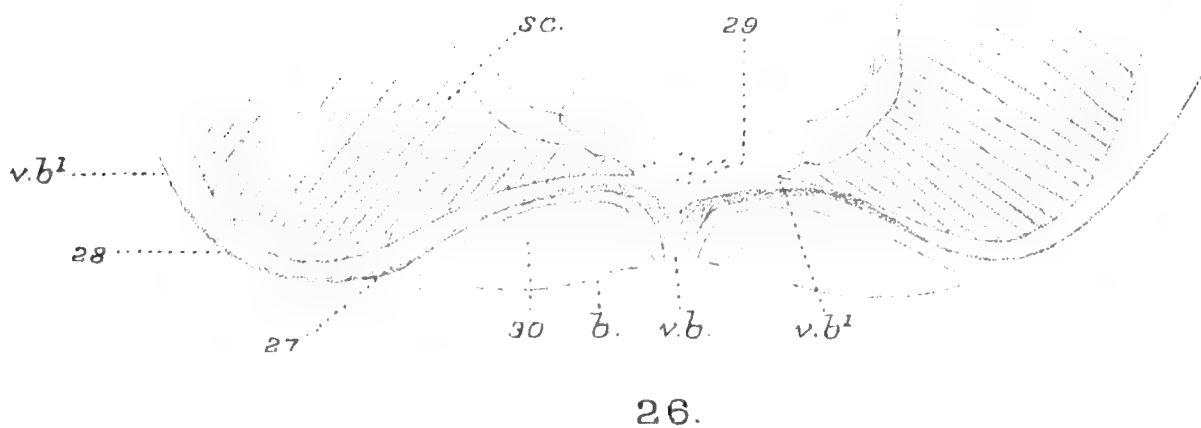
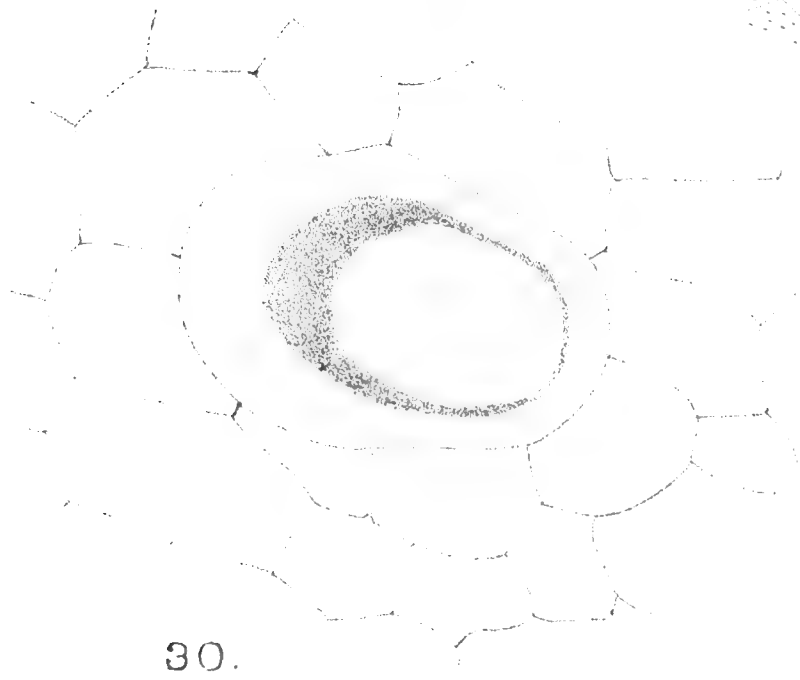
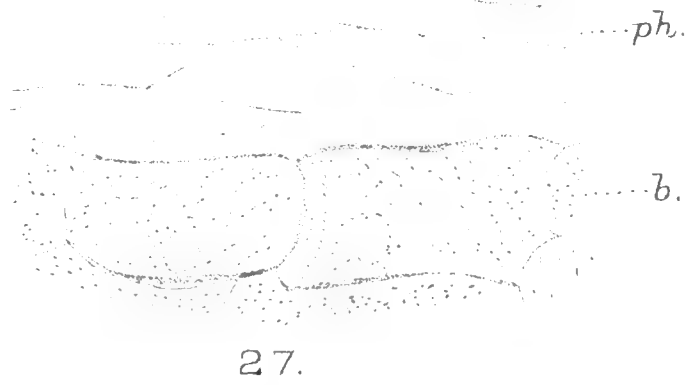
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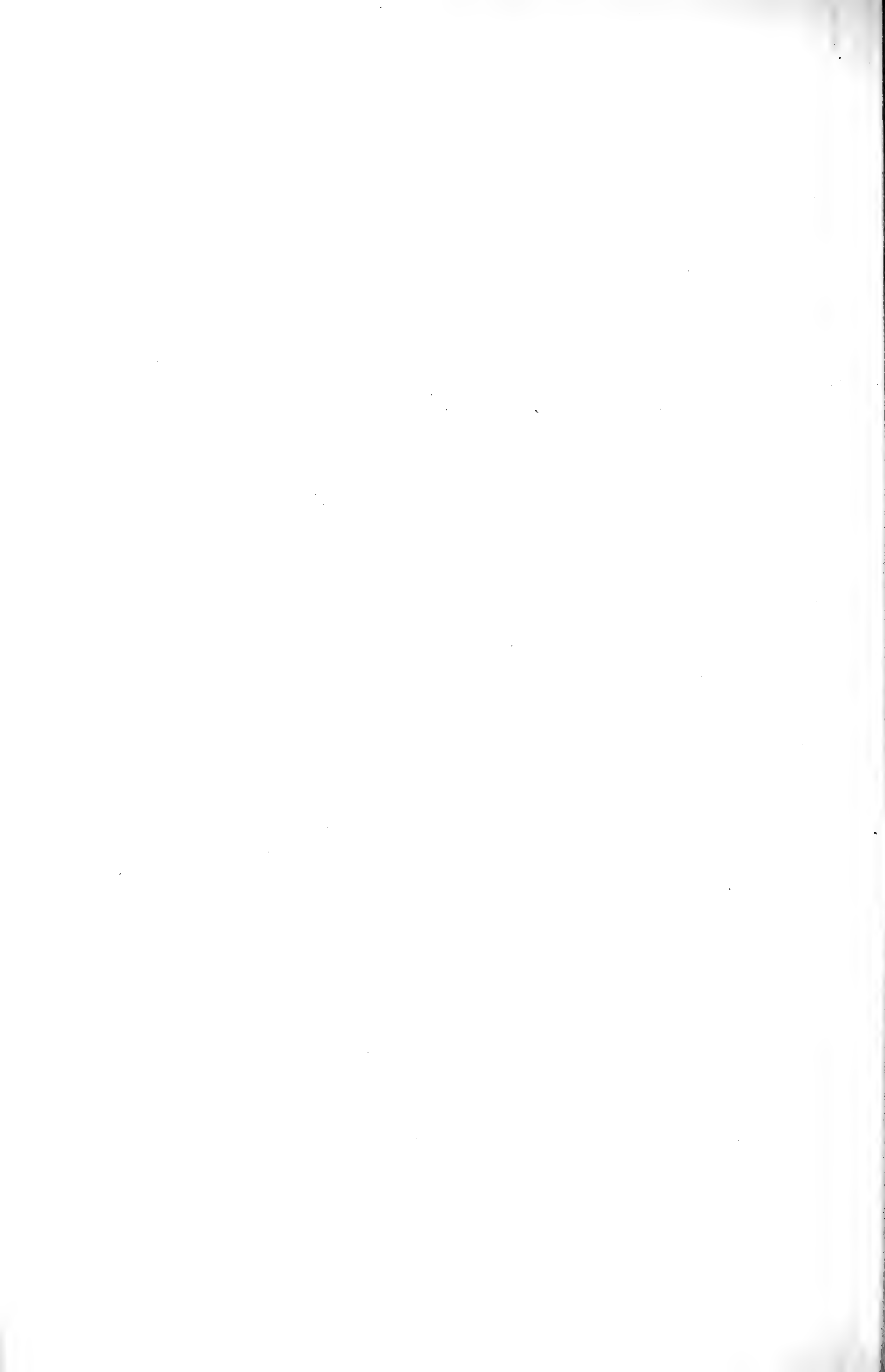






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## EXPLANATION OF PLATES XXXVII-XXXIX.

Illustrating Mrs. Arber's paper on *Mitrospermum compressum* (Will.).

The following abbreviations are used:—

W. = Williamson Collection, British Museum of Natural History; D. H. S. = Dr. Scott's Collection; U. C. L., S. and R. = University College Collection, London; Wat., A. = Mr. D. M. S. Watson's Collection; M., R. = University of Manchester Collection.

### PLATE XXXVII.

Figs. 1-6, 9, 10, photographs by W. Tams; Figs. 7 and 8, photographs by E. A. N. Arber.

Fig. 1. Transverse section, probably near apex of seed, showing sclerotesta (*sc.*), and wing (*w.*), the latter preserved on one side only. U. C. L., S. 48 (Shore).  $\times 20$  circa.

Fig. 2. Longitudinal section in secondary plane (i. e. plane at right angles to plane of flattening, (see p. 493) passing through the micropyle (*mi.*). This section is remarkable for the great development of the sarcotesta (*sa.*) and inner flesh (*i.f.*). *sc.* = sclerotesta. U. C. L., S. 70 (Shore).  $\times 33$  circa.

Fig. 3. Longitudinal section of another seed cut in the same plane as that shown in Fig. 2. The micropyle (*mi.*) is more widely open, and the sarcotesta (*sa.*) less developed. *sc.* = sclerotesta. U. C. L., S. 37 (Shore).  $\times 46$  circa.

Fig. 4. Transverse section of a decorticated sclerotesta (*sc.*), showing a stigmarian rootlet (*stig.*) wedging the valves apart. U. C. L., S. 45 (Dulesgate).  $\times 15$  circa.

Fig. 5. Base of oblique longitudinal section passing through the hilum (*h.*). *sa.* = sarcotesta; *sc.* = sclerotesta; *n.* and *m.* = nucellus and megaspore; *m.c.* mucilage cells of sarcotesta. U. C. L., S. 60a (Shore).  $\times 21$  circa.

Fig. 6. Obliquely transverse section, passing through the hilum (*h.*) on one side, and one wing (*w.*) on the other. *sc.* = sclerotesta; *m.c.* = mucilage cells of sarcotesta; *v.b.'* = branch vascular bundle. U. C. L., S. 67 (Shore).  $\times 22$  circa.

Figs. 7 and 8. Two seeds, or casts, described in Williamson's manuscript catalogue as 'Possibly a nucleus of some of the Sammaropsid forms of Lesquereux'. W. 1427 (Little Hutton, near Peel, Delph, Lancashire).  $\times 1\frac{1}{2}$  circa.

Fig. 9. Longitudinal section in the principal plane (or plane of flattening), showing the origin of the branch vascular bundles (*v.b.'*) from the main bundle (*v.b.*). The wing (*w.*) is partly preserved on the right-hand side. *b.* = seed base with large secretory cells; *sc.* = sclerotesta; *sa.* = sarcotesta, showing very clearly the division into *m.c.* = mucilage cells, and *i.c.* = smaller inner cells. U. C. L., S. 65 (Shore).  $\times 20$  circa.

Fig. 10. Transverse section close to the base. On the right the section passes through a sinus (*sin.*), but on the left, as it is somewhat oblique, it dips into the sclerotesta (*sc.*) below the sinus. On the right the branch bundle (*v.b.'*) is seen passing off from the main bundle, on the left the branch bundle (*v.b.'*) is cut further out. *r* = median ridge of sclerotesta. U. C. L., S. 43 (Hough Hill, Stalybridge).  $\times 26$  circa.

## PLATE XXXVIII.

## DRAWINGS.

Fig. 11. One end of a transverse section, showing the junction of the two valves of the sclerotesta (*sc.*) and base of the sarcotestal wing (*w.*). The vascular elements (*xy.*) form a row down the middle of the wing. The large outer cells of the sarcotesta (*m.c.*) are poorly preserved. *i.c.* = inner cells of sarcotesta; *n.* and *m.* = nucellus and megaspore. U. C. L., S. 46 (Shore).  $\times 90$ .

Fig. 12. Slightly oblique transverse section of part of sarcotestal wing. *m.c.* = mucilage cells. U. C. L., S. 57 (Hough Hill).  $\times 90$ .

Fig. 13. Edge of a transverse section through sclerotesta (*sc.*) and sarcotesta (*sa.*), showing inner cells of sarcotesta (*i.c.*), mucilage cells (*m.c.*), and small outermost cells (*o.c.*). U. C. L., S. 51 (Dulesgate).  $\times 150$ .

Fig. 14. Transverse section of sclerotesta (*sc.*) and sarcotesta (*sa.*) to show the proportion which the size of the unswollen mucilage cells (*m.c.*) bears to the thickness of the sclerotesta. U. C. L., S. 51 (Dulesgate).  $\times 28$ .

Fig. 15. Transverse section of sclerotesta (*sc.*) and sarcotesta (*sa.*) to show the relative size to which the mucilage cells (*m.c.*) may attain in extreme cases (cf. Fig. 14). U. C. L., S. 50 (Shore).  $\times 28$ .

Fig. 16. Small part of a slightly oblique longitudinal section, showing the edge of the sclerotesta (*sc.*) and the sarcotesta (*sa.*). *i.c.* = small inner cells of sarcotesta; *m.c.* = mucilage cells; *o.c.* = membrane probably representing the remains of the small outer cells of the sarcotesta. Wat., A. 113 (Dulesgate).  $\times 155$ .

Fig. 17. Transverse section of sclerotesta (*sc.*) and nucellus (*n.*), probably from near the top of a seed. Wat., A. 248 (Shore).  $\times 28$ .

Fig. 18. Nucellus from Fig. 17 enlarged. Possibly the section passes through the rather flat roof of the pollen-chamber. Wat., A. 248 (Shore).  $\times 387$ .

Fig. 19. Transverse section of a contracted and carbonized nucellus, probably near its apex, to show the four-angled form. D. H. S. 2259 (Shore).  $\times 42$ .

Fig. 20. Tracheides of branch vascular bundle from transverse section of seed, showing flattening and extension in the principal plane. [The position of the bundle can be understood by reference to Text-fig. 2, c.] D. H. S. 2428 (Shore).  $\times 375$ .

Fig. 21. Apex of nucellus in longitudinal section, showing obliquely cut pollen-chamber (?). U. C. L., S. 47 (Shore).  $\times 46$ .

Fig. 22. Longitudinal section of a small part of the nucellus, from a section in which it is unusually well-preserved, showing nucellar epidermis (*n.e.*), thin-walled nucellar tissue (*n.c.*), and nucellar tapetum (*tap.*). U. C. L., S. 36 (Shore).  $\times 155$ .

Fig. 23. Nucellar epidermis (*n.e.*) (or possibly nucellar tapetum) enclosing megaspore membrane (*m.*) (or possibly carbonized tapetum), from a transverse section. D. H. S. 2428 (Shore).  $\times 90$ .

## PLATE XXXIX.

## DRAWINGS.

Fig. 24. Part of a transverse section of a seed, showing the megaspore membrane (or carbonized tapetum) enclosing a tissue which may be a prothallus. D. H. S. 1803 (Dulesgate).  $\times 57$  circa.

Fig. 25. Thick-walled, pitted elements of the sclerotesta in transverse section. U. C. L., S. 53 (Shore).  $\times 244$  circa.

Fig. 26. Base of a longitudinal section cut in the principal plane. The numbers indicate the position of the details drawn in Figs. 27, 28, 29, 30. *v. b.* = main vascular bundle; *v. b.*' = branch bundle; *sc.* = sclerotesta; *b.* = tissue of seed-base below branch bundles. U. C. L., R. 60 *c* (Shore).  $\times 8\frac{1}{2}$  circa.

Fig. 27. Branch bundle cut longitudinally in its nearly horizontal course through the sclerotesta. (See '27' in Fig. 26.) *sc.* = sclerotesta; *xy.* = xylem; *ph.* = phloem; *b.* = tissue of seed-base. U. C. L., R. 60 *c* (Shore).  $\times 400$  circa.

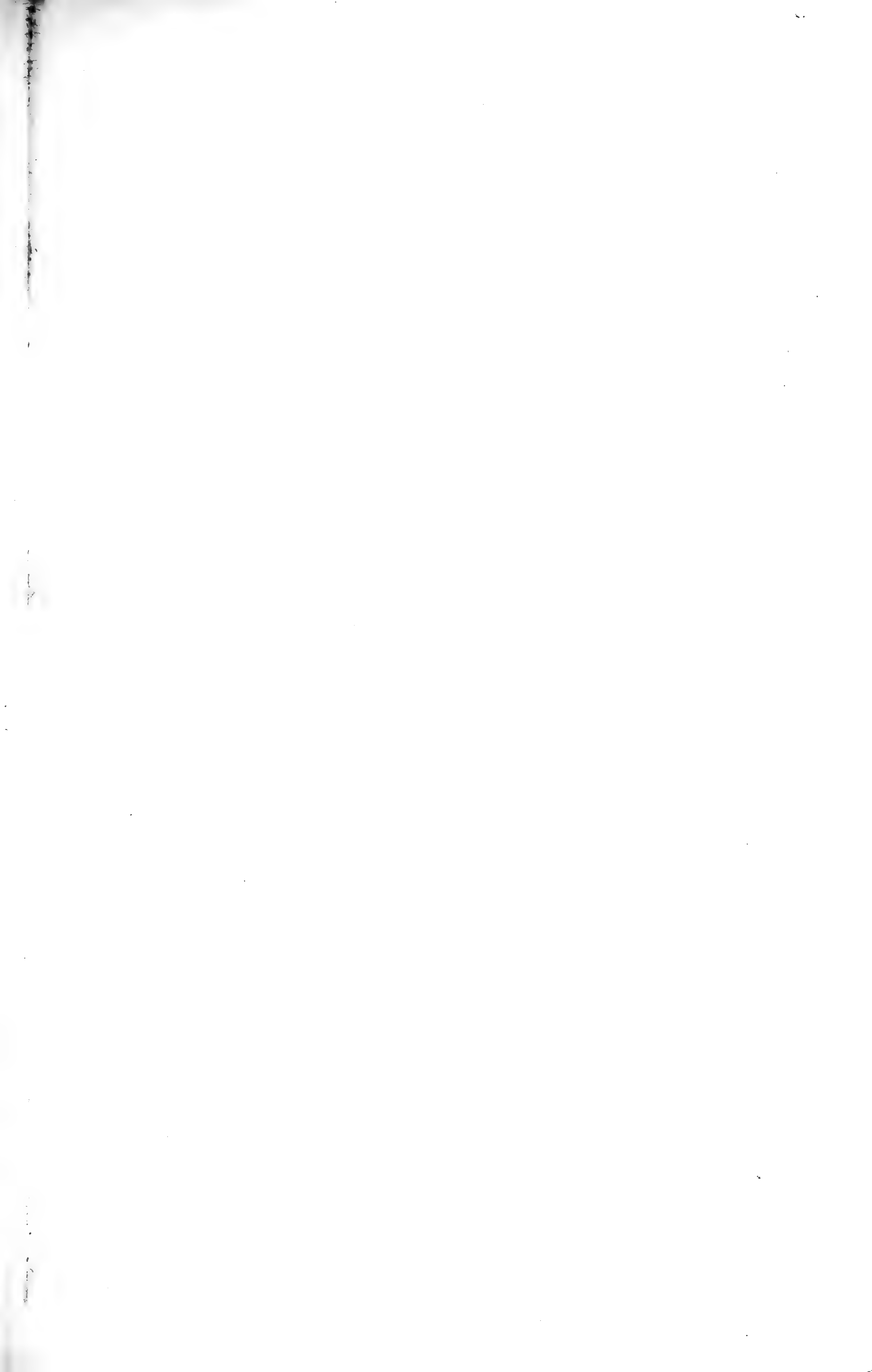
Fig. 28. Small part of the bundle drawn in Fig. 27, but further out, at the point where it is emerging from the shell and turning upwards (see '28' in Fig. 26). Protoxylem occurs on the lower side of the bundle. U. C. L., R. 60 *c* (Shore).  $\times 400$  circa.

Fig. 29. Short reticulate tracheide from just below the nucellus (see '29' in Fig. 26). U. C. L., R. 60 *c* (Shore).  $\times 400$  circa.

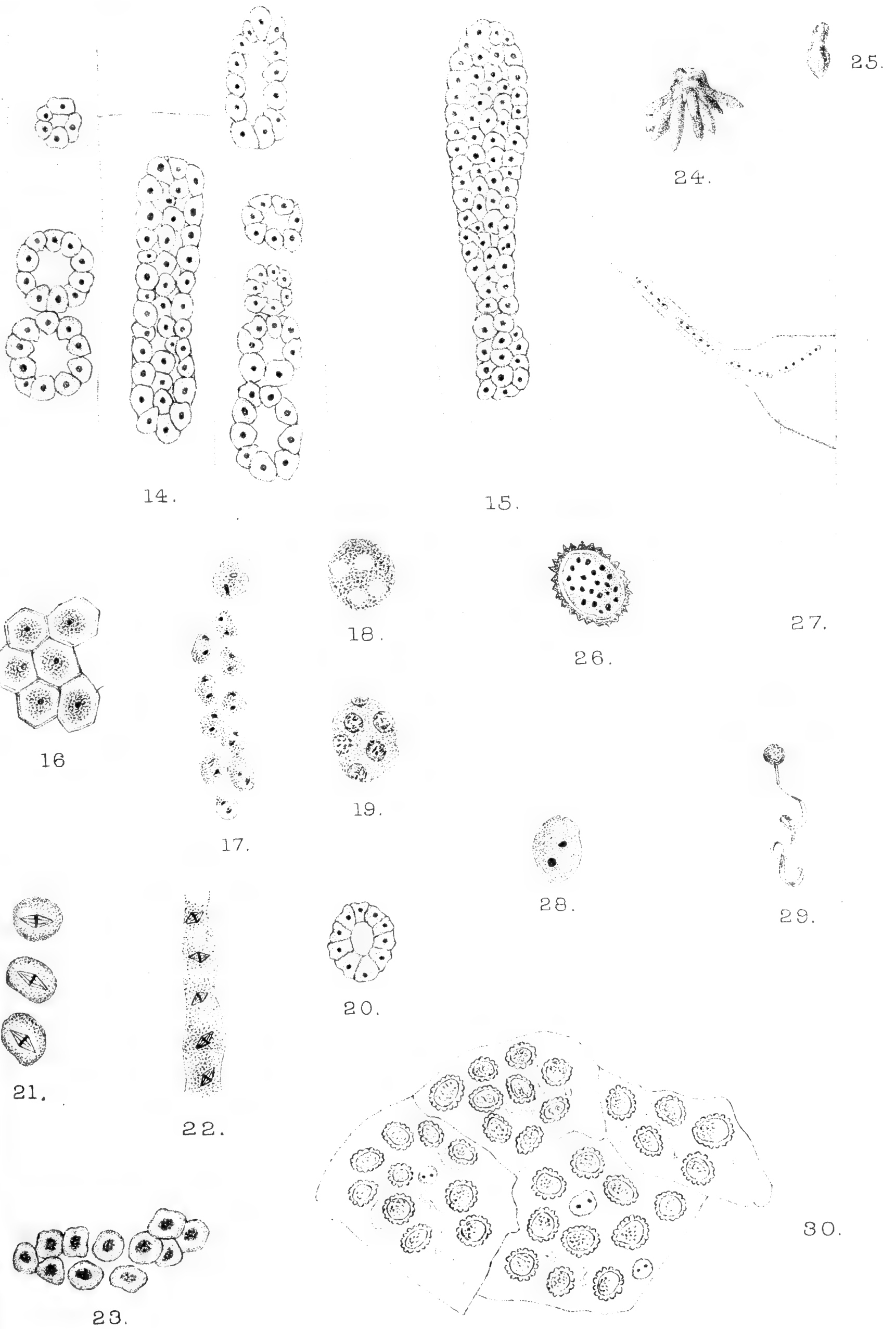
Fig. 30. Secretory cell and surrounding cells from seed-base tissue below the branch bundles (see '30' in Fig. 26). U. C. L., R. 60 *c*.  $\times 400$  circa.













# Parasitic Root Diseases of the Juncaceae.

BY

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

With Plate XL.

AT the suggestion of Professor W. B. Bottomley, to whom I am grateful for advice given during the progress of my work, I decided to undertake an investigation into the nature and structure of the tubercles or swellings which are frequently to be met with on the roots of various species of *Juncus* and *Cyperus*. The present communication (of which a preliminary notice, under the title of 'A new Parasitic Disease of the Juncaceae', appeared in the 'Annals of Botany', January, 1910) is the outcome of that suggestion. On looking up the literature of the subject I found that the structure and cause of the tubercle formations on the roots of *Juncus bufonius* were discussed by C. Weber in a paper entitled 'Ueber den Pilz der Wurzelanschwellungen von *Juncus bufonius*', which appeared in the 'Botanische Zeitung' in the year 1884. In this paper, in which he confines his description and diagrams to the tubercles occurring on *J. bufonius*, Weber states that they may also be found on roots of *Cyperus flavescens*, the plant to which the tubercle-forming fungus owes its name. *Juncus articulatus* (*acutiflorus*) is also mentioned as a host-plant by Magnus, who assigns the fungus causing the tubercle to the genus *Schinzia*. This genus included various uncertain forms, whose sole resemblance to the fungus found in the root-tubercles of the Juncaceae consisted in their being similarly connected with the formation of root-tubercles or swellings, such for instance as *Schinzia Alni* and *Schinzia Leguminosarum*. Weber in his paper arrived at the conclusion that the parasitic fungus found in the tubercles was nearly allied to the Ustilagineae, and he renamed it *Entorhiza Cypericola*. With this conclusion I am entirely in agreement.

I had proceeded but a little way with my investigation when I made the interesting discovery that the *Juncus* roots were at times infected by two distinct parasitic fungi, viz. the one (the *Entorhiza Cypericola*) causing the formation of the root-tubercles, and the other confined to the roots themselves, many of the cells of which were to be seen filled with spherical balls of spores, bearing a striking resemblance to the sorospheres of *Sorosphaera*



*Veronicae*. The latter fungus, which is, I believe, new to Botanical science, I propose to name *Sorosphaera Funci*, as being closely allied to *S. Veronicae*, the Mycetozoan parasite which causes the gall-like swellings on the stems and leaves of *Veronica* plants, which has recently been described by Maire and Tison in the 'Annales Mycologici' (9), and by Blomfield and the present writer in the 'Annals of Botany' (10).

I have found tubercles on the roots of various species of *Funcus*, viz. *bufonius*, *articulatus*, and *lamprocarpus*; these same three species at times were also to be found serving as host-plants for the *Sorosphaera*. Although I have not unfrequently found plants attacked simultaneously by both the *Entorhiza* and the *Sorosphaera*, yet on the other hand I have found no difficulty in obtaining plants the roots of which were infected only with the *Sorosphaera*, from sources which, after prolonged search, were found to be entirely free from plants attacked by the *Entorhiza*, and, vice versa, in obtaining roots with tubercles from sources free from the *Sorosphaera* disease. Of the two diseases, that caused by the *Sorosphaera* was considerably the more common, as far as my experience in finding diseased plants went. Roots of plants of *Funcus effusus* growing in the immediate vicinity of diseased Junci were microscopically examined and found to be free from disease of either kind; roots and stems of *Veronica Beccabunga* were also examined to see if the *Sorosphaera* had attacked them, but were found to be quite healthy. Attempts were made to inoculate plants of *Veronica Chamaedrys* with the *Sorosphaera Funci* by growing them in soil taken from ditches in which the *S. Funci* was abundant; the results, however, were negative, none of the *Veronica* plants forming any galls or showing any trace of disease. Diseased plants of all three species of *Funcus* were kept growing under observation in pots filled with soil which had been mostly taken from infected ditches. A few *Veronica* plants and healthy Junci were planted amongst these; the former, however, kept quite healthy, whereas the latter became diseased. The material which I employed in my investigation was collected by myself from marshy fields, ditches, and the margin of ponds in the neighbourhood of Sevenoaks, where many of the British Junci grow in abundance. I found *F. bufonius* growing in profusion in some trenches draining into the Darent, near Dunton Green, where the soil was a rich alluvium resting on the Gault; only a few ditches, however, were infected by the *Entorhiza*, and in these the diseased plants were very rare, being found in a proportion of about one per cent.; not any plants infected by the *Sorosphaera* were obtained by me from this spot. Plants of *F. articulatus* growing in a sunny position directly on the Gault margin of ponds near to the former position were altogether free from disease of either kind, their roots being white and clean. After considerable further search for infected material I at length came across a marshy meadow in which were some springs breaking out at the junction of the

Folkestone beds (Lower Greensand) with the Gault. Here the water and the rich alluvial soil were highly charged with iron salts dissolved by the former in its passage through the Greensand; the situation also was more shaded than the spot where I had commenced my search, and an altogether more favourable one for the presence of diseased plants. *F. bufonius*, *articulatus*, and *lamprocarpus* were very common, but plants of the first-named were very free from tubercles, about one in three hundred being diseased, although plants of the two latter species growing with them were in almost every case diseased, many possessing large tubercles, whereas those found on diseased *bufonius* plants were mostly small and insignificant. This comparative immunity of the *bufonius* plants might perhaps be accounted for by the fact that *F. articulatus* and *F. lamprocarpus* are perennial plants, whereas *F. bufonius* is an annual; nevertheless, it is difficult to see how this could afford a true explanation, as the ditches are cleaned out every year, and most of the perennial plants are quite young if not first-year plants; the roots, however, of the perennial plants contain large quantities of reserve starch, which is lacking in those of the annual *bufonius* plants. It would seem probable, therefore, that *F. bufonius* is more resistant to the disease, and has perhaps undergone some protective evolution against it.

I have laid stress on the soil and situation in which I found the diseased plants, as these are important factors, and largely determine the question as to the presence or absence of disease. Thus, for instance, it has been stated that *Sorosphaera Veronicae* is of very rare occurrence, but this is by no means the case if the search for diseased Veronicas is limited to those growing in damp shady situations. A sunny situation is apparently inimical to the presence of disease, or it is one in which plants are sufficiently robust to resist attack by parasitic fungi.

The material gathered was fixed in the field in Bouin's fixing fluid made up according to the formula:—

Crystallizable Acetic Acid, 2 c.c.

Formol, 10 c.c.

Aqueous solution Picric Acid (saturated), 30 c.c.

The picric acid was afterwards removed by repeated washings in 50 per cent. alcohol. Absolute alcohol and Flemming's solution were also tried as fixing agents.

Sections were cut with the microtome, and various stains used, the best results obtained being with Benda's Iron Haematoxylin. In the case of those plants gathered in the vicinity of the Folkestone beds it was possible to dispense with the use of the iron solution, as the roots had been already thoroughly impregnated in their habitat. Ehrlich's Haematoxylin and Flemming's triple stain were used in a few cases. Many of the smaller thin diseased roots were not suitable for sectionizing, but stained, teased-

out portions thereof were mounted, and served as well as sections; indeed, they showed the root-hairs to better advantage than the sections did.

#### SOROSPHERA JUNCI.

Whilst examining microscopically a piece of the root of a plant of *F. articulatus* for the purpose of ascertaining whether the hyphae or spores of the *Entorhiza* were present in it, I was surprised and interested in discovering certain spherical balls, which, consisting as they did of a series of wedge-shaped spores surrounding a central cavity, reminded me forcibly of the sorospheres of the terminal phase of *Sorosphaera Veronicae*; the shape of these sorospheres was, however, not always spherical, but more frequently ellipsoidal, and at times they appeared as merely loosely aggregated masses of spores filling the root-cells; in size, too, the spherical sorospheres were mostly smaller than those found in *Veronica* tumours. A more extended search revealed the presence of plurinucleate amoebiform organisms in some of the root-cells of the younger roots, which convinced me that the roots were attacked by a Mycetozoan parasite allied to the *S. Veronicae*, whose life-history I had recently studied. This opinion was strengthened and confirmed by observing the presence of numerous oil-drops in the infected cells.

As the life-history of the parasite closely follows that of *Plasmodiophora Brassicae* and *Sorosphaera Veronicae*, it may, as in their case, be divided into stages, viz. the vegetative or schizont and the reproductive or sporont, each of these stages being characterized by its nuclei, and these two stages being separated by an intervening phase known as the akaryote or chromidial stage.

*Mode of infection.* The earliest stage I have observed of the *Sorosphaera* as a parasite in the root of a *Funcus* plant consisted in the presence of a small plurinucleate amoeba, having six nuclei, in one of the root-hairs of a young root of *F. articulatus*. Each of these nuclei had a large nucleolus, or karyosome, and was surrounded by a definite nuclear membrane, with granules of chromatin at the periphery; the cytoplasm of the amoeba was of the usual granular type, and minute drops of oil were found to be contained in it. An illustration of this infected root-hair is given in Pl. XL, Fig. 10. A microscopical examination of the root-hairs showed that they formed one means by which the parasite gained an entrance into the root, as amoebae in various stages of development were to be seen in them. Part of one such hair filled with the protoplasm and numerous nuclei of the parasite—many of them in an active stage of division—is shown in Fig. 5; a portion of a similar root-hair obtained from a plant uprooted in the late autumn after the onset of frost is represented in Fig. 13, where it is shown filled with the spores of the *Sorosphaera*. It is probable that the actual infection is effected by the entry of a mononucleate amoeba into

a root-hair, not necessarily at its extremity, and by the repeated division of this nucleus a chain of nuclei is produced, with the result that the root-hair is completely filled with the protoplasm and nuclei of the amoeba, and thus the organism reaches the base of the hair and the outer cortical cells of the root. These root-hairs are long and sufficiently strong to resist being torn in the process of removing the plant from the soil. In some roots a considerable number of the hairs exhibit the presence of these amoebae.

It is thus evident that, whereas in the case of *Veronica* plants the infection is a primary one, taking place at the growing apex, in the case of *Funcus* roots it may occur through many root-hairs and at various times during the growth of the root. That the outer cortical cells of the root may be directly infected without the intervention of a root-hair, is no doubt possible, though I have not actually observed it; further, it is probable that the superficial layers at the growing apex may be infected, and so give rise to diseased outer cortical cells; this is rendered likely from the fact that diseased cells are sometimes found in longitudinal rows.

The nuclei of the amoebae divide by a process to be described later, without any division of the amoebae themselves. In this way the amoebae increase in size until they completely fill the root-cells that contain them; at the same time, the granular protoplasm of the organism is clearly differentiated from that of the plant-cell, from which also it may be distinguished by the presence of oil-drops. At times these plurinucleate amoebae behave as schizonts, inasmuch as portions are split off, thus giving birth to uninucleate or multinucleate daughter-amoebae — the so-called 'meronts'. The shape of the amoebae may be rectangular, which is the case when they occupy the whole of the root-cell, or it may be ellipsoidal, or roughly spherical; it is in these latter cases that the splitting off of meronts, or schizogony, as it is called, is usually to be observed. There is no formation of a plasmodium by the coalescence of different amoebae; indeed, a root-cell containing more than a single amoeba is rarely found.

*Nuclear division during the vegetative phase.* As in the cases of *Plasmodiophora Brassicae* and *Sorosphaera Veronicae*, we meet with the same process of nuclear division in *S. Funci*. It is only after the examination of a very large number of nuclei that any of the stages of nuclear division can be discovered. This is probably due to the short time required by the nucleus for the act of division, and also to the difficulty of rapidly fixing a root which has been taken from a muddy soil. In any amoeba the nuclei seem to divide simultaneously, so that all its nuclei may be seen in the same phase of the division. The first change to be observed in the nucleus prior to division is that it becomes somewhat elliptical in shape, then the karyosome elongates, and at the same time the chromatin granules collect into an equatorial plate, and we have the cruciform figure described by various observers in *P. Brassicae* and *S. Veronicae*. Viewed from the



side, this cruciform structure is seen to consist of the elongated karyosome encircled by an equatorial chromatic ring or plate, the relation of the karyosome to the ring being that of an axle to the rim of a wheel. I have not observed any splitting of this plate, though in all probability this occurs. The next stage, which, however, I have not so commonly seen, is similar to the dumb-bell-like stage observed in *S. Veronicae*, and is doubtless formed by the splitting of the plate and the passage of the two halves, one to each extremity of the elongated karyosome, which then becomes very slender. From this point the completion of the division is easy to follow; the equatorial portion of the original nuclear membrane disappears, and the nuclear membranes of the two daughter-nuclei are completed by the formation of a new transverse piece of membrane. Figs. 2, 3, and 4 show the various stages described above.

*The akaryote stage and the sporont.* When the parasite is about to form its sorospheres, i. e. when the schizont is about to be transformed into the sporont, it is observed that the amoebae assume a spherical or ellipsoidal shape, the nuclear membrane disappears, and the karyosome diminishes in size and finally disappears also, so that we have a number of vacuoles more or less circular in outline situated in the spherical mass of plasma. This stage is shown in Fig. 18. In the case of those cells which are completely filled with a single parasitic amoeba a similar change takes place, but in this case the organism retains the shape of the cell. This stage is followed by the appearance of granules and irregular masses of chromatin forming fresh nuclei in the vacuoles referred to. The protoplasm of the amoeba collects around these freshly-formed nuclei, and the amoeba thus separates into a number of well-marked amoebulae, each having a single nucleus and a small quantity of surrounding protoplasm.

In this condition the nature of the parasite is completely altered, and in place of the plurinucleate amoeba we have a number of small uninucleate independent amoeboid organisms, which, while quite distinct from one another, nevertheless tend to aggregate into masses either of more or less spherical shape or to loosely fill the root-cells. These amoebulae are to be seen in Figs. 17 and 23.

The nuclei of these amoebulae then undergo two ordinary mitoses, the spindle formed in the latter of these being markedly smaller than that in the former, thus indicating a reduction division. During the progress of these two nuclear divisions, polar radiations may be seen proceeding from each pole, doubtless due to the presence of centrosomes, which bodies, however, I have not actually been able to see. Owing to the minuteness of the nuclei, which are more difficult to observe than those of *S. Veronicae*, it was found impossible to ascertain the number of the chromosomes. I have observed no conjugation of nuclei either at this or any other stage of the life-history of the parasite, although it might take place after the germina-

tion of the spores; unfortunately, I have not been successful in my attempts to germinate them. After the second of these mitoses each amoebula secretes for itself a wall which gradually thickens, and the amoebula is transformed into a spore; this wall, however, does not give any of the usual cellulose reactions, and probably contains chitin, a substance which, according to Wisselingh (6), is commonly to be met with in fungal cell-walls. The spores are wedge-shaped, with a single nucleus slightly nearer to the broader end; this nucleus stains readily, and especially before the spore coat has thickened much; in no case have I observed binucleate spores. These spores are either collected into sorospheres or loosely aggregated; in either case they are usually enclosed by a common membrane, though this latter is sometimes absent, as, for instance, from the small groups of spores observed in root-hairs. A common form of spore arrangement consists of two rows filling a root-cell, the wedge-like shape of the spores lending itself to close packing. Various forms of sorospheres and spore collections are shown in Figs. 14 and 15.

*Structure of diseased roots.* To the naked eye the roots of diseased plants show scarcely any indication of disease, save that they may be slightly thinner and of a paler hue than those of uninfected plants, this latter distinction being perhaps due to the absence of starch. After some practice I was able to pick out roots in which the disease had advanced to the stage of sorosphere formation, but could only determine those with the earlier stages by microscopical examination. The infected cells are confined to the outer layers of the cortex; these cells, however, are quite normal in size, nor do the roots themselves show any signs of hypertrophy. In the early stages, as shown in Fig. 1, the infected cells are filled with the cytoplasm and nuclei of the parasite; frequently we have a single row of such nuclei, or at other times they form a double row in the root-cell. Rarely are to be found cells containing two or three separate amoebae. The infected cells themselves are never plurinucleate, as was so frequently the case in the hypertrophied cells of the *Veronica* and *Brassica* tumours, nor, as a rule, are the cell nuclei enlarged or degenerated to any extent. In later stages the cortical cells are to be seen filled with sorospheres or with loosely aggregated spores, as is shown in Figs. 12 and 14. The disease apparently does not penetrate deeply into the root, but is confined to the outer cortical layers; as far as my observations go, the amoebae have no power of penetrating the cell-walls, a conclusion which is confirmed by the fact of the disease not spreading deeply into the root from the diseased outer portion of the cortex. As in the cases of the *Veronica* tumours and the clubbed roots of Cabbages, the stores of starch are largely depleted in the infected regions. The fact of there being no hypertrophy of the *Funcus* roots is of considerable interest, because it is contrary to the usual result of a plant becoming a prey to such a fungoid disease, e.g. *P. Brassicae* and



*S. Veronicae.* It is the more strange from the fact that the *Funcus* roots form tubercles or nodules in response to the stimulus of the parasitic *Entorhiza* and also the abnormal stem and leaf structures when attacked by the mite '*Livia Funcorum*'. This freedom from hypertrophy may probably be accounted for by the superficial situation of the disease contrasted with the more deeply-seated cambial infection found in *Veronica*. With but one exception, I have never found any trace of the *Sorosphaera* in the tubercles caused by the *Entorhiza*, nor indeed on the actual roots bearing these tubercles. In the course of my investigations I have met with plants attacked simultaneously by all three diseases, viz. the *Entorhiza*, the *Sorosphaera*, and the *Livia*, and still to all appearance healthy and capable of giving rise to vegetative offspring by rooting at the joints of their prostrate stems; in these instances such of the roots as remain healthy are sufficient for the needs of the plants. Of the three diseases—the club-root of Cabbage, the *Veronica* tumours, and the *S. Funci*—the first is the most destructive, as, by attacking the tap-root in addition to weakening the plant by the formation of the large club, or tumour, the adequate supply of soil water to the plant is intercepted, and in hot weather wilting ensues. In the *Veronica* plants no such destruction occurs, and as the stem only is affected, a minimum amount of harm is done to the plant, which usually possesses other healthy stems. In the present case the *Funcus* plant possesses a number of fibrous roots and not a main or tap-root, so that if a few of its roots become diseased, it probably still has or forms a sufficient number of healthy ones for its needs. In all three instances harm results to the plant, this being evidenced by the considerable depletion of starch which occurs. I see no reason to describe the relationship between the fungus and the plant at any stage of the disease otherwise than that of parasite and host, although with reference to the early stages of *Brassica* tumours it has been spoken of as a symbiotic relationship.

*Systematic position and affinities of Sorosphaera Funci.* Maire and Tison, following Schröter's classification in their paper on the Plasmodiophoraceae (= Phytomyxineae, Schröter), divide them into the three following genera:—

1. *Plasmodiophora*, Wor. Sporont giving rise to free spores.
2. *Sorosphaera*, Schröt. Sporont giving rise to spores collected into spherical balls.
3. *Tetramyxa*, Goebel. Sporont giving rise to spores in tetrads.

Schröter had included a fourth genus, *Phytomyxa*, with free, rod-shaped spores; this was represented by the organism which is now known as *Pseudomonas radicicola*, the bacterium of the leguminous root-tubercles. It is obvious that the parasite of the *Funcus* roots should be classed with the genus *Sorosphaera*, for the wedge-shaped spores are to be found collected

into hollow spherical balls in all respects similar to the sorospheres of *S. Veronicae*. It is true that the spores of *S. Funci* are more frequently collected into ellipsoidal masses or are merely loosely aggregated and fill the root-cells; this, however, is probably due to the non-hypertrophy of the root-cells of the host-plant, which by retaining their normal size do not allow sufficient room for the parasite to form its typical sorospheres. The cramping effect of the narrow root-cells on sorosphere formation is also evidenced by the presence of small sorospheres consisting at times of only eight spores; to the same cause may be attributed the comparative rarity of schizogony, which is of such frequent occurrence in the enlarged cells of *Veronica* tumours. The description of the genus '*Sorosphaera*' should be modified so as to include *S. Funci* into—'Sporont giving rise to wedge-shaped spores either loosely aggregated or collected into spherical or ellipsoidal hollow balls, and usually enclosed by a common membrane.'

The present writer is in agreement with the conclusion arrived at by Maire and Tison in their paper relating to the affinities of the Phytomyxineae, to the effect that these fungi should form a distinct order intermediate between the Sporozoa and the Mycetozoa. They are to be distinguished from the latter by the form of their vegetative nuclear division, their non-cellulose spore membrane, the non-formation of plasmodia, and, lastly, by their parasitic mode of life. The Acrasieae differ from the other Mycetozoa in not forming plasmodia, and to that extent approach somewhat nearer to the Phytomyxineae. The Chytrideae differ in the formation of zoosporangia.

In 1899 Schröter, in his article on the Phytomyxineae in Engler and Prantl's '*Die Natürlichen Pflanzenfamilien*', included the following species:—

1. *Plasmodiophora*—*Brassicae*, Wor.; *Alni*, Moll.; and *Elaeagni*, Schröt.
2. *Tetramyxa parasitica* (parasitic on *Ruppia rostellata* and *Zannichellia palustris*), Goeb.
3. *Sorosphaera Veronicae*, Schröt.
4. *Phytomyxa Leguminosarum*, Schröt.

From this list *P. Alni*, *P. Elaeagni*, and *P. Leguminosarum* have since been removed. The root-tubercles of the Alder and *Elaeagnus*, of which I have cut and examined many sections, show no evidence of the existence of any nuclear parasite, and the organisms seen in them bear only a superficial resemblance to a *Plasmodiophora*. The root-nodules of leguminous plants have now been recognized as due to the symbiotic presence of bacteria.

We have now to add to the list given by Schröter *Tetramyxa Triglochinis*, Moll., parasitic on *Triglochin palustre*, and also recently found on *Triglochin maritimum* by Maire and Tison, and *Sorosphaera Funci*, parasitic on various species of *Funcus*, found by the present writer.

## ENTORHIZA CYPERICOLA.

The life-history of this fungus, which causes the formation of the root tubercles, was studied and described in 1884 by Weber. My own experience has enabled me to confirm the greater number of his observations, though whereas he worked with the tubercles on the roots of *J. bufonius*, I have used those on *J. articulatus* and *lamprocarpus*, finding these easier to obtain. I have had no opportunity of seeing the disease on *Cyperus flavescens*.

The tubercles vary considerably in size ; the largest I found on *J. bufonius* were about 5 mm. long and unbranched, while those on *J. articulatus* were sometimes as much as 10 mm. in length, and frequently branched ; also they were proportionately thicker than the tubercles on *J. bufonius*. The tubercles themselves are the swollen, modified ends of roots ; in section they show a central stele surrounded by an enlarged cortex, all the cells of which, except those comprising the outer layer or perhaps two layers, are dominated by the fungus, being for the most part filled with spores in various stages of development. In longitudinal section it is observed that the oldest spores occupy a position near to the base of the swellings. The mycelium of the fungus is within the cells of the host-plant or partly intercellular ; the *intracellular hyphae* are very delicate and much twisted in corkscrew fashion. The spores are formed as terminal swellings of these hyphae ; at first they are white and glistening and binucleate, afterwards they thicken and take on a brown coloration, and when ripe they have two coats, the outer or episore being spiny, and in general microscopic appearance they resemble typical *Ustilago* spores.

The tubercles are cream in colour when young, but turn brown or black when the spores ripen ; those gathered in the autumn from the annual *bufonius* plants are mostly black. There is no formation of cork round the tubercles of the Juncaceae, as is usually the case in other root-tubercles, e. g. those of *Elaeagnus*.

The mode of infection appears to be by the entry of conidia into the root-hairs as is shown in Fig. 17 ; these conidia multiply in the hair which they thus travel down, and, after entering the root, give rise to hyphae. Most plants from infected ditches show root-hairs of this nature, whereas those from healthy or non-infected places only have root-hairs of the normal type ; it therefore seems probable that these conidia-containing hairs represent the stage of entry of the parasite. Weber does not state how the fungus gains an entry into the root. He was successful in germinating the spores after a winter's rest ; having kept the tubercles in the open in moist sand through the winter, he observed germination in the February of the following year at the moderate temperature of 10° C. These spores germinated when sown in water : Weber, however, obtained better results

by allowing them to do so *in situ* in the tubercle; if kept continuously in the open they germinated early in May.

I regret that all my attempts at germinating the spores were unsuccessful, the more so as in the absence of germination it is impossible to absolutely establish the mode of infection. I kept spores in the open through the winter under about a quarter of an inch of water, which was approximately similar to their natural condition, for many of the nodules are found scarcely beneath the soil under a depth of about one inch of water; I also found tubercles early in March which had wintered in their natural habitat. Spores from both these sources were sown in water and in various culture media containing sugar or plum-juice; temperatures ranging from 10° C. to 25° C. were maintained, but in no case was any good germination observable, the best results being from a spore which produced three small hyphae, but these gave rise to no sporidia.

Weber states that the spores produced promycelia, from the ends of which sporidia were abjuncted; nevertheless, he was unable to cause infection to healthy plants by means of the spores thus germinated.

The result of my observations on these tubercles has been to confirm the conclusions arrived at by Weber, excepting as regards the spore germination, and to suggest the probable mode of infection by the entry of conidia into root-hairs. Struck by the healthy appearance of the aerial parts of *Funcus* plants whose roots may in reality bear three or four large tubercles, one is inclined to think that perhaps the fungus stimulates the plant and increases its power of absorption of food from the soil.

In conclusion, it is interesting to note that the fruits of *F. bufonius* are at times infected by an Ustilaginous fungus, *Tolyposporium Funci*; but whether this is related in any way to the *Entorhiza*, I am not in a position to say, not having been successful in finding it.

My thanks are due to my sister, Miss Alice M. Schwartz, for her assistance in making the drawings.

#### SUMMARY AND CONCLUSIONS.

1. The roots of *Funcus bufonius*, *F. articulatus*, and *F. lamprocarpus* are subject to the attack of two distinct parasites, viz. *Sorosphaera Funci* and *Entorhiza Cypericola*.

2. The life-history of *S. Funci* closely follows that of *S. Veronicae*, to which it is nearly related, the methods of nuclear division being similar.

3. Infection by the *Sorosphaera* occurs by the penetration of an amoeba into a root-hair and thence into the root itself.

4. The roots attacked by the *Sorosphaera* are not hypertrophied, whereas those attacked by the *Entorhiza* form tubercles.

5. Infection by the *Entorhiza* is probably effected by the entry of conidia into a root-hair.

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## EXPLANATION OF PLATE XL.

Illustrating the Paper by Mr. Schwartz on Root Diseases of the Juncaceae.

The drawings were made with the aid of the Camera Lucida.

- Fig. 1. Portion of tangential section of a root, showing the early stage of the disease. × 512.
- Fig. 2. An amoeba with its nuclei in the 'cruciform' stage of their division. × 1100.
- Fig. 3. An amoeba with its nuclei in the 'dumb-bell' stage of division. × 1100.
- Fig. 4, a-h. Various stages of nuclear division. See Text for description.
- Fig. 5. Portion of a root-hair infected by the *Sorosphaera*. × 730.
- Fig. 6. Infected cortical cell. × 730.
- Fig. 7. Enlarged drawing of 'cruciform' stage of nuclear division.
- Fig. 8. Amoeba showing schizogony. × 730.
- Fig. 9. Dumb-bell stage of nuclear division. × 730.
- Fig. 10. Root-hair with infection by a six-nucleate amoeba. × 730.
- Fig. 11. Root-hair infection. × 730.
- Fig. 12. Cortical cell filled with spores. × 730.
- Fig. 13. Portion of root-hair with spores. × 730.
- Fig. 14. Portion of outer cortex of root showing the disease in its reproductive stage. × 730.
- Fig. 15. A sorosphere. × 730.
- Fig. 16. Surface view of a portion of a sorosphere. × 1180.
- Fig. 17. Nuclear division being completed in amoebulae prior to spore formation. × 730.
- Fig. 18. Chromidial or akaryote stage. × 1100.
- Fig. 19. Formation of fresh nuclei in the vacuoles. × 1100.
- Fig. 20. A sorosphere. × 730.
- Fig. 21. First mitosis of newly-formed nuclei. × 730.
- Fig. 22. Second mitosis of newly-formed nuclei. × 730.
- Fig. 23. Amoebulae forming into spores. × 730.
- Figs. 24 and 25. Root-tubercles of *J. articulatus*. Natural size.
- Fig. 26. Ripe spore of *Entorrhiza*. × 730.
- Fig. 27. Conidial infection of root-hair by *Entorrhiza Junci*. × 730.
- Fig. 28. Young binucleate spore. × 730.
- Fig. 29. Formation of young spore at end of twisted hypha. × 730.
- Fig. 30. Portion of section of root-tubercle of *J. articulatus*. × 325.



# The Cones of the Genus *Selaginella*.

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With Plate XLI.

IN this paper we do not propose to give an exhaustive account of the cones throughout the genus *Selaginella*, but merely, by describing a few of the more typical species, to draw attention to the great divergence in the form of the sporophylls found in this genus, and in each case to point out the special adaptations for the secure protection of the sporangia. Some of the more complex forms of cone are found to prevail in species which from their radially distributed leaves have been regarded as more primitive in the genus than are the dorsiventral species; this fact is of some interest when considered in relation to various difficult questions concerning the development of the sporangium-bearing organs among the Pteridophyta.

The material of *S. pumila* was obtained by Professor Pearson at Stellenbosch, Cape Colony, and was handed over to us by Professor Seward; *S. spinosa* and *S. helvetica* were collected by one of us in Switzerland; we also examined *S. Vögeli*, *S. flabellata*, and *S. apus*, var. *elegans*, grown in the Cambridge University Botanic Garden, and *S. caulescens*, of which we used laboratory material. Our thanks are due to Mr. Lynch for kindly sending *S. apus*, var. *elegans*, to Kew for identification.

## DESCRIPTIVE.

*General.* No attempt has hitherto been made to distinguish between the forms of sporophyll characteristic of the various species of *Selaginella*. Besides the simple sporophylls commonly attributed to the genus, closer examination has now revealed several more complex types. Before describing these it may be well to refer to some characters which are common to all or several of the species.

The cones are usually square, with four rows of sporophylls. The sporangium is generally borne in the axil of the sporophyll. As has been



described by Goebel<sup>1</sup> and Bower,<sup>2</sup> it always arises from stem tissue, but its first rudiments may develop either in the axil of the sporophyll, or some little way above the axil. In some species, indeed (e. g. *S. pumila*), even the mature sporangium is never found in the axil of the sporophyll, but is always borne on the stem above the axil.

The line of dehiscence of the sporangium<sup>3</sup> is perpendicular to its plane of symmetry. It extends for some distance down both sides of the sporangium, and, as the latter is slightly inclined towards the main axis, is cut twice by most of the tangential sections of the cone which pass through it (see Fig. 4, Pl. XLI).

There is a well-developed air cavity in the base of many of the sporophylls, a fact which does not seem to have been recorded before; these air cavities appear to us to be of some interest in that they recall the mucilage cavities of *Lycopodium* and the parichnos of fossil genera.<sup>4</sup>

The sporophylls of some species are provided with dorsal outgrowths of a more or less pronounced nature. Hieronymus<sup>5</sup> refers briefly to small projections or 'Öhrchen' developed at the base of the sporophyll in *S. microcladus*, &c., and Lyon<sup>6</sup> has figured a dorsal flap in *S. rupestris*, but even these isolated cases have not been described.

We will now proceed to the description of the various types of sporophyll found in this genus.<sup>7</sup>

*Type 1.* The most interesting and complex type of sporophyll is that found in *S. pumila*. This species has its foliage leaves radially arranged, and there is therefore a presumption that it approaches the original primitive form of the genus *Selaginella*. The sporophylls form a compact cone, and are arranged in four rows; the micro- and megasporangia are somewhat irregularly distributed, and are to a certain extent exposed between the sporophylls (Fig. 1, Pl. XLI). The macrosporangia appear regularly to contain four fertile macrospores; the microsporangia are saddle-shaped. The stalk of the sporangium contains a few more or less distinctly elongated cells, whose walls are slightly lignified;<sup>8</sup> this character, however, is by no

<sup>1</sup> Goebel, K., 1881, p. 697 ff.

<sup>2</sup> Bower, F. O., 1894, p. 522 ff.; also Hieronymus, G., in Engler and Prantl, i. 4, p. 655.

<sup>3</sup> Goebel, K., 1901, has described the method of sporangial dehiscence (also Organography, ii, pp. 580-2). See also Mitchell, G., 1910, pp. 25-39.

<sup>4</sup> See Hill, T. G., 1906. Hill points out that hitherto nothing comparable with a parichnos had been recorded in *Selaginella* (p. 270).

<sup>5</sup> Hieronymus, G., p. 654.

<sup>6</sup> Lyon, F., 1901, Fig. 124, Pl. IX.

<sup>7</sup> A recent article on the strobilus of *Selaginella* (Mitchell, G., Ann. of Bot., Jan., 1910) has dealt with the questions of the relative distribution of the two kinds of sporangia in the cones of the different species, and has revealed some interesting points in connexion with spore dispersal, &c. The stelar anatomy has also been dealt with. The present paper was complete before the appearance of Miss Mitchell's article, but we have tried to avoid repetition by cutting out such portions as are now merely confirmatory observations.

<sup>8</sup> Cf. Sykes, M. G., 1908 (2), *L. serratum*, Fig. 2, Pl. III, and *L. clavatum*, p. 48.

means so prominent as in some others of the species examined. The sporangium originates (Fig. 2, Pl. XLI) from stem tissue, and though it later becomes associated with a so-called sporophyll, it is never in this species placed accurately in its axil, but is always borne on the stem some little way above the axil (Fig. 3, Pl. XLI).

The sporophyll is shortly stalked, and, in addition to the large, upward extending portion of the lamina, there is a downward growing dorsal flap<sup>1</sup> (Figs. 1 *b, c*, 2, 3, Pl. XLI). The upper portion folds round, and to some small extent protects the sporangium associated with it. The dorsal flap protects the sporangium which occurs vertically below the sporophyll,<sup>1</sup> and a groove is also present in the sporophyll stalk into which this sporangium fits (*g*, Figs. 1 *c* and 4, Pl. XLI). Fig. 4, Pl. XLI, represents a tangential section through the cone of *S. pumila*, and clearly shows the relative positions of sporangia and sporophylls.

The line of dehiscence of the sporangium is displaced slightly downwards towards the subtending sporophyll; dehiscence thus occurs in the position which in this species best ensures the liberation of the spores opposite the narrow gap between the sporophylls (see Fig. 3, Pl. XLI), at the point most convenient for their dispersal. There is some feeble lignification at the base of the ligule.

It may be of some interest to note that small projections occur on the dorsal surface of the lowest whorl of sporophylls forming the cone, although below these there are of course no sporangia (Figs. 1 and 1 *a*, Pl. XLI).<sup>2</sup> No such down-growths are developed in connexion with the vegetative leaves.

The stalk of the sporophyll contains no air space, unless we should include in this category a very minute space sometimes found accompanying the stele for a short distance on its lower side (*a*, Fig. 3, Pl. XLI).

*S. rupestris* is the only other form which has yet been described whose sporophylls are similar to those of *S. pumila*. In the cone of this species also, according to Lyon,<sup>3</sup> a large dorsal flap is present, which protects the sporangium vertically below it. The whole sporophyll here appears to be composed of loosely arranged tissue containing numerous intercellular spaces.<sup>4</sup>

It is interesting to find that these two species are placed near one

<sup>1</sup> The arrangement of sporophylls and sporangia in this cone forcibly recalls *Lycopodium cernuum* (Sykes, M. G., 1908 (2), pp. 48-9; Lang, W. H., 1908); the sporophylls are perhaps even more strictly comparable with those of *L. alpinum* (Sykes, M. G., 1908 (2), p. 48, Fig. 8 *a* and *b*, Pl. II).

<sup>2</sup> In *S. Preissiana* Goebel found that the lowest sporophylls resembled the foliage leaves instead of being intermediate in character, as in this species (Organography, ii, p. 506).

<sup>3</sup> Lyon, F., 1901, Fig. 124, Pl. IX.

<sup>4</sup> Miss Mitchell (1910, p. 21) refers to the presence of a 'strongly recurved portion' of the sporophyll 'at the junction of the leaf base and the upturned lamina' in both *S. rupestris* and *S. Lyallii*; she regards the 'strongly recurved portion' in these two species as a special protective adaptation, but does not add any description.

another in the most primitive group of the genus, by both Baker<sup>1</sup> and Hieronymus,<sup>2</sup> on the ground that neither species is dorsiventral.

A section of the cone of an *unknown species* was found in a cabinet of slides at the Cambridge Botany School; unfortunately we have not been able to identify it. The sporophylls of this cone had a remarkably well developed free dorsal flap, relatively larger than that described in *S. pumila*, and quite solid without any air cavity.

*Type 2.* The only other species examined which is not dorsiventral is *S. spinosa*. This form is also placed by Baker in his first group, along with the other non-dorsiventral species, but its many peculiarities make it difficult to regard it as primitive.<sup>3</sup>

Its cone differs from the cones of the other species in being cylindrical; it is very lax and is composed of large simple sporophylls with ciliate margins.<sup>4</sup>

The sporophyll is not folded round the sporangium growing in its axil, and hardly gives it any protection. The sporangium is thus exposed on a sporophyll which is almost flat (Fig. 5, *a* and *b*, Pl. XLI), except for a slight depression into which the lower surface of the sporangium fits. There is no definite dorsal flap visible to the naked eye, but minute examination shows a very slight dorsal swelling, most prominent in the median plane of the sporophyll; the interior of this swelling is occupied by a small air cavity (Fig. 5 *d*, Pl. XLI).

The megasporangia each contain four megaspores. The microsporangia are very large, much elongated laterally, and saddle-shaped (Fig. 5 *c*, Pl. XLI), recalling the sporangia of some of the Lycopodiums. There are occasionally a few lignified cells in the stalk of the old sporangia, but they are not present in the younger stages.

At the base of the ligule is a cup of tracheides, similar to those recorded by Gibson<sup>5</sup> in the vegetative leaves of the species he examined. We did not, however, find this cup of tracheides in the sporophylls of most of our species.<sup>6</sup>

*Types 3 and 4* (dorsiventral). Among the dorsiventral species a number of forms have been examined. Type 3 is somewhat isolated and is described first: the forms included in Type 4 constitute a series which appears to us to suggest that there has been a progressive change in the form of the sporophyll. From the complex sporophyll with its freely projecting dorsal flap, such as we have described in *S. pumila*, and such as is probably present in *S. Lyallii*,<sup>7</sup> there have been derived sporophylls in which this free

<sup>1</sup> Baker, J. G., 1887.

<sup>2</sup> Hieronymus, G., pp. 653-664.

<sup>3</sup> e. g. the absence of a foot in the embryo; cf. Bruchmann on *S. spinosa*, 1897, with Pfeffer on *S. Martensii*, 1870. Gibson appears also to regard the extremely simple stele in *S. spinosa* as peculiar rather than primitive (1894, pp. 171-173).

<sup>4</sup> Glück, H., 1895, p. 355.

<sup>5</sup> Gibson, R. J. H., 1896.

<sup>6</sup> See Mitchell, G., 1910, p. 32.

<sup>7</sup> Figured by Hieronymus, G., in Engler and Prantl, i. 4, p. 708, Fig. 408, and referred to by Mitchell, G., 1910, p. 21.

projection is much reduced, but is still to some extent adapted to the protection of the sporangia below it. Finally, there are sporophylls in which this dorsal projection is entirely absent; each sporangium is closely enfolded and protected by the sporophyll which subtends it. It would seem as if this close enfolding of the sporangium by its own sporophyll has gradually taken the place of its protection by outgrowths from other sporophylls.

*Type 3. S. helvetica.*<sup>1</sup> The young cone is here composed of compactly arranged sporophylls, but in the older cone the internodes become much elongated, and the sporophylls no longer overlap (Fig. 6, *a* and *b*, Pl. XLI). Each sporophyll has a prominent dorsal swelling, which is decurrent; in the young cone the two sporangia, which are borne on the alternating sporophylls of the whorl below, are appressed on either side of this swelling (Figs. 6 *b* and 7, Pl. XLI).<sup>2</sup> In the old cone the sporangia are considerably exposed, being only partially protected by the folded edges of the sporophyll in the axil of which they are borne. A large air cavity occupies the decurrent swollen base of the sporophyll; it extends upwards for a short distance into the lamina, being bisected by the entering bundle into two portions; it is crossed by a few trabeculae (Fig. 8, Pl. XLI) which increase in number in its upper portion. The surface of the sporophyll is provided with a thin cuticle, which is less strongly developed in the region covering the swollen base.

It is obvious from the above description and figures that in this species the dorsal projection is of no value for the protection of the sporangium except in its youngest stages, and its small size makes it of very little use even in the young cone. It may possibly represent the rudimentary remains of a well-developed dorsal flap, such as is present in *S. pumila*, and which has here become fused with the stem.

The macrosporangium in *S. helvetica* contains four macrospores. The microsporangium is tangentially somewhat elongated, or saddle-shaped.

An interesting feature is a row of cubical cells with strongly lignified but unpitted walls, which stretch across the stalk of the sporangium from one epidermis to the other, and are already present even at a stage when the sporangium is still quite immature (Figs. 8 and 9, Pl. XLI). In some cases more than one layer of these peculiar cells may be present. The epidermis of the sporangium stalk is also strongly cuticularized. The significance of this feature is at present obscure.

*Type 4 a.* In *S. flabellata* and *S. caulescens* the cone is more compact than in the last species, though the sporangia are still to some extent visible between the sporophylls. Each sporophyll is here more closely

<sup>1</sup> Hieronymus, G., p. 687, Fig. 405 (no dorsal projection is visible in this figure); Luerssen, C., Fig. 225, p. 864.

<sup>2</sup> This arrangement is essentially similar to that found in the cone of *Lycopodium inundatum*, though less effective (Sykes, M. G., 1909).



folded round its subtending sporangium (Fig. 10, Pl. XLI) than in any of the species yet described. The base of the sporophyll in *S. flabellata* is provided with a dorsal ridge which is elongated at right angles to the long axis of the sporophyll, and projects freely downwards (Fig. 10, *b* and *c*, Pl. XLI). The ridge is strongly curved, and is a little more prominent in the median than in the lateral planes; its ends turn upwards so that in a transverse section of the sporophyll at its base it is cut once, but higher up it is cut twice. It is traversed by an air cavity, and its surface is uncuticularized, while the rest of the sporophyll is covered by a remarkably thick cuticle (Fig. 10 *e*, Pl. XLI). The degree of development of the ridge is very variable, and in some cases it is almost entirely absent. The lowest sporophylls have generally a small dorsal projection (Fig. 10 *d*, Pl. XLI).<sup>1</sup>

In *S. caulescens* the sporophyll has a much smaller dorsal transverse ridge which does not project freely downwards (Figs. 11 *a* and 11 *b*, Pl. XLI). It is curved, and is a little less prominent in the median than in the other planes, so that to the naked eye it appears to constitute two small bulging projections. There is here no definite air cavity.

The microsporangium is round or oval in all the species included in Type 4, and is never saddle-shaped. The line of dehiscence is along a slit placed much as in the more primitive species.

The stalk of the sporangium of *S. flabellata* is composed of several rows of cells with strongly lignified walls (Fig. 10 *e*, Pl. XLI); these are well developed even when the sporangium is still quite immature. In *S. caulescens* there is a row of cells with lignified walls which are strictly confined to the base of the sporangium stalk, and are much less prominent than in the other species.

*S. flabellata* shows a tendency to variation in the number of the megaspores.<sup>2</sup> In one sporangium two large and two small megaspores occurred; in another there were one large spore, two smaller ones, and two very small ones.

*Type 4 b.* *S. Vögelii*, like others of the dorsiventral species of *Selaginella*, has square cones which are fairly compact; in the young cone of this species the sporangia are entirely concealed.

The cones are very small; each sporophyll is unusually long and so closely folded that in transverse section it appears to be V-shaped; thus it protects efficiently its own subtending sporangium. There is no dorsal flap or ridge adapted for the protection of any other of the sporangia, and no air cavity is present in the sporophyll (Fig. 12, Pl. XLI).

The epidermis of the sporangium stalk is strongly cuticularized, and

<sup>1</sup> See p. 525, footnote 2. *S. Preissiana* (Goebel, K., Organography, ii, p. 505 and Fig. 338) probably belongs to Type 4.

<sup>2</sup> Variation in the number of the megaspores in this and other species is recorded by Mitchell, G., (1910, pp. 24, 25).

there are a few cells with feebly lignified walls in the stalk of the sporangium, but they are not arranged in rows stretching across it. In *S. Vögeli* the sporangium has an unusually long stalk.

*S. apus*, var. *elegans*, probably also belongs to this group. In the young cone the sporophyll enfolds the sporangium as in other species of this type; the tip of the sporophyll is also turned inwards over the young sporangium in order the better to protect it. The mature microsporangium projects from the enclosing sporophyll, and the association of the mature megasporangium with the sporophyll is not at all close; indeed, in many cases it is even difficult to be sure which leaf is the one by which the megasporangium was enfolded when young.

As in *S. Vögeli*, the sporophyll has no dorsal projection at its base. A narrow longitudinal ridge (*m*, Fig. 13 *e*), occupied by an air cavity, is however present on the dorsal surface, beginning a little above the base and running, at the back of the midrib, nearly up to the tip of the sporophyll.<sup>1</sup> This dorsal ridge with its air cavity crossed by trabeculae is very characteristically seen in the V-shaped transverse sections of the microsporophyll.

The distribution of the two kinds of sporangia is a feature of some interest in this species. In the plants grown in the Botanic Garden microsporangia were rare, and at least one plant occurred on which megasporangia only were found, while in another plant there was only one mature microsporangium, but numerous megasporangia; in all cases the megasporangia were largely in excess. A single megasporangium is generally borne at the base of a terminal cone, which otherwise consists only of a tuft of leaves each associated with an abortive sporangium. In a few cases one or two mature microsporangia occurred in the same tuft as the megasporangium. Abortive sporangia were frequent in all cases, and one cone consisted of aborted sporangia only.<sup>2</sup> At first it was thought possible that the mega- and microsporangia in this species might ripen at different times on the same plant, but it was found that in a short time the few microsporangia had dehisced; at the end of two months all the megaspores had fallen, and there was no sign of further development of microsporangia.<sup>3</sup>

The number of megaspores in the megasporangium is extremely variable;<sup>4</sup> in several cases the sporangium contained a single large fertile

<sup>1</sup> It appears probable that the small ridge here described is comparable with the median longitudinal wing recorded by Goebel in some of the sporophylls of species whose cones are dorsiventral (Goebel, K., 1901, p. 225; and Organography, ii, p. 508, Fig. 340 (*S. suberosa*)).

<sup>2</sup> It is unnecessary to append figures of these abortive microsporangia, as exactly similar ones have been figured by Miss Mitchell in *S. Kraussiana* (1910, Fig. 12, Pl. IV).

<sup>3</sup> Lyon, F., 1901, p. 183, refers to the limited number of microsporangia in her material of *S. apus*. It is interesting to find that in *S. rupestris* also, the other form recorded as exhibiting reduction in the number of megaspores in the megasporangium, Hieronymus remarks that he was unable to find any microsporangia; he tentatively suggests that the embryos in this form may be parthenogenetic. It is hoped to investigate the problem later in *S. apus*.

<sup>4</sup> Cf. Lyon, F. (1901). In *S. apus* she found four megaspores, but in *S. rupestris* she found a varying number.



megaspore, round the base of which were clustered three minute aborted spores (Fig. 13c, Pl. XLI). In other cases the sporangium contained two large and two small spores, or two large and one small spore, &c.

There are two or three rows of cubical cells with feebly lignified walls in the stalk of the mature sporangium. There is also slight lignification at the base of the ligule in this species.

#### GENERAL REMARKS.

##### A. Comparison with *Lycopodium* and other genera.

(i) *The form of the sporophyll.* The method of protecting the sporangia by means of sporophylls with both upward and downward extending portions is well known to have been common among the ancient Pteridophyta. The sporophyll both of *Lepidostrobus*<sup>1</sup> and of *Spencerites*<sup>2</sup> had a more or less well-developed dorsal lobe, and the sterile segments of the sporophylls of *Cheirostrobus*<sup>3</sup> had a downward extending portion. Mr. Watson asserts that in *Mesostrobus*<sup>4</sup> also the sporophyll had a similar form, but this statement does not appear to be supported by his figures; consequently it is not possible to make any clear comparison between this and the other genera, and the discussion on pp. 392-3 of his paper is somewhat obscured.

When we find such a series of forms in a single genus as has now been demonstrated in both *Lycopodium*<sup>5</sup> and *Selaginella*, it becomes difficult to place entire confidence in any comparison, based on the forms of the sporophyll, between different genera of widely separated geological ages.

Watson<sup>6</sup> has grouped together *Miadesmia*, *Bothrodendron*, and *Selaginella*, and it is true that these genera resemble one another to some extent in the position of the attachment of the sporangium. But here it must be remembered that while we have no certain evidence as to where the sporangium originated in the fossil genera, we do know that in *Selaginella* it arises from stem tissue, and in some species, even in the adult cone, is inserted on the stem above the leaf.

On the other hand, the presence of the dorsal lobe in two probably primitive species of *Selaginella* suggests a comparison with *Lepidostrobus*, *Spencerites*, *Lycopodium alpinum*, &c. It is, of course, possible that this method of protecting the sporangia may have been evolved over and over again; still it would appear to be a character at least as valuable for purposes of comparison as is the particular point of attachment of the sporangium.

<sup>1</sup> Williamson, W., 1893; Maslen, A. J., 1899, p. 371, Fig. 22, Pl. XXXVII; Fig. 36, Pl. XXXVIII.

<sup>2</sup> Berridge, E. M., 1905; Watson, D. M. S., 1909, Text-fig. 4, p. 389.

<sup>3</sup> Scott, D. H., 1897, p. 7 (Diagram).

<sup>4</sup> Watson, D. M. S., 1909, p. 393.

<sup>5</sup> Sykes, M. G., 1908 (2).

<sup>6</sup> Watson, D. M. S., 1909, p. 391.

Unfortunately our knowledge of the fossil *Selaginellites*<sup>1</sup> is insufficient to make it possible to decide to which of the *Selaginella* types they most closely approximated. The exact relation of the sporophylls was not apparent in Halle's specimens. *Selaginellites Suissei*<sup>2</sup> is more reminiscent of *Selaginella pumila* than of the other types, though we do not think much value can be attached to the suggestion. Its multiseriate cones and more numerous megaspores mark this fossil species sharply off from *Selaginella*.

A more detailed comparison between the series of forms of sporophyll found in the two genera *Lycopodium* and *Selaginella* may prove to be of some interest. The remarkable resemblance between the sporophyll of *S. pumila* and that of *Lycopodium cernuum*, *alpinum*, &c., has been pointed out above. In both cases the dorsal flap of the sporophyll and the groove on the under surface of its stalk<sup>3</sup> efficiently protect the sporangium vertically below it. The main difference in the sporophylls of these species consists in the nature of the flap, which in *L. alpinum* and *S. pumila* is entirely a free down-growth, as it appears to have been in *Lepidostrobus*, while in *L. cernuum* it is further modified by adaptations connected with the large mucilage cavity.<sup>4</sup>

One of us<sup>5</sup> has already expressed the conviction, as one of the results of a comparative study of the sporophylls of *Lycopodium*, that this dorsal flap is a primitive feature in that genus; its occurrence in two avowedly primitive species of the genus *Selaginella* appears to us to be suggestive.

*S. helvetica* may be compared with *L. inundatum*. In both forms the dorsal flap is no longer developed, but a small ridge or swelling is now present which appears to us to represent its reduced remains. *S. helvetica* seems to stand between *S. pumila* and such forms as *S. Vögeli* much in the same way as does *L. inundatum* between *L. cernuum* and the simpler types of *Lycopodium*. On the other hand, we know of no forms of sporophyll in the genus *Lycopodium* which are so clearly intermediate as are the sporophylls of *S. flabellata* and *S. caulescens* in the genus *Selaginella*.

The simpler *Lycopodiums* differ however from those species of the fourth type of *Selaginella* in which there is no sign of a dorsal projection, and are more closely comparable with *S. spinosa*, the sporophyll of which appears to be reduced; in them there has been no development of any fresh adaptation for the protection of the sporangium such as has occurred in the dorsiventral *Selaginellas*.

<sup>1</sup> Halle, T. G., 1907.

<sup>2</sup> Zeiller, R., 1906.

<sup>3</sup> See Lang, W. H., 1908, p. 360, Fig. 2. This groove is hardly comparable with the curious furrow on the lower surface of the sporophyll stalk in *Bothrodendron* (Watson, D. M. S., 1908, p. 51, Text-fig. 1).

<sup>4</sup> Lang, W. H., 1908; Sykes, M. G., 1908 (2). It appears as yet uncertain to which of these two forms *Spencerites* may be most closely compared (Watson, D. M. S., 1909), since the evidence as to whether or no there was cohesion between the sporophylls of that genus is not yet conclusive. In either case it does not seem necessary to discuss Mr. Watson's unauthorized extension of Dr. Lang's explanation of *Spencerites* to *Mesostrobus* and other genera.

<sup>5</sup> Sykes, M. G., 1908 (2).

(ii) *The origin and development of the sporangium.* The chief contrast between *Selaginella* and *Lycopodium* is in the development of the sporangia, which arise from stem tissue in the former, and generally from leaf tissue in the latter. At present we are very much in the dark as to the meaning of this feature. In *Miadesmia*<sup>1</sup> the position of the sporangium appears to indicate that it was there developed from leaf tissue. Perhaps in *Selaginella* the sporangium has never been taken up on to the leaf, or it may be that both here and in *Lycopodium Selago* the development of that portion of the sporophyll which intervenes between the sporangium and the axis has been dropped out and the sporangium has become reinserted on the stem.<sup>2</sup> At present such suggestions are mere conjectures.

(iii) *The air cavity.* The air cavity recorded in the projecting base of the sporophyll of *S. helvetica*, *S. flabellata*, *S. caulescens*, &c., is of some interest; it forcibly recalls the mucilage cavity in the genus *Lycopodium*, and the parichnos of fossil genera.<sup>3</sup>

(iv) It is perhaps also worth mentioning the *lignified cells* found in the stalk of the sporangium of *S. helvetica* and other species. They are, however, hardly comparable with those occurring in the genus *Lycopodium*,<sup>4</sup> since their walls, unlike the lignified walls of the cells in the sporangium stalk in species of that genus, have no pits that are visible under the magnifications which we have employed. The lignified walls in both genera stain with iodine green and methyl violet.

(v) The tangential elongation of the microsporangium in *S. pumila*, *S. helvetica*, and *S. spinosa* recalls the *saddle-shaped sporangium* of so many of the Lycopods,<sup>5</sup> while the rounded microsporangia of the other forms is more like the sporangium of *Bothrodendron*,<sup>6</sup> *Miadesmia*,<sup>7</sup> &c. It appears probable that the shape of the sporangium is a character largely dependent on the relations between the sporophylls and the adaptations for the dispersal of the spores, and is not one which can be relied on for comparative purposes.

B. *A tendency towards reduction in the number of megaspores in the sporangium* appears to be prevalent in the genus *Selaginella*, and is found in such widely separated species as *S. rupestris*, *S. flabellata*, and *S. apus*. This may possibly represent a survival from Palaeozoic times, when it was very highly expressed in such forms as *Miadesmia*.

<sup>1</sup> Benson, M., 1908 (1), p. 420.

<sup>2</sup> Sykes, M. G., *New Phyt.*, 1908; and *Ann. of Bot.*, 1908.

<sup>3</sup> Hill, T. G., 1906, p. 270.

<sup>5</sup> Sykes, M. G., 1908 (2); Benson, M., 1908 (2), p. 145.

<sup>7</sup> Benson, M., 1908 (2), pp. 420, 421.

<sup>4</sup> Sykes, M. G., 1908 (2).

<sup>6</sup> Watson, D. M. S., 1908.

SUMMARY.

There have now been described in this genus four main types of sporophylls.

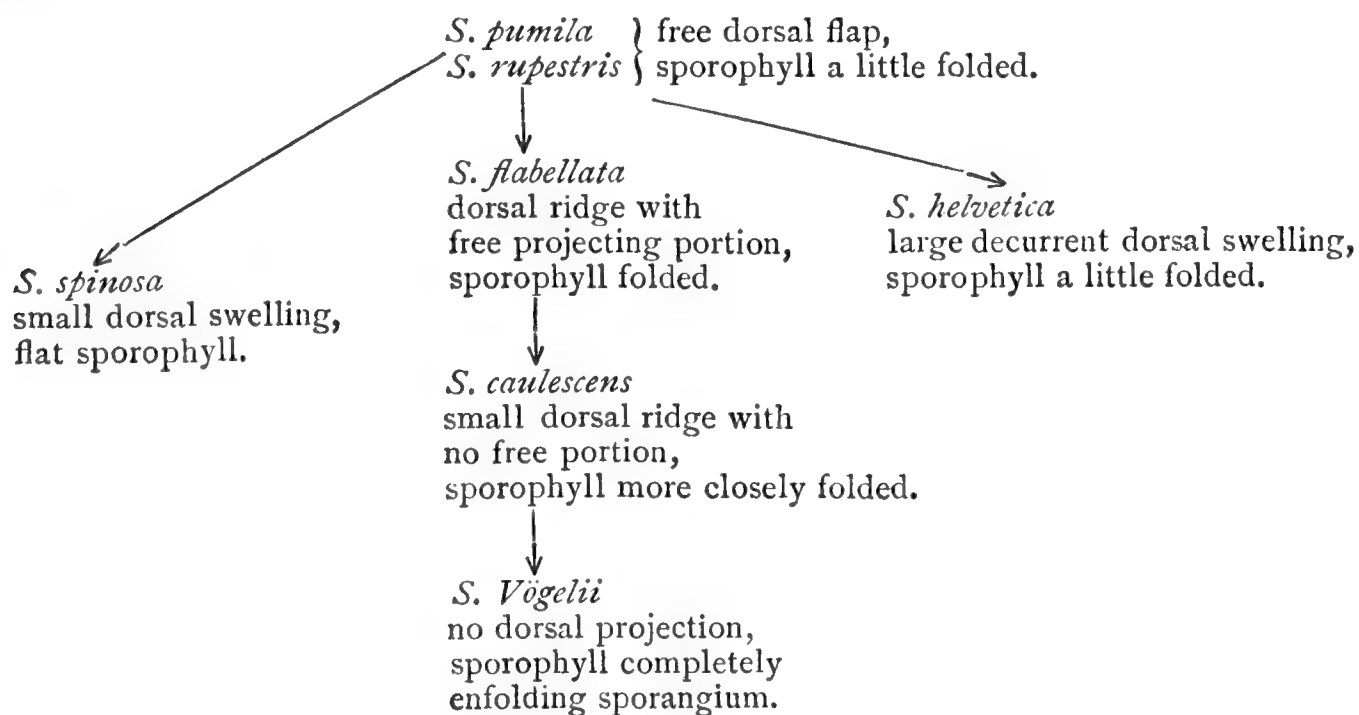
I. In *S. pumila*, *S. rupestris*, an unknown species, and probably also *S. Lyallii*, the sporophyll has a well-developed dorsal flap extending freely downwards, and protecting the young sporangium immediately below it. Two of the species have radially arranged leaves, and are presumably among the more primitive members of the genus.

II. In *S. spinosa*, the only other radial species examined, there is nothing which can certainly be compared with this free dorsal flap. A slight dorsal swelling is present, occupied by an air cavity; it is possible that this may represent the reduced remains of such a flap. In this species the sporophyll is flat and the sporangium exposed.

III. In *S. helvetica*, one of the dorsiventral species, there is a well-developed dorsal projection which is however not free but decurrent. It is especially prominent in the young cone where the two alternating sporangia of the whorl below are appressed against it. It is suggested that it may be homologized with the free dorsal flap in *S. pumila*, here fused with the stem.

IV. The species placed in Type 4 form a series, in which the dorsal outgrowth, which originally served to protect the sporangia below, is gradually reduced and lost, while at the same time each sporophyll more and more completely enfolds and protects its own subtending sporangium. In *S. flabellata* there is a transversely elongated dorsal projection the median portion of which extends freely downwards; in *S. caulescens* the free median portion is lost, and only a small curved ridge is left; in *S. Vögelii* and *S. apus* all signs of a dorsal projection at the base of the sporophyll are lost.

The following table is meant to illustrate this comparison of the sporophylls, but is not intended to indicate phylogenetic relationships:—



Finally, it appears to us that we find in this genus a series of forms of sporophyll, the most complex form prevailing in one of the more primitive species. This complex form presents a remarkable resemblance to the form of the sporophyll in *Lycopodium alpinum* and *cernuum*, and in *Spencerites*,<sup>1</sup> and though it would of course be inadvisable to suggest any kind of *close* relationship between forms otherwise so divergent, we cannot refrain from inferring some significance underlying so close a resemblance.

The development of a complex sporophyll differentiated into dorsal and ventral portions in genera separated in time and in general features so widely as are these three, seems to point to the conclusion that we are here dealing with a very ancient character. We realize, however, that in the light of the instances of parallel development now so frequently revealed, it is not yet possible to regard such a view as definitely proved.

BOTANY SCHOOL, CAMBRIDGE.

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## DESCRIPTION OF FIGURES IN PLATE XLI.

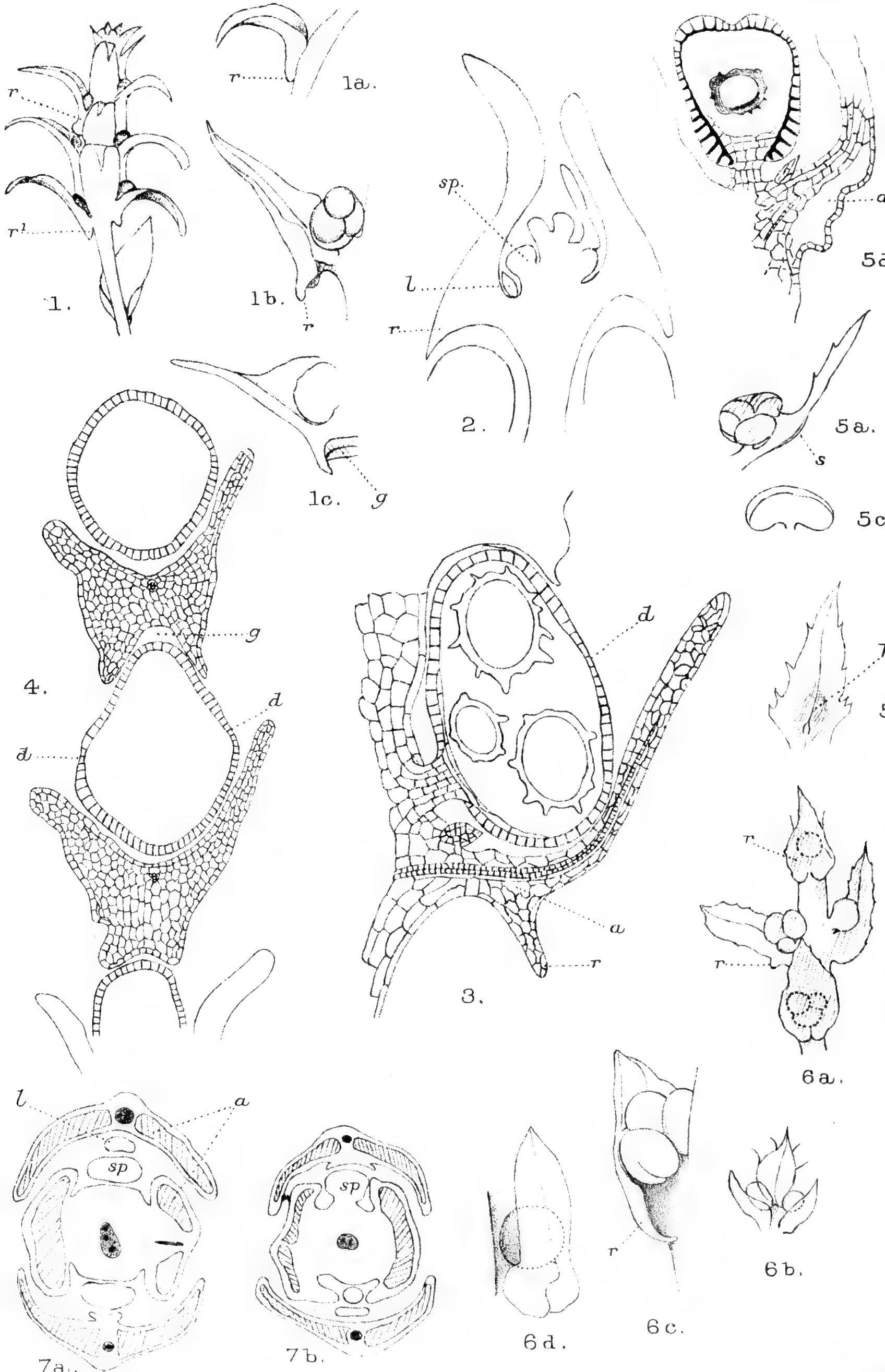
Illustrating the paper on the cones of *Selaginella* by Miss Sykes and Mr. Stiles.

- Fig. 1. Cone of *S. pumila*. *r* = dorsal flap of sporophyll; *r'* = projection on lowest sporophyll. × 10.
- Fig. 1 *a*. Lowest sporophyll of cone of *S. pumila*. *r* = dorsal flap. × 12.
- Fig. 1, *b*, *c*. Sporophylls from middle region of cone of *S. pumila*. *g* = groove in sporophyll stalk. × 20.
- Fig. 2. Longitudinal section of the apex of a cone of *S. pumila*, showing origin of sporangium (*sp*) from stem tissue. *l* = ligule. × 54.
- Fig. 3. Radial section of sporophyll of *S. pumila*. *r* = dorsal flap; *a* = small cavity below stele; *d* = line of dehiscence. × 54.
- Fig. 4. Tangential section of cone of *S. pumila*, to show groove in stalk of sporophyll, more pronounced in proximal portion (upper sporophyll), less pronounced in the distal portion (lower sporophyll). The line of dehiscence (*d*) is cut twice in a tangential section of the sporangium. *g* = groove in sporophyll stalk into which the sporangium fits. × 64.
- Fig. 5, *a* and *b*. Front and side views of sporophyll of *S. spinosa*. *s* = small dorsal swelling; *h* = ventral depression. × 20.
- Fig. 5 *c*. Saddle-shaped sporangium of *S. spinosa*.
- Fig. 5 *d*. Section of sporophyll of *S. spinosa*. *a* = air cavity in dorsal swelling. × 40.
- Fig. 6, *a* and *b*. Parts of young and old cones of *S. helvetica*. *r* = dorsal projection. 6 *b* shows two young sporangia appressed on either side of *r*. × 10.
- Fig. 6, *c* and *d*. Sporophylls of *S. helvetica*, back and side views. × 20.
- Fig. 7. Transverse sections of young cone of *S. helvetica*. *sp* = sporangium; *l* = ligule; *a* = air cavity. × 54.
- Fig. 8. Radial longitudinal section of sporophyll of *S. helvetica*, showing (*a*) cavity in dorsal ridge traversed by rows of cells or trabiculae, (*b*) the row of cells with lignified walls across the base of the sporangium stalk. × 54.
- Fig. 9. Ditto from another sporophyll more highly magnified. Note also cuticularization of the epidermis (*e*) of the sporangium stalk. × 170.
- Fig. 10. Sporophyll of *S. flabellata*, enfolding megasporangium. × 10.
- Fig. 10 *a*. Contents of macrosporangium.

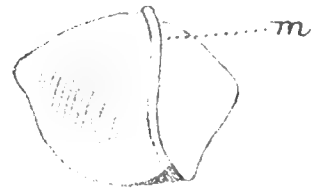
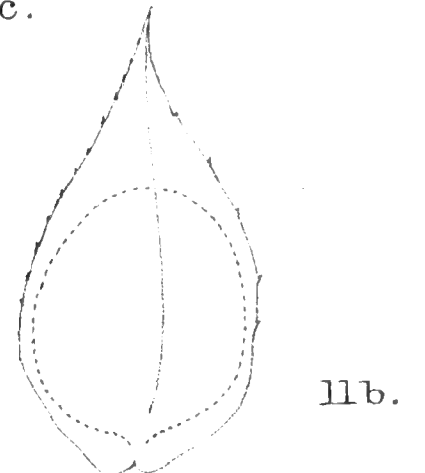
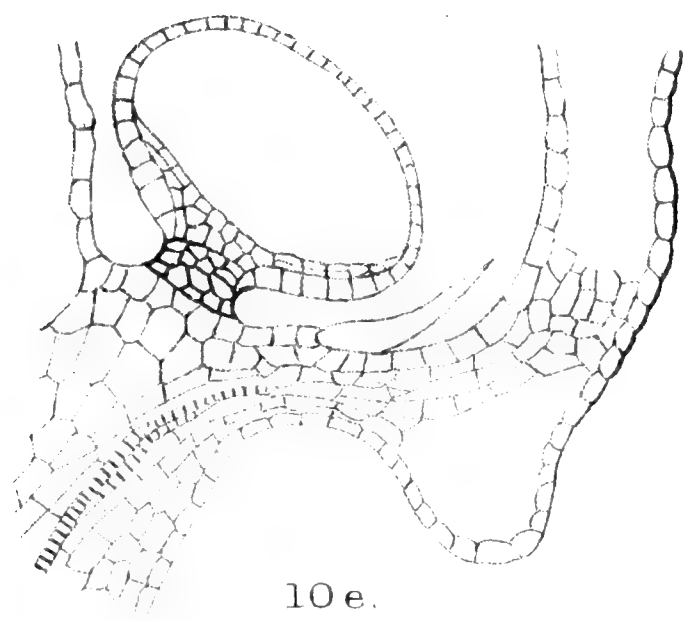
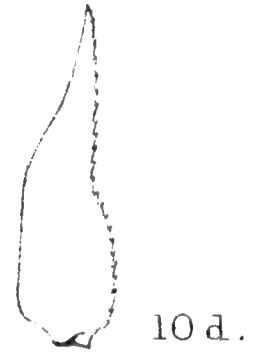
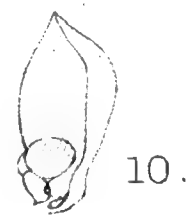
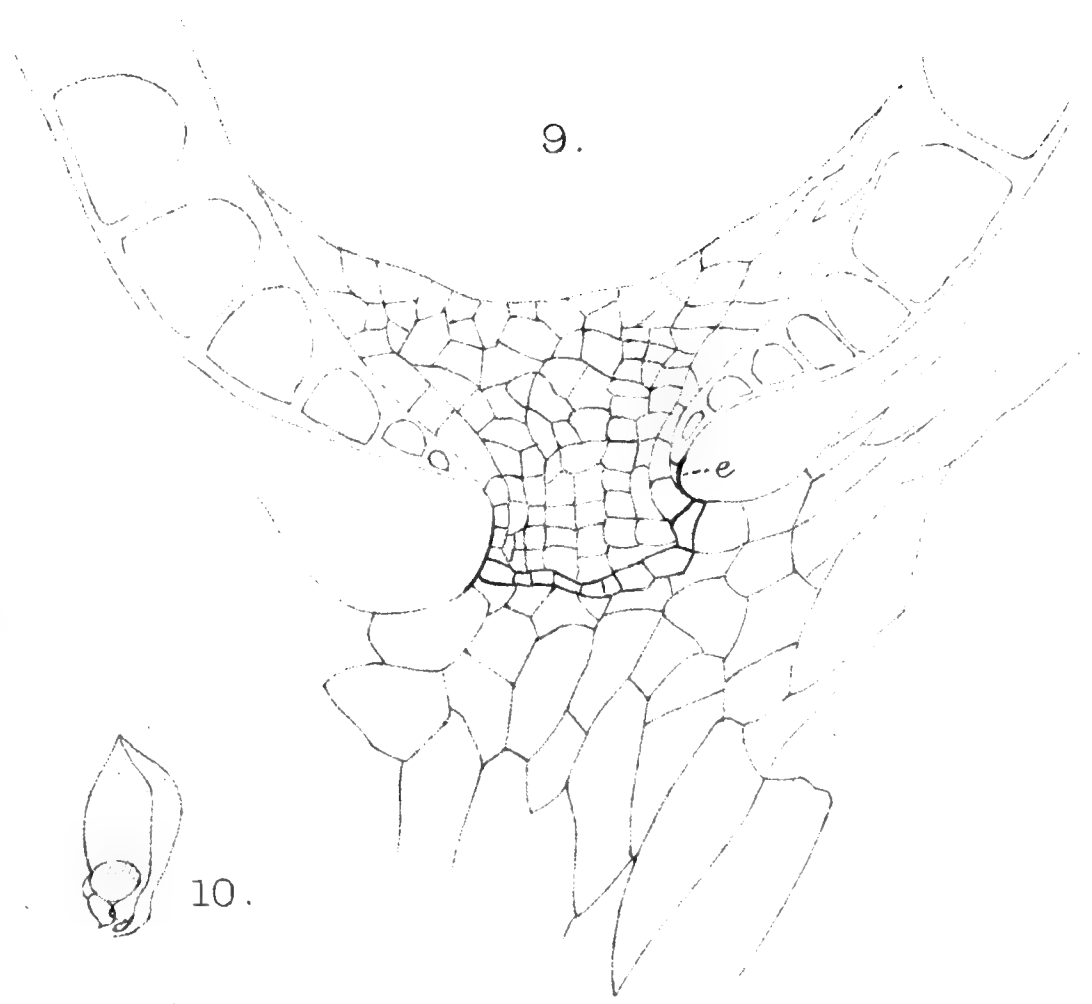
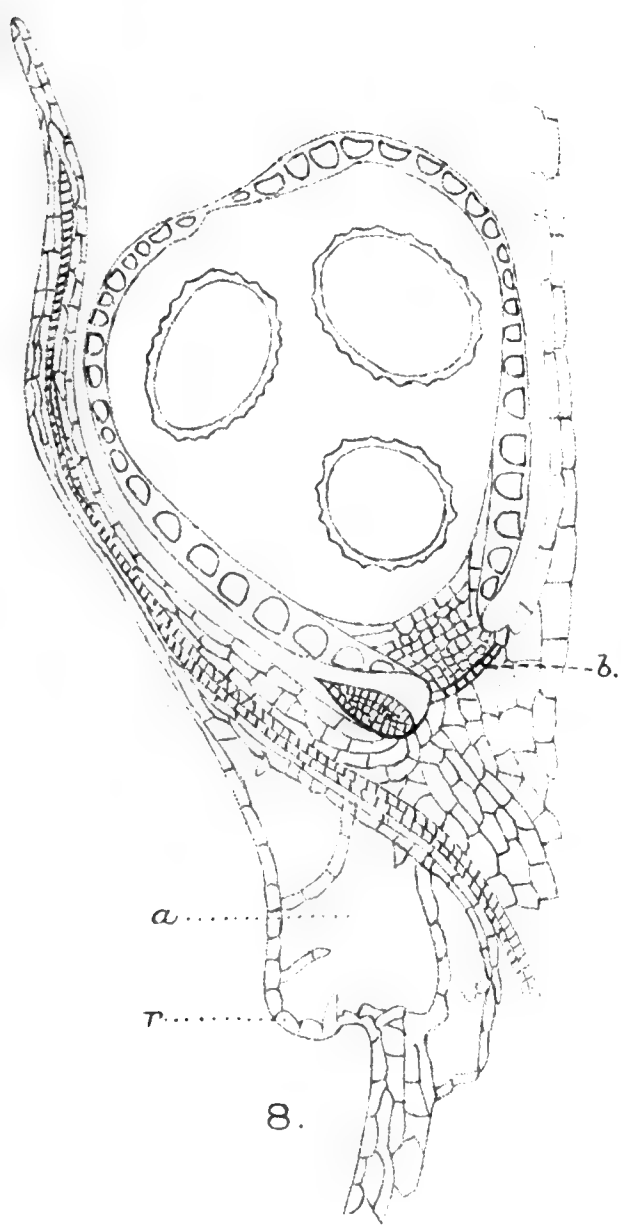


- Fig. 10, *b* and *c*. Two sporophylls of *S. flabellata* in side view. *r* = dorsal projection.  $\times 20$ .  
Fig. 10 *d*. Lowest sporophyll of cone, showing minute dorsal projection.  
Fig. 10 *e*. Section of sporophyll of *S. flabellata*, showing dorsal projection containing air cavity, and rows of lignified cells in sporangium stalk.  
Fig. 11 *a*. Sporophyll of *S. caulescens*, side view.  $\times 10$ .  
Fig. 11 *b*. Ditto, back view.  $\times 20$ .  
Fig. 12. Sporophyll of *S. Vögeli*.  $\times 10$ .  
Fig. 13 *a*. End of a fertile branch of *S. apus*, var. *elegans*. The cone contained only two fertile microsporangia and abortive sporangia.  $\times 10$ .  
Fig. 13 *b*. Ditto, bearing one megasporangium only and some abortive sporangia.  $\times 10$ .  
Fig. 13, *c* and *d*. Spores from megasporangia of *S. apus*, var. *elegans*.  
Fig. 13 *e*. View of sporophyll of ditto from above, showing longitudinal median dorsal ridge (*m*).  $\times 20$ .





M.G.S. and W.S. del.



12.

13a.

13d.

13b.

13c.

13e.



# On the Anatomy of some Tubers.

BY

T. REED, A.R.C.S.

*Biology Department : St. Mary's Hospital Medical School.*

With Plates XLII and XLIII and four Diagrams in the Text.

THE investigation of the anatomical structure of tubers has been much neglected. Hitherto no account appears to have been published dealing with the anatomy of the potato, *Solanum tuberosum*, or with that of the artichoke, *Helianthus tuberosus*.

Several attempts have been made to determine the causes which bring about tuberization in the potato. In a long series of papers N. Bernard (1) has attempted to show that tuberization in the potato is a result of the infection of the plant by a fungus. By inoculating the cortex of the roots of young plants of *S. tuberosum* with the spores of the fungus *Fusarium*, he claims that he obtains a greater yield of tubers than he does in the case of plants which are not artificially inoculated with the fungus spores. He does not explain why infection should cause tuberization in a totally different part of the plant from that which is actually infected. It will be difficult to explain why infection of the root by the fungus should cause tuberization in the stems, for in no case has the fungus been traced from the root to the tubers. In the case of attacks of fungi on plant tissue it is usual to find the part actually infected becoming meristematic and forming tubercular growths such as we get in the cases of galling, witches' brooms, &c. Bernard tentatively suggests that it may be an *action à distance* by the fungus, brought about by its giving rise to soluble products which in some mysterious way cause the underground stems to swell up and accumulate vast reserves of carbohydrates, &c.

H. Jumelle (9) has caused some doubt to be thrown on the work of Bernard. Repeating some of Bernard's experiments, Jumelle does not appear to confirm his results, and he says that the results, in his opinion, are not sufficiently clear to convince him of the conclusions drawn by Bernard.

In Bernard's third experiment he describes a case in which the potatoes were grown *en pleine terre*. The first lot were assured of infection by being



manured with dung which was contaminated with *Fusarium*; in the second lot the infection was *irrégulière et tardive*. Since he obtains a greater yield of tubers from the first lot than he does from the second, the inference is drawn that the infection by *Fusarium* is responsible for the difference in yield. Bernard appears to have overlooked the fact that the manuring of the soil of the first lot of tubers would probably account for very considerable differences between the harvests of the two lots. This probability is strengthened in view of the work of L. Dufour (6), who has shown that plants of *S. Commersonii* are very susceptible to changes in the physical and chemical condition of the soil. Dufour concludes that moist earth favours the production of tubers, and that sandy soil is more favourable to their formation than is sandy soil mixed with varying percentages of calcium.

None of these authors has given us any account of the anatomical structure of the potato, and very few accounts of the anatomy of other tubers are available.

E. Bucherer (2) has dealt with the anatomy of several tubers of Dioscoreaceae, but he deals more particularly with the origin of the tuber and the features of its vascular anatomy, and he makes no reference to the tissues which are concerned with the formation of the tuberous tissue.

A. De Bary (4), dealing with the tubers of Dioscoreaceae, states that in those tubers which exhibit secondary thickening the origin of the cambium is unknown. In the specimens investigated, however, a cambium surrounds the whole lateral surface of the tuber inside the cortex. *This cambium gives rise to interfascicular tissue consisting of thin-walled parenchyma which forms the main mass of the tuber.*

Leclerc du Sablon (10) also deals with the tuber of a member of the Dioscoreaceae, *Tamus communis*. He deals more particularly with its development and morphological nature than with its anatomy or the origin of the tuberous tissue, and his figures give us no clue as to the tissue which is responsible for forming the main mass of the tuber.

Miss E. Dale (3), dealing with the tuber of *Dioscorea sativa*, mentions that it is made up of parenchymatous tissue, but does not discuss its origin.

Marcel Dubard and Renè Viguier (5) give an interesting account of the tuber of *Euphorbia Intisy*. This is a root tuber. The root shows a central cylinder consisting of a hexarch stele. Secondary thickening commences in the usual way by a cambium which surrounds the xylem portions of the vascular cylinder. This cambium, however, forms few secondary xylem elements, but gives rise to long radial rows of parenchyma cells which compose the bulk of the tuber. At infrequent intervals a few wood elements may be formed, but owing to the very extensive development of parenchyma these become cut off as '*îlots vasculaires*'. The pith also contributes to the tuberous tissue.

Theo. Holm (7) gives an account of the root tubers of *Rhexia virginica*.

Whilst he does not deal particularly with the origin of the tuberous tissue, his figures suggest that it is formed from wood parenchyma of the secondary hadrome or wood. It is worth noticing that although these tubers are the principal means of propagation of this plant, neither the tuberous tissue nor the cortex contains any starch, and other deposits appear to be scarce. The cortex also appears to contribute some part of the tuberous tissue.

F. J. F. Shaw (11) describes the anatomy of the tuberous seedlings of *Araucaria Bidwillii*, but he gives us no account of how the tuberous tissue arises.

#### ANATOMY OF TUBERS OF *SOLANUM TUBEROSUM*.

The tubers of *S. tuberosum* arise as terminal swellings on long underground stems or stolons. These stems arise in the axils of the lower leaves of the main shoot and grow more or less horizontally outwards, and sooner or later they swell up at their tips to form tubers. That these structures are stems is shown by their origin and their anatomical and morphological structure.

The plants used in this investigation were grown in soil, and at various stages of their development tubers were cut off and preserved in alcohol.

To determine the course of the vascular bundles in the tuber, series of transverse sections were cut by hand from the stolon to the tip of the tuber.

The stolon presents a typical stem structure. There is a ring of bundles consisting of four larger groups of xylem elements with a few smaller groups scattered between them; each group is accompanied on its outer side by a group of phloem elements. Each bundle is also accompanied on its inner or medullary side by a group of phloem elements, so that the structure of the bundles is bicollateral, a structure which appears to be quite typical for the Solanaceae (4). The vascular cylinder is surrounded by an easily recognized endodermis (Pl. XLII, Fig. 1, *En.*). It is worthy of notice that this endodermis is entirely devoid of starch grains. This appears remarkable when one is reminded that the cortex and pith contain very abundant quantities of starch grains. This absence of starch from the endodermis of the stolon of *S. tuberosum* is remarkable in that in most plants the endodermis is the tissue which almost invariably contains starch. It may possibly be explained that the absence of starch is due to the fact that the whole of the protoplasm of the endodermal cells is used up in their formation, for Millon's test and the xanthoproteic reaction failed to reveal the presence of protoplasm. Since then all the protoplasm is apparently used up in the formation of the cells, in their adult stage they would be incapable of any further activity.

The endodermis is surrounded by about eight or ten layers of parenchymatous cortical cells. These parenchymatous cells of the cortex contain abundant starch grains and protoplasm, and usually a nucleus as well. The

pith is composed of large parenchymatous cells, and these also contain large quantities of starch grains. The outer surface of the stolon is covered by a single-layered epidermis, the cells of which have a thin cuticle on their outer surface. There is no development of cork on the stolon.

The change from underground stolon to tuber is quite abrupt; there is no gradual transition from stolon to tuber such as obtains in the case of

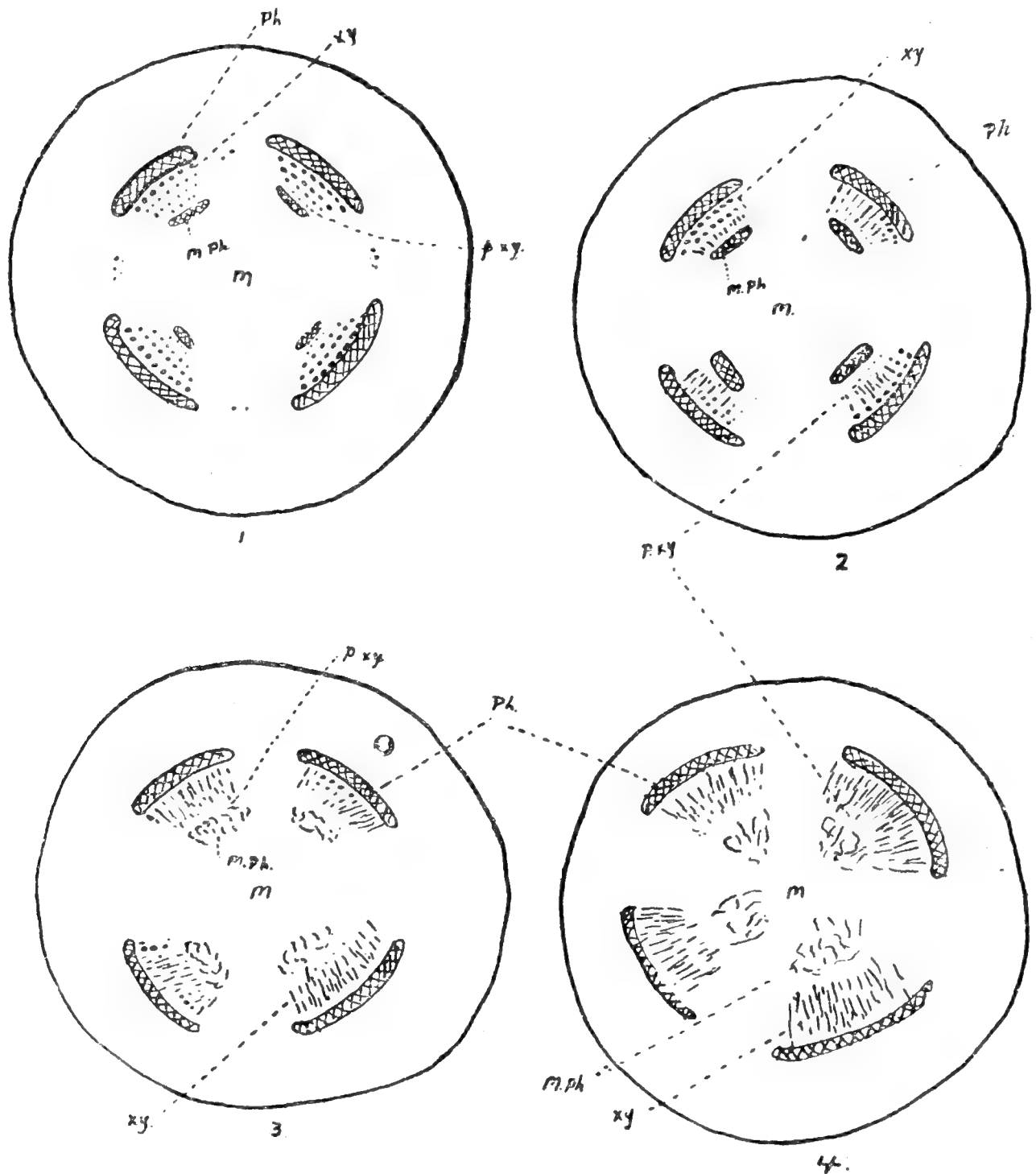


DIAGRAM I. *M.*, medulla. *M.Ph*, medullary phloem. *p.xy.*, protoxylem. *xy.*, xylem. *Ph.*, phloem.

*Helianthus tuberosus*. This circumstance makes it difficult to trace clearly the course of the vascular bundles from stolon to tuber.

Passing from the stolon to the base of the tuber it will be seen (Diagram I, Fig. 1) that the ring of bundles begins to lose its definite arrangement into groups, as seen in transverse section, and to become 'sprayed' outwards (Diagram I, Figs. 2 and 3). Thus it will be seen that a transverse section of a tuber at this level would cut the vascular elements in an oblique direction. Still farther in the tuber the 'spraying' has gone

on to such an extent that the wood elements are now seen to be cut longitudinally even in a transverse section (Diagram I, Fig. 4).

This derangement of the vascular tissue is a direct result of the tuberization of the stolon, and is brought about in the following manner:—

Just about the region where the stolon joins on to the tuber it will be noticed (Pl. XLII, Fig. 4) that the pith cells are becoming meristematic. Transverse sections a little farther in the tuber show that almost every cell of the pith is dividing. As a result of the activity of these pith cells a very considerable amount of parenchymatous tissue is added to the pith. It is this increase of medullary tissue which is responsible for the deranging of the vascular tissue. As the medullary cells continue their divisions they produce what is essentially a wedge of tissue which forces the xylem elements out of their normally vertical course and compels them to pursue a more or less oblique course.

Diagram II shows the effect of the meristematic medulla on the strands of xylem.

It must be fairly obvious that the large increase in quantity of the pith elements, and the consequent dilatation of the stolon to form a tuber, would under ordinary circumstances also result in a rupture of the cortex, so that *pari passu* there is a corresponding division in the cells of the cortex.

It is thus seen that the pith contributes a very large portion to the tuberous tissue of the tuber.

It now remains to trace the distribution of the medullary phloem in the tuber.

The medullary phloem consists of the usual elements, sieve tubes, companion cells, and phloem parenchyma (Pl. XLII, Figs. 1 and 2).

At the time when the medullary cells of the stolon begin to become meristematic it is seen that the parenchyma cells between the protoxylem of the bundle and the medullary phloem also commence to divide. Simultaneous with this the phloem parenchyma of the medullary phloem begins to divide. As a result of these divisions it is found that the medullary phloem becomes broken up into a large number of small strands isolated from each other by pockets of parenchyma (Pl. XLII, Fig. 3, and Diagram II, *M.Ph.*).

The phloem strands which are distributed amongst the medullary

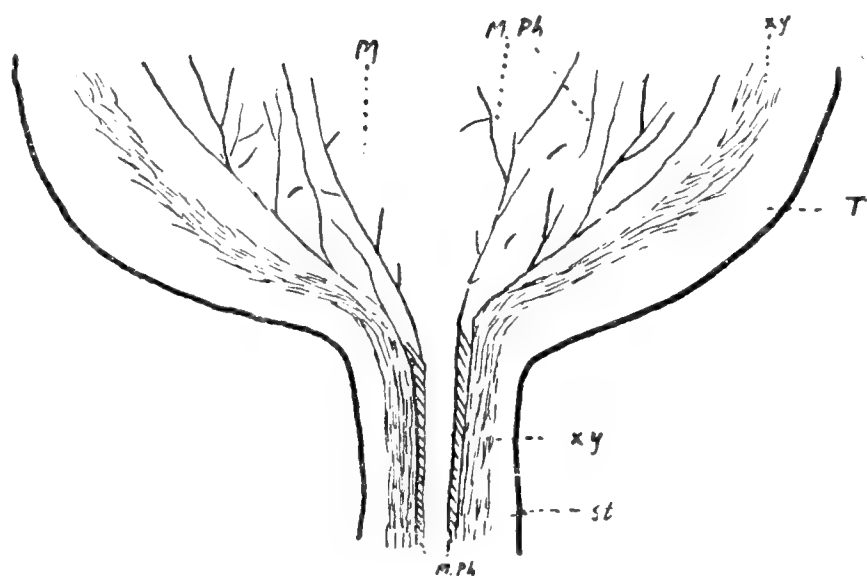


DIAGRAM II. *M.*, meristematic medulla. *xy.*, xylem. *st.*, stolon. *T.*, tuber. *M.Ph.*, medullary phloem. Longitudinal section of tuber at junction with stolon.

parenchyma cells pursue a very irregular course (Pl. XLII, Fig. 6). This is because the isolated strands are surrounded by meristematic cells which are not dividing at a uniform rate, so that in one region the strands will be pushed in one way and elsewhere in a different direction.

It is interesting to find that the medullary phloem is apparently used for the conduction of carbohydrates from the parent plant to the developing tuber. This conclusion can hardly be resisted when one considers the manner in which the phloem elements ramify amongst the tuberous elements of the medulla.

From the above it is seen that the tuber of *S. tuberosum* is built up from three kinds of tissue: (1) medulla, (2) phloem parenchyma, and (3) to a smaller extent, cortex. Xylem parenchyma does not contribute any considerable portion to the tuber. In some few cases it may be seen that the protoxylem is partly separated from the later-formed xylem by a wedge of parenchyma, but this is not generally the case (Pl. XLII, Fig. 4). This fact is interesting when we compare it with what obtains in *Helianthus tuberosus*, where it is found that the tuber is built up largely from xylem parenchyma together with medullary parenchyma.

It is also worth noticing that the tuber forms few, if any, new wood elements. In ripening fruits, &c., L. Jost (8) states that under natural conditions the amount of mechanical tissue increases in proportion to the increase in weight. The case of these tubers is quite in harmony with this observation, for although there is an increase in the size and the weight of the tuber as growth continues, this weight has not to be borne by the plant, since the tubers are subterranean.

It will be recalled that the stolon which gives rise to the tuber was covered with a single-layered epidermis which has a slightly developed cuticle (Pl. XLII, Fig. 1, *Ep.*). Almost as soon as the stolon commences to dilate to form the tuber the cells of the hypoderma begin to divide tangentially and give rise to a cork cambium (Pl. XLII, Fig. 5, *Hyp.*). In the case of *S. Dulcamara*, De Bary (4) states that the periderm arises in the epidermis, and Solereder (12) makes the same statement for *some* species of *Solanum*, but does not include any species of *Solanum* in the group in which the periderm arises in the sub-epidermal layer. It may possibly be that the more deep-seated origin of the cork cambium in the tuber of *S. tuberosum* is due to the fact that since the tuber is a subterranean structure, the epidermis would be liable to injury.

The cork cambium gives rise to several layers of cork on the outside and phelloderm on the inner side (Pl. XLII, Fig. 5 *a, ck., P.*) The development of a covering of cork for such a structure as the potato is almost a necessity, since the tuber contains a high percentage of water.



ANATOMY OF TUBERS OF *HELIANTHUS TUBEROSUS*.

These tubers, like those of *Solanum tuberosum*, arise as swellings on underground stems, or stolons, which spring from the axils of scale leaves at the base of the main shoot. The stolon may at once swell up and become a tuber, or it may greatly elongate and give rise to a number of lateral tubers, finally terminating itself in a tuber. So far as these observations go this is different from what obtains in the potato, in that in the case of the latter the tubers do not arise laterally, as they may in *H. tuberosus*.

Figs. 7 and 8, Pl. XLII, show two young tubers. From these it will be seen that the tuber develops from the apex backwards.

The anatomical structure of an untuberized stolon is that of a typical dicotyledonous stem. Fig. 9, Pl. XLII, shows a portion of such a stolon, as seen in transverse section. It consists of a ring of a dozen or more vascular bundles, each of which is accompanied on the outer side of the phloem by a group of sclerenchymatous fibres (Pl. XLII, Fig. 9, *scl.*). Between the groups of xylem vessels there can be made out even at this stage small groups of xylem parenchyma (Pl. XLII, Fig. 9, *xy.p.*). The pith and cortex are made up of parenchymatous elements of the usual type.

As has been pointed out above, the transition from stolon to tuber is here very gradual, so that the manner in which the vascular elements are distributed in the tuber can be made out quite clearly. Tuberization is effected by two classes of tissue: (1) medulla, and (2) xylem parenchyma.

Just at the region where stolon structure passes into that of the tuber, the cells of the pith commence to divide tangentially (Pl. XLIII, Fig. 12, *M.*). These divisions commence around the periphery of the medulla, and gradually extend inwards until almost the whole of the pith is involved.

The pith cells first enlarge considerably in a radial direction, and very soon tangential walls appear, and then the process is repeated again and again. As a result of these cell divisions in the medulla a very considerable amount of new parenchymatous tissue is added to the developing tuber. The cells resulting from the activity of this new meristem become arranged in definite radial rows (Pl. XLIII, Fig. 13).

For a time the parenchymatous cells of the medulla are the only cells which contribute to the formation of the tuber.

After a time, however, divisions are seen to commence in the xylem parenchyma (Pl. XLII, Fig. 10, and Pl. XLIII, Fig. 12, *xy.p.*). These divisions are mainly tangential, and first make their appearance in those pockets of xylem parenchyma which are nearest the protoxylem side of the bundle (Pl. XLII, Fig. 10). As a consequence of these tangential divisions the protoxylem becomes pushed towards the centre of the stem. A little later other pockets of xylem parenchyma become similarly meristematic, with the result that the whole xylem portion of the bundle becomes separated into small groups



of three or four vessels each, with intervening pockets of parenchyma. A transverse section of a portion of a tuber at this stage is shown in Pl. XLIII, Fig. 14, where, as a result of the activity of the xylem parenchyma, the xylem appears to be broken up into islands (*xy.*). Strictly speaking, these are not true vascular islands, for in longitudinal section they are seen to be continuous, and not pinched off, as they would be were they true *îlots vasculaires*. The following diagram shows a longitudinal section of such a tuber:—

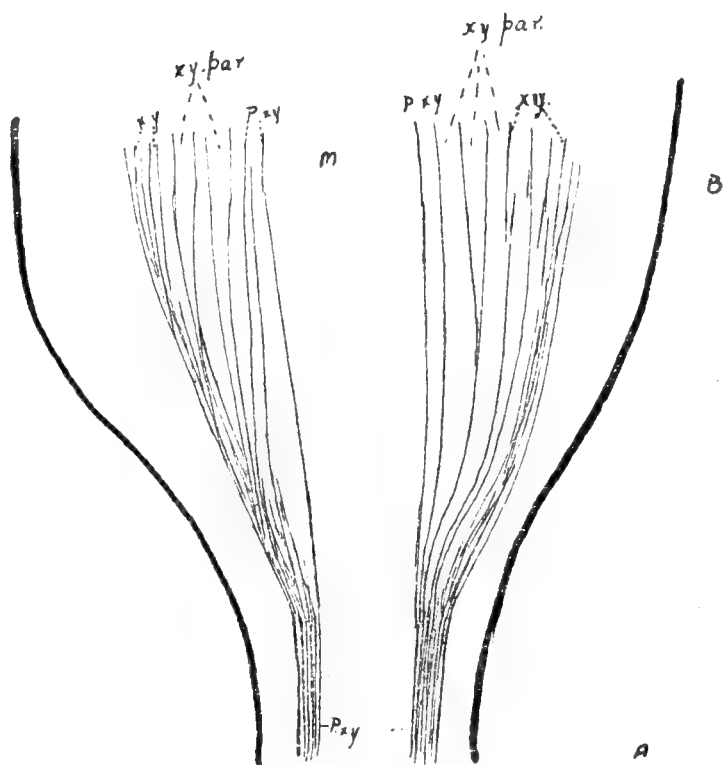


DIAGRAM III. *xy. par.*, xylem parenchyma. *P.xy.*, protoxylem. *xy.*, xylem. *M.*, pith.

A series of transverse sections passing from *A* to *B* would present the features as shown in Diagram IV.

In the above description the term dilatation parenchyma has been purposely avoided. We have no evidence to show that the xylem parenchyma in this case is true dilatation parenchyma. This term is restricted to parenchyma which has ordinarily ceased to be active, but which may be recalled to activity by pathological conditions caused by fungal attacks, mechanical injury, &c.

At the stage represented by Fig. 9, Pl. XLII, the cambium does not extend the whole way round the stem, but presently this extension is effected. The fascicular cambium produces very few new lignified elements, in which respect it agrees with *S. tuberosum*; most of the tissue to which it gives rise is xylem parenchyma which goes to form part of the tuberous tissue. The interfascicular cambium gives rise to *no* new xylem vessels, but restricts itself to the production of parenchymatous cells. The broad parenchymatous medullary ray tissue thus produced is arranged in regular radial rows as shown in Pl. XLII, Fig. 11, *M.ry.*, and contributes largely to the tuberous tissue. A similar tissue is described as occurring in tubers of *Euphorbia Intisy* by MM. Dubard and Viguier (5).

A few remarks might conveniently be added at this point with reference to the distribution of inulin in these tubers and its influence on cell division. The inulin is seen in young tubers as masses of sphere crystals surrounding the xylem elements of the bundles and 'welling' over into the immediately adjacent parenchyma. Tuberization does not appear to commence until these crystals are deposited in the young tuber, but as soon as

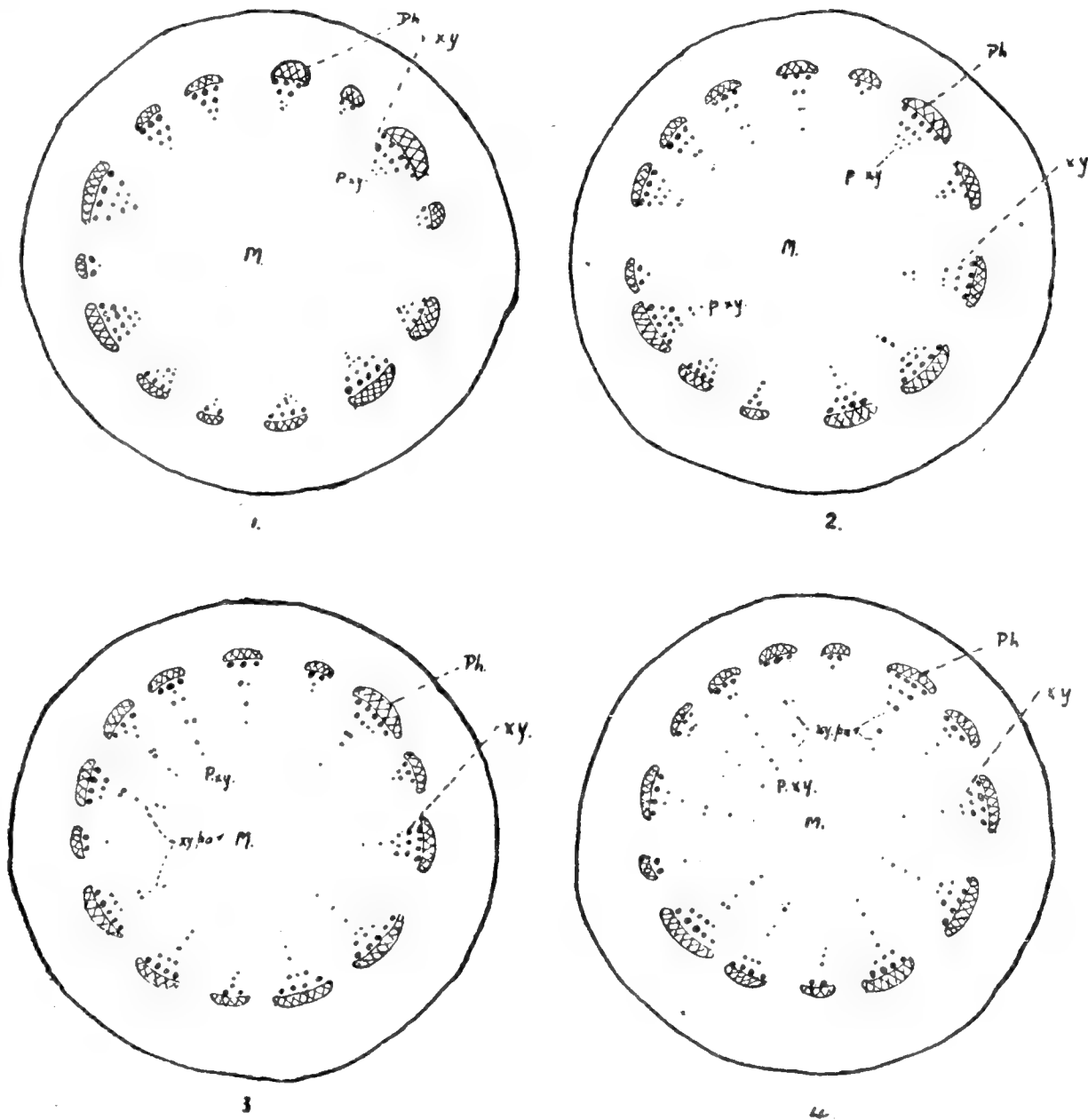


DIAGRAM IV. *Ph.*, phloem. *xy.*, xylem. *P.xy.*, protoxylem. *M.*, pith. *xy.par.*, xylem parenchyma.

they appear divisions commence in the parenchyma in their immediate vicinity. In some few cases the distribution of the inulin crystals was irregular; one side of the young tuber might contain an abundance of inulin, whilst the other side had comparatively little. When such was found to be the case the region devoid of inulin deposits had not become meristematic. It would appear then that the presence of the food reserve has a stimulating effect on the cells in which it is deposited, or it may possibly be the reverse, i. e. the activity of the cells demands a supply of food material.

From the above it is seen that the tuber of *Helianthus tuberosus* is made up of parenchyma derived from the activity of the medullary tissue and

xylem parenchyma, together with parenchyma derived from the division of the interfascicular cambium. The cortex is not concerned with the addition of any appreciable amount of tissue to the tuber.

Tubers of the White and Red Fir Apple Potato (Sutton and Sons, Reading) were also examined. These tubers more nearly resemble ordinary stem structures in their external characters than does either of the two tubers already described. Their anatomical structure corresponds with their external appearance and more nearly approximates to that of a normal stem.

As in *S. tuberosum* the tissues concerned with tuberization are the pith and to a certain extent the cortex, together with the parenchyma which separates the medullary phloem from the protoxylem. The development of tuberization parenchyma, however, does not go on to such an extent as in any way to render obscure the normal stem structure, so that on the whole we may say that it represents what we have in the case of *S. tuberosum*, only it does not go quite so far with its tuberization, particularly with respect to the formation of parenchyma from the tissue separating the medullary phloem from the xylem.

#### SUMMARY OF RESULTS.

1. The tuber of *Solanum tuberosum* is formed mainly from medullary parenchyma and from the parenchyma between the xylem and the medullary phloem. This latter source is largely responsible for the scattered distribution of the medullary phloem strands in the tuber.

2. The medullary phloem probably serves as the channel for supplying food material to the parenchymatous portion of the tuber.

3. The tuber is covered with a layer of cork which is developed from the hypoderma.

4. The structure of the tubers of the White and Red Fir Apple Potatoes is similar to that of *S. tuberosum*, but much less tuberous tissue is developed.

5. The tuber of *Helianthus tuberosus* is made up of medullary parenchyma, xylem and medullary ray parenchyma. In this latter respect it differs from *S. tuberosum*.

6. The distribution of inulin appears to have some influence on the meristematic activity of the cells.

7. In tubers of *S. tuberosum* and *H. tuberosus* few, if any, secondary lignified elements are formed.

In conclusion I wish to express my thanks to Prof. J. B. Farmer, F.R.S., for the suggestion that this work should be undertaken, and for his help and advice during the progress of this research; and also to Mr. Hales, the Curator of the Chelsea Physic Garden, where this work was carried out.

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## EXPLANATION OF FIGURES IN PLATES XLII AND XLIII.

Illustrating Mr. Reed's paper on the Anatomy of some Tubers.

Abbreviations used: *cam.*, cambium; *co.*, cortex; *ck.*, cork cells; *c.c.*, cork cambium; *En.*, endodermis; *Ep.*, epidermis; *Hyp.*, hypoderma; *M.*, medulla; *M.Ph.*, medullary phloem; *M.xy.*, medullary ray; *P.*, phelloderm; *P.xy.*, protoxylem; *xy.*, xylem; *xy.p.*, xylem parenchyma; *st.g.*, starch grains; *S.*, stolon; *T.*, tuber; *s.lv.*, scale leaves.

Figs. 1-6. *Solanum tuberosum*.

- Fig. 1. Transverse section of stolon, showing portion of vascular cylinder.  
 Fig. 2. Longitudinal section of stolon, showing medullary phloem.  
 Fig. 3. Longitudinal section of tuber, showing isolated strands of medullary phloem.  
 Fig. 4. Longitudinal section of tuber at its junction with stolon, showing the isolated protoxylem and the meristematic pith cells.  
 Figs. 5 and 5 a. Transverse section of tuber, showing origin and development of cork cambium.  
 Fig. 6. Longitudinal section of tuber, showing isolated strand of medullary phloem.

Figs. 7-14. *Helianthus tuberosus*.

- Figs. 7 and 8. Drawings of young tubers. *T.*, developing tuber; *s.lv.*, scale leaves; *S.*, stolon.  
 Fig. 9. Transverse section of stolon, showing portion of the vascular cylinder.  
 Fig. 10. Transverse section of tuber, showing isolation of protoxylem by developing wood parenchyma.

Fig. 11. Transverse section of tuber, showing interfascicular cambium (*intf. cam.*) forming radial rows of parenchyma cells (*M.ry.*).

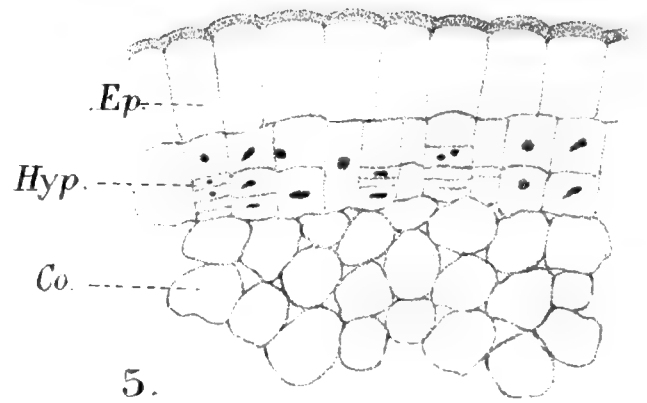
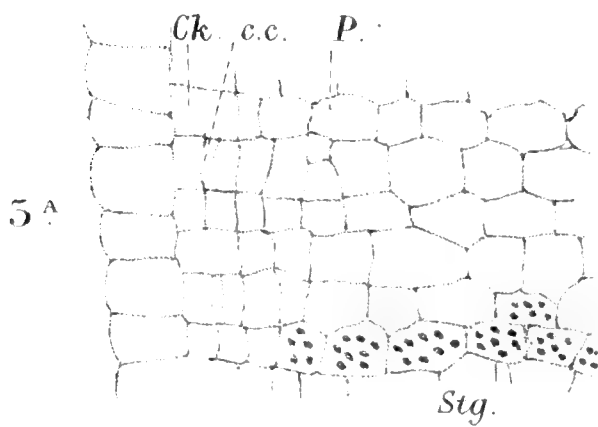
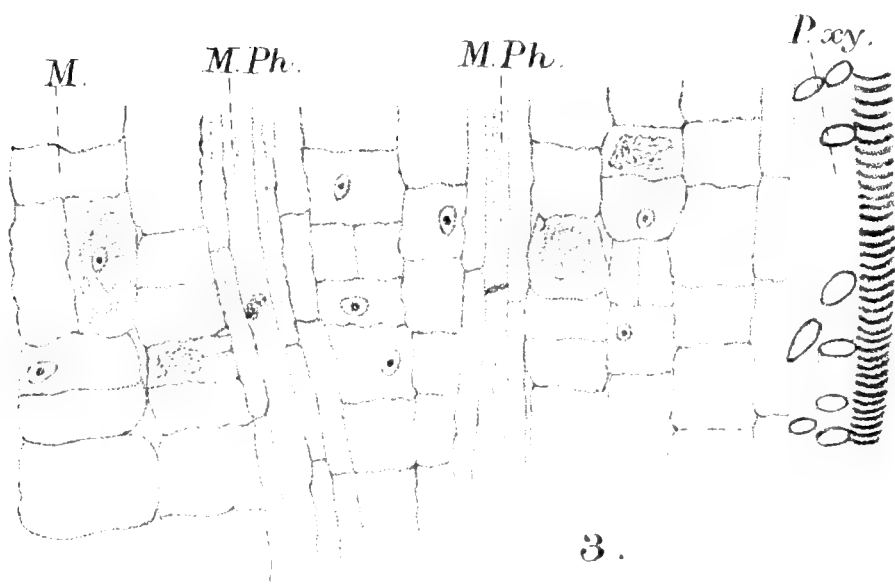
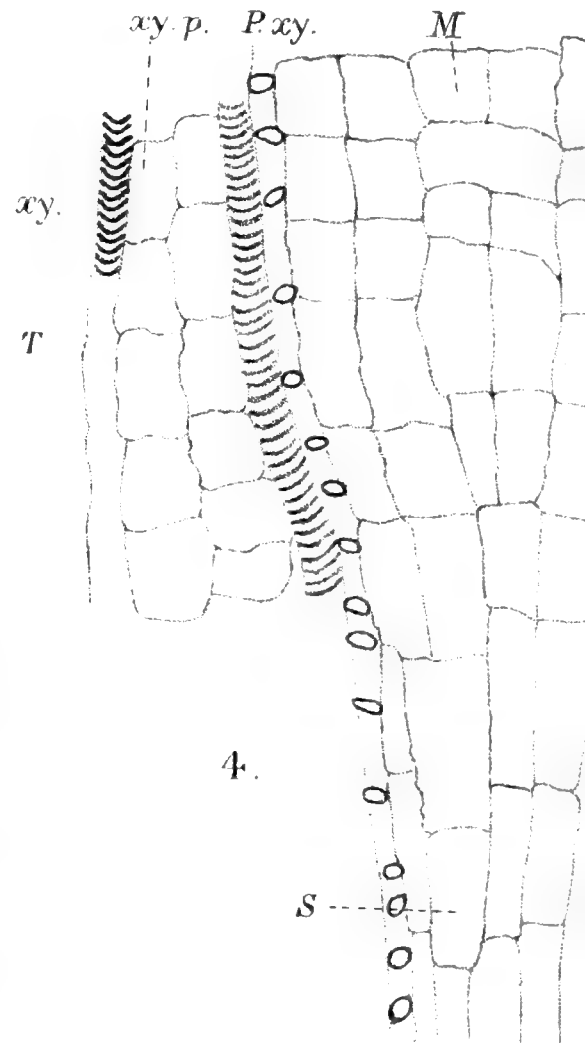
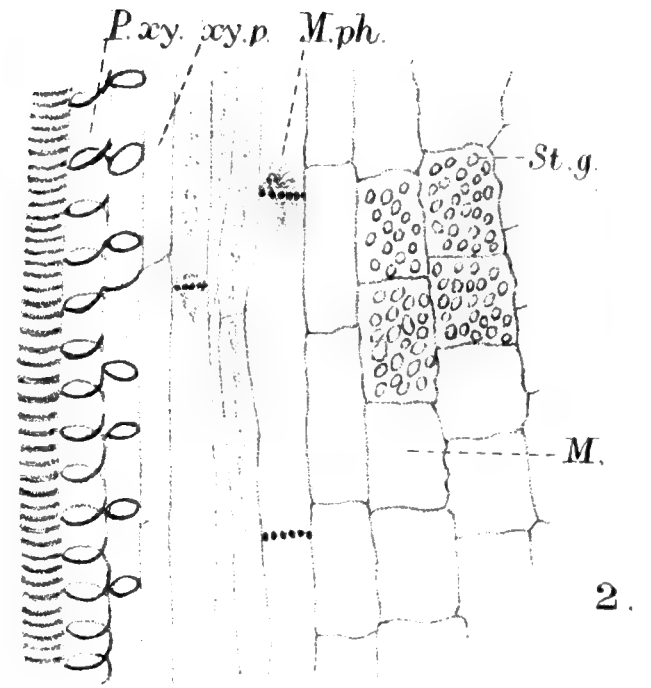
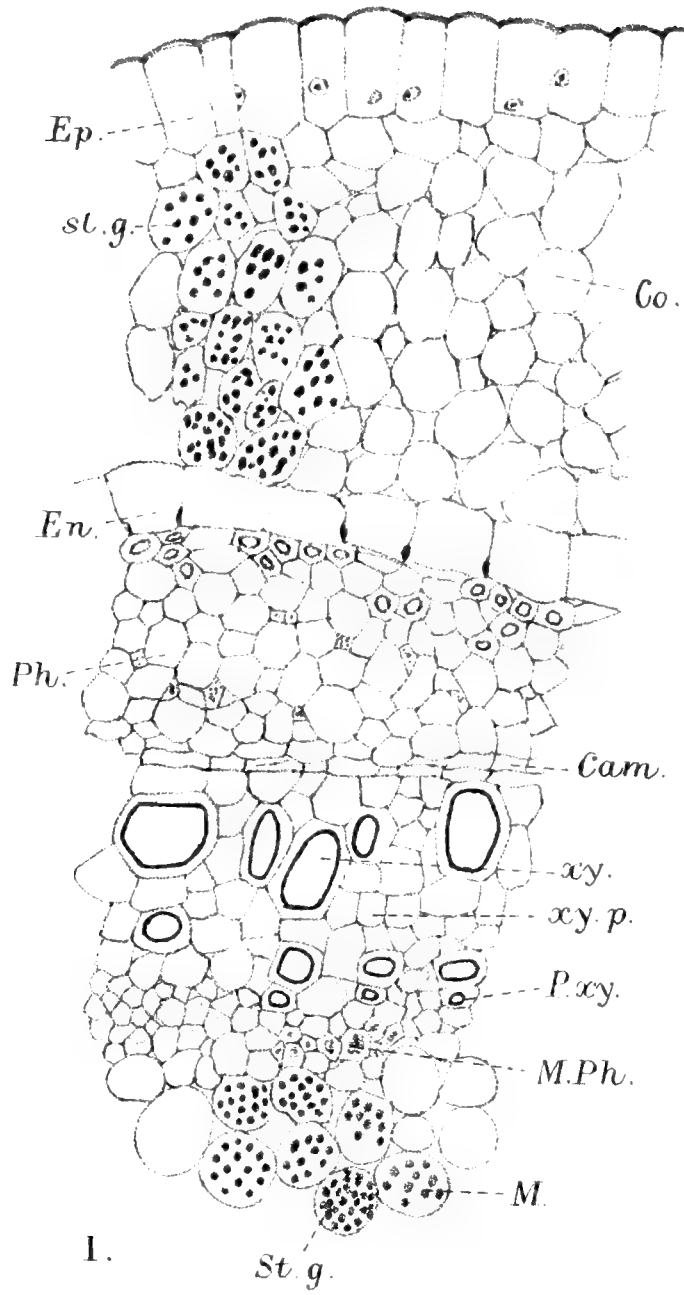
Fig. 12. Transverse section of young tuber, showing the early stages of cell division at periphery of medulla.

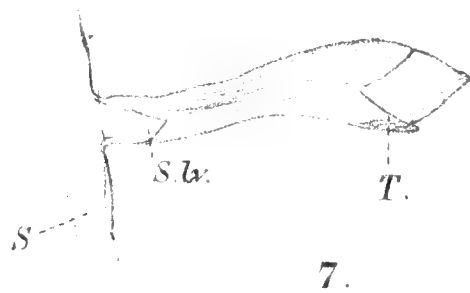
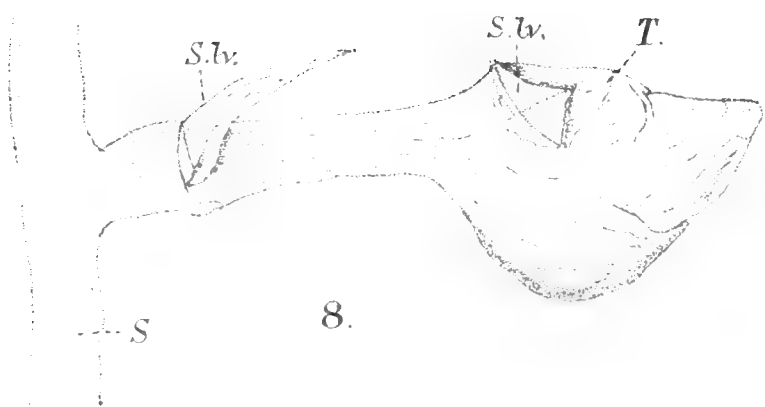
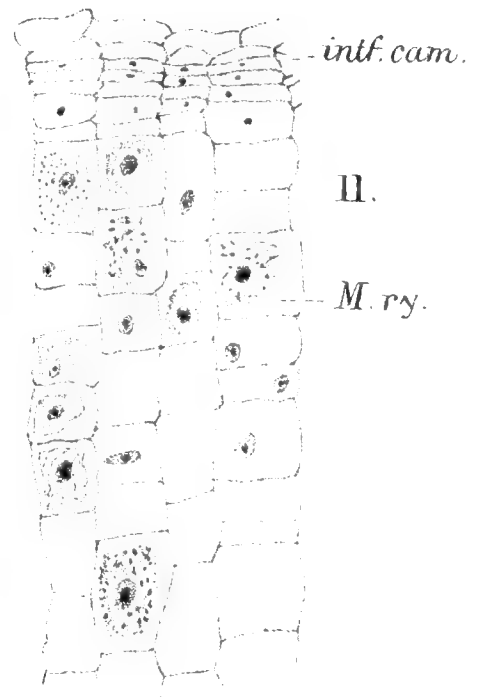
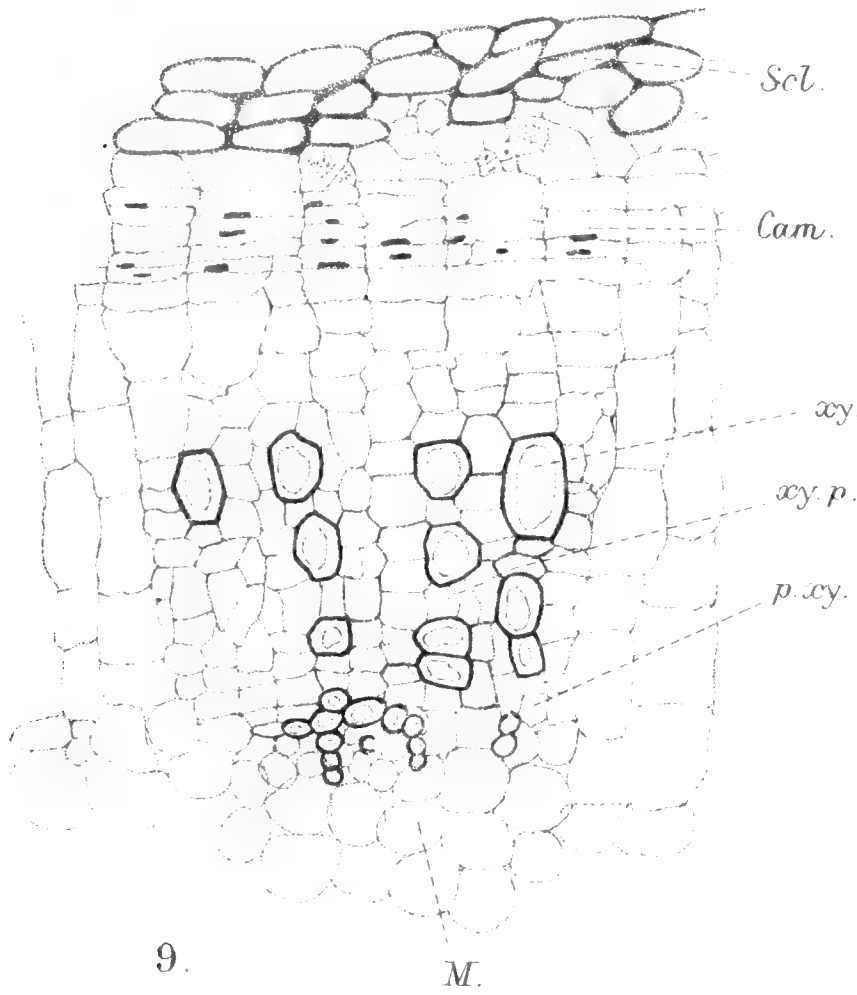
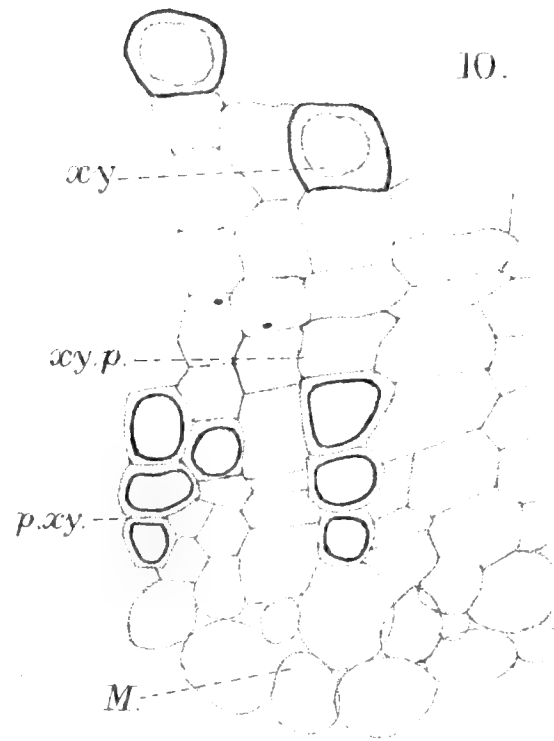
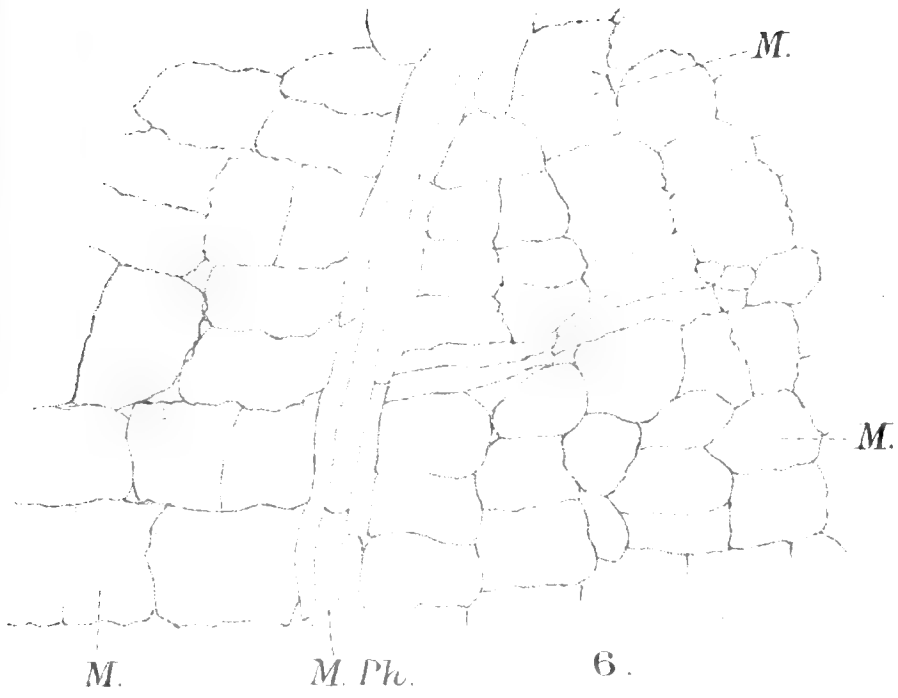
Fig. 13. Transverse section of older tuber, showing the radially arranged medullary parenchyma.

Fig. 14. Transverse section of old tuber, showing the isolated strands of xylem and extensive development of xylem parenchyma.

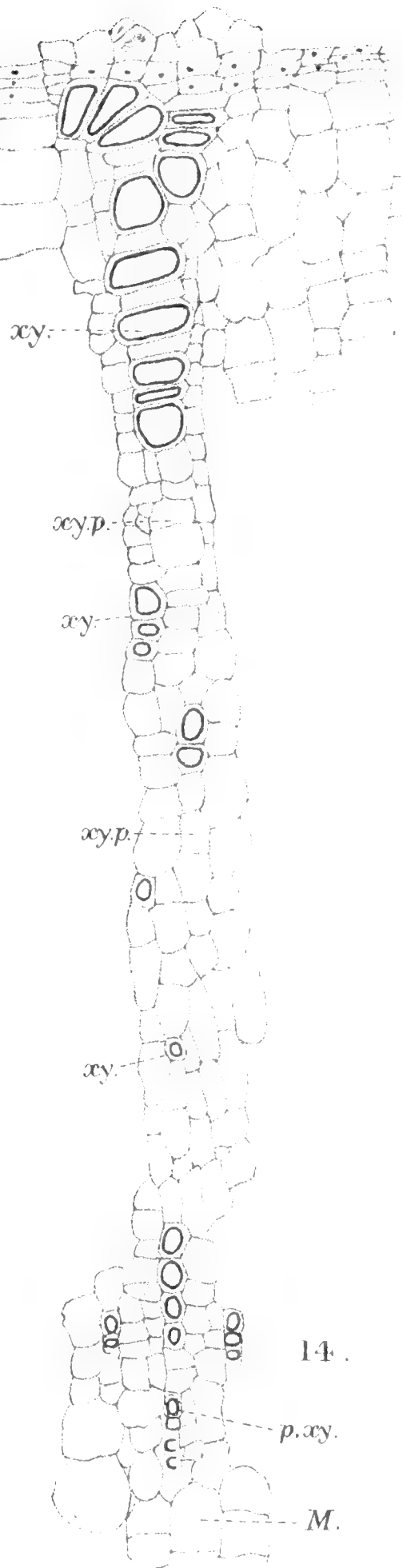
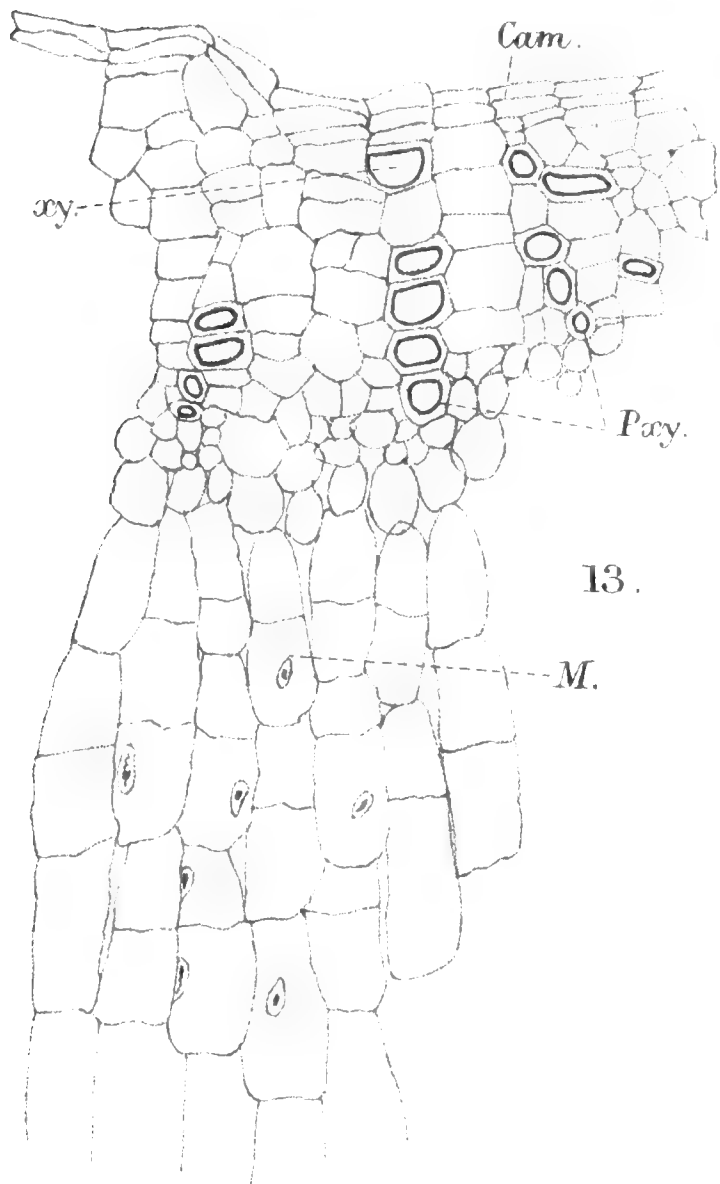
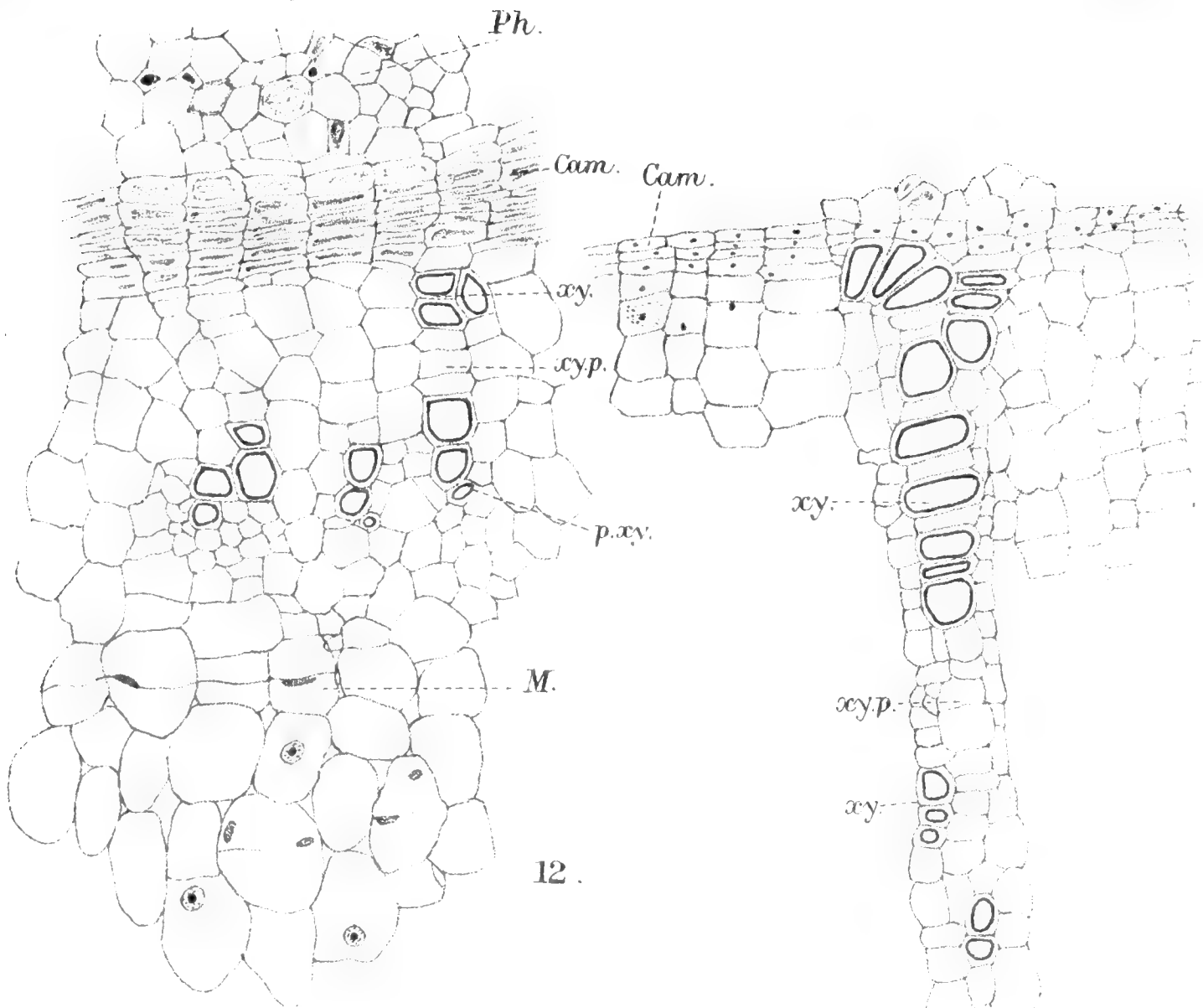












Huth, lith et imp.

REED — HELIANTHUS.



# Utricularia emarginata, Benj.

BY

BERTHA CHANDLER, M.A., B.Sc.

With Plate XLIV.

THOUGH the genus *Utricularia* has received more attention than many others, owing to peculiarities of form, development, and to the possession of characteristic bladders, which features distinguish them from other plants, yet of the 150 or so species which are comprised in this interesting genus, only a very small fraction have been thoroughly examined, except from a systematic point of view. As early as 1848 Benjamin<sup>1</sup> gave his results of a minute examination of *Utricularia vulgaris*, *U. minor*, &c., only, however, from a morphological and physiological standpoint; and in this direction also are the recent investigations of Kamienski,<sup>2</sup> Pilger,<sup>3</sup> Brown,<sup>4</sup> Stapf,<sup>5</sup> Small,<sup>6</sup> Ostenfeld,<sup>7</sup> Goebel.<sup>8</sup> Other investigators such as Buchenau,<sup>9</sup> Cohn,<sup>10</sup> Darwin,<sup>11</sup> Pringsheim,<sup>12</sup> have greatly contributed to our knowledge of the structure and function of the various parts of the plant. From a comparative standpoint, however, the germination of the Utricularias is perhaps the most interesting and the most important; and among the first investigators in this direction was Kamienski,<sup>13</sup> who took as his type the common bladderwort, *Utricularia vulgaris*. Goebel<sup>14</sup> has described and figured other species, e. g. *U. oligosperma*, *U. reniformis*, &c., with a more detailed description of *U. vulgaris*. More recently,<sup>15</sup> he has examined other species in greater detail, such as *U. flexuosa*,

<sup>1</sup> Botanische Zeitung, 1848.

<sup>2</sup> Supplement II, Ann. du Jard. Bot. de Buitenzorg, 1898. Botanische Jahrbücher. Engler, xxxiii, 1902.

<sup>3</sup> Botanische Jahrbücher. Engler, xxx, 1901-2.

<sup>4</sup> Trans. Linn. Soc., London (Ser. 2), vi, 1901.

<sup>5</sup> Dyer : Flora Trop. Afric. 4, Sect. II, 1906. Dyer : Flora Cap. 4, Sect. II, 1904.

<sup>6</sup> Flora of the United States. New York, 1903.

<sup>7</sup> Repert. nov. sp. regni veget. Berlin., ii, 1906.

<sup>8</sup> Gartenflora, 40, 42, 45.

<sup>9</sup> Botanische Zeitung, 1865.

<sup>10</sup> Über die Funktion der Blasen . . . Pflanzen, 3. Heft, 185.

<sup>11</sup> Insectivorous Plants.

<sup>12</sup> Monatsberichte der Berliner Akademie, 1869.

<sup>13</sup> Botanische Zeitung, 1877.

<sup>14</sup> Goebel : Pflanzenbiol. Schilderungen, 2, p. 127.

<sup>15</sup> Ann. du Jard. Bot. de Buitenzorg, 9, 1890-I.



*U. exoleta*, *U. stellaris*, *U. coerulea*, &c., particularly from the standpoint of the relations of the various species one to another, and the homologies of the various parts, but also from the standpoint of germination. Glück,<sup>1</sup> in his comprehensive study of water and marsh plants, has classified the *Utricularias* that have been studied to some extent, according to their mode of germination, into three series.

1. Those having a number of primary leaves, for example :—*U. vulgaris*, *U. oligosperma*, *U. reniformis*.

2. Those having two primary leaves, for example :—*U. exoleta*.

3. Those having no primary leaves, for example :—*U. bifida*, *U. laterifolia*, *U. montana*.

*Utricularia emarginata*, a Mexican species, is one hitherto practically unmentioned by the various authorities on *Utricularia*. It is given as a distinct species in Index Kewensis ;<sup>2</sup> no mention is made of the species in Engler-Prantl, but being a true aquatic, it would fall under section B, in the classification of *Utricularias*, 'Wasserformen mit geteilten oder gefiederten Blättern.' The material used in the description was grown from seeds obtained in the Royal Botanic Garden, Edinburgh, from a specimen in Pringle's Mexican collection of 1904.

*Conditions of germination.* Mr. L. Stewart, foreman of the glass department of the Royal Botanic Garden, Edinburgh, has proved, as mentioned in a previous note,<sup>3</sup> that the seeds flourish best in a shallow pan, having a thin layer of mud at the bottom with enough water to cover the mud. As the plant is insectivorous and the presence of Microcrustacea is essential for food, experience has shown that the best method of cultivation is to keep the plant in partial shade in still water. The water soon becomes alive with Microcrustacea, and the plant thrives and grows rapidly. The young seedlings from which some of the drawings were made at the different stages were kept merely in a small jar of water, maintained at a constant temperature by being placed on top of a jacketed embedding oven. The only difficulty here is the rapid evaporation of the water, and the danger of chilling when adding a fresh supply. Also, the water added must not be clean, as in that case the supply of Microcrustacea is soon reduced, and the plant dies for want of nourishment.

*Seed and germination.* The seeds of *Utricularia emarginata* are minute, about 0.5 mm., round, and covered with a dark brown, net-like testa, extending beyond the seed itself into a wing-like expansion with irregular edges (Pl. XLIV ; Fig. 1, *a*). This outer covering is easily detached by means of a fine needle, revealing the round embryo in a light brown tegmen,

<sup>1</sup> Glück : Biologische und morphologische Untersuchungen über Wasser- und Sumpfgewächse. Teil ii, Jena, 1906.

<sup>2</sup> Linn., xx, 1847.

<sup>3</sup> Ann. of Botany, vol. xxiii. Preliminary Note on *Utricularia emarginata*.

with a darker spot at the micropylar end, opposite to which all later development takes place. This dark spot indicates the place of attachment of the seed to the mother plant. When the tegmen is also removed, the naked embryo is seen to be not quite round in shape, but slightly flattened at the base, that is, at the micropylar end, where the primordium of the root would normally develop in other embryos. *Utricularia emarginata*, like *U. vulgaris*, is, however, rootless. In order to see the very early stages of germination, while the testa is not yet burst, the seeds must be carefully examined by dissecting off the outer coverings. Germination was regular in all the material examined: in this respect *Utricularia emarginata* differs from *U. exoleta* described by Goebel,<sup>1</sup> with which the present species would occupy a place in the second series of Glück's classification.<sup>2</sup>

The embryo, which is of a pale green colour, at first shows no differentiation, but very soon at the apex four primordia appear (Fig. 2, *a, b*). Of these, the two outer develop more rapidly than the two inner, thus showing the same order of development as in *U. vulgaris*, but to a more limited extent (Figs. 3-5, *a, a*). In that species, as Kamienski<sup>3</sup> has pointed out, the organs appear in the following order: the oldest primary leaves are at the outside, the leaves gradually becoming younger as one proceeds inwards; the secondary shoot (*Adventivsprosse*) is formed from the youngest protuberance in the centre, the main stalk from the youngest but one, and the first bladder from the youngest but two. Though differing so much in the number of primary leaves, yet *U. emarginata* follows the same order of development. The outer two protuberances form the primary leaves, and develop most rapidly. Of the remaining two protuberances, one forms the main shoot (Figs. 3-5, *b*) and at first develops, not quite so rapidly as the two primary leaves, but more rapidly than the youngest outgrowth (Figs. 3-5, *c*), which forms another shoot—analogueous to Kamienski's *Adventivsprosse*. Thus we have, although to a much more limited extent, the same order of development as in *U. vulgaris*.

From its earliest stages, the main shoot possesses circinate ptyxis (Figs. 4, 5, *b*), a feature noticeably lacking in the development of the two primary leaves (Figs. 3, 4, *a*). Though its growth is at first not so rapid as the two mentioned outgrowths, it soon outdistances them in length, and when the two primary leaves have ceased further development, the main shoot, having unlimited growth, still continues to increase at its rolled up apex (Figs. 6-9, *b*). By continued dichotomy of the growing point, as in other *Utricularias*, linear lateral appendages ('leaves' of authors) are given off (Figs. 6-9, *b*). The first bladder appears in a super-axillary position on the first formed of these appendages (Figs. 6, 7, 9, *e*). The younger shoot (*Adventivsprosse* of Kamienski) also elongates, but more slowly, and in its

<sup>1</sup> Ann. du Jard. Bot. de Buitenzorg, 9, 1890-1.

<sup>2</sup> p. 2.

<sup>3</sup> Botanische Zeitung, 1877.

turn branches and produces lateral appendages and bladders. The two oldest outgrowths ('primary leaves') develop and increase in size until they attain a length of some two to three times that of the original seed, when further development, at least for a period, ceases. Investigation remains to prove whether these two primary outgrowths develop into ordinary water-shoots after the seed has fallen away. Of what physiological importance these so-called primary leaves are to the plant it is difficult to determine. That they are leaves and not cotyledons Goebel<sup>1</sup> affirms, and he asserts that if one be cut off, it will develop into an ordinary water-shoot. In his later paper,<sup>2</sup> however, he sees no reason why these two outgrowths should not be designated as 'cotyledons', though shoots can and do replace them.

The seedling with its growing points develops rapidly, the water-shoots reaching a great length, so that the plant covers a large area in a comparatively short space of time, always remaining submerged.

The general appearance of *Utricularia emarginata* therefore, which has been growing some time, is that of a matted mass of submerged water-shoots, with here and there flower-stalks arising out of the water.

The external appearance of the water-shoots have already been described.

The aerial flower-stalk does not develop for some time. In its origin it does not differ from that of *Utricularia vulgaris*, arising from a bud on the upper surface of the main stem. Only one example was seen, for it is difficult to discern the small bud on the water-shoot. At the base of every well-developed flower-stalk, however, is to be found a bud which in its turn gives rise to a flower-stalk, and it is from secondary flower-stalk buds that observations and figures were made. The bud arises at the base of the old flower-stalk in the axil of the youngest water-shoots formed, and is easily distinguishable to the naked eye, even in its young stages, by its solid appearance and reddish colour (Figs. 10, 11, *b*). It is early differentiated into stalk and flower-bud. It develops and in its turn bears a younger bud at its base. The old flower-stalk rises to a height of about 15–20 cm. and bears about 2–3 small flowers (Fig. 12). The flower (Fig. 13) does not differ very much markedly from that of *U. vulgaris*. The lower and upper lips of the corolla (Figs. 13, *a*, *b*) and the pouched petal (Fig. 13, *c*) are all yellow, while the tongue (Fig. 13, *d*) is white tipped with yellow.

The fruiting of *U. emarginata* is somewhat characteristic. While still young the fruit is green and opaque, and half-hidden under the persistent sepals. Later, however, it increases in size, becoming transparent, and the seeds show as dark spots through the ovary-wall. When the fruit is ripe, the flower-stalk bends over and lies flat on the surface of the water; the fruit, breaking away from the flower-stalk, opens, and floats on the surface of the water, the seeds in mucilage still adhering to the fruit wall. Finally

<sup>1</sup> Goebel: Schilderungen, 2.

<sup>2</sup> Ann. du Jard. Bot. de Buitenzorg, 1890–1.

the seeds, freed from the decaying mucilage, sink to the bottom of the water, and remain until ready to germinate.

Looking again at the base of the flower-stalk we find, as in *U. vulgaris*, limited shoots, usually about three in number, with small white protuberances (Fig. 11, *d*). These are rhizoids; though from the frequent transition at the tip into ordinary water-shoots, one might easily mistake their significance. The length of these rhizoids are from 5-15 mm. They possess circinate ptyxis and usually develop into normal water-shoots after the production of five or six rhizoid segments.

The rhizoid segments to the naked eye present the appearance of small whitish protuberances on the main shoot (Figs. 11, *d*, 15, *a*). Under the microscope they appear as divided into segments, six or nine segments being a very usual number (Fig. 16). These segments are caused by the dichotomizing of the main side branch of the rhizoid, one portion usually dichotomizing however, in advance of the other.

The rhizoid segments (Fig. 16) are in general shape not unlike those figured by Glück<sup>1</sup> for *Utricularia vulgaris*, but the individual segments are themselves more similar to those of *U. intermedia* or *U. neglecta*, for the ends of the segments are not pointed and bristle-tipped as in *U. vulgaris*.

The ends of the segments of the rhizoids of *U. emarginata*, also, do not broaden out, but preserve a uniform thickness, though they show a curling apex like those of other species (*U. intermedia*, &c.). The apices of the segments are thickly covered with glandular hairs (Fig. 16), but, as already remarked, there is no bristle present as in *U. vulgaris*.

The rhizoids of *U. emarginata*, as in *U. vulgaris*, are very reduced. They are metamorphosed water-shoots, and unlike *U. vulgaris* (according to Glück), the tip in *U. emarginata* is nearly always transformed into an ordinary water-shoot. Up till now, rhizoids have only been found on the two species, *U. flexuosa* and *U. exoleta* for the tropical submerged species, and the rhizoids of *U. emarginata* seem to differ in no marked way from the rhizoids of these two species.

Glück has worked out the function of rhizoids in land and water species of *Utricularia*, and has shown that in the case of submerged species, the rhizoids naturally lose their anchoring function to a great extent, and therefore become almost or entirely degenerate. The frequent metamorphosis of the rhizoids of *U. emarginata* into ordinary water-shoots should be noticed.

Examining the tip of even a very young rhizoid, bladders and leaf segments are to be seen formed in the usual way by the dichotomy of the growing point, one portion of the segment forming a roundish knob which will develop into a bladder, the other a more pointed portion which develops into a leaf segment ended by a sharp bristle (Figs. 15 *b*, 20 *a*, *b*). Sometimes the rhizoid segments have been changed into bladders and leaf

<sup>1</sup> Glück, loc. cit.



segments and the other half remain unchanged at the base. Various transitions are noticeable, all showing that the rhizoids are metamorphosed water-shoots (Figs. 17–20).

As fresh water-shoots are produced on the main stem, every well-developed flower-stalk is covered at its base by a cluster of water-shoots and rhizoids, possibly for the purpose of giving support and stability to a stem itself so much thicker and stronger than one of the frail water-shoots. The usual number of shoots at the base of a well-developed flower-stalk seems to be between twenty and thirty (Figs. 10, 11).

*Bladders.* The position of the first bladder has been observed. In its subsequent development it does not differ from that of *U. vulgaris*. The development of this organ is perhaps best watched in the region of the rolled-up apex of a water-shoot, where it appears in various stages. It arises as a roundish knob on a short stalk. Later, a slit is produced which gradually becomes larger until the whole becomes hollow. The bladders of *U. emarginata* only differ from those of *U. vulgaris* in the shape of the quadrifid processes (Fig. 22).

*Morphology.* The internal structure of the water-shoot is simple. A transverse section of the main stem (Fig. 21) or of one of the leaves shows a structure very similar to that of the floating leaf of *Salvinia natans*, and needs but little comment. The vascular system is weakly developed, and consists mainly of spiral vessels in the centre of the strand. These spiral vessels were very readily seen on treatment of the shoot with caustic potash, especially in the examination of the rhizoid segments, into which subsidiary vascular strands run (Fig. 16).

The internal structure of the flower-stalk (Fig. 14) is more complicated than that of the water-shoot. It differs mainly in the reduction of the aerenchyma, a feature which might be expected from its aerial nature. Though larger in its diameter (1–1.5 mm.) a transverse section of *U. emarginata* does not differ much in its essential features from that of the peduncal of *U. brachiata* recently described.<sup>1</sup>

The epidermis is composed of regularly shaped cells slightly concave and thickened on their outside walls. Inside the epidermis is a small amount of aerenchyma. A ring of large cells forms the endodermis. In the ground-tissue the cells are small immediately behind the endodermis, but increase in size towards the centre, the cells of the pith being as large, if not larger than those of the endodermis. As in *U. brachiata*, five or six groups of vascular tissue are irregularly distributed, sometimes bordering on the pith and even in some cases occurring just behind the endodermis.

<sup>1</sup> New Phytologist, April, 1908. *U. brachiata*, R. H. Compton.

## EXPLANATION OF PLATE XLIV.

Illustrating Miss Chandler's paper on *Utricularia emarginata*, Benj.

Fig. 1. Seed of *Utricularia emarginata*. *a*, wing-like testa.

Fig. 2. Naked seed showing four primordia. *a, a*, the two outer; *b, b*, the two inner.

Figs. 3-9. Embryo in various stages of development. *a, a*, the two primary outgrowths; *b*, the main shoot; *c*, secondary shoot (*Adventivsprosse*); *d*, linear lateral appendage (leaf); *e*, first formed bladder.

Fig. 10. Base of aerial flower-stalk showing development of—*a*, old flower-stalk; *b*, bud of new flower-stalk; *c*, water-shoot.

Fig. 11. Same, later stage. *a*, old flower-stalk; *b*, bud of new flower-stalk; *c*, water-shoot; *d*, rhizoid segment.

Fig. 12. Flower-stalk (nat. size) showing—*a*, flower; *b, b*, buds; *c*, bud of new flower-stalk; *d*, water-shoot.

Fig. 13. Flower in detail. *a*, upper lip of corolla; *b*, lower lip; *c*, pouched petal; *d*, tongue.

Fig. 14. T. S. of flower-stalk showing—*a*, epidermis; *b*, aerenchyma; *c*, endodermis; *d*, ground-tissue; *e*, vascular tissue.

Fig. 15. Rhizoid showing—*a*, rhizoid segments; *b*, tip of rhizoid metamorphosed into ordinary water-shoot.

Fig. 16. Rhizoid segments showing glandular tips and curled apices.

Figs. 17-19. Development of bladders and leaf tips from rhizoid segments.

Fig. 20. Segment of leaf dichotomizing. *a*, pointed apex which forms the leaf bristle; *b*, rounded apex which forms the bladder.

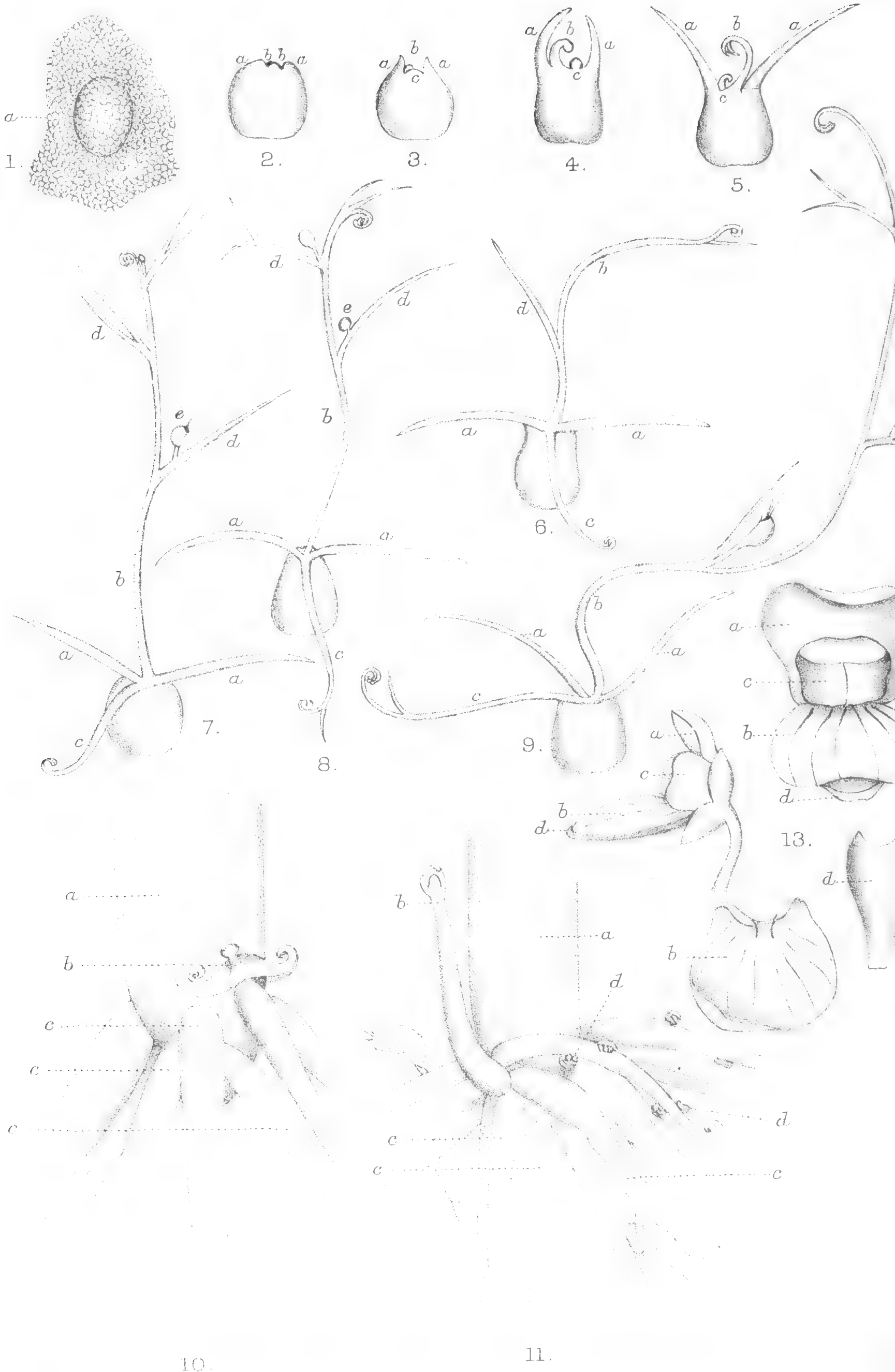
Fig. 21. Transverse section of water-shoot.

Fig. 22. Quadrifid processes inside the bladder of *U. emarginata*.



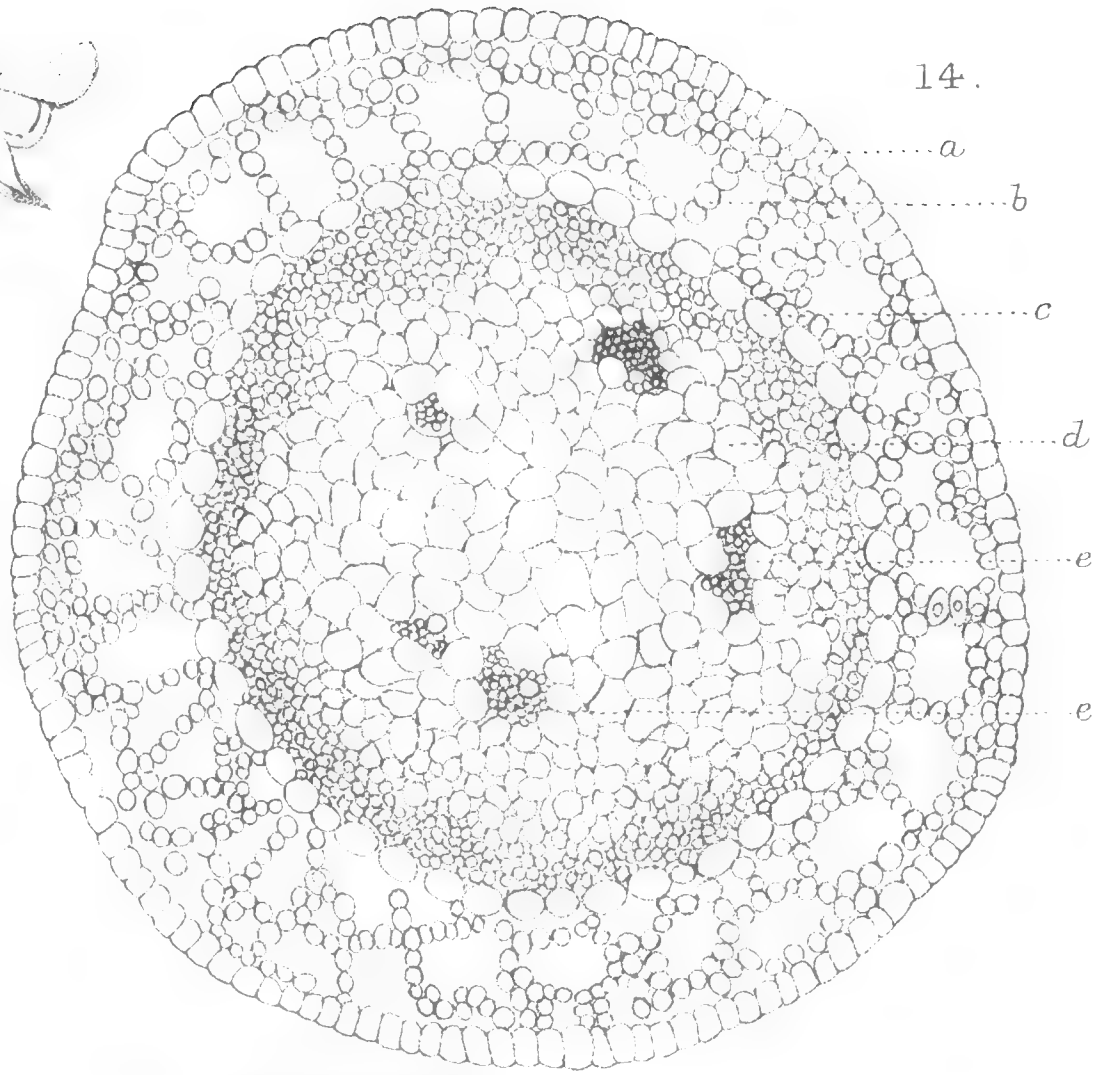






CHANDLER — UTRICULARIA EMARGINATA.

14.



b

a

a

b

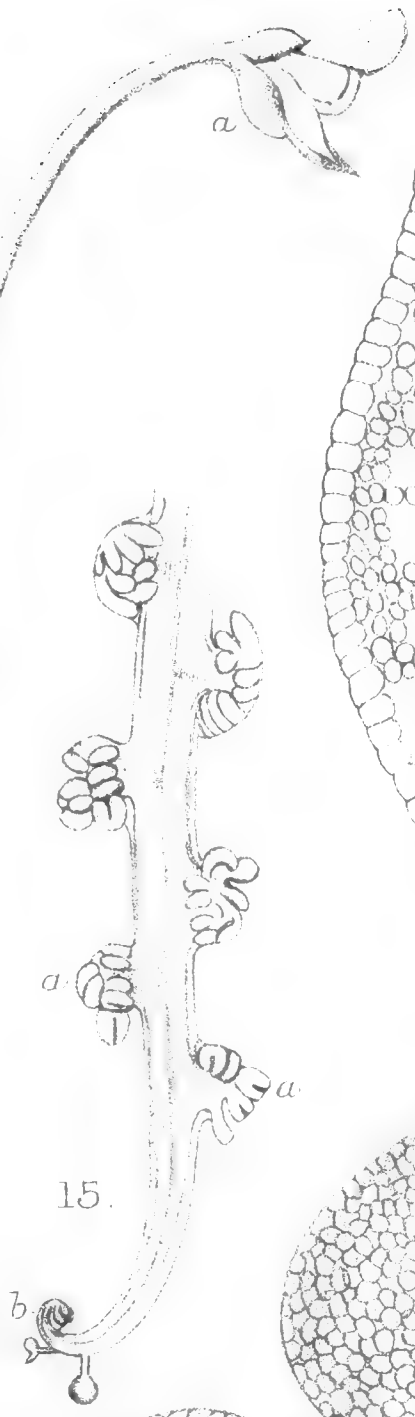
c

d

e

e

15.



b

a

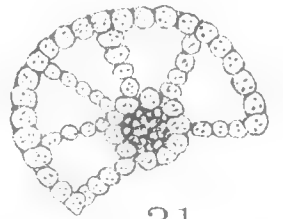
a

a

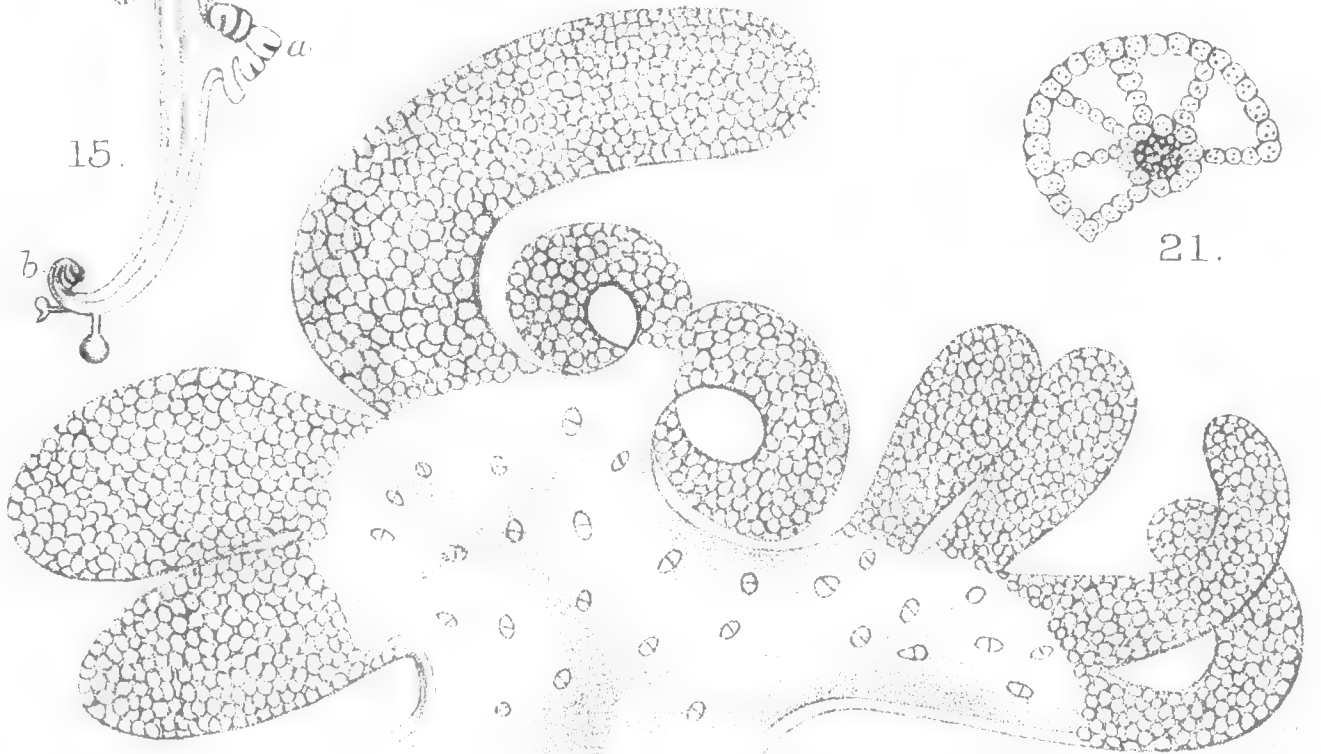
a

a

21.



16.



12.

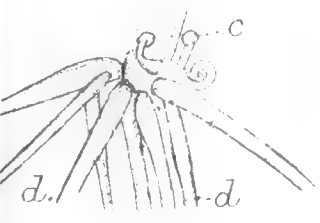
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18.

19.

20.

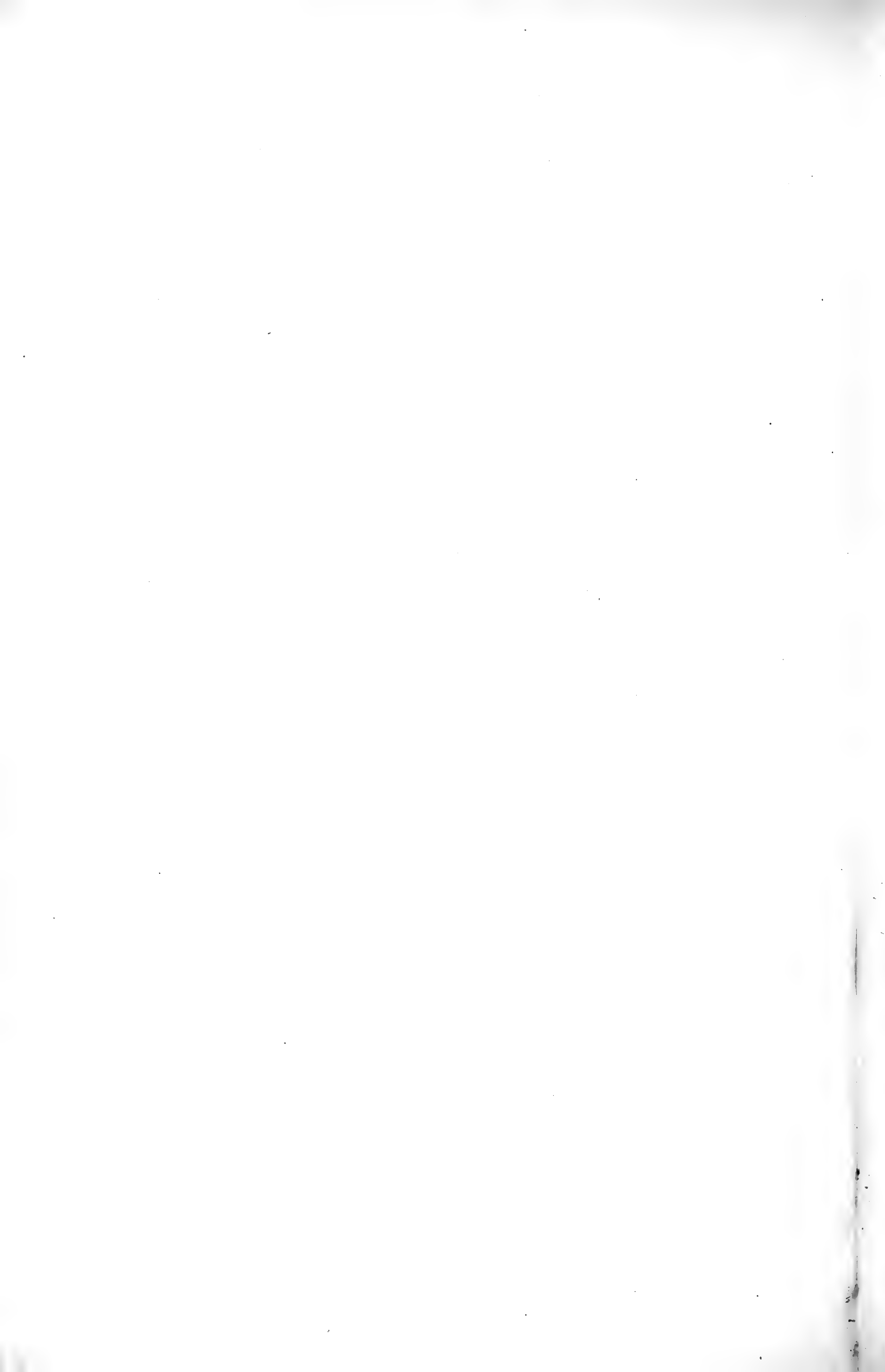
22.



d. d.

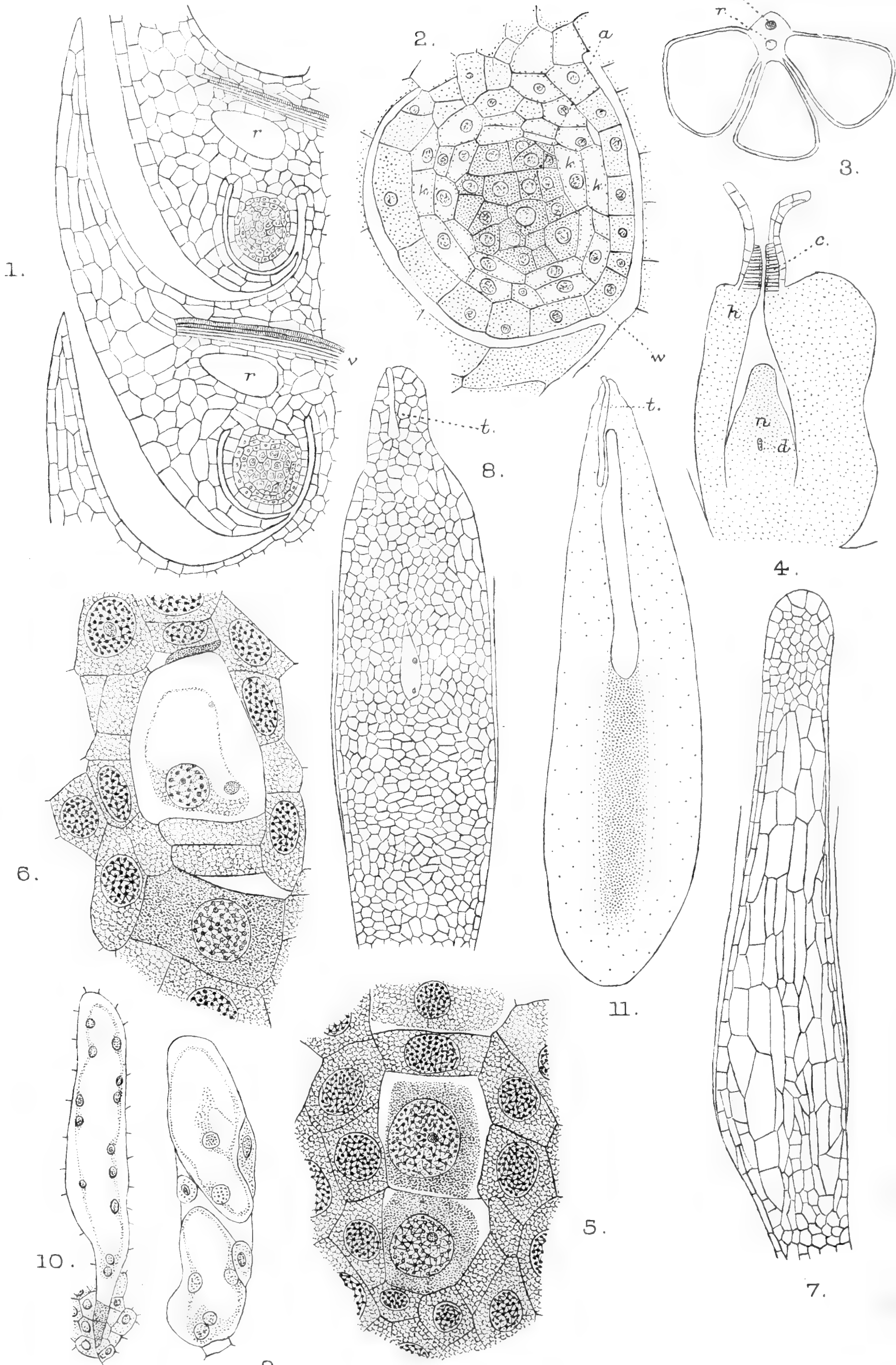
a b

a b

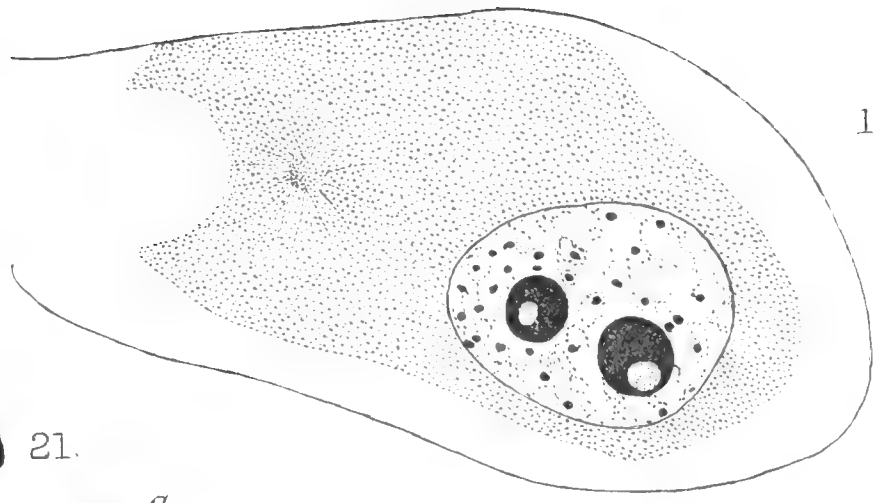
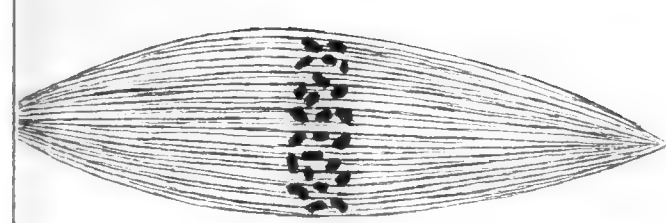






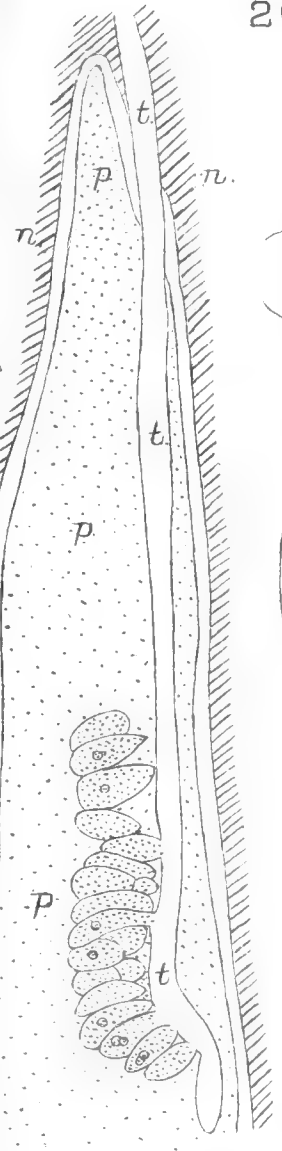


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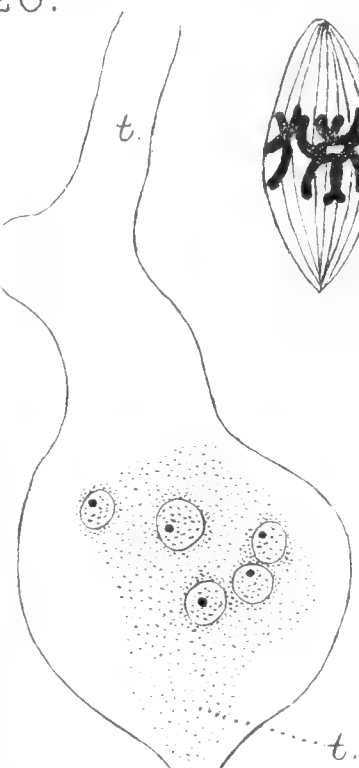


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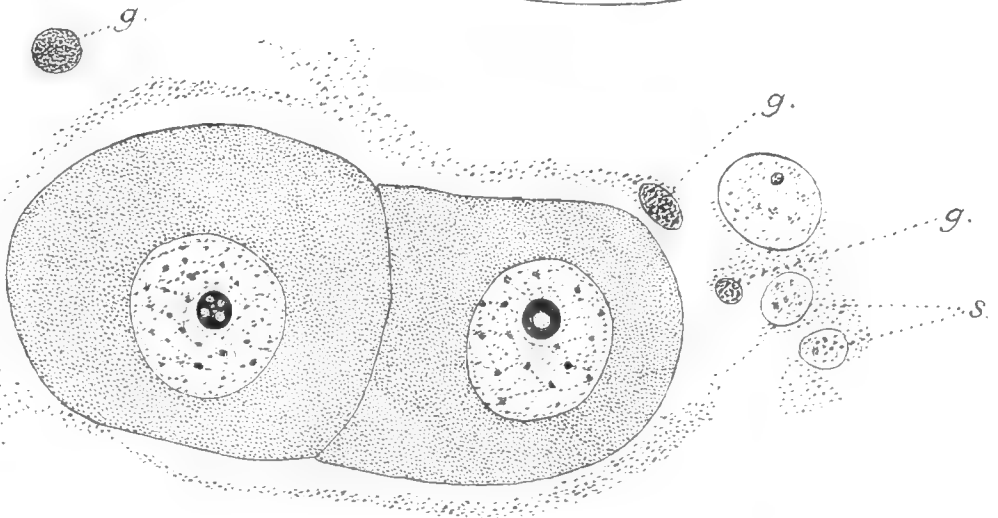
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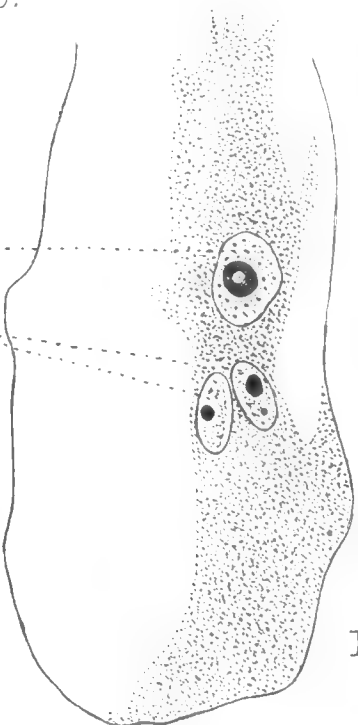
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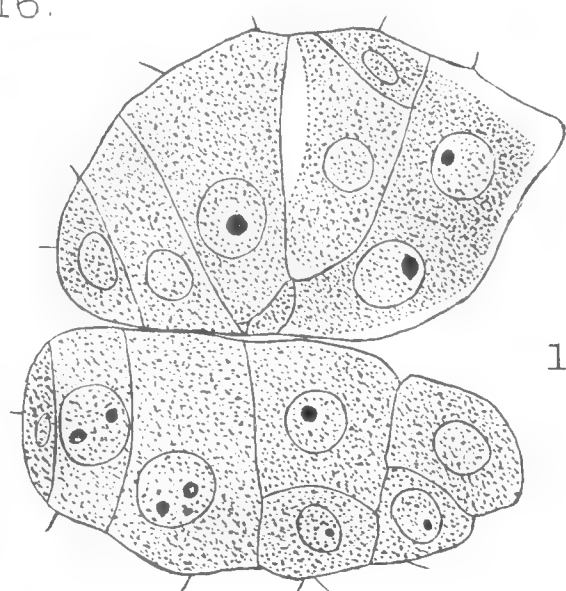
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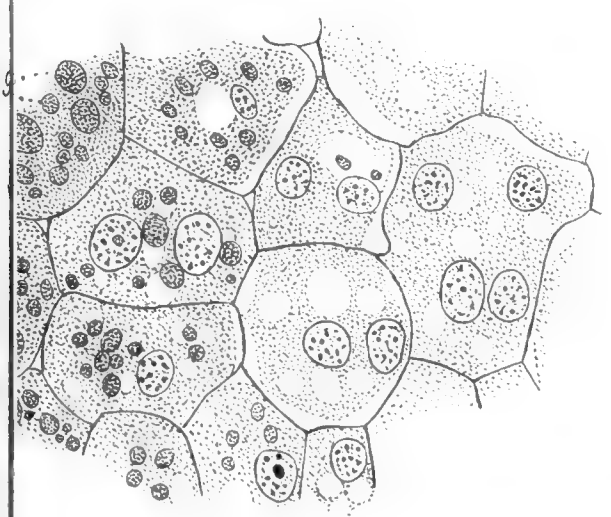
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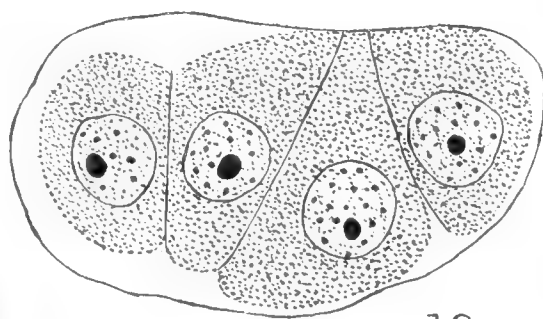
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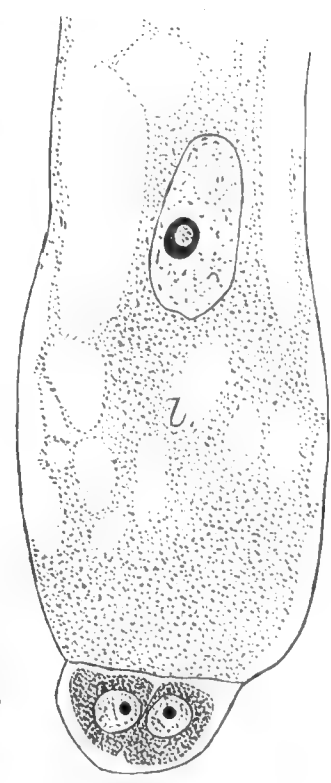
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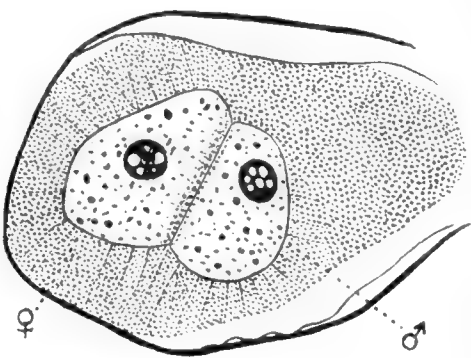
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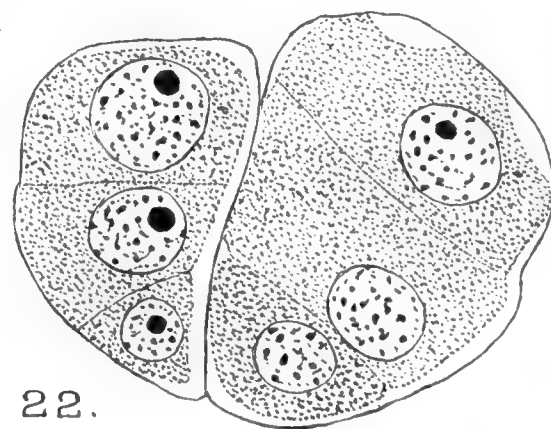
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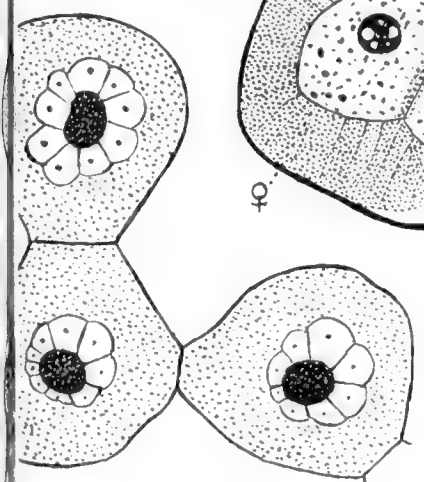
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17.

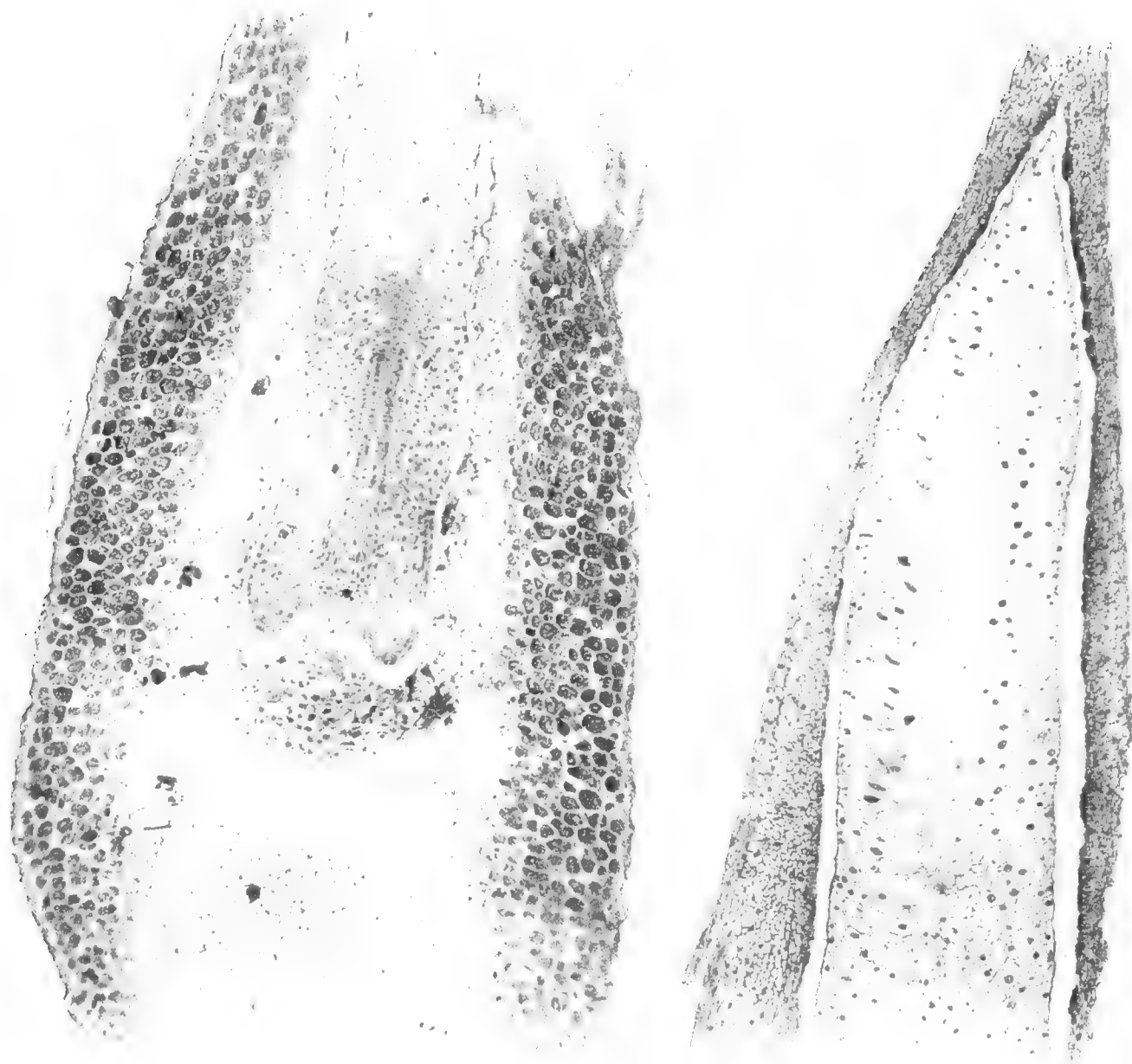


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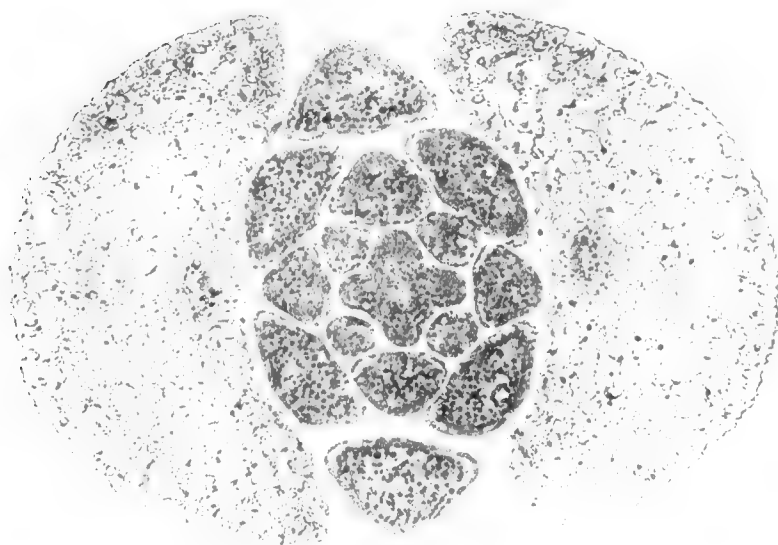
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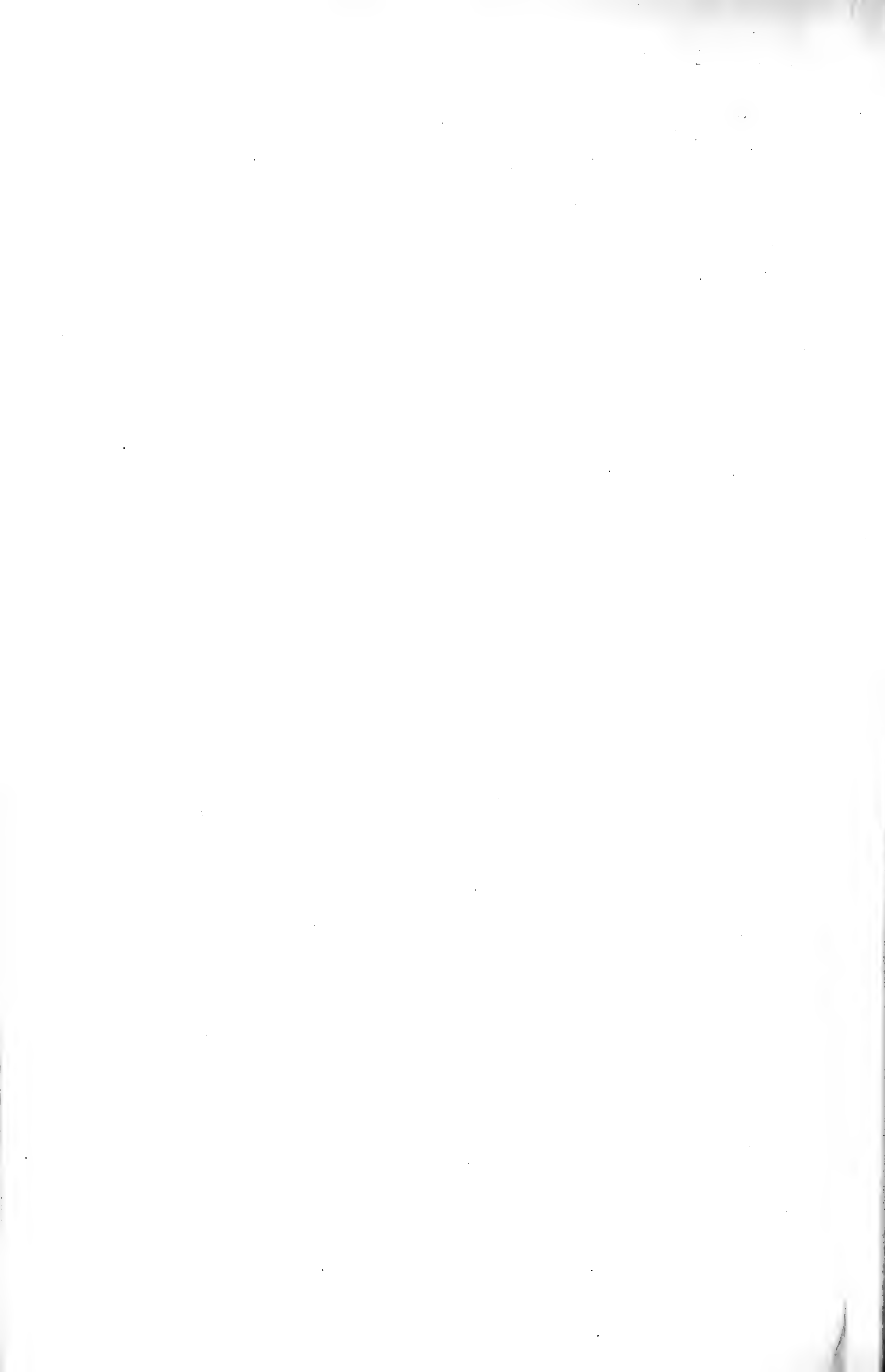
1.

3.



2.

SAXTON - CALLITRIS



# Contributions to the Life-History of *Callitris*.

BY

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With Plates XLV and XLVI.

## INTRODUCTION.

THE genus *Callitris* was incidentally referred to in two accounts of the life-history of *Widdringtonia* recently published by the writer (13, 16). The present study has been carried on conjointly with that of *Widdringtonia*, and, as was stated in the last published account of the latter genus (16), the facts there reported will be discussed in connexion with the corresponding phases in the life-history of *Callitris* in the present communication.

The methods employed are similar to those reported in the study of *Widdringtonia* (13) and need not be repeated here.

*Callitris* is an Australasian genus consisting of about a dozen closely allied species. So closely, in fact, do different species approximate that even with ample material it is no easy matter to discriminate between them. The material used has been collected entirely in the Government plantations in the neighbourhood of Cape Town, mainly in those at Tokai.

I desire to express my thanks to the following gentlemen who have assisted me in the collection of material, &c. :—

To Mr. J. J. Boocock for bringing material from the Tokai plantations on twelve occasions; to Mr. W. C. Worsdell, F.L.S., for collecting and fixing material for me on three occasions during a visit to the Tokai Forestry School; to Mr. G. A. Zahn for bringing material on two occasions from the Tokai plantations; to Mr. G. A. Wilmot, M.F., Forest officer in charge of the Tokai plantations, for giving me every facility for collecting material while staying at the Tokai Forestry School; to Mr. G. H. Ridley, curator of the Municipal Gardens, Cape Town, for germinating seed of *Callitris* and *Widdringtonia* for me in the municipal greenhouses.



Several species of *Callitris* have been examined from time to time. All are so much alike that it may safely be said that the following account would apply equally to any of these. That, however, which has been mainly studied is *Callitris verrucosa*, chiefly because it cones freely and contains a larger proportion of fertile ovules than most species. In a few cases material was fixed in the field, but more usually the cones were collected one afternoon, placed in moist packing material and fixed the following day, either morning or afternoon.

The preparations from such material proved quite as satisfactory as those from material fixed immediately.

#### DESCRIPTION.

1. *The Male Cone.* The sporophylls are arranged, like the leaves, in alternating whorls of three. Each sporophyll is peltate when mature, but when young the proximal part of the blade is curved inwards round the central sporangium (Pl. XLV, Fig. 1). A single vascular strand passes into each sporophyll and terminates a little beyond the large resin cavity. The wall of each sporangium (Fig. 2) consists of a single layer of cells; within this are two layers of tapetal cells probably formed by periclinal divisions in the sister-cells to those forming the wall. The mature sporangium closely resembles that of *Widdringtonia*, the output of spores being about the P. 558, l. 21. For 500 read 5000 §, however, from that of *Widdringtonia* in bearing only three (rarely two or four) sporangia (Fig. 3).

2. *The Female Cone and the Ovule.* The young female 'cone' consists of six radiating sporophylls in two whorls. Each scale of the upper whorl bears about fifteen ovules. The scales of the lower whorl are considerably smaller, as a rule, and each bears about five ovules. Thus the total number of ovules in a cone is about sixty. The ovules are situated on the proximal part of the upper surface and close to the median ridge. Ovules prior to pollination have not been seen. The micropyle closing cells, like those of *Widdringtonia*, remain non-septate. The micropyle is more widely spreading than in *Widdringtonia*, as shown in Fig. 4, and its wall is either one or two cells wide, whereas that of *Widdringtonia* is three cells wide. A whole ovule in longitudinal section has the structure indicated in Fig. 4. (The asymmetric appearance of the section is due to it being nearly in the plane of the wings.)

Pollen tubes may be seen in the nucellus at this stage but have not been indicated in the figure. The position of the sporogenous tissue is shown.

3. *Megasporogenesis.* Only a very small number of megaspore mother-cells are found (one to three) and they lie about half-way down the nucellus (Fig. 4). Their structure is shown in Fig. 5. They are sharply differen-

tiated from the surrounding cells of the nucellus, having large and lightly staining nuclei and dense cytoplasm, while those of the nucellus have smaller, deeply staining nuclei and less dense cytoplasm.

Only a very limited number of preparations show at all clearly the stages following this. Neither of the reducing divisions has been seen. Fig. 6 indicates that the upper of the two megaspore mother-cells may develop, but as this represented the only preparation in which the point could be definitely ascertained, it is impossible to say if there is any constancy in the position of the functional mother-cell. This figure also shows that of a row of four megaspores the second from the top became functional. Other preparations indicate considerable variation in this respect (see Figs. 8 and 10).

A considerable proportion of ovules become abortive while quite young. The structure of the nucellus of such an ovule is shown in Fig. 7, about the same age as the normal ovule shown in Fig. 8. No pollen-tube is found and all the central tissue of the nucellus is made up of very large cells with scanty cytoplasm.

4. *The Female Gametophyte.* The general structure of the nucellus after the first division of the megaspore nucleus is shown in Fig. 8. In this case traces of sterile megaspores can be seen above but not below the prothallus; i. e. the lowest of the row of megaspores has become functional. The relative size of the pollen-tube is shown in the tip of the nucellus.

Instances are occasionally met with where more than one megaspore begins to develop. Such a case is drawn in Fig. 9. The upper embryo-sac here contains two nuclei, the lower contains four.

It could not be definitely ascertained whether these two megaspores developed from the same or different megaspore mother-cells.

The next figure (10) shows a somewhat older sac, containing sixteen nuclei. In this case the three sterile megaspores are below the embryo-sac, and it must therefore have been the uppermost which became functional.

At this time and throughout the development of the prothallus, the absence of any kind of tapetal layer is a noteworthy feature. Physiologically its place is possibly taken by the basal part of the nucellus, the cells of which are always densely packed with contents; growth of the nucellus takes place mainly in this basal part, as is indicated in Fig. 11. This represents the structure of the nucellus at a time when 128 nuclei are found in the prothallus. The basal meristematic tissue is shaded. It consists at this time of a number of exceedingly regular rows of tabular cells, somewhat resembling the 'Pavement tissue' in *Gnetum Gnemon* recently described by Coulter (4). The upper part of this tissue is absorbed later as the chalazal end of the sac penetrates more deeply into the base of the nucellus, but its basal part persists. As far as the species available to the writer are concerned this has proved a constant character. Cell-formation in the pro-

thallus of *Callitris* has not been seen, but doubtless takes place in the normal manner.

The archegonia are formed in a single group abutting on the side of the pollen-tube (Fig. 12), except where two pollen-tubes are present, when two such groups are organized. No other archegonia, not in contact with the pollen-tube, are found. The number of archegonia in a group is usually about seventeen to twenty. The archegonia are considerably smaller than those of *Widdringtonia*, but probably their development is the same. The neck soon disappears, and the adjacent part of the pollen-tube breaks down, so that the contents of the archegonium are freely exposed to those of the tube (Fig. 13). The oosphere nucleus has one, or sometimes two, large hollow nucleoli, and is situated near the base of the archegonium, no vacuole being present as a rule. Occasionally an aster-like structure is evident near the top of the archegonium (Fig. 13). This may possibly represent the remains of the ventral canal nucleus.

5. *The Male Gametophyte.* Some of the microspore mother-cells, probably shortly before division, are shown in Fig. 14. Unfortunately stages to illustrate microsporogenesis have been missed. The wall of the mother-cell is rather thick, and between this and the central nucleus is a space packed with starch grains (Fig. 14). The wall of the mature pollen grain is, however, much thicker than that of the mother-cell. As far as has been seen, the microspore nucleus does not divide before the spores are shed; this point has been investigated in *C. verrucosa* and *C. robusta*. It is not very easy to make out the structure of the mature grain owing to the thick hard wall, which is difficult to penetrate and to cut, but in the thousands of spores examined not one has appeared to contain a second nucleus.

This does not agree with the statement made by Coker (3) that two nuclei are present at the time of shedding in a species of *Callitris*, but no statement is made by him as to which species was the subject of investigation.

I have found it impossible at present to follow the early development of the pollen-tube. The nucellar beak seems to offer great resistance to the microtome knife, and it is comparatively seldom that a clear view even of the tube itself is obtained, much less of the contents. Later stages, after the cutting-off of the two sterile nuclei, are quite easy to follow. These two nuclei are invariably equal in size and staining-capacity and are usually abreast of one another in the tube (Fig. 15), so that it is impossible to distinguish between 'tube' and 'stalk' nuclei. They are always to be seen in the tube and do not tend to disappear as they do in *Widdringtonia*. When the male cells are formed, starch grains are found in the tube, contrary to what is the case in *Widdringtonia*.

The two male cells have very dense cytoplasm with a distinct limiting membrane or wall and a large central nucleus, with a single large, hollow or reticulate nucleolus (Fig. 16). In this figure the two sterile nuclei are quite

distinct, and, in addition, a large nucleus of quite a different character is present. This must represent some abnormal structure.

6. *Fertilization and Embryogeny*. Only one preparation has been obtained which undoubtedly shows the sexual nuclei in contact in two adjacent archegonia. (These are the lowest but one, and the lowest but two of the group of archegonia in Fig. 12.) One of these is shown in detail in Fig. 17. The structure of the two nuclei is exactly identical, each containing a large reticulate nucleolus. This figure closely resembles Lawson's (7) fig. 29 of *Thuja*, but in the present case a number of plasmic fibres may be seen radiating from the outside of both nuclei. These are a conspicuous feature in both the fertilized archegonia, although nothing of the kind has been described in similar stages of other Conifers. The male nucleus is also relatively larger here than in Lawson's figure cited above.

The cytoplasm is denser and more homogeneous towards the apex of the archegonium, probably due to the presence of the cytoplasm of the male cell. No starch can be seen in the archegonium. A comparison of Figures 13 (archegonium), 16 (male cells), and 17<sup>1</sup> (fertilization) will show that the size of male and female nuclei before fertilization is almost precisely identical, but the male nucleus seems to become a little smaller inside the archegonium.

Early stages of the proembryo are wanting. The mature proembryo, like that of *Widdringtonia*, completely fills the archegonium. The arrangement of the cells shows considerable variation. Two mature proembryos are shown in Fig. 18 in median longitudinal section, and another in tangential section in Fig. 19. More than eight cells are present in a mature proembryo.

A dividing nucleus from a mature proembryo is drawn in Fig. 20 (not all chromosomes shown). The large spindle and short chromosomes are in striking contrast to the much shorter spindle and long chromosomes of the divisions in the prothallus cells (Fig. 21, not all chromosomes shown). The latter are frequent at this time, giving rise to the typical bi- and multinucleate cells of the mature prothallus. In a former paper (13) the opinion was expressed that the number of chromosomes in gametophyte and sporophyte respectively were in the neighbourhood of 12 and 24. I am now convinced that the numbers are somewhat less than that, the thickness of the chromosomes tending to make the number appear more than is actually the case, and the estimation difficult. For the same reason it is impossible to represent more than a few of the chromosomes in a drawing. The numbers are probably nearer 10 and 20 or perhaps 8 and 16.

In the same paper (13) a comparison was provisionally suggested between the multinucleate prothallus cells of *Widdringtonia* and *Callitris*

<sup>1</sup> Figure 13 is drawn on a considerably larger scale than 16 and 17.



and those of *Welwitschia*. The origin of the latter has now been definitely ascertained (Pearson (11)), and is so radically different from that of the same cells in the former genera that any comparison between them must be abandoned.

It is certain that at least three separate embryos are derived from a single proembryo. From the material examined it has not been possible to determine precisely how this takes place, but it seems likely that the whole proembryo is inclined to partially separate into groups of two or three cells, of which the smallest becomes the embryo initial and a larger one the single suspensor cell. Indications of such a state of affairs are seen in the upper proembryo of Fig. 18, and especially in Fig. 22, which shows part of a proembryo in longitudinal section, in which two such groups, of three cells each, seem clearly indicated. It is merely suggested that this is what happens. The evidence is by no means conclusive. The next stage seen shows a tortuous mass of thick-walled suspensors below the crushed remains of the archegonia, the free ends of which protrude in all directions from this mass (except immediately above, and especially below). About a dozen of these embryos may be found in a single ovule. Since more than two pollentubes never reach maturity (usually only one) it is only possible for four archegonia to be fertilized. In confirmation of this inference (if any were required) four is the maximum number of proembryos found in an ovule. Hence the presence of a dozen or so embryos in an ovule proves conclusively that at least three must be formed from a single proembryo, as stated above.

The free tip of one of these conspicuous suspensors, bearing a four-celled embryo, is drawn in Fig. 23. The first two divisions of the embryo cell are constantly vertical, giving rise to a single tier of four cells. This is exactly the opposite to what occurs in *Pinus*, where the first division is always (?), and the second nearly always, transverse, but it agrees with *Sequoia*, as described by Arnoldi (1).

The next division in each cell is transverse, giving two tiers of four cells each. In its further development, which has been followed fairly closely, the embryo agrees essentially with that of *Pinus*, as recently described by the writer (14). The meristem which gives rise to embryonal tubes, root-cap, and periblem, appears perhaps a little earlier than in *Pinus*. The region traversed by the suspensors is also relatively shorter than in other conifers, this being probably a necessary consequence of the position of the archegonia. The part of the prothallus above the archegonia develops no further, and in advanced stages of the embryo very little can be seen of it.

Fig. 1, Pl. XLVI shows an embryo shortly after the differentiation of the cotyledons. The region in which starch is present is clearly shown. Fig. 24 is a drawing of a few cells just on the boundary line of the starch-containing

region, showing the persistently multinucleate cells of the prothallus. This character can be clearly seen in the photograph from which Fig. 1, Pl. XLVI is reproduced, but is not likely to be apparent in a half-tone reproduction.

The mature embryo has invariably been found to possess two cotyledons. The germination is of the same type as in *Widdringtonia*, but the seedling is very much more slender than in that genus. The succession of plumular leaves is also the same in the two genera; that of *Callitris* is shown in Fig. 2, Pl. XLVI.

7. *Abnormalities*. One case has already been mentioned where a supernumerary nucleus was present in the mature pollen-tube (Fig. 16), but the case shown in Fig. 25 is still more remarkable. Here the dilated tip of the pollen tube has put out a slender tube below, suggestive of a haustorial function, and in the swollen part are found five nuclei, all practically alike in size and structure. In the prothallus, surrounding the enlarged part of the tube, are a considerable number of archegonium initials.

Fig. 3, Pl. XLVI represents a prothallus which is only abnormal in the fact that no pollen-tube has yet penetrated it, and yet cell-division is complete and archegonium initials have appeared. The latter are clearly seen to be endogenous in origin. This figure will be referred to again later.

8. *Discussion and Conclusions*. As was stated above (p. 557) this discussion and the conclusions arrived at are based partly on the facts here reported, and partly on the corresponding facts in the life-history of *Widdringtonia*, as recently described by the writer.

The main conclusion reached from these studies is that *Callitris* and *Widdringtonia* must be placed in a tribe apart from those Cupressineae which have been fully investigated, and no longer included as a sub-tribe of Cupressineae.

Masters (8) has already separated these two genera together with *Tetraclinis* and *Actinostrobus* as the sub-tribe Callitrineae, but has included them in the tribe Cupressineae. Most other authors (e. g. Rendle (12)) have not recognized even the subtribal distinction.

The writer suggests that the Callitrineae as defined by Masters (loc. cit.) should be raised to the rank of a tribe, co-ordinate with the Cupressineae, although the inclusion of *Tetraclinis* and *Actinostrobus* must be merely provisional until their life-histories have been worked out.

It is hoped that at a future date material of these genera may be available for investigation in order to ascertain whether or not their life-history does conform to that of *Callitris* and *Widdringtonia*.

In Table I, showing the chief differences between the Callitrineae and the Cupressineae, it has been assumed that certain points only definitely ascertained for one genus will be found to hold good for both. It may be pointed out that where this is done (fertilization and post-fertilization stages) there is considerable evidence that the two genera will be found to agree,



but, the series not having been very close in either case, some stages are missing in the one genus which are present in the other and vice versa.

TABLE I.

<i>Organ or Stage.</i>	<i>Callitrineae.</i>	<i>Cupressineae.</i>
Female cone.	All cone scales fertile.	Some cone scales sterile.
Ovule.	<i>Widdringtonia</i> : many megaspore mother-cells. <i>Callitris</i> : very few megaspore mother-cells.	Very few megaspore mother-cells.
Prothallus.	Cells eventually bi- and multi-nucleate.	Cells finally uni-nucleate. Temporarily bi-nucleate in <i>Cryptomeria</i> . <sup>1</sup>
Archegonia.	Never situated at apex of prothallus. Number may be under 20 ( <i>Callitris</i> ) or over 100 ( <i>Widdringtonia</i> ).	Always situated at apex of prothallus. Number usually few (6-12). Rarely up to 20 or 30.
Jacket cells.	Seldom recognizable.	Usually well marked.
Fertilization.	Male and female nuclei almost identical in size.	Male nucleus smaller than female.
Proembryo.	Not more than five, possibly only two, free nuclei before wall-formation. Proembryo fills archegonium.	Eight free nuclei before wall-formation. Proembryo never fills archegonium.
Embryo.	Three or four from single proembryo.	One from each proembryo.

Of these differences the position of the archegonia and the development of the proembryo seem most important, while the bi- and multinucleate prothallus cells of *Callitrineae* also constitute an interesting feature. In a recent paper, Coulter (5), referring to the position of the archegonia in *Sequoia* as reported by Lawson (6), and in *Widdringtonia* as found by the writer (13), considers the point of only secondary importance on account of the fact that it is correlated with the position of the pollen-tube, but it seems scarcely reasonable to conclude that because two unusual features are correlated with one another, therefore they are of little importance. Moreover, it has been shown in *Callitris*, that while ordinarily the archegonia are organized in relation to the pollen-tube, yet, in the case of the pollen-tube being arrested in its development, the archegonia still develop in relation to the position which a normal pollen-tube would have occupied. It seems to the writer that this makes it probable that although,

<sup>1</sup> In certain other Conifers, e. g. in *Podocarpus* (Coker 2), multinucleate cells are formed in the prothallus.

in the individual development, the position of the archegonia is usually a correlated character, yet phylogenetically it may well have been otherwise. Nevertheless evidence from abnormalities is not always reliable, and may be easily used beyond its legitimate extent, so this suggestion is put forward with some hesitation.

The position of the archegonia in *Araucaria* and *Agathis*, especially in the latter, is similar to that here described, and Seward and Ford (17) regard this as a primitive character, with which view the present writer is entirely in agreement.

In this connexion something may be said in regard to the points of resemblance with *Sequoia sempervirens*, as worked out by Lawson (6). Other writers had previously mentioned some of the peculiarities in this remarkable Conifer, but the life-history was fully described for the first time by Lawson, and it is therefore found most convenient to refer to his account only.

Two points of resemblance stand out sharply. (i) The position of the archegonia, and (ii) the similarity in size and organization of male and female nuclei.

A possible relationship between *Sequoia* and the Cupressineae has been suggested before by Arnoldi (1), and in many respects the Callitrineae may be considered to supply the missing link, agreeing with Cupressineae in their more obvious, with *Sequoia* in some of their more recondite, characters.

This relationship, if admitted, would tend to the view that the Callitrineae represent a relatively ancient type as compared with the Cupressineae, and other considerations support this view. There can be no doubt that *Callitris* is more nearly related to the Cupressineae than in *Widdringtonia*, and there are many indications that *Callitris* is the more specialized type of the two. Evidence of this would be largely a repetition of Table II below (which see). It seems quite clear to the writer that specialization in the Cupressineae must have proceeded on similar lines, although a wide gap, as shown above, separates the two tribes. The main change must have been increase in the number of cone-scales, accompanied by sterilization of some of them, the culmination of the process being reached in *Cupressus*.

The second important conclusion is that, although fairly closely allied, the genera *Widdringtonia* and *Callitris* are quite distinct, and should undoubtedly be kept apart, as recently urged by the late Dr. Masters (9). It is unfortunate that, in the paper cited, a well-known species of *Callitris* should have been described by the author as a new species of *Widdringtonia*. The necessary correction was made by the author himself shortly after (10), but in the meantime it had been admitted in the first paper (*loc. cit.*) that the supposed new species to some extent broke down the distinction between the genera, and in consequence the conclusion lost much of its weight.

The writer has already expressed (13) the opinion that the two

genera should be kept apart, and has cited the opinions of some other botanists both for and against this view, which need not here be repeated. The evidence was not brought forward at that time, as it was thought better to postpone it until the life-history of *Callitris* had been more fully worked out.

Table II gives a list of the chief differences noted, including both those which are more obvious and those more recondite. Since not all the species of either genus have been examined, it may prove that not quite all of the differences given will prove constant, but it is highly improbable that many of the distinctions will be broken down.

TABLE II.

<i>Organ or Stage.</i>	<i>Widdringtonia.</i>	<i>Callitris.</i>
Leaves.	Opposite and decussate.	In alternating whorls of three.
Female cones.	Two decussate pairs of scales. All scales equally fertile.	Six scales in two alternating whorls. Outer scales smaller and bearing fewer ovules.
Ovules.	Not more than about thirty in a cone. Most develop up to free nuclear conditions of the prothallus (except when not pollinated).	About sixty in a cone. Many abort very early and appear merely as a flat scale, slightly thicker in the middle, and eventually of almost the same size as the fertile seed, but apparently containing no nucellus.
Megaspore mother-cells.	About sixty-four at base of nucellus.	One or very few half-way up the nucellus.
Young prothallus.	Appears in lower half of nucellus. No 'pavement tissue'.	Appears in upper half of nucellus. 'Pavement tissue' below.
Mature prothallus.	Relatively large.	Relatively small.
Archegonia.	More than twenty-five in more than one group.	Less than twenty-five in one group.
Seeds.	Relatively large. Wings not very broad or may be obsolete.	Relatively small. Wings broad.
Male cone.	Sporophylls in decussate pairs.	Sporophylls in alternating whorls of three.
Microsporophyll.	Bears four sporangia.	Bears three sporangia.
Chromosomes.	Six and twelve.	More than six and twelve. (Perhaps eight and sixteen).
Habitat.	South and Central Africa and Madagascar.	Australasia.

The anatomy of leaf and stem also shows considerable differences between the two genera. An account of this has been published elsewhere by the writer (15).

In a former paper (13) the name Actinostrobeae was used for the four genera here named Callitrineae. I had at that time failed to note that Masters (8) had already applied the latter name to the same four genera in preference to Endlicher's name which included also *Libocedrus*. In the same paper a comparison was provisionally suggested with the *Gnetales*, especially with *Welwitschia*, but further research has shown that the resemblances noted are apparent rather than real. Nevertheless it does seem likely that of all Conifers *Widdringtonia* come a trifle nearer the *Gnetales* (at any rate *Welwitschia* and *Gnetum*) than any other genus, at any rate in gametophyte characters. Coulter (5) regards the *Gnetales* as derived from the Cupressineae.

#### SUMMARY.

The microsporophylls are arranged in alternating whorls of three, and each bears three microsporangia.

The six megasporophylls are in two alternating whorls of three, the outer somewhat smaller and bearing fewer ovules.

The young embryo-sac is situated in the apical half of the nucellus.

The archegonia are never situated at the apex of the prothallus, but in a single group of about, or just under, twenty, arranged along the inner side of the pollen-tube near its apex. If two pollen-tubes are present, two such groups are organized.

The pollen grain is uninucleate at the time of shedding in *C. verrucosa* and *C. robusta*.

The later development of the male gametophyte is normal.

The proembryo completely fills the archegonium, but the arrangement of the cells is variable.

More than one embryo is formed from a single proembryo.

The first two walls in the embryo are longitudinal.

The mature embryo has two cotyledons.

The cells of the mature prothallus are all binucleate or multinucleate.

It is concluded that *Widdringtonia* and *Callitris* undoubtedly represent two distinct genera and must be made the types of a separate tribe of Coniferae, the Callitrineae, co-ordinate with Cupressineae. The characters of the Callitrineae are in many respects intermediate between those of Cupressineae and those of *Sequoia*.

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November, 1909.

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## EXPLANATION OF FIGURES IN PLATES XLV AND XLVI.

Illustrating Mr. Saxton's Paper on *Callitris*.

All figures were drawn with Zeiss Camera lucida, microscope and lenses. In all:—

*A* = archesporium; *C* = micropyle-closing cells; *D* = megaspore mother-cells; *G* = Starch grains; *H* = integument; *K* = tapetum; *L* = suspensor; *N* = nucellus; *P* = prothallus; *Q* = body-cell nucleus; *R* = resin cavity; *S* = sterile nuclei; *T* = pollen-tube; *V* = vascular bundle; *W* = wall of microsporangium; ♂ = male nucleus; ♀ = Female nucleus.

## PLATE XLV.

Fig. 1. Part of median longitudinal section of young male cone showing position of sporophyll microsporangium, resin cavity, and vascular bundle. × 135.

Fig. 2. Microsporangium from a similar cone. × 420.

Fig. 3. Transverse section of mature microsporophyll, showing three microsporangia, resin cavity, and vascular bundle.  $\times 40$ .

Fig. 4. Longitudinal section of a young ovule, almost in the plane of the wing. [Wing partly cut on right, missed on left, of figure.]  $\times 58$ .

Fig. 5. Part of Fig. 4, showing the two megaspore mother-cells.  $\times 930$ .

Fig. 6. A somewhat later stage showing the functional megaspore, one disorganizing megaspore above and two below, and the non-functional mother-cell below these.  $\times 930$ .

Fig. 7. Median longitudinal section of the nucellus of an abortive ovule. About same age as Fig. 8.  $\times 135$ .

Fig. 8. Median longitudinal section of normal nucellus after first division of megaspore nucleus. [cf. Fig. 7.]  $\times 135$ .

Fig. 9. Development of two embryo-sacs.  $\times 400$ .

Fig. 10. Embryo-sac with 16 nuclei, and 3 disorganizing megaspores below.  $\times 230$ .

Fig. 11. Sketch of nucellus in longitudinal section when 128 nuclei are present in the embryo-sac, showing positions of pollen-tube, embryo-sac and 'pavement tissue'.  $\times 40$ .

Fig. 12. Longitudinal section of upper two-thirds of prothallus, showing position of pollen-tube and archegonia. Two adjacent archegonia near the base have just been fertilized.  $\times 58$ .

Fig. 13. Mature archegonium. The neck-cells have broken down, and the archegonium opens directly into the pollen-tube.  $\times 930$ .

Fig. 14. Microspore mother-cells connected to form a net-work.  $\times 930$ .

Fig. 15. Tip of fairly young pollen-tube.  $\times 400$ .

Fig. 16. Contents of tip of mature pollen-tube.  $\times 545$ .

Fig. 17. Archegonium after fertilization. [One of the archegonia of Fig. 12.]  $\times 545$ .

Fig. 18. Two adjacent mature proembryos, in nearly median longitudinal section.  $\times 400$ .

Fig. 19. Tangential section of a similar proembryo.  $\times 400$ .

Fig. 20. Dividing sporophyte nucleus. Not all chromosomes shown.  $\times 1,500$ .

Fig. 21. Dividing gametophyte nucleus from same series of sections. Not all chromosomes shown.  $\times 1,500$ .

Fig. 22. Part of a single proembryo in longitudinal section.  $\times 570$ .

Fig. 23. A suspensor bearing a four-celled (two in section) embryo.  $\times 400$ .

Fig. 24. Multinucleate prothallus cells on the border of the starch-containing region.

Fig. 25. Tip of abnormal pollen-tube.  $\times 135$ .

#### PLATE XLVI.

Fig. 1. Longitudinal section of seed, showing embryo with cotyledons differentiated.

Fig. 2. Transverse section of seedling, showing cotyledons and plumular leaves.

Fig. 3. Longitudinal section of ovule, containing prothallus.





# The Influence of Copper Sulphate and Manganese Sulphate upon the Growth of Barley.

BY

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With Plate XLVII, and four Figures in the Text.

**D**URING recent years a great deal of attention has been directed to the physiological action of certain chemical substances upon the growth of plants, particularly with regard to plant poisons. A theory has been put forward that all chemical substances which are deleterious to plant growth act universally as stimulative agents if they are only available in exceedingly minute quantities. An examination of the literature on the subject indicates that many of the experimental results are self-contradictory and that, as a general rule, little can be regarded as definitely settled.

In 1907 investigations were begun at the Rothamsted Laboratory to determine, if possible, the limits of concentration of a few specified salts necessary to produce either a toxic or stimulative effect upon the growth of certain of the higher plants. The experiments were carried on by means of water-cultures, as they afford the most ready method of controlling the nutrient and toxic substances supplied to the plants. Great care was observed to eliminate, as far as was practicable, any disturbance of the experimental results by outside causes, such as dirty bottles, corks, &c. The plants were grown singly in clear glass bottles fitted with bored corks to support the plants. These corks were either new or else were sterilized in an autoclave previous to use in order to kill off any adherent mould spores or green algal cells. The bottles were filled with culture solution to within an inch from the top, and the plants after germination in fresh damp sawdust were lightly held in position in the cork by means of non-absorbent cotton wool. Great care was taken to keep the latter dry, as damp wool, especially in the early stages of growth, would prove a fruitful source of loss of plants on account of disease and bacterial action. The bottles were then covered with closely fitting brown paper coats to exclude light from the solutions and roots.

The Laboratory distilled water used for ordinary chemical purposes exerts a deleterious action on the growth of plants, especially upon the roots, probably on account of the fact that it is delivered through copper pipes (Pl. XLVII, Fig. 1). All such water was rejected for experimental purposes, and that used in the preliminary investigations was specially made with a glass still.

COPPER SULPHATE.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ .

Experiments were made to ascertain the effect of varying concentrations of Copper as Sulphate on the growth of Barley:—

- (a) in the absence of any nutrient salts ;
- (b) in the presence of a full complement of nutrient salts.

(a) *No nutrients.*

Duplicate series of plants were grown with seven dilutions of copper sulphate<sup>1</sup> in distilled water, varying from 1 : 12,500 to 1 : 1,000,000 C.S.,<sup>2</sup> two duplicate checks being also made, one in pure glass distilled water, one in laboratory distilled water (called for convenience Cu distilled water). In these very early experiments, dealing with small numbers of plants, the individuals were periodically measured, root and shoot. Later on this was abandoned, as the results did not repay the time and labour expended ; the method of comparison of dry weights was adopted in its stead and proved far more satisfactory.

In the series under consideration the plants were allowed to grow for a month, and it was found that the root and shoot behaved somewhat differently with regard to similar concentrations of C.S. *Root* development was entirely checked by every concentration of C.S. from 1 : 12,500 to 1 : 1,000,000. A little growth had taken place in the Cu distilled water, but this did not in any way approach that in glass distilled water. The *shoot*, on the other hand, showed approximately a gradual increase in growth with decreasing strength of C.S., the best growth occurring in the glass distilled water. These results seem to indicate that the barley root is very sensitive to minute traces of copper sulphate, even one part in 1,000,000 being sufficient to check growth. It is evident, though, that even the strongest concentration, 1 : 12,500, does not actually kill the plant, at any rate at once, since the shoot continues its growth to a certain extent, living on the reserve materials in the grain. Similar results were obtained from a second set of plants grown later on in the year.

(b) *With nutrients.*

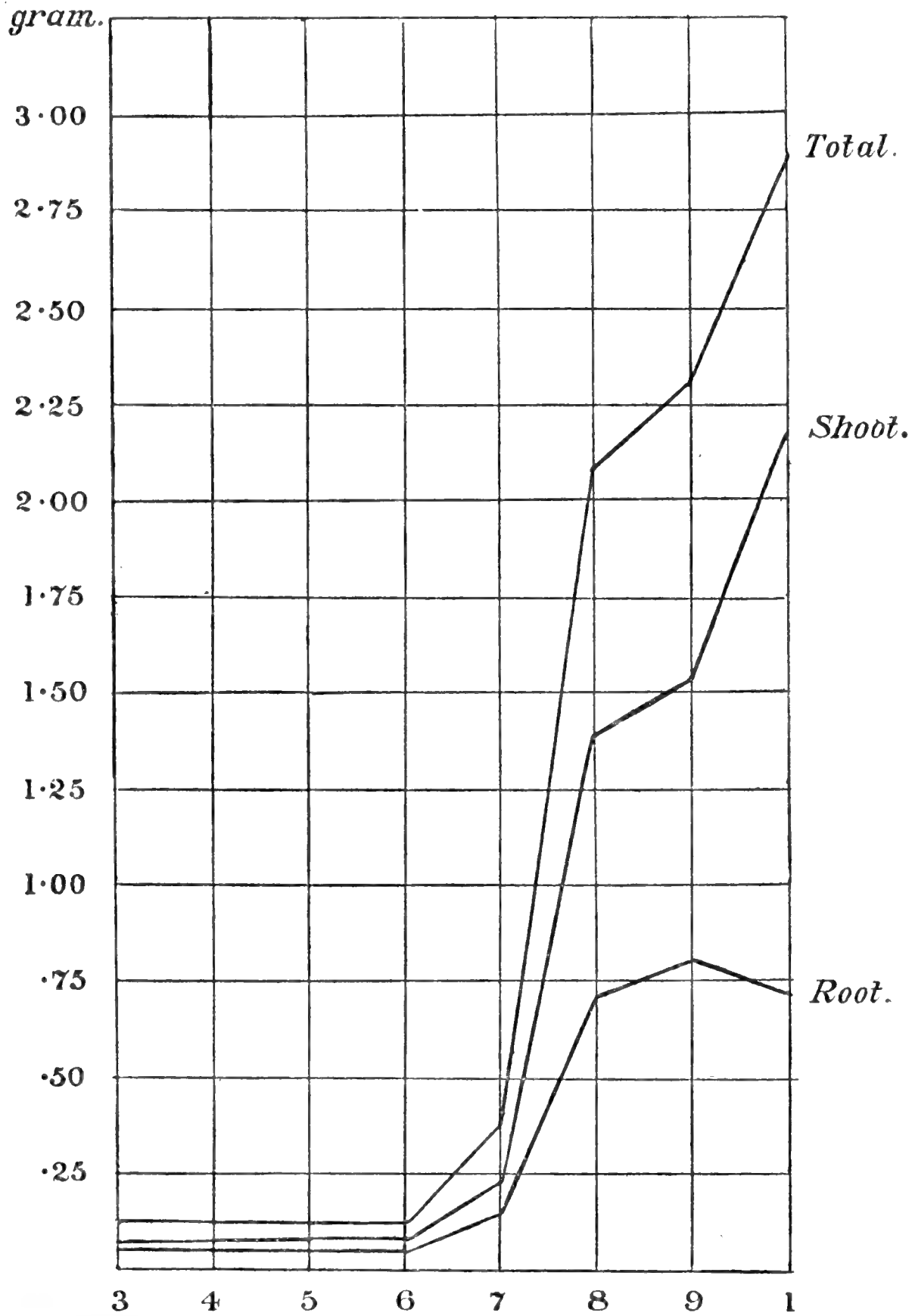
Duplicate series of plants were grown with similar concentrations of C.S. as under (a), but containing a definite quantity of nutrient salts in addition.

<sup>1</sup> For convenience  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  is expressed throughout as C.S.

<sup>2</sup> All concentrations are expressed in grams of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  to cubic centimetres water. Thus 1 : 12,500 C.S. means 1 gram  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  crystals to 12,500 c.c.  $\text{H}_2\text{O}$ .

Each litre of culture solution<sup>1</sup> contained, besides the requisite amount of copper sulphate:

Potassium Nitrate . . . . .	1 gram.	Sodium Chloride . . . . .	0.5 gram.
Magnesium Sulphate . . . . .	0.5 „	Potassium Phosphate . . . . .	0.5 „
Calcium Sulphate . . . . .	0.5 „	Ferric Chloride . . . . .	Trace (0.04 gram).



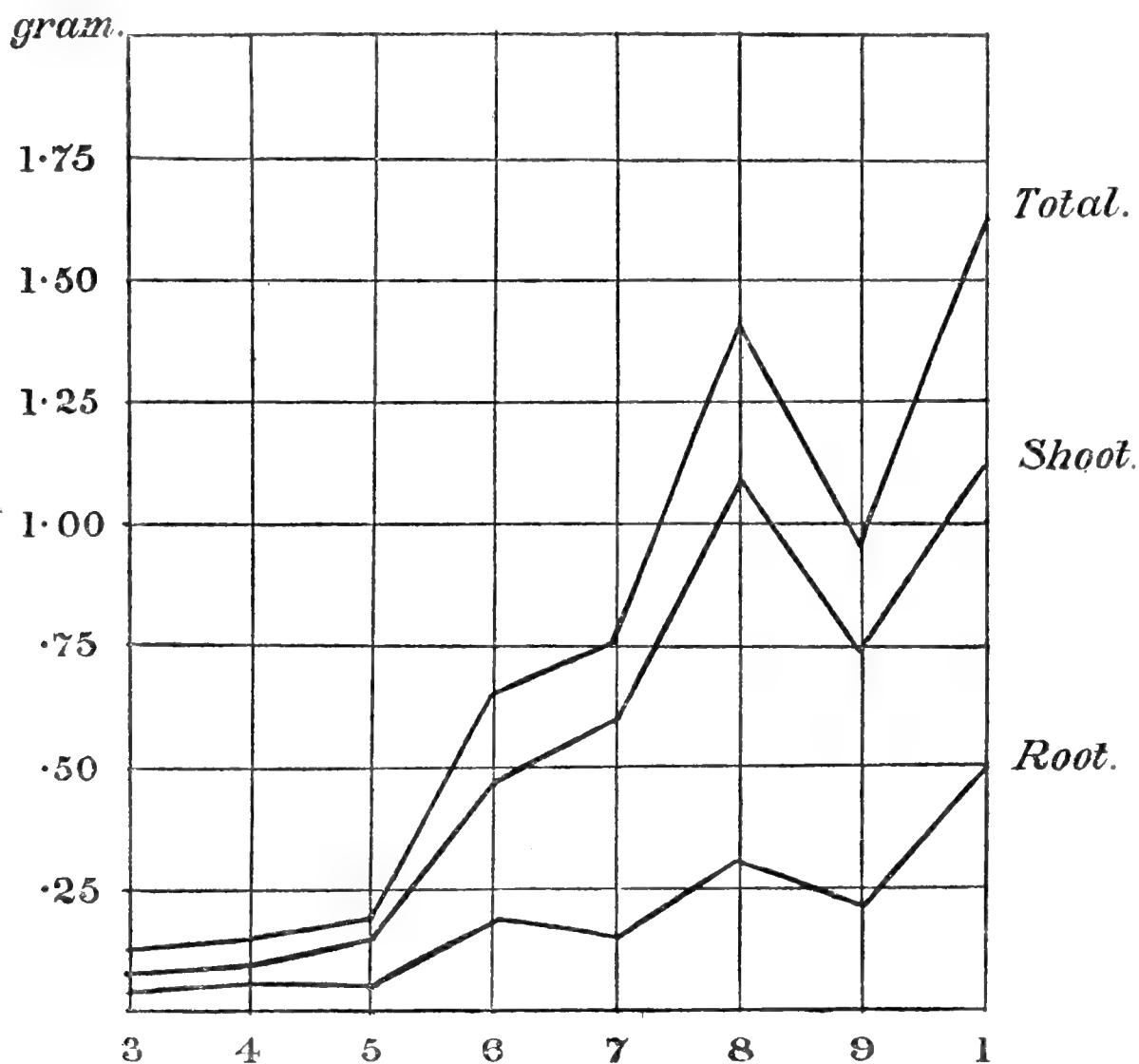
Curve I. Showing the result of the initial experiment on the action of copper sulphate on the growth of barley in the presence of nutrient salts.

- |                    |                                   |
|--------------------|-----------------------------------|
| 3. 1 : 12,500 C.S. | 7. 1 : 250,000 C.S.               |
| 4. 1 : 25,000 „    | 8. 1 : 500,000 „                  |
| 5. 1 : 50,000 „    | 9. 1 : 1,000,000 „                |
| 6. 1 : 100,000 „   | 1. Control—glass distilled water. |

<sup>1</sup> D. H. Scott, Structural Botany (Flowering Plants.), p. 202.

These plants were measured during growth for about seven weeks, and at the close of the experiment those in one series were divided into root and shoot, separately dried for two or three days in a steam oven at 100° C., and weighed, in order to find the amount of dry matter present.

1 : 12,500 to 1 : 100,000 C.S. solution checked the root growth entirely, as both measurements and weights indicated. The shoots had made a certain slight increase in length, but the dry weights were insignificant. 1 : 250,000 C.S.



Curve 2. Showing the mean values of the dry weights of three series of barley plants grown in the presence of copper sulphate with nutrient salts.

3. 1 : 50,000 C.S.	7. 1 : 1,000,000 C.S.
4. 1 : 100,000 ,,	8. 1 : 2,500,000 ,,
5. 1 : 250,000 ,,	9. 1 : 5,000,000 ,,
6. 1 : 500,000 ,,	1. Control—glass distilled water.

seemed to be a transition point, as below this concentration marked improvement resulted, though none of the plants grown with C.S. reached the level of those in pure glass distilled water with nutrient salts (Pl. XLVII, Fig. 2 : Curve 1).

Further experiments made later in the year afforded similar results. In this case the concentration of the toxic salt was further reduced to 1 : 5,000,000, but even at this dilution the C.S. seemed to exercise a deleterious action, as the plants did not make as much growth as in its absence. (Curve 2.)

Since it was anticipated that the manufacture of sufficient glass distilled water would prove a difficulty when the investigations were extended, tests were made to find a satisfactory substitute. Pure glass distilled water was shaken up with pieces of metallic silver and aluminium respectively, and allowed to stand on the metals for at least an hour. Food solutions were then made up with the water so prepared, and test plants were grown in them, with parallel controls in pure-water culture solutions. It was found that there was little or no difference in the growth of the two sets of plants, the toxic effect of the metals, if any, being completely masked by the nutrient salts (Pl. XLVII, Fig. 3). Acting on this discovery a Brown's 'chemist' still with a condensing arrangement of pure silver was obtained, in which the condensed water came into contact with nothing but the pure silver until it passed into a glass delivery tube. The water was finally filtered through charcoal, which has been found to remove the toxic effects of metals. All the later water-culture work was carried out with water thus prepared. At the close of the experimental work in 1907, two points stood out very clearly:—

(1) Owing to the individuality of the plants, investigations dealing only with small numbers cannot be depended upon to give really reliable information as to the effect of various strengths of a toxic salt upon plant growth. To this end repeated experiments dealing with groups of 5, 10, or 20 plants for each concentration are necessary to give accurate information. In these experiments the question of individuality in barley was eliminated to the greatest practicable degree, as the seed used was a pure strain (obtained from Mr. E. S. Beaven, Warminster) which had been raised from a single seed. Further, the seeds were graded before being sown, so that they only varied within 0.02 gram from one another.

(2) The presence of nutrient salts in a culture solution exercises a definite masking effect upon the action of the toxic salt, enabling a plant to make satisfactory growth in contact with a much more concentrated solution of the poison than it can otherwise endure, i. e. whereas a concentration of 1:1,000,000 (and possibly less) copper sulphate solution alone entirely inhibits growth in barley, yet in the presence of nutrient salts a concentration of 1:250,000, at least four times as great, does not prevent growth, though it decidedly checks it.

The preliminary year's work had shown that the best results were obtained with plants grown between February and July, as during the early winter months practically no growth is made, and seedlings raised after the early part of June are very apt to fail.

Early in 1908 extended experiments were started with twenty parallel series, each consisting of eight concentrations of C.S. ranging from 1:50,000 to 1:10,000,000, with a control in distilled water, nutrient salts being added in each case. The seeds were graded between 0.04 and 0.06 grams and the



plants were allowed to grow on for seven or eight weeks. The series was very good, but even here the plants showed great individuality, as is seen in Table I, the variation from the mean being as much as 30% to 40% in some cases. The curve of the means (Curve 3) shows that 1:50,000 C.S. to 1:250,000 C.S. practically checked growth in nearly every case, 1:500,000 C.S. marking the critical point after which growth progressed rapidly. A possible stimulus is indicated with 1:5,000,000 C.S., but with such large variations it was recognized that this might easily be due to experimental error. To

	2.	3.	4.	5.	6.	7.	8.	9.	1.
	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.	Gram.
A	.123	.132	.135	.175	.416	.965	.476	.747	.324
B	.100	.105	.076	.107	.389	.758	1.018	.787	.987
C	.106	.092	.102	.137	.566	.732	.800	.761	.818
D	.132	.083	.150	.109	.641	.901	.795	.932	.771
E	.097	.098	.112	.196	.659	.736	.767	.759	.958
F	.102	—	.106	.113	.283	.529	.662	.718	.884
G	.110	—	.099	.290	.239	.921	.632	1.030	.984
H	.083	—	.096	.304	.447	.718	.718	.832	.869
I	.111	—	.145	.162	.344	.762	.960	.793	.501
K	.088	—	.099	.210	.337	.318	.698	.672	.958
L	.073	.102	.107	.183	.408	.886	1.236	.442	.283
M	.089	.114	.080	.157	.331	.901	.984	.839	.936
N	.105	.111	.147	.355	.375	1.104	1.088	.928	1.029
O	.118	.111	.145	.117	.472	.734	.980	.833	1.074
P	.116	.082	.112	.189	.323	.183	.883	.868	.916
Q	.105	.129	.131	.146	.716	1.182	1.261	.932	1.033
R	.104	.079	.116	.152	.126	1.039	.951	.844	1.120
S	.085	.101	.114	.295	.164	.193	.899	.837	1.114
T	.179	.120	.168	.268	.547	.907	1.061	.976	1.211
U	.124	.123	.086	.234	.321	.598	.854	1.025	.670
Mean.	.107	.105	.116	.194	.405	.753	.881	.827	.872

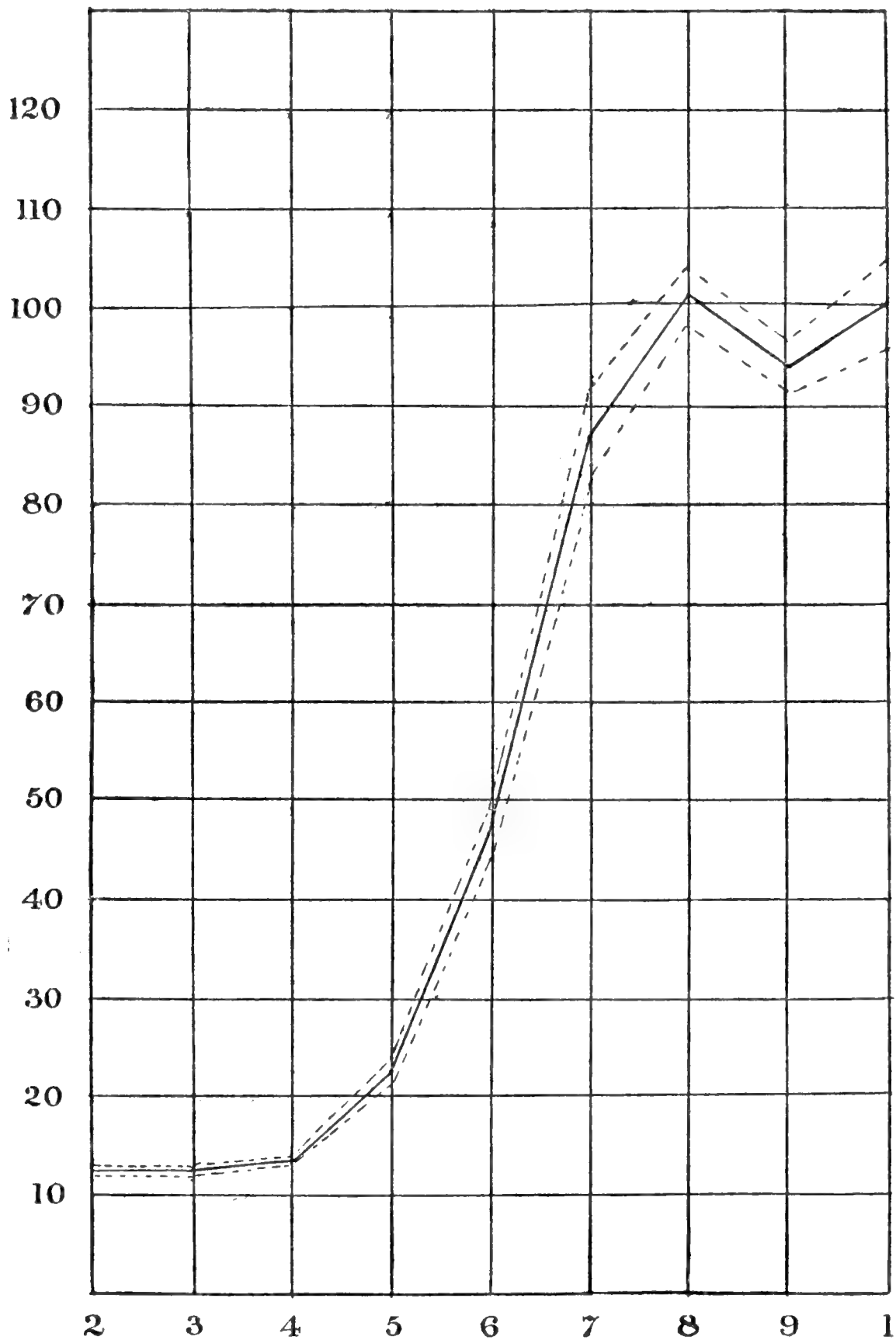
Table I. Actual dry weights of twenty series of barley plants, A-U, grown in varying strengths of copper sulphate with nutrient salts. The mean dry weight for each concentration of the toxic salt is also given.

2. 1:50,000 C.S.	7. 1:2,500,000 C.S.
3. 1:100,000 ,,	8. 1:5,000,000 ,,
4. 1:250,000 ,,	9. 1:10,000,000 ,,
5. 1:500,000 ,,	1. Control—distilled water.
6. 1:1,000,000 ,,	

determine the magnitude of the latter factor the usual method of least squares was followed, the individual results being first recalculated by taking the mean of the control series as 100. In this way the following table was obtained:—

Concentration.	Value of Result.
2. 1:50,000	12.25 ± 0.25
3. 1:100,000	12.0 ± 0.25
4. 1:250,000	13.5 ± 0.5
5. 1:500,000	22.0 ± 1.5
6. 1:1,000,000	46.5 ± 2.5
7. 1:2,500,000	86.5 ± 4.5
8. 1:5,000,000	101.0 ± 3.0
9. 1:10,000,000	94.0 ± 2.5
1. Control	100.0 ± 4.5

From this table and the resulting curve (Curve 3) it is evident that the possible stimulus with 1:5,000,000 C.S. does fall within the range of experimental error. This was confirmed by recalculating the means of



Curve 3. The *black* line shows the mean of the dry weights of twenty series of barley plants grown with copper sulphate and nutrient salts. The *dotted* lines indicate the limits of probable experimental error for each concentration of the toxic salt. (See Table I for concentrations.)

1:5,000,000 C.S., 1:10,000,000 C.S. and the control results after rejection of the individual results below 60. This corrected mean fell within the range of experimental error in the first two cases, and above it in the control, and indicated that no stimulus occurred with 1:5,000,000 C.S.

To sum up, it appears that copper, as sulphate, does not exert a stimulative action on barley, however small the concentration, but that the toxic effect of the salt gradually decreases with the concentration, until strengths of 1:5,000,000 and under have no appreciable effect, either toxic or stimulant, upon the growth of the plant.

#### MANGANESE SULPHATE.

The effect of Manganese on plant growth has recently been attracting considerable attention, and much work has been reported on the subject. Manganese occupies a different position from copper, for while the latter is a definite poison, the former is not, and minute traces of it are found in the ash of almost all plants. Manganese salts in sufficient concentration are well known to exert a toxic influence upon plant growth, and the tendency has been to assume that sufficiently dilute strengths of the salt have a consistently stimulative effect upon growth. Several investigators, notably M. Bertrand and some of the Japanese scientists, have reported results in support of this view, but nearly all the work has dealt with the action of manganese salts on plants growing in soil, either in pots or in the open field. This at once introduces a point of difficulty, as it is impossible to determine how far the results obtained are due to the direct action of the manganese salts themselves on the plants, or how far interaction has taken place between the manganese salts and the soil, thereby influencing the growth of the vegetation. Also, manganese is frequently an element contained in the soil, so one cannot be certain what concentrations of salts are really being dealt with.

In order to eliminate, as far as possible, this element of uncertainty due to unknown external factors, experiments with water-cultures have been made at Rothamsted. The initial series of plants, in 1908, were carried right through from seed to seed, as the barley plants were allowed to grow till they had set and ripened the grain in the ear. Barley grains of an absolutely pure strain were graded, only varying between 0.04 and 0.06 gram. These grains were germinated in damp sawdust as usual, and were then watered with dilute food solution to promote growth. As soon as the seedlings were large enough they were transferred on March 7 to bottles containing the following nutritive solution with the addition of manganese sulphate (=  $\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$ . = M.S.) in concentrations varying from 1:10,000 to 1:1,000,000,000, with a control containing no M.S.

Potassium Nitrate . . .	0.5 gm.	Sodium Chloride . . .	0.1 gm.
Potassium Phosphate . . .	0.25 „	Ferric Chloride . . .	Trace.
Magnesium Sulphate . . .	0.25 „	Water . . . . .	1,000 c.c.
Calcium Sulphate . . .	0.25 „		

Five similar sets were grown to put a check on individuality. As ordinary ferric chloride usually contains a certain small percentage of

manganese, Kahlbaum's sublimed ferric chloride was used to ensure its purity.

By April 8, about four weeks after the start, marked differences were manifest in the plants. Those growing in the strongest toxic solutions 1 : 10,000 M.S., had very brown roots, especially near the seed, probably on account of some deposit of manganese. These same plants gave every indication of being diseased, as the lower leaves were also very brown and appeared to be badly infected with rust, which died out gradually towards the upper leaves.

The next concentration 1 : 100,000 M.S. showed very little brown coloration on the roots and much less discoloration of the lower leaves. At a weaker strength still, 1 : 1,000,000 M.S. and less, the roots were strong, healthy, and white, while the indications of possible disease were very slight and died out altogether lower down the range of concentration.

This appearance of 'rust' so early in the season was rather unexpected, so, as no other plants in the greenhouse seemed to be attacked, further investigations were made. A microscopical examination showed no signs of any fungal disease, but indicated that the cells were simply dead and brown while retaining their normal shape and size, at any rate in the early stages. The dead cells at first occurred in small patches which gradually spread and coalesced till finally the whole leaf was involved. Appearances pointed to death on account of manganese poisoning. Some of the affected leaves were detached and fused with a mixture of sodium carbonate and potassium nitrate. The resulting mass when dissolved in water gave a green coloration, indicating the presence of manganese.

It is thus evident that the manganese is taken up by the plant roots and finally deposited in the cells of the leaves, which are killed if the concentration of the salt is sufficient. Whether the manganese is further excreted by the leaves and deposited on the outer surface is a point that is not as yet cleared up.

The plants grew steadily, one series being photographed on May 1, eight weeks from the beginning of the experiment (Pl. XLVII, Fig. 4). After eleven weeks, as the food solutions were getting exhausted and unable to support growth, the roots were carefully washed in distilled water, and the plants were placed in fresh nutritive solutions with a new quota of manganese sulphate, the concentrations remaining the same. At this date it was evident that manganese was deposited in the leaves even at so low a concentration as 1 : 1,000,000 M.S. and in some cases traces could even be observed in 1 : 10,000,000 M.S. By June 2, after three months' growth, most of the plants began to show ear, and by July 14, when the plants were harvested, a large proportion of the ovules had set and ripened to fully developed grains.

An examination of the plants at the time of harvesting indicated that

the varying concentration of toxic salt had apparently not influenced the actual number of ears produced, but the grains had reached various stages of ripeness. The grains from the controls and from the manganese solutions of 1 : 1,000,000 and less were to be regarded as ripe, being hard and yellow. The ears resulting from a concentration of 1 : 100,000 M.S. yielded a mixed crop, some of the grains being ripe, some half ripe, and others green. With 1 : 10,000 M.S. practically all the grains were green. It is thus evident that the stronger concentrations of manganese sulphate have a definite retarding action on the ripening of the grain in the ear, and the indications are that as the strength of the toxic salt decreases this retarding influence gets less. Possibly the lowest strengths of the salt have no influence in this respect one way or the other.

On being finally removed from the culture solutions the roots of the plants were carefully washed in two or three changes of distilled water to remove adherent extraneous salts as completely as possible. The grain was shelled out from the ears, and the roots, shoots, and grain were dried separately for about three days at 100° C., all the plants from a similar concentration of toxic salt being placed together. It is to be regretted that the individual plants were not kept apart and weighed separately, as has been done in the more recent experiments.

The dry weights obtained are shown in the following table :—

	Shoot.	Root.	Shoot + Root.	Grain.	Shoot + Root + Grain.
1.	39.140	6.282	45.422	7.464	52.886
2.	26.670	4.409	31.079	3.852	34.931
3.	37.140	5.020	42.160	5.996	48.156
4.	40.565	5.890	46.455	1.709	48.164
5.	47.900	5.780	53.680	4.635	58.315
6.	45.925	6.630	52.555	6.440	58.995

A consideration of the total dry weight, including the grain, indicates a very considerable depression in (2), which is obviously beyond the limits of experimental error. A possible slight depression seems to be shown in (3) and (4), and a stimulus in (5) and (6) as compared with the controls.

As the dry weight of the grain varies so much throughout the series, a truer estimate may perhaps be obtained by dealing only with the dry weights of the roots and shoots, which are fairly comparable.

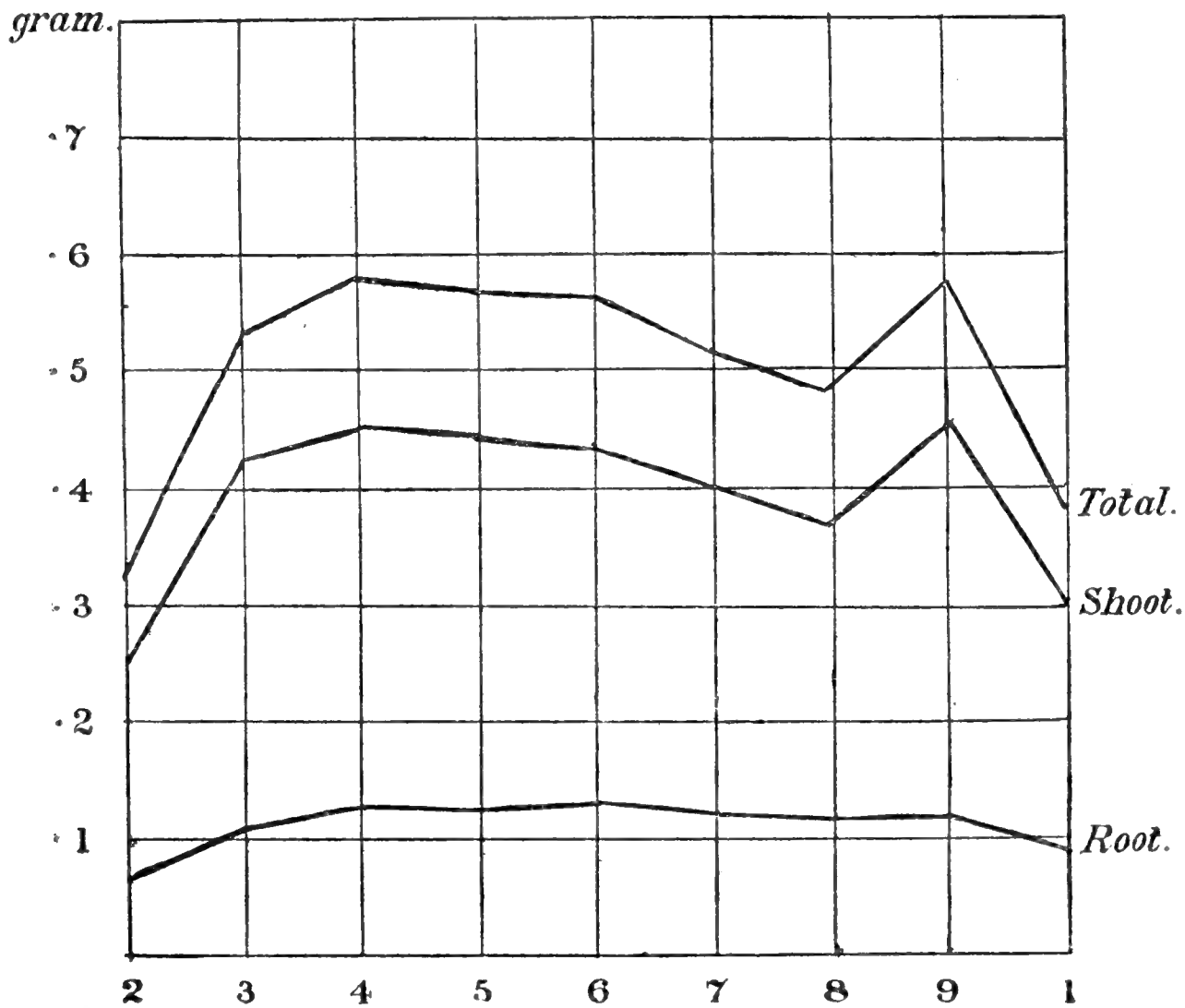
A rather different estimate of the state of the case is obtained from these figures. The striking depression with 1 : 10,000 M.S. is still evident, and also a possible slight depression with (3) 1 : 100,000 M.S., but in (4) the apparent depression was due to the failure of the grain, as the new figures show that this concentration has little effect upon the growth, the balance possibly being in favour of a slight stimulus. The stimulative effect of (5) and (6) is real.

Two points stand out clearly from this set of results :—

(1) The higher concentrations of manganese sulphate have a decided retarding effect upon the ripening of the grain.

(2) While strong solutions of manganese sulphate exert a toxic influence upon the growth of barley, very dilute solutions have a definite stimulative effect.

A later series of tests was carried out with pedigree 'Plumage' Barley, with the seeds graded to 0.05 and 0.07 gram, in which the plants were only



Curve 4. Showing the mean value of the dry weights of ten series of barley plants grown with manganese sulphate and nutrient salts.

- |                      |                             |
|----------------------|-----------------------------|
| 2. 1 : 10,000 M.S.   | 7. 1 : 50,000,000 M.S.      |
| 3. 1 : 100,000 ,,    | 8. 1 : 100,000,000 ,,       |
| 4. 1 : 1,000,000 ,,  | 9. 1 : 1,000,000,000 ,,     |
| 5. 1 : 5,000,000 ,,  | 1. Control—distilled water. |
| 6. 1 : 10,000,000 ,, |                             |

grown for a limited period, not being allowed to reach the flowering stage. In these tests the normal food solution was used (see copper sulphate experiments) at about double the concentration of the early manganese sets.

Five weeks from the start the 'browning' of the roots and discoloration of the lower leaves with 1 : 10,000 M.S. was manifest, and also at this date it was evident that all strengths of solution from 1 : 100,000 M.S. downwards were supporting plants which appeared better than the controls. This phenomenon was consistent through ten similar sets, and continued up to



the time of harvesting, after about seven weeks' growth, as was borne out by means of the dry weights (Curve 4).

Although the plants with 1 : 10,000 M.S. had very brown roots and showed the characteristic deposit in the leaves, yet, so far as weight went, there was practically nothing to choose between them and the controls, in fact, in some cases, the plants grown with the strong concentration of manganese were rather better than those without it.

Parallel results were obtained from a similar series of ten sets of plants grown later in the year.

It is conceivable that the discrepancy in the results obtained for barley was due to the difference in the length of the growing period. In the initial experiments the plants were taken through the whole cycle of growth, finally ripening their seeds, while in the later tests only the earlier stages of purely vegetative growth were passed through. The stimulative effect of manganese sulphate of 1 : 100,000 strength and downwards makes itself felt from the beginning, and can be traced by means of the periodical notes made during growth. The stronger concentration of 1 : 10,000 apparently has no effect on the earlier stages of growth, but seems to exert a very decided depressing influence when the plants pass through the reproductive phases of existence. This depressing effect of manganese is shown by its retarding action upon the ripening of the grain, even in concentrations which are weak enough to cause stimulation.

Probably the variation in the results of the earlier and later experiments is partly due to the food solutions used. In the early trials the nutritive solution was only half the normal strength, and it may be that the masking action of the food salts on the manganese sulphate was less than in the later experiments. If this was the case, it is evident that a weaker concentration of M.S. would cause depression than with the stronger normal nutritive solution. Under these circumstances it may have happened that while 1 : 10,000 M.S. exercised a depressing influence with the weaker food solution, the same effect with the normal amount of salts would only have been obtained with a concentration of manganese sulphate outside the range of the experiments under consideration.

#### SUMMARY.

1. The action of plant poisons in dilute solutions is masked by the presence of nutrient salts, which thus enable plants when grown in such solutions as water-cultures to endure a much greater concentration of the toxic substance than in the absence of nutrients.

2. Copper sulphate, which is a definite poison to Barley, does not have any stimulative effect in very dilute solutions, even at so low a concentration as 1 : 10,000,000 C.S.

3. Manganese sulphate, though not an actual toxic to Barley, retards the growth very considerably if supplied in moderate quantities. Minute traces of the salt have a decided stimulative action both on the root and shoot.

4. When supplied in sufficient concentration manganese is taken up by the plant and deposited in the lower leaves.

In conclusion, I have to express my indebtedness to Mr. A. D. Hall for the valuable advice he has given me during the progress of the experiments, and also to Dr. N. H. J. Miller for assisting me by taking the photographs reproduced in this paper.

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### EXPLANATION OF PLATE XLVII.

Illustrating Miss Brenchley's Paper on the Influence of Poisons on Barley.

Fig. 1. Photograph showing the deleterious effect of copper distilled water on the growth of plants (Peas).

- 1, 3. Peas growing in glass distilled water.                      2, 4. Peas in Cu distilled water.  
(No nutrient salts.)

Fig. 2. A series of barley plants about six weeks old grown in different concentrations of copper sulphate in the presence of nutrient salts.

- |                            |                     |
|----------------------------|---------------------|
| 1. Glass distilled water.  | 6. 1 : 100,000 C.S. |
| 2. Copper distilled water. | 7. 1 : 250,000 „    |
| 3. 1 : 12,500 C.S.         | 8. 1 : 500,000 „    |
| 4. 1 : 25,000 „            | 9. 1 : 1,000,000 „  |
| 5. 1 : 50,000 „            |                     |

Fig. 3. Photograph showing the effect of distilled water, differently prepared, upon the growth of plants—Peas. (No nutrients.)

- |                           |  |
|---------------------------|--|
| 1. Glass distilled water. | 3. Glass distilled water which had been allowed    |
| 2. Cu distilled water.    | to stand in contact with pure silver for one hour. |

Fig. 4. A series of barley plants grown with varying concentrations of manganese sulphate, in the presence of nutrient salts.

- |                             |                       |
|-----------------------------|-----------------------|
| 1. Control—distilled water. | 4. 1 : 1,000,000 M.S. |
| 2. 1 : 10,000 M.S.          | 5. 1 : 10,000,000 „   |
| 3. 1 : 100,000 „            | 6. 1 : 100,000,000 „  |





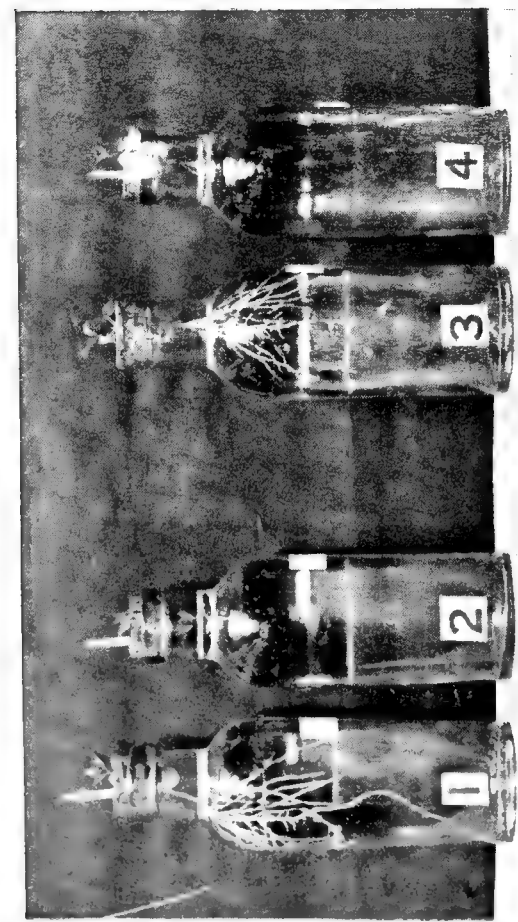


FIG. 1.

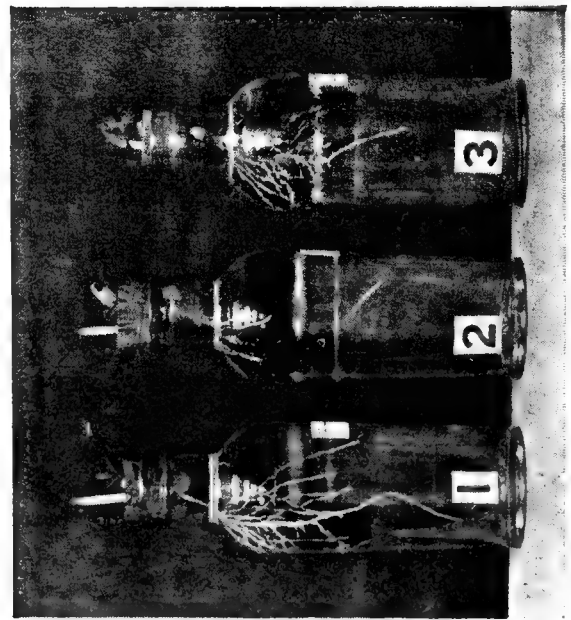


FIG. 3.

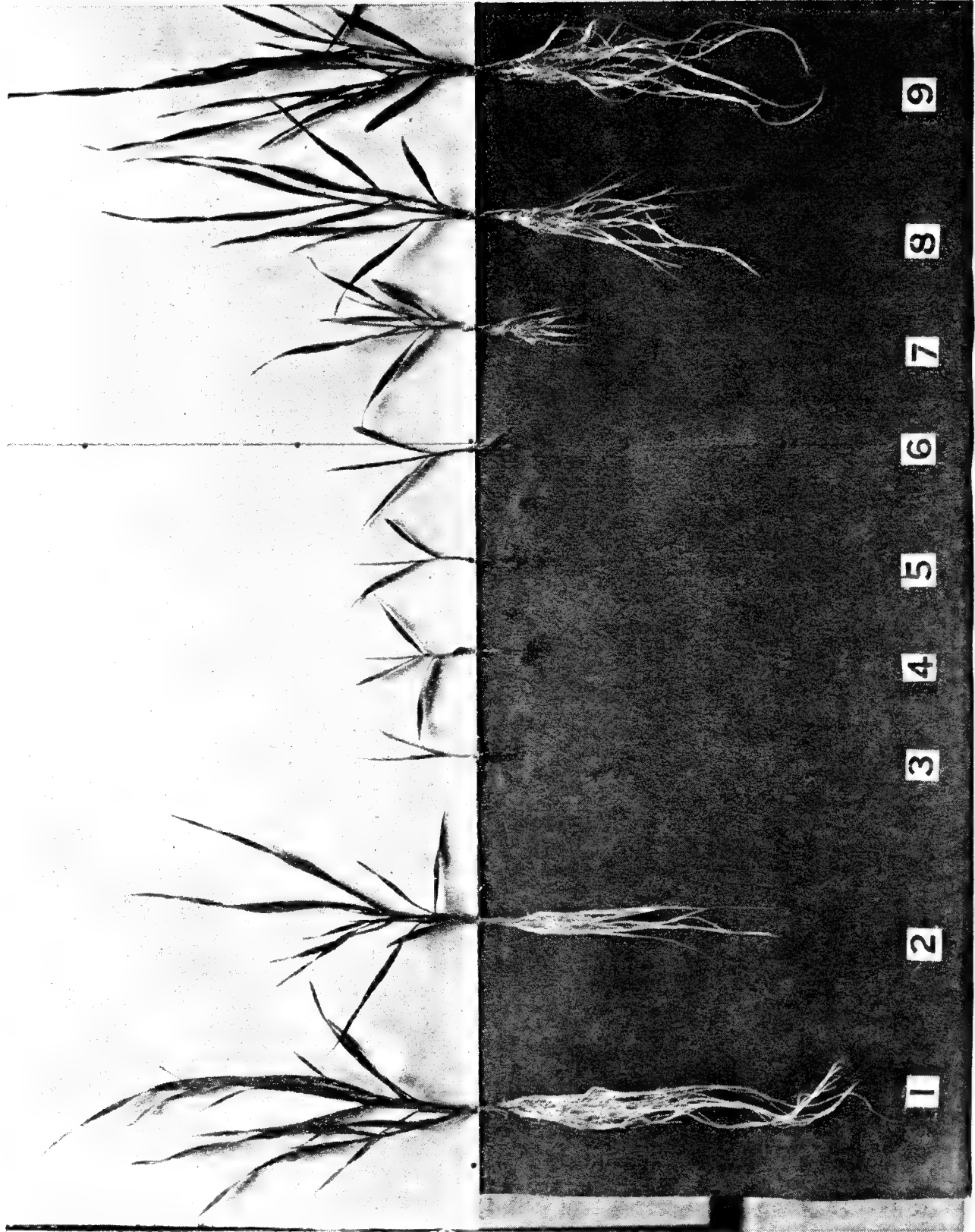


FIG. 2.



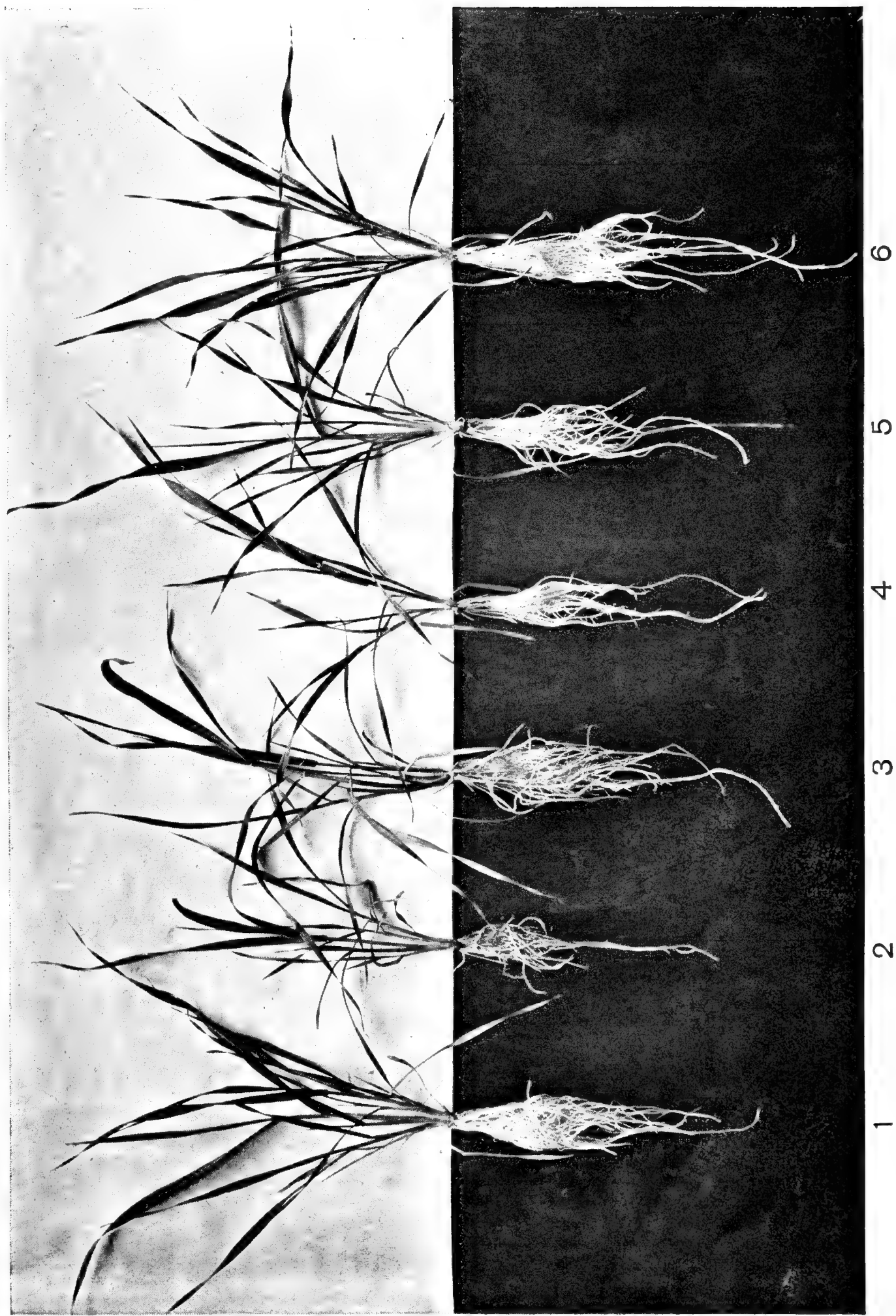


FIG. 4.

BRENCHLEY—INFLUENCE OF COPPER SULPHATE, ETC.





# The Development of *Gnomonia erythrostoma*, Pers. The Cherry-Leaf-Scorch Disease.

BY

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With Plates XLVIII and XLIX.

IN 1886 Frank (17) gave a short account of the development of this Pyrenomycete and showed that it was the cause of an epidemic disease of Cherry trees in the district of Altenlande in Germany. The chief features of the life-history of the fungus as described by Frank may be summarized as follows:—

Infection of the leaves first takes place in the spring, and the mycelium which develops in them passes towards the base of the leaf-stalks where it prevents the formation of the absciss layer. By this means infected leaves do not fall in the autumn but remain hanging on the trees to cause infection of the new foliage in the following spring. Where the mycelium is present in the leaves, spermogonia giving rise to spermatia, and coiled structures with trichogynes, are found. Frank considers that the spermatia fertilize the coils, after which the latter develop into perithecia which liberate their spores in the spring. Frank's brief account only dealt with the grosser development of the fungus, and it was obvious that a cytological investigation was to be desired. The absence of figures of the ascogonia, trichogynes, &c., in Frank's account, and the lack of detailed knowledge of the development of the perithecium of the Pyrenomycetes generally, made this all the more imperative.

The present investigation was begun at the suggestion of Professor V. H. Blackman, to whom I am much indebted for advice and criticism.

Material was obtained from the Cherry-growing districts of Kent, where the disease caused by *Gnomonia erythrostoma* is known as the 'Cherry Leaf Scorch' on account of the appearance presented by the retained foliage of infected trees in autumn and winter. At the outset of the investigation attempts were made at Cambridge to cause infection of some of the varieties of Cherries most susceptible to this disease. For this purpose specimens of such trees were obtained, and in the spring, at the

time when the new foliage was appearing, cherry-leaves containing the ripe perithecia of the fungus—likewise obtained from Kent—were damped and hung on the trees. Frank has shown that the perithecia when alternately damped and dried liberate their spores by successive ejaculations. I confirmed this by placing damp leaves bearing perithecia in Petri dishes; when the leaves had become dry it was found that the ascospores had collected in groups of eight on the lid of the dish. Thus when leaves bearing mature perithecia were damped and hung on the trees it was considered that the conditions approximated to those under which infection ordinarily occurred in nature. Later on in the summer it was found that a few leaves had become infected. The number of infected leaves, however, was insufficient to provide material in different stages, so several journeys were made to Kent for the purpose of obtaining further supplies. During these visits some observations were made on the relative susceptibility and immunity of different cultivated varieties of Cherry trees. It was found that the following kinds of sweet cherries are attacked by the disease to the greatest extent:—Florence, Waterloo, and Frogmore, while Elton Heart and Black Heart are only slightly attacked, and Amber, Turk, and Crown varieties are apparently immune from the disease.<sup>1</sup> These observations are in agreement with those of Salmon (27).

Thus the 'Cherry Leaf Scorch' is another of those diseases which affect different cultivated varieties of the host species to very different degrees. As yet, practically nothing is known as to the factors which confer immunity upon some varieties.

Upon making inquiries I found that the 'Scorch' disease is now much less prevalent in Kent than formerly. This is due to the commendable practice in many orchards of destroying from year to year the leaves found hanging on the trees in winter. It has been well known since Frank's first account of the disease, that the leaves which hang on the trees in winter are the only means by which a new infection can take place.

In one or two plantations visited, these measures had not been taken for the destruction of the disease. It was here noticeable that the trees susceptible to the disease produced only a very meagre quantity of fruit—the natural consequence of the weakening of the trees by the presence of the fungus for several years—and the cherries that were formed became hard, distorted, and spotted. Such cherries never became properly ripe.

#### EXTERNAL APPEARANCE OF LEAVES AFFECTED BY THE PARASITE IN ITS EARLY STAGES OF DEVELOPMENT.

Great difficulty was experienced at first in finding leaves in the early stages of attack. Little help was obtainable from Frank's account as

<sup>1</sup> Acid cherries also remain free from attack.

he describes the external features of the disease with extreme brevity. After considerable trouble, however, I was able to find Cherry leaves which contained the parasite in the very young stages of development. The first signs of disease are the presence of faint yellowish patches either on the margin or near the midrib of the leaves, though when these patches are seen for the first time they hardly suggest disease of a serious nature.

On cutting hand sections through leaves affected in this way the characteristic mycelium of *Gnomonia erythrostoma* could be discerned. These yellowish patches are first recognizable in the early part of July. They increase in size and become more clearly yellow in colour. Towards the end of August spermogonia become visible. They are formed in enormous numbers, and appear externally as tiny circular spots on the under surface of the yellowing parts of the leaves and just visible to the naked eye. With the production of spermogonia the diseased portion of the leaf becomes brown.

#### METHODS.

Portions of diseased cherry leaves were fixed in various fluids. Fleming's weaker and stronger solutions, Gilson's fluid, Juel's fluid, and acetic alcohol were tried, but as the first-named solution proved to be by far the most satisfactory fixative it was generally used. An air-pump to aid fixation was employed when necessary. The material was cleared in chloroform or in cedar oil and embedded in paraffin of about 52° C. melting point. Microtome sections were cut from 5  $\mu$ –15  $\mu$  in thickness and were stained either in gentian violet and orange G, or in Heidenhain's iron-alum-haematoxylin, with a counter-stain of orange G, congo red, or erythrosin.

#### VEGETATIVE MYCELIUM.

Frank (17) states that the ascospore on germinating upon the surface of the leaf gives rise to an appressorium, a structure which doubtless performs mainly an anchoring function. The appressorium puts out a short tube which penetrates the cuticle directly, thus entering an epidermal cell. The parasite enters the intercellular spaces of the mesophyll by means of another short hypha which penetrates the inner wall of the epidermal cell. Evidence of the production of appressoria and of the penetration of epidermal cells was also seen in my preparations of the young mycelium.

The subsequent development of the mycelium is intercellular. A general view of it is seen in Plate XLVIII, Fig. 1. The mycelium consists of broad hyphae which are septate and branch at intervals; it ramifies most abundantly in the spongy parenchyma, but not infrequently branches are put out which force their way between the closely packed palisade cells. Narrowings

of the hyphae occur frequently when they intrude between the host cells. It is often observed that cells of the parasite are very closely attached to cells of the host. In such cases it appears that the wall of the fungus cell becomes somewhat gelatinized so that the contact can be more intimate. Haustoria, however, were never seen. After a time the host cells, which are in intimate contact with the fungus, shrivel and die. Such disorganization of the host cells is seen particularly well where a branch of the general mycelium penetrates the palisade region of the leaf. It is obvious that the fungus kills the host cells gradually. On the other hand, the parasite does not remain in intimate connexion with living cells of the host over such a long period of time as is the case with Rust Fungi, where, of course, haustoria are developed. The chlorophyll granules of the cells of the Cherry leaves in the neighbourhood of the mycelium are smaller in size than granules in healthy parts, and they are yellowish in colour.

On cutting hand sections of fresh material, it is seen that the hyphae of the parasite are filled with extremely granular protoplasm. In stained preparations the cells are found to be multinucleate, as will be seen by an examination of Fig. 1. Some cells contain as many as fifteen nuclei, but the general number is from five to eight. The multinucleate nature of the cells of this fungus is thus the same as that found in *Humaria granulata* (3), *Aspergillus herbariorum* (20), and many other Ascomycetes, though different from the uninucleate condition of the cells in the Mildews (22). It is very doubtful, however, whether any phylogenetic significance is to be attributed to the uninucleate or multinucleate nature of vegetative cells in the Ascomycetes.

The nuclei of *Gnomonia erythrostoma* are small, but, in spite of this, they show a fair amount of structural detail—much more in fact than the vegetative nuclei of many other fungi as yet examined. The vegetative nucleus contains several small, deeply staining, granules found generally near the nuclear membrane, and in some cases a distinct though faint reticulum can be seen (Fig. 2); a nucleolus may or may not be present. In many other fungi (e. g. the Rusts), the only portion of the vegetative nucleus which takes up the stain with ease is the nucleolus, so that, apart from the latter, the nucleus appears homogenous.

Apart from the divisions in the ascus and ascospore to be described later, the nuclei have been only rarely seen undergoing the process of division, and in these cases details could not be made out.

After the mycelium has ramified in the leaves for some weeks, spermogonia and the coiled structures (Knäuel verflochtener Fäden) described by Frank are formed. Although the two sets of structures overlap in development, some spermogonia always appear first.

## THE SPERMOGONIA.

These are produced in enormous numbers on the under surface of the affected leaf-areas. Their external appearance has been already described. Occasionally two spermogonia coalesce to form a compound structure. The wall of the spermogonium consists of very closely compacted hyphae which form a kind of pseudoparenchyma. From the interior of the spermogonium, hyphae grow out towards the small circular orifice. These hyphae even at the base are much narrower than the vegetative hyphae, and they quickly taper to a point. The spermata are formed by abstriction from the extremities of these hyphae. A general view of a section through a spermogonium is seen in Fig. 3, a portion of which is shown on a more highly magnified scale in Fig. 4. The cells which give rise to spermata are uninucleate, the single nuclei being larger and showing a more clearly defined network than the vegetative nuclei. The hyphae from which the spermata originate, are often unbranched, though sometimes branching may occur as seen in Fig. 4. There is some evidence that more than one spermatum can be abstricted successively from the apical region of the spermogonial hyphae. In a mature spermogonium a few hyphae are seen to be distinguished from those giving rise to spermata by growing further towards the orifice, and by their more deeply staining character. It is likely that these hyphae aid in the extrusion of the spermata.

The material was not favourable for observing details of the nuclear division which occurs prior to the abstriction of a spermatum. After this division, the daughter-nucleus which will become the nucleus of the spermatum rapidly becomes elongated. After separation of the spermatum from the hypha, the nucleus of the former is long and thread-like, and occupies a large part of the space within the wall. A relatively small amount of cytoplasm is present, most of which is found towards the lower end of the spermatum (Fig. 6). Under high magnifications the thread-like nucleus is seen to be not quite homogeneous, some portions staining more deeply than other (Fig. 6).

The spermata are straight at the time of formation, but most of them become curved to different degrees upon liberation from the spermogonium. Fig. 5 shows the diversity of form assumed by the spermata. Sometimes the spermatum has the hooked shape of the spermatum of *Polystigma rubrum* (15), but more often the amount of curvature is only slight. The spermata are from  $8\ \mu$ – $18\ \mu$  in length by about  $0.5\ \mu$  broad. Frank gives the length of the spermata as  $14\ \mu$ – $16\ \mu$ , but my experience is that the diversity in length is much greater than this. They are very slightly broader at one extremity than at the other, but the difference in this respect is much less marked than is the case with the spermata of *Polystigma rubrum*.



A very large number of spermatia are produced within a spermogonium. They extrude from the orifice in a short coiled thread. The pycnidia of many other Pyrenomycetes (e. g. *Valsa*) and of certain members of the Sphaeropsidales (e. g. *Cytosporina*) liberate their spores in the same way, but whereas the coiled thread in these cases is frequently as long as 7 mm., the thread in *Gnomonia* is less than a millimetre in length. When one of these threads of spermatia is put into a drop of water it becomes broken up into the innumerable spermatia of which it is composed. Doubtless in nature spermatia are scattered over the under surface of the affected area of the leaf by the action of rain or dew.

Spermatia have been frequently seen adhering to the trichogynes whose structure will be described later (cf. Figs. 7, 8). It may be said here, however, that the trichogynes pass out of the stomata in compact groups of two to five. In most cases observed, not one, but several, spermatia were found to be attached to one group of trichogynes. When one considers the enormous numbers of spermatia produced and the close proximity of the gelatinous trichogynes to the spermogonia, one recognizes that it is difficult for the trichogynes to escape having spermatia adhering to them.

A large number of sections prepared during two successive years have been examined, but no definite evidence has been obtained for the passage of spermatial nuclei down the trichogynes. Occasionally what appear to be spermatial nuclei are seen embedded in the disorganizing matrix of the trichogynes and even at some considerable distance from their extremities. It is, however, a matter of great difficulty to be certain of the identity of such bodies at this stage because the degenerating nuclei of the trichogynes become elongated and, when stained, simulate the spermatial nuclei themselves.

Attempts have been made to induce the spermatia to germinate, but without success. After prolonged immersion in water and various nutritive solutions they did not show the least indication of further development. Indirect evidence as to the lack of germinative power possessed by these spermatia is obtained from the circumstance that no newly infected areas appear on the leaves after spermogonial formation. Of course one must not lay too much stress upon the apparent inability of these spermatia to germinate, because it is well known that the spores of many parasitic fungi are often erratic in their germinative capacity.

Upon considering the above data one is compelled, I think, to conclude that the spermatia of *Gnomonia erythrostoma* are abortive structures of the nature of male cells. Frank (17) came to the conclusion that they were male cells chiefly upon the ground that he had seen them attached to the trichogynes. The above account of their cytological characters and other facts cited in regard to them demonstrate more clearly what their nature is. V. H. Blackman (1) has shown that the spermatia of the Uredineae possess

the cytological characteristics of male cells—viz. a relatively large nucleus, a small amount of cytoplasm, and absence of food-reserves. The cytological features of the spermatia of *Gnomonia* are identical with these. One can also point to the striking similarity in general structure between the spermogonia of *Gnomonia* and those of the Uredineae. The fact that no success has attended one's efforts to induce the spermatia of *Gnomonia* to germinate is in keeping with the inability exhibited by the spermatia of the Uredineae to undergo further development under all conditions approximately normal.

In this connexion it is interesting to compare the cytological characters of the pycnidiospores of a species of *Phyllosticta* frequently found associated with *Gnomonia* on old Cherry leaves. *Phyllosticta* is a genus belonging to the Sphaeropsidales. The pycnidiospores of this genus readily germinate. Fig. 6a shows some of stained spores of the species of *Phyllosticta* found on old Cherry leaves. It will be observed that the relation between the size of the nucleus and the amount of cytoplasm is radically different from that holding in the spermatium of *Gnomonia*.

The fact that in *Gnomonia* some spermogonia are always produced before the formation of the coiled structures (to be described later) is what one would expect if the spermatia at present or in the course of recent phylogeny performed a fertilizing function. In the Rusts, too, one finds that the formation of spermogonia precedes that of aecidia.

Some authorities, e. g. Brefeld (4) and Christman (5), hold that the spermatia of the Uredineae are simply asexual spores which have lost the power to germinate; but their cytological characteristics, as first demonstrated by Blackman, seem to negative this view.

#### TRICHOGYNES AND ASCOGONIA.

Soon after the beginning of the formation of spermogonia, groups of hyphae towards the lower epidermis begin to entwine themselves closely together and so form the 'coils' (i. e. the 'Knäuel verflochtener Fäden' of Frank) which are the earliest stages of perithecial development (Fig. 8). The hyphae which become compacted into these 'coils' cannot be distinguished from vegetative hyphae. The 'coils' are roughly spherical in shape, but they differ much in size, their diameter varying from 40  $\mu$ –100  $\mu$ . It is generally found that certain hyphae connected with the periphery of the 'coils' pass out through a stoma. These hyphae, the trichogynes of Frank (17), are most often grouped together in a compact bundle of two to five which protrudes slightly from the stoma. The trichogynes passing through any one stoma are closely compacted together in the apical region (Figs. 7, 8). The group becomes somewhat narrowed while passing through the stoma, but when clear of it expands again and terminates as a dome-shaped mass (Fig. 7). The cell walls at the tips of the trichogynes become

swollen and apparently mucilaginous, as do also the lower lateral walls to a lesser extent.

The tufts of trichogynes are generally placed laterally in relation to the 'coils'. Thus it is rare to see a group of trichogynes over the middle of a 'coil'. It is often evident that at least two series of trichogynes are associated with a single 'coil', but only very rarely are two such groups seen in a single section. In all cases examined it was only possible to trace a connexion between the trichogynes and the outermost cells of the 'coil'. The trichogynes could never be traced to any structures resembling ascogonia, so must therefore be looked upon as mere continuations of ordinary vegetative hyphae belonging to the 'coil'. Fisch (15) and Frank (16) state that in *Polystigma rubrum* the trichogyne is the termination of a clearly differentiated archicarp. In this respect, therefore, *Gnomonia* differs considerably from *Polystigma*.

The apical cell of each trichogyne is long and narrow, and generally contains a single nucleus (Fig. 11); occasionally a second nucleus is present. The first septum below the extremity of the trichogyne is approximately at the level of the inner wall of the epidermal cells, and below this region the identity of the trichogyne is lost, so that as a well-marked structure it is only one cell in length. It may be pointed out that the uninucleate condition of the apical cell of the trichogyne is the same as that found by Baur (*a*) in the corresponding structure of *Collema*, and by Darbishire (12) in *Physcia*. On the other hand, all the cells of the hyphae beneath the terminal cells of the trichogyne are multinucleate and, as has already been said, are scarcely distinguishable at the time of their formation from cells of the general mycelium.

At a later stage a few cells in the midst of the 'coil' become differentiated from those around by their larger size, their denser protoplasm, and by their larger and less numerous nuclei (Figs. 9, 10, 11, 14). Though these cells often appear to be isolated from each other in a single section they really belong to one or more twisted filaments, as is ascertained by examining successive sections. No pores such as occur in the archicarps of *Ascobolus* (32) and *Ascophanus* (7) have been observed between the contiguous cells of these filaments.

The nuclei of these special cells usually possess a distinct nucleolus, but no reticulum is visible, while the nuclei of the cells around contain several small chromatin granules and the nucleolus when present is very small. Furthermore, they are about twice the size of the vegetative nuclei. Nuclei of an intermediate character are sometimes visible in the larger cells in the centre of the 'coil'. Occasionally one or two exceptionally large nuclei are found in these differentiated cells. Such nuclei contain several deeply staining chromatin granules, but no well-marked nucleolus (cf. Fig. 11). It may be that these nuclei have grown immensely in size prior to division, but on the other hand they may be hypertrophied.

It is considered that the nuclei present in the differentiated part of the 'coil' have become larger in size by a process of growth, no fusion having been observed before their occurrence. By comparison with certain Lichens and with *Polystigma* these larger cells in the midst of the 'coil' are to be interpreted as ascogonial cells, while the surrounding cells are purely nutritive and protective in function. On the other hand the ascogonial cells are not so clearly defined in *Gnomonia* as in the Lichens and in *Polystigma*. There can be no doubt that in *Gnomonia* more than one ascogonium is usually present in the 'coil', which later gives rise to a single perithecium; while in *Polystigma*, according to Fisch and Frank, the perithecium generally develops in association with a single ascogonium.

It has already been mentioned that spermatia in considerable numbers have been seen attached to the trichogynes. No evidence was obtained that the nucleus of a spermatium fused with the single nucleus usually present in the terminal cell of a trichogyne, or that it passed downwards to the larger ascogonial cells. In fact the single nucleus of the apical part of the trichogyne often shows signs of disorganization as soon as the trichogyne has passed beyond the stoma, and this unhealthy condition frequently extends to several of the nuclei in the cells immediately below. Frank states in his account of *Polystigma rubrum* that where spermatia adhered to the trichogynes the former seemed to be poorer in contents. He concluded from this that the nuclei of the spermatia passed into the trichogynes and performed a fertilizing function. It would appear, however, that the apparent diminution of protoplasmic contents might be just as readily explained by the process of disorganization which the spermatia would naturally undergo if they remained for any length of time on the exposed trichogynes. It is clear that the mere adhesion of spermatia to the trichogynes is no evidence for their fertilizing function.

During this investigation of *Gnomonia*, coils, apparently normal, have been frequently seen, to the trichogynes of which no spermatia were adhering. A more surprising phenomenon has been observed in that the trichogynes sometimes grow out from small groups of hyphae in which no ascogonia can be seen, and which therefore cannot be considered to be of the same nature as the 'coils'. Trichogynes have also been seen growing from ordinary hyphae which were not massed together (Fig. 12). Frank also mentions this fact, but appears to attach no significance to it. The nuclei of the cells in immediate connexion with such trichogynes are in no way different from ordinary vegetative nuclei. It was not only in material gathered early in the season that trichogynes were found apart from the 'coils', but that obtained much later showed the same phenomenon. Hence the view that the vegetative cells below these trichogynes develop later into 'coils' cannot be entertained.

I observed also a group of trichogynes in connexion with the margin



of a spermogonium. In addition to this anomalous behaviour, trichogynes have also been seen undergoing further development as vegetative hyphae (Fig. 13). Such behaviour of the trichogynes militates strongly against the view that they are functional receptive organs. The question of their present function will be considered later.

Careful search was made amongst the ascogonial cells of the 'coil' to see if there was any fusion of nuclei in pairs similar to that which takes place in the ascogonium of *Humaria granulata* described by Blackman and Fraser (3), or in the more similar multicellular ascogonium of *Ascophanus carneus* recently described by Cutting (7). No such process, however, was observed.

The 'coils' may develop into perithecia in the autumn or may remain throughout the winter in the same condition. Fig. 15 shows a section through a portion of a 'coil' in the resting stage. It will be noticed here that two types of cells are present, viz. those with dense cytoplasm and large nuclei, and those with highly vacuolate protoplasm and smaller nuclei. I conclude that the former are the cells previously described as being ascogonial in nature. Fig. 16 also represents a section of a 'coil' in the resting condition.

#### THE DEVELOPMENT OF THE PERITHECIUM.

Frank states that the perithecia develop from the 'coils' in the autumn, though he suggests that the asci do not ripen until later. My experience has been, however, that there is no one definite time for the development of the perithecia from the 'coils'. The change of 'coils' into perithecia has been found to occur as early as November, while in other cases resting coils have been seen as late as March.

The first thing to be observed in the further development of the 'coil' is the growth of cells in the lower middle part, i. e. the region which is remote from the trichogynes. The cells in this position send out branches which grow towards the lower epidermis of the leaf. A few of the cells above are often seen to be disorganizing. About the same time the cells towards the periphery of the 'coil' increase in number, thicken their walls, and thus become differentiated at an early stage to form the wall of the perithecium. These cells contain food reserves which are probably of an oily nature, since they blacken with Flemming's fixative; the protoplasmic contents, however, of these cells are poor. A cavity has by this time appeared towards the upper part of the developing perithecium. This is formed probably as the result in the main of the greater expansion of the outer tissues.

It is a matter of great difficulty at this stage to trace the remains of the ascogonia. Only occasionally does one find at the periphery of the perithecium an unusually large cell containing nuclei of about the same size

and general appearance as those described above in ascogonial cells. The difficulty of tracing the ascogonium at this period arises from the fact that in *Gnomonia* it is such a poorly differentiated structure. In spite of prolonged search no evidence could be obtained for the development of asci from ascogonia, and in fact after this stage the latter cannot be traced at all. It would seem, therefore, that in *Gnomonia* the ascogonial cells are no longer functional. Fisch (15) states that in the allied fungus *Xylaria polymorpha* the ascogonial cells disorganize during the young stages of perithecial development, and that the ascogenous hyphae arise *de novo*. Traces of functionless ascogonia have also been found in *Otidea aurantia* (20 a) and in certain Lichens.

The branches previously put out from the lower middle part of the ascocarp continue their growth towards the region which later becomes the neck of the perithecium. These branches vary considerably in width, but usually they are nearly as broad as the asci which are formed later. They are occasionally septate and are of irregular growth (Fig. 17). Their cells are poor in cytoplasm, and their nuclei are not to be distinguished from the usual vegetative nuclei. These threads disorganize before the asci come to maturity. The width, the irregular growth, and the early disorganization of these threads all indicate that they cannot be looked upon as typical paraphyses. In fact, *Gnomonia erythrostroma* has no ordinary paraphyses when the asci are mature. The appearance of these outgrowths is striking, and at first sight they suggest asci which have developed abnormally; their function is obscure, although they may take a part in the formation of the cavity of the perithecium. They do not appear to have been described elsewhere.

At a later stage, short hyphae with dense cytoplasm and several deeply staining nuclei become distinguishable towards the base of the perithecium (Plates XLVIII and XLIX, Figs. 18, 19). The asci invariably take their origin from these hyphae. Though it was difficult to obtain certainty in the matter, close investigation pointed strongly to the conclusion that these ascogenous hyphae grow out directly from vegetative cells at the base of the perithecium. Such a development is the natural corollary of the disappearance of the ascogonia in earlier stages. In the process of differentiation of the ascogenous cells from vegetative cells the cytoplasm becomes denser; the nuclei become larger, and contain a more definite chromatin network and also a distinct nucleolus. No fusion has been observed prior to the formation of these clearly differentiated nuclei.

The gradual increase in size of the nuclei in these ascogenous cells would thus appear to be due to a process of growth alone. Fig. 18 shows some of these nuclei which are becoming differentiated from ordinary nuclei.

Sometimes the larger nuclei of these ascogenous cells are arranged irregularly in relation to each other, but more often they appear to be



associated in pairs (Figs. 20, 22). This state possibly indicates a conjugate condition of these nuclei, but in the absence of preparations showing nuclear divisions at this stage—apart from one doubtful case (Fig. 21), it is impossible to speak with certainty. In this doubtful case the difficulty of determining the connexion of the cell in which it occurs prevents one from deciding whether this conjugate division is the one immediately before the formation of the young ascus or one at an earlier stage. The cells that contain the larger nuclei branch, and so the number of ascogenous cells is increased. Fig. 22 shows this branching. The ascogenous cells cannot be traced very far because of the intricate way in which all structures at the base of the perithecium are interwoven. Hence to follow the course of the ascogenous hyphae in *Gnomonia* is a more difficult matter than in *Discomyces* generally.

There is a considerable difference in regard to the manner in which the asci arise from the ascogenous cells. Crozier formation, so frequently described for other Ascomycetes, has been seen in *Gnomonia* also (Fig. 25). Often, however, although the end of the ascogenous cell begins to bend over, this process is not continued, and the terminal part, containing two nuclei which fuse, forms the ascus. Sometimes there is not even a trace of bending over (Figs. 22, 23) before ascus formation. In Fig. 22 it is evident that the penultimate cell grows out laterally to form another ascus. The same process may no doubt go on indefinitely. This mode of ascus formation is similar to that described by Maire (26 a) in *Galactinia succosa*. Faull (14) has also pointed out the differences to be observed in the mode of ascus formation in other Ascomycetes.

The two nuclei present in the young ascus cell lie in contact with each other and soon fuse together (Fig. 27). They seem to melt into each other. The nucleoli, however, do not become united for some time. Immediately after fusion, the nucleus thus formed increases in size and the ascus grows in length.

It should be mentioned here that while the first asci have been in process of formation, the neck of the perithecium has been differentiated by the outgrowth of cells which originally formed the part of the coil towards the trichogynes. The periphyses develop as narrow outgrowths from cells on the inside of the upper part of the ascocarp. As stated earlier, no typical paraphyses are developed.

#### THE CYTOLOGY OF THE ASCUS.

When the ascus nucleus has reached its maximum size, i. e. shortly after fusion has taken place, the chromatin thread becomes densely aggregated towards one side of the nuclear membrane (Fig. 28). At a later stage the thread becomes looser and more generally distributed. It has been

often observed that the chromatin network is not readily stainable at this stage (Fig. 29). This condition apparently persists for some time. The exact method of formation of the chromosomes of the first division could not be made out. No indications of loop formation have as yet been observed. Fig. 30 shows one of the few stages seen in the formation of the chromosomes. It will be observed that four lumpy masses are being elaborated within the nuclear area. Spindle formation takes place in the same manner as that described by Harper (24) and Fraser (19) in other Ascomycetes. Distinct centrosomes of a discoid shape are present at the two extremities of the spindle (Fig. 33). While the chromosomes are in process of formation the nucleolus becomes vacuolate (Fig. 30), and the nuclear membrane less clearly defined. Figs. 31-33 show the metaphase of the first division. In some cases the shape of the chromosomes of this division is strikingly like that of the heterotype chromosomes of pollen mother-cells (Fig. 34). They are short and thick, and in metaphase show a middle portion projecting equatorially and two limbs directed along the spindle. Fig. 35 shows a late stage in the first division. This nuclear division is therefore to be interpreted as being one of reduction, though whether it is heterotypic or brachymeiotic one cannot say with certainty, since the exact details of chromosome-formation have not been ascertained. The shape of the chromosomes and the fact that the second division follows rapidly upon the first furnish analogies with meiosis rather than with brachymeiosis.

In the reconstruction of the two daughter-nuclei a distinct nucleolus is formed (Fig. 36). Preparations showing the metaphase and anaphase of the second division have not yet been obtained, but the telophase is represented in Figs. 38, 39.

The four nuclei thus formed rest for some time before undergoing the next division. The chromatin network of these nuclei is clearly marked. The nuclei often show a certain amount of polarity at this stage and a little later. Thus in Fig. 40 one nucleus shows the attachment of the chromatin net to one end of the nuclear membrane. Such a condition reminds one of the ascus nuclei of *Phyllactinia*, where Harper found very distinct polarity. A slight polarity has also been seen in the bi-nucleate stage of the ascus of *Gnomonia*. No clear contraction of the chromatin network, such as has been described as occurring in some cases in connexion with brachymeiosis, was seen in the prophase of the third division nor indeed at any stage subsequent to the first division. In the third division, the spindle is generally orientated at right angles to the long axis of the ascus, but not invariably so. During the metaphase of this division the chromosomes are arranged on the equatorial plane of the spindle in the manner usual in karyokinesis (Fig. 42). The anaphase is represented in Fig. 43, and the telophase in Fig. 44. In reference to Fig. 43 it should be stated that the preparation was made from material which had been lying damp in the laboratory for some days, whereas

most of the other preparations have been obtained from material fixed directly upon arrival at Cambridge. There is no reason, however, for thinking that this preparation represents an abnormality.

From an examination of these figures there would appear to be no reduction in number of chromosomes at this stage such as there is in the brachymeiotic phases described by Miss Fraser in *Humaria rutilans* and by Miss Fraser and Miss Welsford in *Otidea aurantia*, &c.

The number of chromosomes appears to be usually four after the contraction of the first division. This number certainly seems to fit the phenomena observed better than any other number or numbers. On the other hand, there seems to be some evidence—not very definite as yet it is true, that the chromosome number in *Gnomonia* is not strictly constant. Still the preparations necessitate the consideration of this view. Furthermore, the shape of the chromosomes would appear to be variable in this fungus. Without considering the chromosomes of the first division, one has seen that they are sometimes long and thread-like, and at other times short and thick. In addition the extraordinary power of increase in size possessed by the nuclei is worthy of mention.

After the reconstruction of the eight nuclei each puts out a beak-like process similar to that described by Harper in *Lachnea seutellata* and other Ascomycetes. The astray rays become more conspicuous and bend down towards the other end of the nucleus. The exact mode of delimitation of the spores, however, could not be made out. A certain amount of epiplasm is left as in other Ascomycetes. After the spore membrane has been laid down, the nucleus undergoes a period of rest subsequent to which another nuclear division is effected. This division usually takes place towards the base of the spore. Fig. 45 shows this division. The number of chromosomes would appear to be four at this stage also. One of the daughter nuclei thus formed passes to the extremity of the spore which is towards the base of the ascus, and a septum is laid down between the two nuclei cutting the spores into cells of unequal size (Fig. 46). The nucleus of this tail-cell gradually disorganizes. It is interesting to note in this connexion that in all experiments made upon the germination of the ascospores it was only the larger cells of the latter which put out germ-tubes. The larger cell of each ascospore contains two conspicuous oil drops at maturity.

In considering the nuclear phenomena in the ascus as a whole, one is led to the conclusion that there is a single process of reduction which is effected during the first division. There is no evidence yet that a second reduction is effected at a later stage. Nor is there any obvious pairing of chromosomes which might obscure such a process.

On the evidence yet available one cannot decide whether the first division is heterotypic or brachymeiotic in nature, though certain phenomena already mentioned would appear to favour the former interpretation.

The single process of reduction in the ascus is in harmony with the single nuclear fusion which appears to occur in this form.

#### GENERAL CONSIDERATIONS.

From the observations recorded earlier in the paper, one is led to conclude that the 'coils' develop into perithecia without undergoing any process of fertilization by means of the spermatia. It is possible, of course, that this process has been missed. Even if such a fertilization did occur, it would be extremely difficult to demonstrate, both on account of the collective grouping of the trichogynes and the intricacy of the coiled structures. In view, however, of the care which has been taken in order to obtain the earliest stages of development of the sexual organs, I do not think this process can have been missed. As has been said before, the mere adhesion of spermatia to the trichogynes is no evidence that fertilization occurs, for under the conditions of growth it would be surprising if most of the trichogynes did not have spermatia attached to them. Besides, it is difficult to imagine how the spermatia can pass down to the ascogonial cells in view of the fact that no pores appear to exist in the cell-walls between the trichogynes and the cells beneath.

The most probable view is that the spermatia were originally the agents of fertilization. Their cytological characters and other evidence in regard to their behaviour point to this conclusion. The spermogonium of *Gnomonia* is to be looked upon as a sorus of functionless male organs similar to that which is so often found in the Uredineae. Such a collection of male organs may perhaps be compared to the male conceptacles of certain Florideae (e. g. *Corallina*).

The trichogynes are looked upon as having originally functioned as receptive organs for the ascogonial cells, though my observations indicate that they do not thus function at present. Other functions have been attributed to the trichogynes which are present in certain Lichens, e. g. *Collema*, *Physcia*. Thus Van Tieghem (30) considered that the trichogynes of these Lichens were really respiratory organs. It is difficult to conceive that this was the original function of such trichogynes because there are many Lichens and innumerable Rusts, and other fungi parasitic on leaves, which at present exhibit no trace of such structures. Lindau (26), on the other hand, assumes that the trichogynes in the case of the Lichens serve a mechanical function in preparing apertures for the ascocarps which arise later. This explanation will not account for the occurrence of trichogynes in *Gnomonia* where the perithecia are not developed until after the 'coil' has undergone a prolonged resting stage. Though I consider the original function of the trichogynes was that of receptive organs, it is possible that they serve at present some secondary purpose. Thus, the production of trichogynes which have no connexion with 'coils' may be explained by their



functioning in a secondary manner as respiratory organs. One naturally supposes that as the leaf of the host becomes first yellow, and then brown, the assimilatory process dwindles, until no oxygen at all is liberated, while the evolution of carbon dioxide continues for a time. Such phenomena may very well necessitate the growth of certain hyphae through the stomata to the outside atmosphere. Vuillemin (31) records the interesting fact that in the genus *Hypostomum*, parasitic on the leaves of Conifers, structures considered by him to be analogous to trichogynes are produced, although no spermogonia are present. This also points to the possibility that the trichogynes may now perform a function which was not their original one.

This investigation leads one to look upon the ascocarp of *Gnomonia* as a structure formed in relation to one or more ascogonia in close connexion with one another. This association of two or more ascogonia before the development of the perithecium is paralleled in the Discomycetes by *Pyronema confluens* (23) in which several pairs of sexual organs co-operate to give rise to a single apothecium. Stahl (28) states also that in the Lichen genus *Physma*, several archicarps go to produce one apothecium. Many other Pyrenomycetes show the presence of definite archicarps before the formation of perithecia, but in these the female organs normally arise separately one from the other. Fisch (15) states, however, that in *Polystigma rubrum*, two ascogonia with their trichogynes may arise in close association. The ascogonia of *Gnomonia erythrostoma* seem to be more closely allied to those of *Polystigma rubrum* than to those of any other Ascomycete as yet investigated. Coiled ascogonia have been described also by Kihlmann (25) as occurring in *Melanospora parasitica*, by Miss Dawson (13) in *Poronia punctata*, and by Fisch (15) in *Xylaria polymorpha*. In *Poronia*, however, the trichogyne portion is not always present, and in *Xylaria* it is constantly absent. Fisch states, in regard to the latter, that the asci arise independently of the ascogonia as they would appear to do also in *Gnomonia*. He found that in *Claviceps purpurea* there was no trace of an ascogonium, so that in the series mentioned there is an evident reduction in the formation of the female organs. When one considers the Lichen genera *Collema* and *Physcia*, one sees some resemblance between the general form of their ascogonia and those of *Polystigma* and *Gnomonia*. These four genera are all characterized too by the production of spermogonia. The cells of the ascogonia of these Lichen genera are uninucleate as described by Baur (a) and Darbishire (12) respectively, a condition in marked contrast to the multinucleate nature of the ascogonial cells of *Gnomonia*. Baur states that in *Collema crispum* the successive cells of the trichogyne are clearly connected by strands of protoplasm at the time which he considers to be that of fertilization. He infers that the pores through which these strands pass enable the spermatial nucleus to pass down to one of the ascogonial nuclei below. In *Gnomonia* no such phenomenon has been observed, and the fact that the ascogonia at a later stage

can no longer be traced is against such a possibility. There is circumstantial evidence in the case of *Collema crispum* in favour of fertilization by means of spermatia, because Baur (a) found that of the two kinds of thallus present in this species, that which bore both spermogonia and archicarps gave rise to apothecia, whereas the thallus which did not possess spermogonia only very rarely developed the ascus fructifications. It would seem, however, that until a fusion of nuclei has been observed it is not safe to conclude that fertilization has taken place by an external means. When the pores are produced between the cells of the ascogonium of *Collema*, that structure becomes a coenogamete, and development may proceed by the fusion of nuclei in pairs as occurs in *Humaria granulata*. Another possibility is, that only one nuclear fusion, that immediately prior to the development of the ascus, occurs.

Claussen's recent work on *Pyronema confluens* (6) which, however, requires confirmation, must be taken into consideration. He considers that in this species the male and female nuclei do not actually fuse in the ascogonium but merely remain closely associated together, while in the ascogenous hyphae these paired nuclei divide in a conjugate manner, nuclear fusion only occurring just before the formation of the ascus. Blackman and Miss Fraser have, however, both pointed out that even if Claussen's work holds good for *Pyronema* a mistake in the interpretation of a first nuclear fusion in the ascogonium can scarcely have been made in the examination of the uninucleate sexual organs of the Mildews, *Sphaerotheca* (22) and *Phyllactinia* (24). Besides, so many observers have independently recorded the fusion of nuclei in pairs in multinucleate ascogonial forms, e. g. *Humaria granulata* (3), *Lachnea stercorea* (18), *Aspergillus repens* (9), *Ascophanus carneus* (7), &c., that stronger evidence than has yet been urged by Claussen (6) will be needed in order to disperse the usual view of the presence of two nuclear fusions in many forms. The process of brachymeiosis following that of meiosis discovered by Miss Fraser (19) also supports the occurrence of two nuclear fusions in the life-history of those Ascomycetes in which this process of reduction has been observed.

As has already been stated, there is no evidence that the ascogonia of *Gnomonia* are functional in that they give rise to ascogenous hyphae, the latter indeed appearing to arise by the subsequent differentiation of ordinary cells. It will be remembered that Fisch has described the degeneration of ascogonia in *Xylaria polymorpha*, so that these two Pyrenomycetes would appear to be similar in this respect. In *Claviceps* and *Pleospora*, according to Fisch and Bauke<sup>1</sup> respectively, the formation of female organs is entirely in abeyance.

The absence of any indications of nuclear fusion in *Gnomonia erythrostroma* until ascus development occurs induces one to entertain the view that

<sup>1</sup> Bauke : Zur Entwicklungsgeschichte der Ascomyceten. Bot. Zeit., 1877.



in this species only a single nuclear fusion takes place.<sup>1</sup> It would seem that the sexual fusion which doubtless originally occurred in the ascogonia of *Gnomonia* has been replaced by a fusion immediately before the formation of the asci. There is a slight indication that a period in which the nuclei may be conjugately arranged precedes the actual fusion. If this is really the case, the commencement of the sexual process must be looked upon as beginning with the association of nuclei in pairs, as Blackman (1) has pointed out for the Uredineae. It would be premature at present to discuss the possible means by which the transference of the sexual process might have been brought about in the course of phylogeny. Only a single process of reduction in the ascus of *Gnomonia* has been observed. This harmonizes with the single nuclear fusion which has alone been seen.

The nuclear phenomena described for *Gnomonia* seem to indicate a closer parallelism between *Gnomonia* and the Uredineae than has yet been determined for other Ascomycetes. The origin of the binucleate phase in the Uredines has been shown to take place in more than one way, and in some forms possessing deeply-seated aecidia it is possible, from the recent work of Olive,<sup>2</sup> that the binucleate phase originates in multinucleate cells at the base of the aecidium. If this prove to be the case, the cytological behaviour would appear to be somewhat similar to that of *Gnomonia*. Such a similarity between the nuclear phenomena of some of the Uredineae and a member of the Ascomycetes is of great interest, but in the present state of our knowledge it can hardly be made the basis for phylogenetic speculations as to the relationship of these two groups. On the other hand, the closest relationships of the Uredineae are certainly with the other Basidiomycetes. It is recognized that expressions of opinion on the phylogeny of the fungi must be highly speculative from the very nature of these organisms. Therefore one hesitates to state the possibility that the Uredineae—true Basidiomycetes as they undoubtedly now are—may have been connected with the Ascomycetes in the course of phylogeny.

#### SUMMARY.

1. The vegetative mycelium consists of multinucleate cells. It is intercellular. Haustoria are not developed.

2. The spermogonia are similar in structure to those of the Uredineae. The spermatia are long and thread-like, and possess the cytological characters of male cells. They are considered to be now functionless, there being no evidence that fertilization is effected by their agency as Frank supposed.

3. The trichogynes arise in tufts of 2-5, and do not invariably arise in

<sup>1</sup> It is possible that an earlier fusion has been missed, though in view of the careful search made for such a process this is unlikely.

<sup>2</sup> Olive, E. W.: Sexual Cell Fusions and Vegetative Nuclear Divisions in the Rusts. *Ann. Bot.*, xxii, 1908.

association with 'coils'. It is suggested that the trichogynes, though originally the receptive organs, now perform a different function, possibly a respiratory one.

4. The 'coils' are the first beginnings of perithecial development. In the centre of each 'coil' one or more slightly differentiated hyphae are found. The latter are considered to be of the nature of ascogonia. No clear connexion can be traced between the trichogynes and the ascogonia.

5. The stages in the development of the perithecium from the 'coil' are described. During these processes it is no longer possible to trace the ascogonia. The ascogenous cells appear to arise by differentiation from ordinary cells.

6. The only nuclear fusion that has been observed takes place in the young ascus.

7. The nuclear divisions in the ascus are described. Only a single process of reduction has been seen. This occurs in the first division. It is not possible to decide whether this division is heterotype or brachymeiotic, though the analogies seem to be with the former rather than the latter.

8. Thus *Gnomonia erythrostoma* would appear to be an Ascomycete in which only a single nuclear fusion and a single reduction occur normally in the life-cycle.

BOTANY SCHOOL, CAMBRIDGE,  
June, 1910.

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#### LIST OF PAPERS.

- a. BAUR, E. ('98): Zur Frage nach der Sexualität der Collemaceen. Ber. d. Deut. Bot. Gesell., xvi.
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2. BLACKMAN, V. H., and FRASER, H. C. I. ('05): Fertilisation in *Sphaerotheca*. Ann. Bot., xviii.
3. ————— ('06): On the Sexuality and Development of the Ascocarp in *Humaria granulata*. Proc. Roy. Soc., London, B. lxxvii.
4. BREFELD, O. ('72 et seq.): Untersuchungen aus dem Gesamtgebiete der Mykologie. Leipzig.
5. CHRISTMAN, A. H. ('05): Sexual Reproduction in the Rusts. Bot. Gaz., xxxix.
6. CLAUSSEN, P. ('07): Zur Kenntniss der Kernverhältnisse von *Pyronema confluens*. Ber. d. Deut. Bot. Gesell., xxv.
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11. ————— ('04): Recherches sur le développement du Périthèce chez les Ascomycètes. Le Botaniste, ix.

12. DARBISHIRE, O. V. ('99): Ueber die Apothecienentwicklung der Flechte *Physcia pulverulenta*. Jahr. f. wiss. Bot., xxxiv.
13. DAWSON, M. ('00): On the Biology of *Poronia punctata*. Ann. Bot., xiv.
14. FAULL, J. H. ('05): Development of the Ascus and Spore Formation in Ascomycetes. Proc. Boston Soc. Nat. Hist., xxxii.
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16. FRANK, A. B. ('83): Ueber einige neue und weniger bekannte Pflanzenkrankheiten. Ber. d. Deut. Bot. Gesell., i.
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18. FRASER, H. C. I. ('07): On the Sexuality and Development of the Ascocarp in *Lachnea stercorea*. Ann. Bot., xxi.
19. ————— ('08): Contributions to the Cytology of *Humaria rutilans*. Ann. Bot., xxii.
20. FRASER, H. C. I., and CHAMBERS, H. S. ('07): The Morphology of *Aspergillus herbariorum*. Ann. Myc., v.
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21. HARPER, R. A. ('95): Zur Kenntniss der Kerntheilung und Sporenbildung im Ascus. Ber. d. Deut. Bot. Gesell., xiii.
22. ————— ('95): Entwicklung des Peritheciums bei *Sphaerotheca Castagnei*. Ber. d. Deut. Bot. Gesell., xiii.
23. ————— ('00): Sexual Reproduction in *Pyronema confluens*. Ann. Bot., xiv.
24. ————— ('05): Sexual Reproduction and the Organisation of the Nucleus in certain Mildews. Publ. Carnegie Inst., Washington, No. 37.
25. KIHLMANN, O. ('85): Zur Entwicklungsgeschichte der Ascomyceten. Acta Soc. Sc. Fennicae, xiv.
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29. STOPPEL, R. ('07): *Eremascus fertilis*. Flora, xcvi.
30. VAN TIEGHEM, Ph. ('84): Traité de Botanique.
31. VUILLEMIN, P.: Les bases actuelles de la systématique en mycologie. Progressus Rei Botanicae, 1907, p. 46.
32. WELSFORD, E. J. ('07): Fertilisation in *Ascobolus furfuraceus*. New Phyt., vi.

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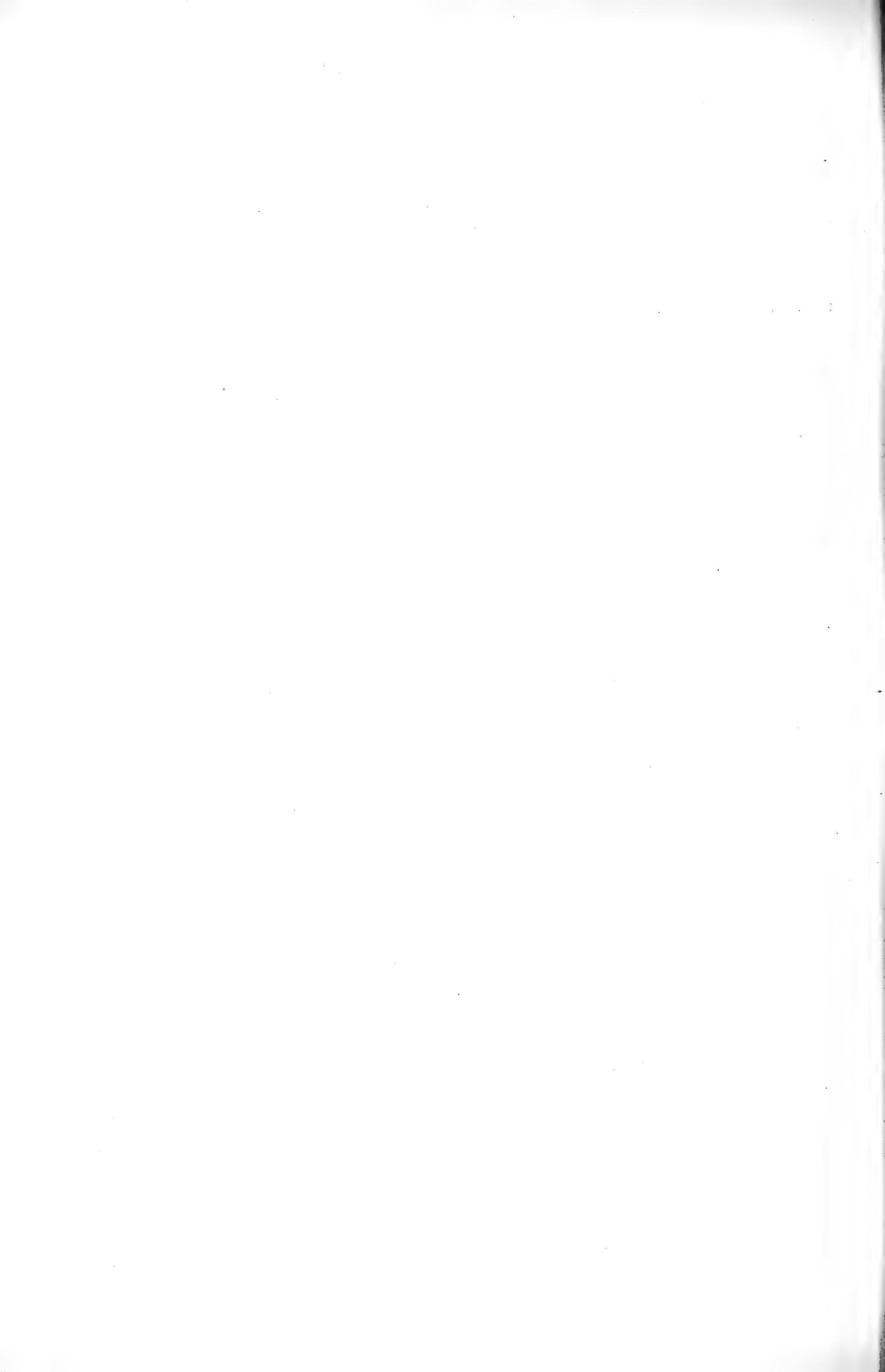
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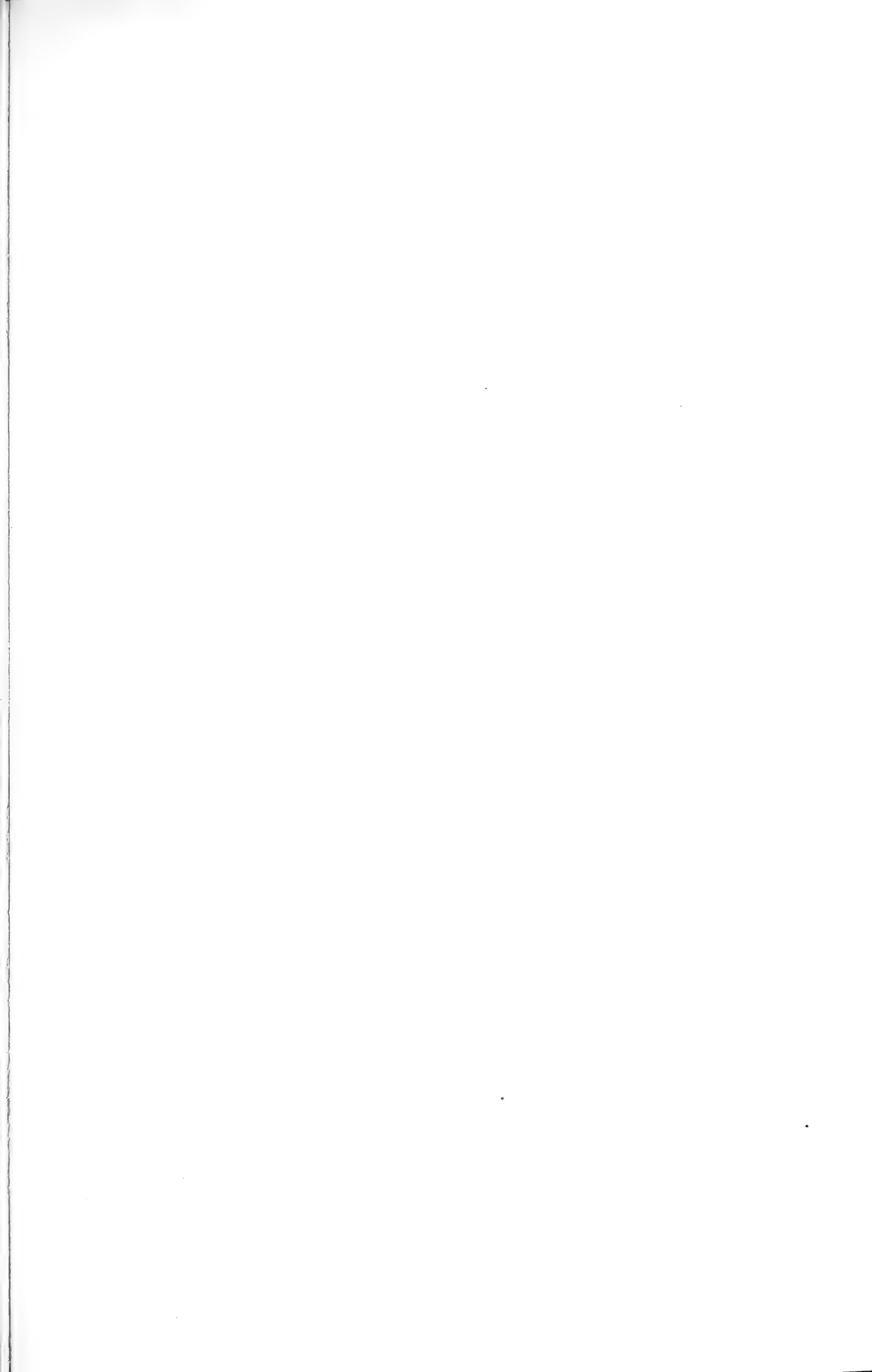
Illustrating Mr. Brooks's paper on *Gnomonia*.

All the figures have been drawn with the aid of the camera lucida.

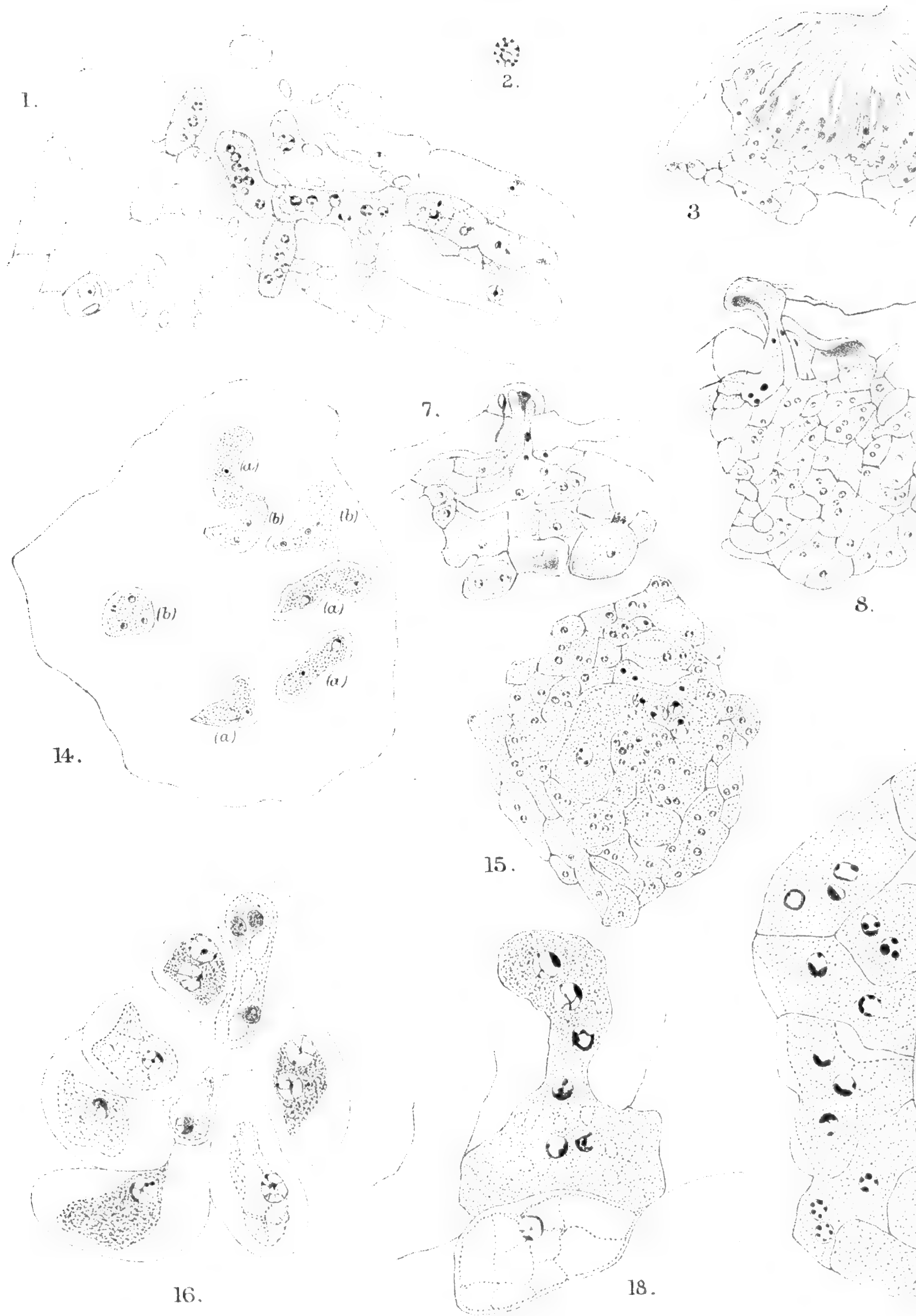
- Fig. 1. Mycelium of *Gnomonia erythrostroma*. × 680.
- Fig. 2. Single vegetative nucleus. × 2,500.
- Fig. 3. General view of spermogonium. × 380.
- Fig. 4. Part of spermogonium. × 1,300.
- Fig. 5. Fresh spermatia. × 400.
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- Fig. 7. Tuft of trichogynes. × 680.
- Fig. 8. Tuft of trichogynes with 'coil' below. × 680.
- Fig. 9. 'Coil' with ascogonium. × 680.
- FIG. 10. Central portion of 'coil' showing ascogonium. × 680.

- Fig. 11. Central portion of 'coil' showing ascogonium.  $\times 2,500$ .  
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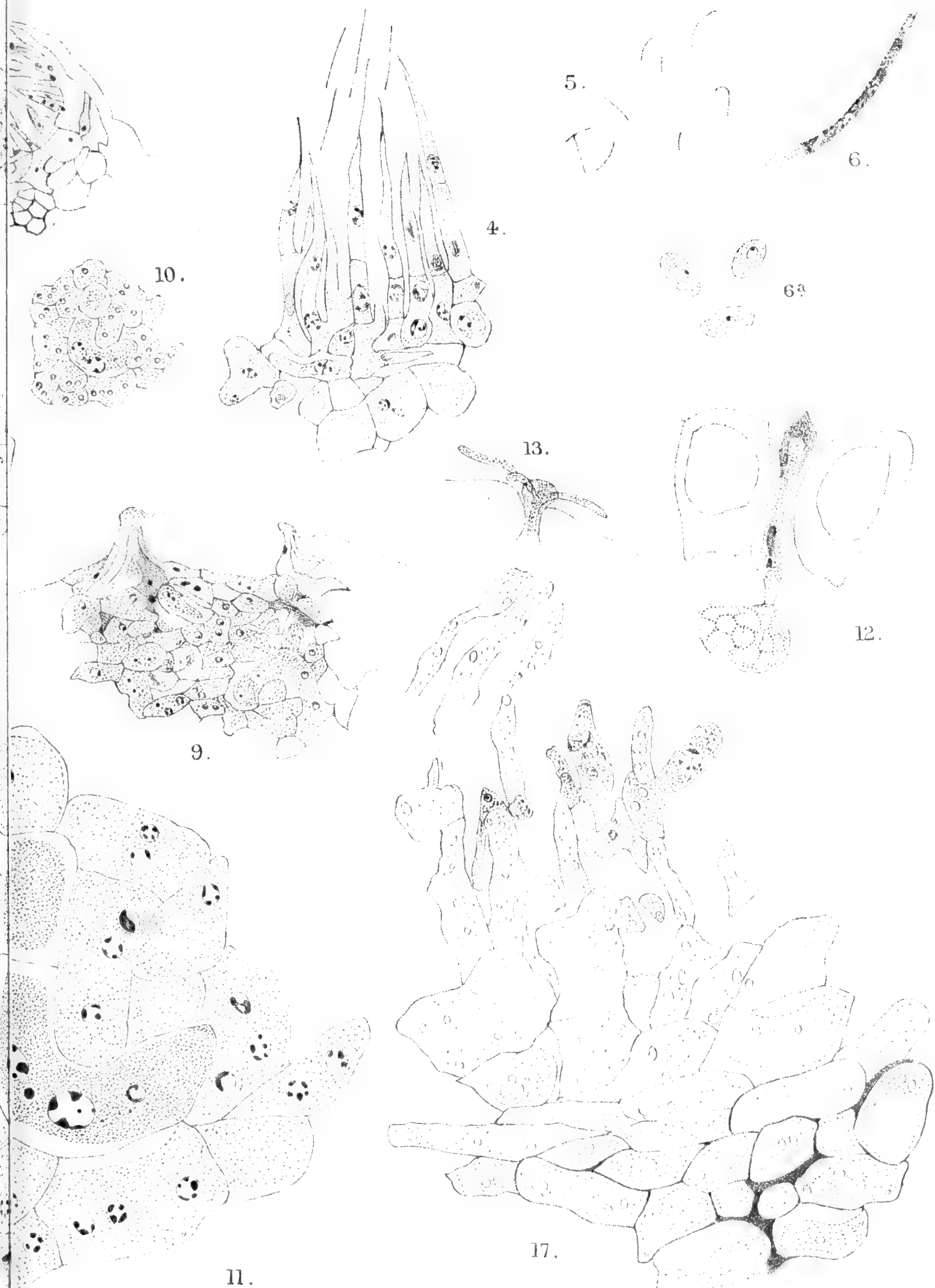






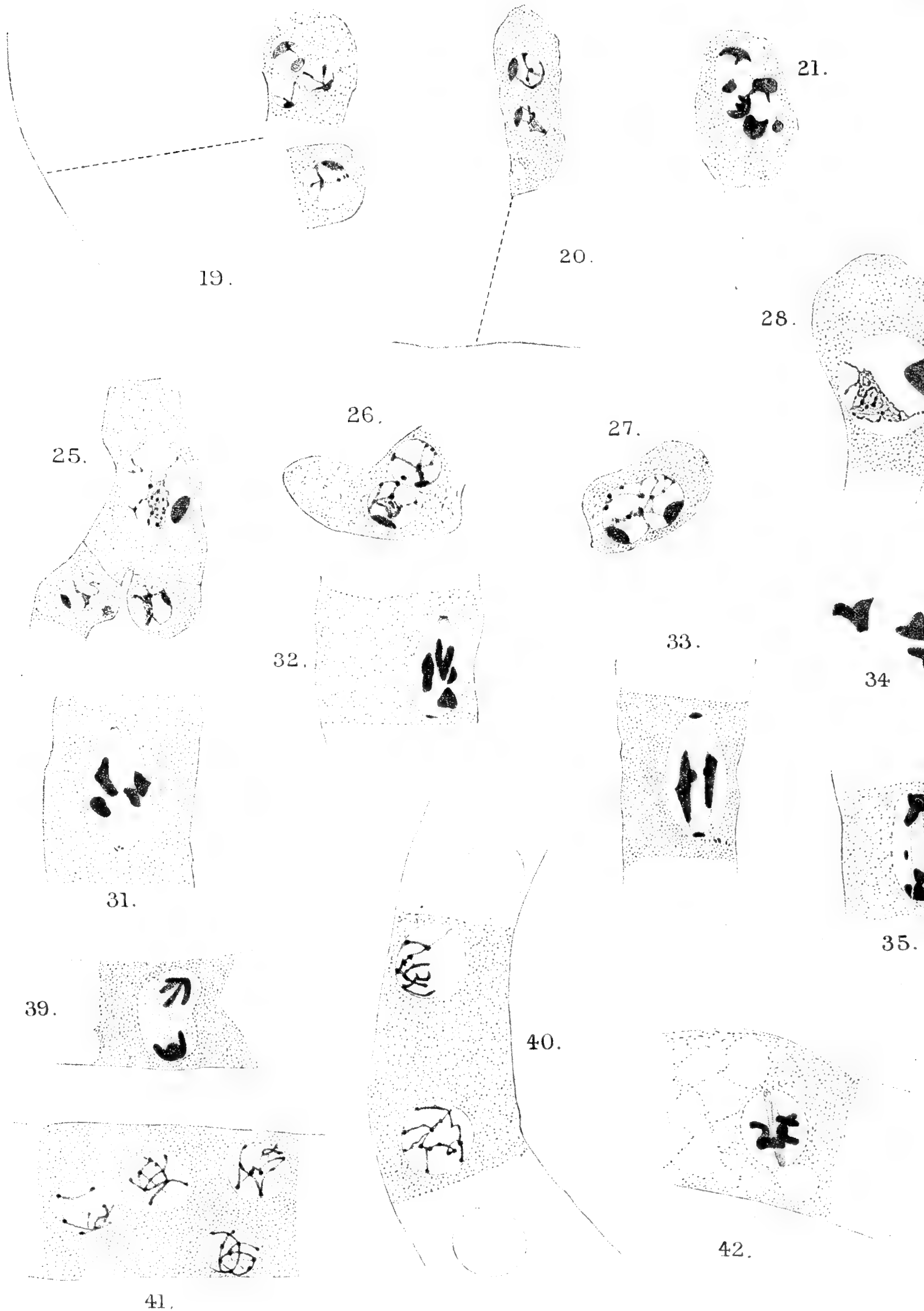


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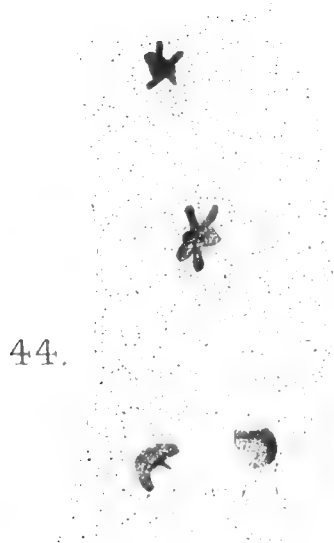
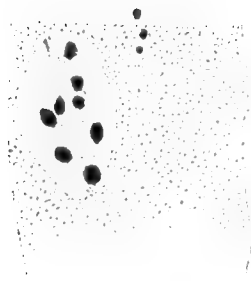
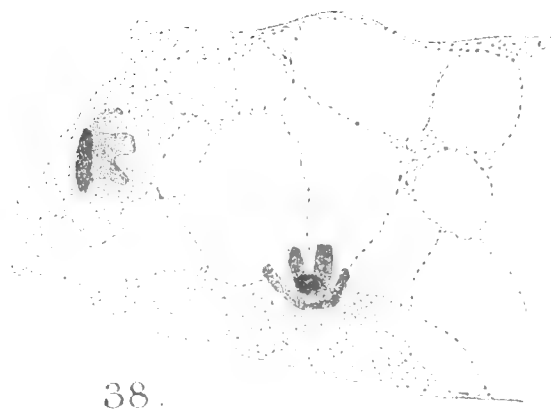
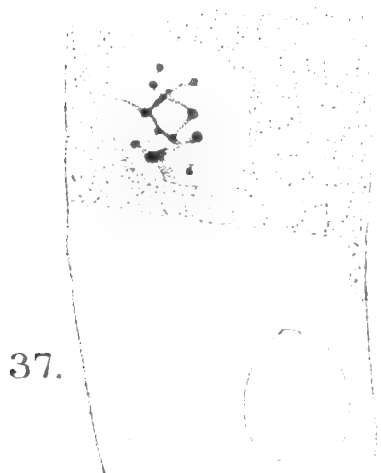
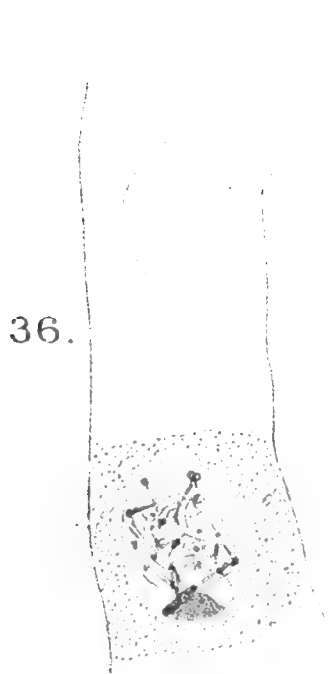
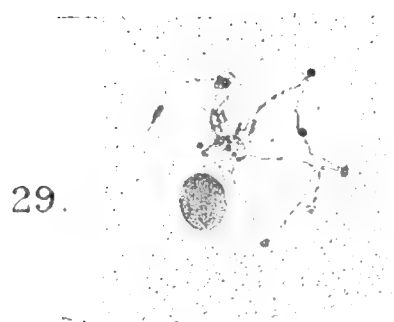
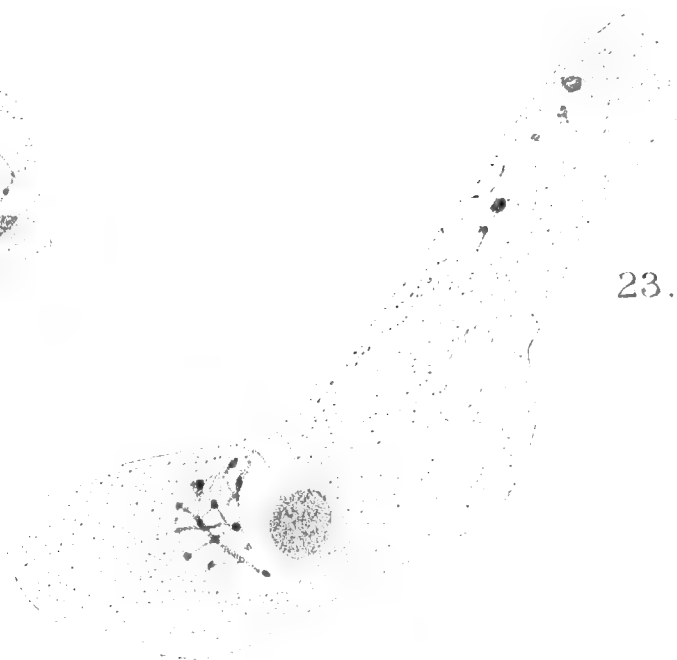
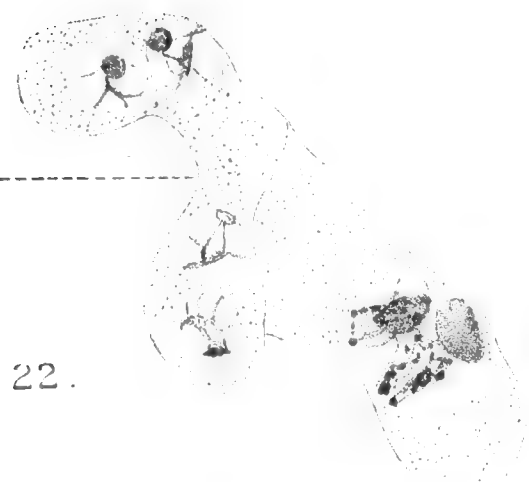




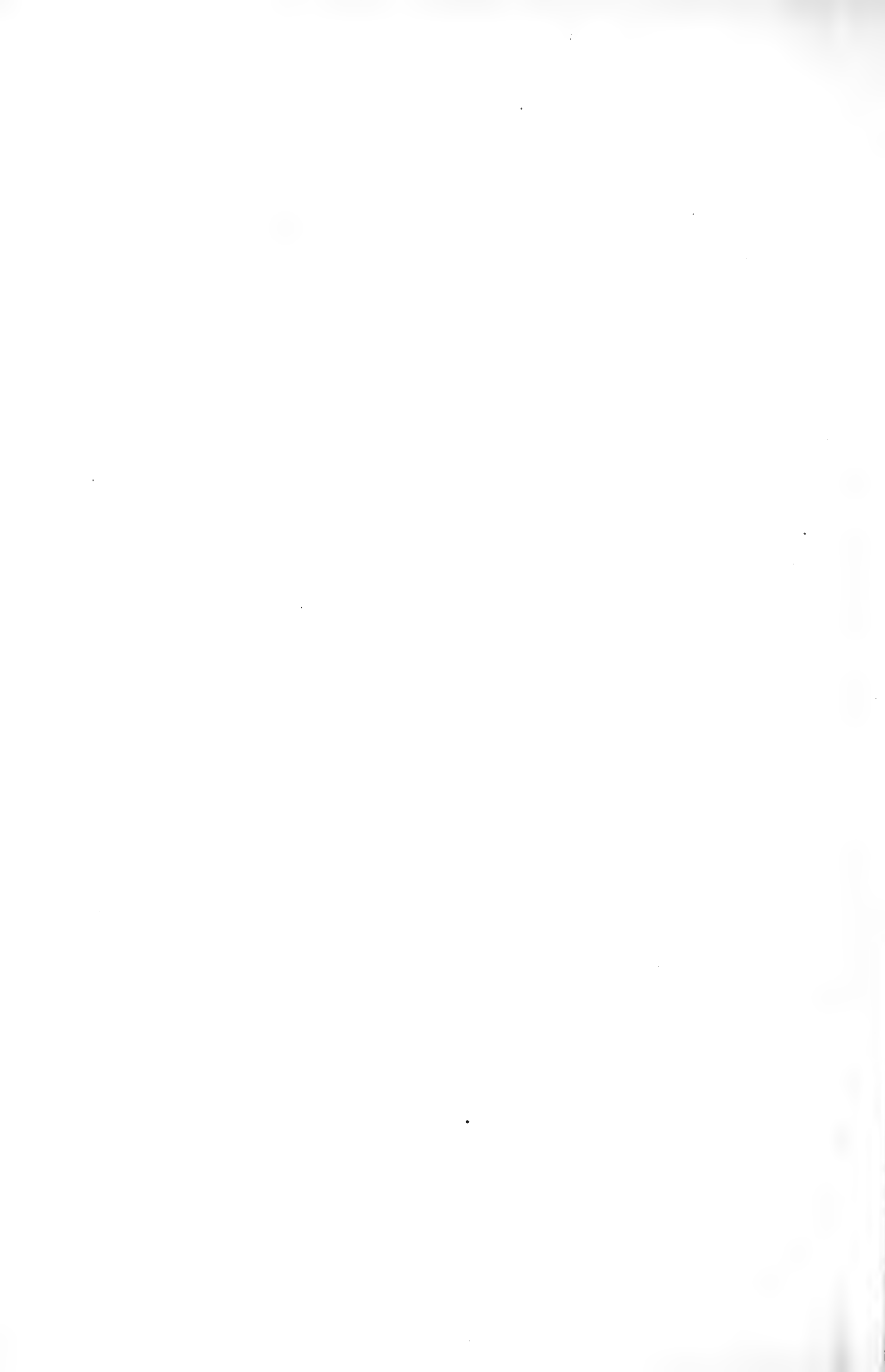




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## NOTES.

### THE DEVELOPMENT OF THE SEED-COAT OF *CARICA PAPAYA*.<sup>1</sup>—

The seed-coat of many species of *Carica* shows a complicated structure, the highest degree of differentiation being reached in the seeds of *C. microcarpa*, *C. hastaefolia*, and *C. Papaya* (the Paupauw). Klebs<sup>2</sup> has described the constitution of the mature seed-coat in the two former species, but has not followed up its development from the unfertilized ovule—a development which in the case of *C. Papaya* shows points of interest. Material of the fruit and seeds of this species, at various stages before and after fertilization, was obtained in Angola during the recent Percy Sladen Memorial Expedition by Dr. H. H. W. Pearson, who handed it over to me for examination.

The fruit of *C. Papaya* is a one-celled berry, with seeds lining the wall. These seeds, when mature, are oval in form and about 8 mm. long. Their coat consists of two separable layers—a hard reddish-brown endotesta, covered by a soft white sarcotesta.<sup>3</sup> The endotesta rises into irregular ridge-like outgrowths, which run down the length of the seed (Fig. 1, A) and appear in transverse section as pyramidal projections (Fig. 1, B). The sarcotesta fills up the hollows between these ridges, so that the surface of the seed, while still enclosed within the fruit, is smooth and shiny; but when it is set free, the sarcotesta quickly loses its watery contents and shrivels down into the hollows.

In the young ovule, the two integuments develop as usual, and at the time of pollination consist each of four or five layers of ordinary parenchyma (Fig. 2). Immediately after pollination, these layers begin to show the following series of changes:—

#### A. *The inner integument.*

The cells of the inner epidermis (*a*) are the only ones in this integument which retain their original form. As the seed matures, they enlarge, and a cuticular layer is formed on the outer side where they are in contact with the nucellus. At the same time, tannin is deposited in their cytoplasm, so that the contents of each cell become converted into a tanniferous block (Fig. 4). All the other cells in the integument begin to show sliding growth immediately after pollination (Fig. 3), the cells of the outer epidermis (*b*) elongating in a direction parallel to the long axis of the seed, while those of the underlying layers lengthen tangentially. Their walls become thickened

<sup>1</sup> Percy Sladen Memorial Expedition in S.W. Africa, 1908–1909; Report No. 3.

<sup>2</sup> Klebs: Beiträge zur Morphologie und Biologie der Keimung.

<sup>3</sup> This sarcotesta was formerly described as an arillus (Baillon: Natural History of Plants, iv, 293, London, 1880).

and deeply pitted, and they are gradually transformed into characteristic sclerenchymatous fibres (Fig. 4). The epidermal fibres are elliptical in transverse section; their lumina are larger than in the underlying layers, and their walls much thicker.

B. *The outer integument.*

Immediately after pollination, this integument begins to increase in thickness, until it attains to the relatively considerable size shown in Figs. 1 and 4. This increase is due to the appearance of bands of meristem, running down the integument

FIGS. 1-4. Sections through the seed and ovule of *C. papaya*, to show the structure and development of the seed-coat. Lettering in each figure :

Inner Integument	{	<i>a</i> = inner epidermis	}	= endotesta.
		<i>b</i> = outer "		
Outer Integument	{	<i>c</i> = inner "	}	= sarcotesta.
		<i>d</i> = outer "		
		<i>e</i> = ridges of the endotesta.		

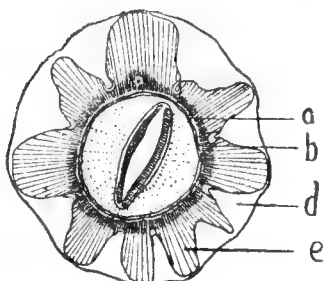
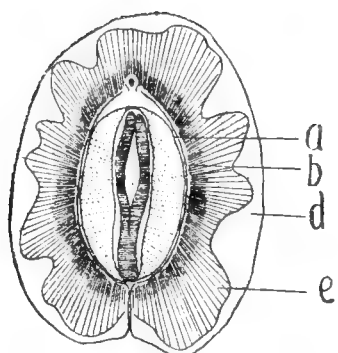


FIG. 1, A and B. Median longitudinal and transverse sections of the mature seed.  $\times 3$ .

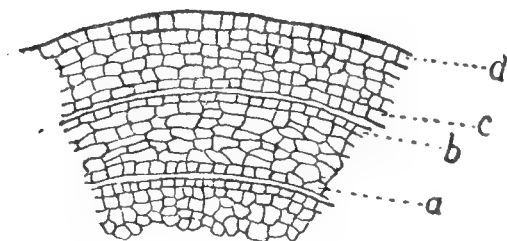


FIG. 2. Transverse section through integuments and nucellus of ovule before pollination.  $\times 120$ .

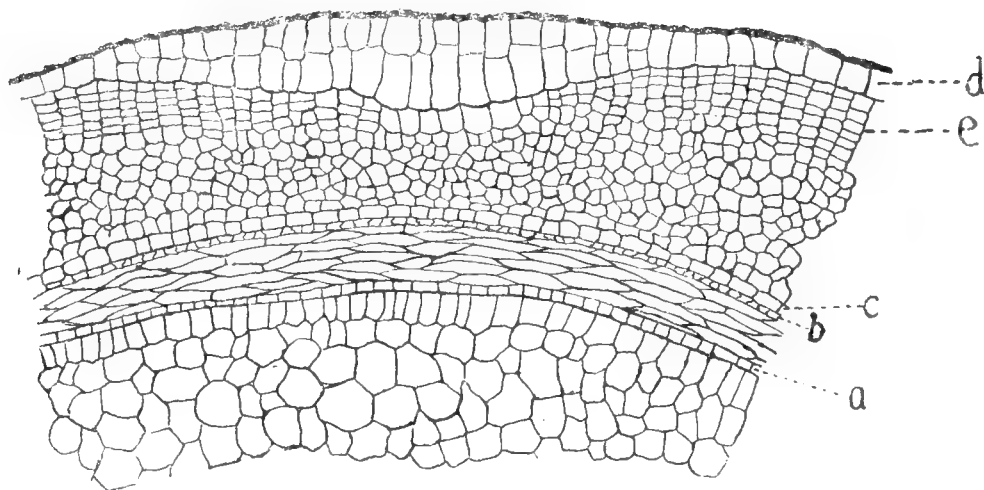


FIG. 3. The same, a short time after pollination, showing a developing ridge of the endotesta on each side of the section.  $\times 120$ .

in the layer immediately underlying the outer epidermis, and marking the future position of the ridges of the endotesta. By the divisions of these meristems, a ridge gradually arises along each band. These ridges, like the meristems, generally run roughly parallel in a longitudinal direction, but are often quite irregular. Two of these developing ridges are shown in transverse section in Fig. 3, and it will be noted that at the base of the depression between them, six or seven adjacent cells of the hypodermal layer have remained undivided, merely increasing in size. A similar row of about six hypodermal cells is found in transverse section at the base of each depression between the ridges, and these cells never divide, but continue to increase

greatly in size and elongate vertically, so that in the mature seed they form a pavement, six to ten cells broad, running along the bottom of these depressions (Figs. 4 and 1, B).

As these ridges develop, the cells composing them increase greatly in size, while those of the original integument remain small. The mature integument (excluding

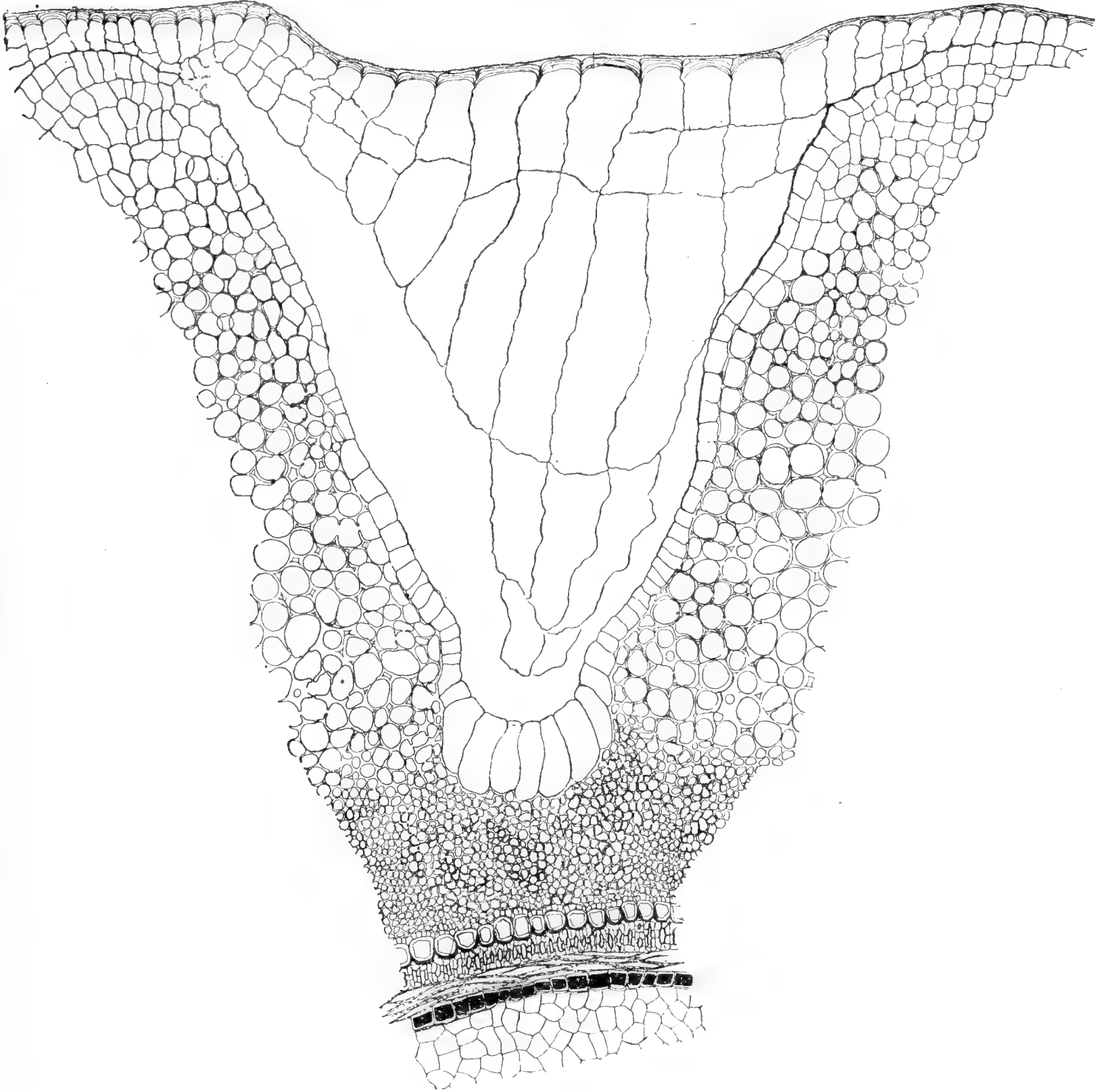


FIG. 4. Transverse section through mature seed-coat, showing further development of the ridges of Fig. 3.  $\times 60$ .

the epidermal layers) thus consists of a region of cells with small lumina passing at the ridges into cells with much larger lumina (Fig. 4). As the seed-coat matures, these cells all begin to lose their contents, and their walls become thicker and rather hard (but not lignified) and take on a yellowish colour. The response of these walls to the ordinary tests for cellulose, both staining and microchemical, grows fainter, until in the mature seed only the outermost still unaltered layers give any reaction with these tests. With a solution of iodine in potassium iodide and with Schulze's

solution their yellow tinge deepens, and after treatment with iodine and sulphuric acid they become brown; this coloration in each case passing in the outermost layers into the ordinary bluish tinge of a cellulose reaction. From these facts, it seems probable that they are composed of a hardened horny variety of cellulose, like the 'reserve cellulose' described by Gardiner and Hill<sup>1</sup> in the endosperm of *Tamus*, and by Grüss<sup>2</sup> in that of *Phoenix* and other plants, which is also unaffected by ordinary cellulose tests, and reacts similarly with iodine in potassium iodide and with iodine and sulphuric acid. As in *Tamus*, moreover, the middle lamella is not evident in most of the walls when mature, though in the outermost layers, and fairly often among the other large cells in the ridges, it can still be recognized as a fine line. Otherwise, the layers of the walls of adjoining cells seem to coalesce to form a homogeneous intercellular matrix.

A similar transformation takes place in the outer wall of the inner epidermis (*c*), which also becomes thickened and loses its cellulose reactions. Otherwise, this epidermal layer remains unaltered, except that the cells increase in size and a crystal of calcium oxalate appears in each.

Meanwhile, the outer epidermis (*d*) is developing into the sarcotesta, which is formed entirely from this layer. Over the ridges it remains single, the cells merely increasing in size, but between the ridges the cells elongate perpendicularly, and divide several times by transverse walls. By continued elongation as the ridges of the endotesta rise up, they fill up the hollows between them, so that the surface of the seed remains smooth. In the latest stages they become separated from the endotesta towards the bottom of the hollows (Fig. 4). As the epidermis develops, its outer wall becomes much thickened and striated.

As the water in the seed-coat evaporates, the sarcotesta shrivels up, and sinks into the hollows between the ridges of the endotesta, whose thick-walled cells retain their shape. When moistened, both layers rapidly absorb water. The cells of the sarcotesta expand to fill up the hollows once more, and the outer epidermal wall swells up considerably, so that the surface of the seed becomes smooth and shiny. The cell-walls of the endotesta also expand slightly, and water replaces the air in their lumina, so that the whole seed-coat becomes a spongy reservoir for the developing embryo to draw upon.

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<sup>1</sup> Gardiner, W., and Hill, A. W.: The Histology of the Endosperm during Germination in *Tamus communis* and *Galium tricorne* (Proc. Camb. Phil. Soc., xi, 445-57, Pl. V, 1902).

<sup>2</sup> Grüss, J.: Studien über Reserve-Cellulose (Bot. Centralbl., lxx, 242-61, 1897).

**ON A SUSPENSOR IN HELMINTHOSTACHYS ZEYLANICA.**—In an account of the prothallus and young plant of *Helminthostachys* published in vol. xvi of the *Annals of Botany* the description of the embryo was omitted owing to lack of material. The few arrested embryos which were found only showed that 'the young embryo soon becomes deeply seated'. Re-examination of these embryos suggested that their likeness to those of *Botrychium obliquum*, as figured by Bower<sup>1</sup> from preparations of H. Lyon, might be due to the development of a suspensor in *Helminthostachys*. The study of serial sections of a number of young plants still attached to the prothallus has fully confirmed this interpretation. A multicellular suspensor, in which two tiers can be distinguished, appears to be constantly present. It closely resembles that of *B. obliquum* as figured by Lyon,<sup>2</sup> but in *Helminthostachys* the plant is attached to the prothallus by a large foot.

A full account of this feature of the young plant will shortly be given. It seems advisable, however, to complete the account of the young plant already published, by recording the fact of the existence of a suspensor without delay. This is especially the case since the discovery of a suspensor in the embryos of *Botrychium obliquum* and *Danaea*<sup>3</sup> has shown that this organ is not a peculiarity of the Lycopodiales among the Vascular Cryptogams, and directed attention to its significance.

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**PRELIMINARY NOTE ON PERIDERM FORMATION IN FILICINEAN PETIOLES.**—The discovery of a typical wound periderm in a Medullosean petiole, an account of which is to appear shortly, has led to the investigation of a number of Filicinean petioles, mostly of the Polypodiaceae, with a view to determining whether a similar response is exhibited by these.

The results have proved extremely interesting, a surprisingly large number showing a well-marked wound cambium, often several cells in depth.

As the experiments are still being continued with a view to obtaining as complete a series as possible, and will take some considerable time, it has been thought advisable to publish this preliminary note in order to briefly indicate the results.

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UNIVERSITY COLLEGE, NOTTINGHAM.

<sup>1</sup> *Land Flora*, p. 472, Fig. 266.

<sup>2</sup> *Bot. Gazette*, xl. 1905, p. 455.

Campbell, *Ann. of Bot.* xxiii, p. 691.





# The Journal of Botany

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# ANNALS OF BOTANY

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# The Function and Fate of the Cystidia of *Coprinus atramentarius*, together with some General Remarks on *Coprinus* Fruit-bodies.

BY

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*Professor of Botany at the University of Manitoba.*

With Plates L and LI.

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### I. INTRODUCTORY REMARKS.

CYSTIDIA are certain specialized cells, usually much larger than the paraphyses and basidia, of peculiar shape, which form part of, and project from, the hymenium of certain species of Hymenomycetes and Gastromycetes.

The form and size of cystidia vary much in different species, but for any one species they are fairly constant. Cystidia are unbranched and as a rule unicellular, but in the two species of the genus *Bonia*<sup>1</sup> they are represented by numerous short multicellular bristles. Cystidia may be cylindrical as in *Coprinus atramentarius*, fusiform as in *Peniophora quercina*,<sup>2</sup> ventricose as in *Inocybe geophylla*,<sup>3</sup> spear-shaped as in *Hymenochaete Cacao*,<sup>4</sup> or club-shaped as in *Polyporus mollis*.<sup>5</sup> The wall of the part of a cystidium which is exposed to the air may remain smooth as in the Coprini, or it may become covered with crystals of calcium oxalate as in species of *Peniophora*,<sup>6</sup> or it may become crowned with mucilage as in *Inocybe geophylla*.<sup>7</sup>

<sup>1</sup> Engler und Prantl, Die nat. Pflanzenfamilien, Teil 1, Abteil. 1\*\*, p. 123.

<sup>2</sup> Ibid., Fig. 68 B, p. 122.

<sup>3</sup> G. Masee, A monograph of the genus *Inocybe*. Annals of Botany, vol. xviii, 1904, p. 477 and Pl. XXXII, Figs. 7, 8, and 9.

<sup>4</sup> Engler und Prantl, l. c., Fig. 68 E, p. 122.

<sup>5</sup> R. Hartig, Die Zersetzungserscheinungen des Holzes, Berlin, 1878, p. 51, also Taf. IX, Fig. 10.

<sup>6</sup> M. C. Cooke, Introduction to the Study of Fungi, 1895, p. 143.

<sup>7</sup> G. Masee, l. c., p. 463 and Pl. XXXII, Figs. 7 and 8.

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The distribution of cystidia among species and genera is very irregular. Thus for instance, in the Thelephoreae, whilst cystidia are present in the genera *Peniophora* and *Hymenochaete*, they are absent in *Corticium*, *Stereum*, *Coniophora*, and *Craterellus*.<sup>1</sup> But in a single genus some species may possess cystidia, and others not. Thus *Psathyra spadiceo-grisea*<sup>2</sup> and *Fomes nigricans*<sup>3</sup> are provided with cystidia, whereas *Psathyra nolitangere*<sup>4</sup> and *Fomes annosus*<sup>5</sup> are without them. Even in closely allied species which have cystidia, the distribution of these structures may differ considerably. Thus in *Coprinus comatus* the cystidia are restricted to the free margins of the gills, whilst in *C. atramentarius* they are not only present in these situations but project in large numbers from the general hymenial surfaces. Cystidia occur in species of the Hymenogastrineae,<sup>6</sup> but, so far as I am aware, they have not been found in any other group of Gastromycetes.

Brefeld<sup>7</sup> occasionally found cystidia bearing sterigmata and spores in *Coprinus stercorarius*, and he also noticed that the cystidia alternated with the paraphyses like the basidia. He drew the conclusion that the cystidia are metamorphosed basidia. In support of this view may be mentioned the fact that the cystidia on the basidia-free gill-margins of *Coprinus comatus* are distributed among the paraphyses in precisely the same manner as the basidia on the general gill-surfaces.<sup>8</sup>

The different types of cystidia are so diverse in size, structure, and distribution that it seems certain that they cannot all have the same function. Cystidia are analogous to the epidermal hairs of the higher plants, and probably like these they perform various functions for which they are fitted by their peculiar structure. So far, however, comprehensive physiological studies of these functions have not been made, so that we are still almost entirely in the dark as to what they are and what part they play in the fruit-body economy.

A few decades ago it was thought that cystidia were male organs. This view was held by Corda, Hoffmann,<sup>9</sup> and Worthington Smith.<sup>10</sup> The last-named writer thought that the cystidia were antheridia which produced spermatozoa. He believed that the spermatozoa found their way to the spores with which they fused, thus bringing about their fertilization.

Earlier writers, Corda and others, stated that the cystidia of fleshy Fungi discharge their contents through their apices in the form of drops, but

<sup>1</sup> Engler und Prantl, l. c., pp. 117 and 118.

<sup>2</sup> O. Brefeld, Untersuchungen, Heft 8, p. 47.

<sup>3</sup> G. Masee, British Fungus-Flora, vol. i, p. 221.

<sup>4</sup> O. Brefeld, l. c., p. 48.

<sup>5</sup> Ibid., p. 161.

<sup>6</sup> Engler und Prantl, l. c., p. 297, also Fig. 150 E.

<sup>7</sup> O. Brefeld, Untersuchungen, Heft 3, p. 54.

<sup>8</sup> A. H. R. Buller, Researches on Fungi, London, 1909, Pl. III, Fig. 13.

<sup>9</sup> H. Hoffmann, Die Pollinarien und Spermarien von *Agaricus*. Bot. Zeit., Bd. xiv, pp. 137-8, 153-63.

<sup>10</sup> W. Smith, Reproduction in *Coprinus radiatus*. Grevillea, vol. iv, 1875-6, pp. 53-63.

De Bary<sup>1</sup> states that both he and Brefeld could never satisfy themselves that this is done spontaneously. However, Masee<sup>2</sup> has upheld the older view. In one of his first papers he stated that cystidia, when mature, contain glycogen, which is emitted through the nipple-like openings at their apices and poured over the surrounding hymenium, where it serves as food. In his recent monograph on *Inocybe*, he<sup>3</sup> states that in this genus the cystidia become crowned with mucilage, which escapes from the interior after the deliquescence of the thin portion of the walls at their apices.

Masee<sup>4</sup> remarks that in *Peniophora* 'the cystidia are colourless, thin-walled, and eventually become rigid, due to a superficial crust of particles of lime. When young the cystidia appear to act as organs of transpiration; very minute drops of water containing the lime salt in solution are liberated by the cystidia, and as the water evaporates, the lime is deposited as a superficial crust. In *Hymenochaete* the cystidia are brown in colour, thick-walled, and not at all encrusted with lime.' From these observations it seems not unlikely that in some cases at least cystidia have excretory functions comparable with those of certain epidermal hairs of flowering plants; but we shall see that such functions cannot possibly be attributed to the cystidia of *Coprinus atramentarius*.

Worthington Smith,<sup>5</sup> twenty-nine years ago, figured the cystidia of *Coprinus atramentarius*, *Gomphidius viscosus*, and *Agaricus radicans* as large flask-like structures with narrow necks, each provided with an operculum. He stated that the opercula drop off when the cystidia are mature, and thus permit the cell-contents to escape. It will become evident from what follows that these observations, in so far as they concern *Coprinus atramentarius*, were in the main erroneous.

The best suggestion for the function of the large cystidia which occur on the gill-surfaces of many Coprini was made by Brefeld.<sup>6</sup> In his masterly account of the life-history of *Coprinus stercorarius*, he recorded the observation that during the development of the gills the cystidia grow towards the neighbouring gills and often press into them; and he adds: 'Man möchte fast glauben, als ob sie dazu dienten, die Lamellen in gemessener Entfernung von einander zu halten, damit sie mit der Streckung, während welcher die Sporenbildung vor sich geht, sich nicht stören und gegenseitig bedrücken.' He further remarks: 'Der Gedanke, dass die Cystidien seitliche Schutzpfosten sind, deren Gestalt sie nachahmen, könnte vielleicht noch darin eine Stütze finden, dass die kürzeren Lamellen an ihrer Spitze keine Cystidien ausbilden wie die grossen, die bis zum Stiel reichen.' From

<sup>1</sup> De Bary, Comparative Morph. and Biol. of Fungi, 1887, p. 304.

<sup>2</sup> G. Masee, Journ. Roy. Micr. Soc., 1887, p. 205.

<sup>3</sup> G. Masee, A Monograph of the genus *Inocybe*. Ann. of Bot., vol. xviii, 1904, p. 462.

<sup>4</sup> G. Masee, A Textbook of Fungi, London, 1906, p. 350.

<sup>5</sup> W. Smith, Grevillea, vol. x, 1881, p. 304; also Gardeners' Chronicle, 1881, p. 367.

<sup>6</sup> O. Brefeld, Untersuchungen, Heft 3, 1887, pp. 57 and 58.

these quotations it is evident that Brefeld regarded his suggestion as tentative, and that he did not feel justified in laying much emphasis upon it. Notwithstanding the fullness of his illustrations, he did not show cystidia stretching between the gills; nor did he say anything about their ultimate fate.

In the present paper I hope to prove that the cystidia of *Coprinus atramentarius* do actually serve the purpose suggested by Brefeld. I shall further endeavour to explain why the gills should be kept a certain distance apart, and finally, how the cystidia are got rid of, when by their presence they would constitute a hindrance to the escape of the spores from between the gills. A study of the comparative anatomy of *C. atramentarius* and *C. comatus* will enable us to understand why cystidia should be present on the general gill-surfaces of the former species and absent from those situations in the latter.

In a recent publication I<sup>1</sup> have given a somewhat detailed account of the beautiful mechanism of the fruit-body of *Coprinus comatus*. It was shown that the spores ripen on the gills from below upwards, and that there is a zone of spore-discharge that passes from the bottom to the top of each gill in the course of about forty-eight hours. It was further shown that 'deliquescence' is a process of auto-digestion that serves to remove those parts of the gills which have already shed their spores and which, if they continued in existence, would hinder the fall of the remaining spores. I stated that the arrangements for spore-liberation in *Comatus atramentarius* were essentially the same as those in *Coprinus comatus*.<sup>2</sup> Whilst this statement is true, we shall see that the presence of the cystidia on the general surfaces of the gills in the former species and their absence in the latter are associated with some very interesting differences in detail in the respective fruit-body mechanisms. In order to explain the function of the cystidia of *Coprinus atramentarius*, it will be necessary to describe the general structure of the fruit-body and the mode of spore-liberation.

## II. INVESTIGATIONS ON COPRINUS ATRAMENTARIUS.

*Coprinus atramentarius* is one of the largest and commonest species of its genus. It is widely distributed over the earth's surface, and has been found in Europe, North America, Australia, and Kerguelen Island.<sup>3</sup> The genus *Coprinus*, according to Hennings,<sup>4</sup> contains about 175 species. Whilst most of them grow on dung or dunged ground, a few occur on dead stems or wood. *Coprinus atramentarius* belongs to the minority, and,

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, London, 1909, part i, chap. xix. The *Coprinus* Type of Fruit-body, pp. 196-215.

<sup>2</sup> *Ibid.*, p. 208.

<sup>3</sup> G. Masee, *A Revision of the genus Coprinus*. *Ann. of Bot.*, vol. x, 1896, p. 143.

<sup>4</sup> P. Hennings, in Engler u. Prantl, *Die nat. Pflanzenfamilien*, Teil I, Abteil. 1\*\*, p. 205.

so far as my observation has gone, is most frequently found on partly buried stumps and logs of wood. In England I have often noticed the fruit-bodies at the foot of gate-posts and the supports of fence-rails. Probably the mycelium is specialized for the destruction of wood.

The fruit-bodies of *Coprinus atramentarius* are usually clustered, four or more individuals often being united by the bases of their stipes. The pilei are silvery grey, ashen grey, or sometimes brownish in colour.<sup>1</sup> They are usually more or less scaly, frequently plicate or lobed, and at first egg-shaped, although later on during auto-digestion they become expanded (Pl. L, Figs. 1-4). The gills are exceedingly thin, and at the same time very broad; they are somewhat less than 0.2 mm. thick and often 10 or 12 mm. wide. On account of their thinness and breadth they are mechanically remarkably weak, and in this respect differ much from the gills of the Mushroom and of fruit-bodies of the same type. When two gills are torn apart, the cystidia can be seen with the naked eye as pellucid processes projecting in large numbers from the hymenial surfaces.

If a median cross-section be taken through the gills (i. e. in the direction *c-d* in Pl. L, Fig. 1), the cystidia can readily be made out, extending across the interlamellar spaces. They present the appearance shown in Pl. L, Fig. 5. A thin cross-section more highly magnified (Pl. L, Fig. 8) shows that each cystidium consists of a single large cylindrical cell which has a narrowed end inserted in the hymenium from which it has originated, and a rounded apex which has come to be partly embedded in the hymenium of the opposing gill. The connexion of the cystidia with more or less cylindrical subhymenial cells can often be made out (cf. Pl. L, Figs. 8, 9, 10, and 11). There can be no doubt that the apical end of each cystidium becomes fixed into the hymenium against which it has come to press. This is proved by the fact that when the gills are mature, one cannot pull any adjacent two apart without tearing them. The cystidia hold the gills tightly bound together. When one succeeds in forcibly separating parts of two neighbouring gills, one finds that although the cystidia have mostly separated from one of the gills at their apical ends, and have thus remained attached to the other gill by their basal ends (Pl. LI, Fig. 19, *i*), not infrequently the reverse happens; the cystidia which have broken away at their basal ends and have remained attached at their apical ends then have the appearance shown in Pl. LI, Fig. 19, *j*. Worthington Smith<sup>2</sup> doubtless saw cystidia projecting in this manner, for his figure shows one upside down. He evidently thought that the narrowed end of each cystidium was the apical end, and that it projected freely into the interlamellar space. It may well have been this error which suggested to him that a cystidium is really

<sup>1</sup> Cf. G. F. Atkinson, *Studies of American Fungi*. Ithaca, 1901, p. 40. Some excellent photographs of *Coprinus atramentarius* are given in his Figs. 39-42.

<sup>2</sup> W. Smith, *Cystidia in the Mushroom Tribe*. Grevillea, vol. x, 1881, p. 78.



like a flask in function, that the wall closing the end of the neck acts as a stopper which drops out when the cystidium is ripe, and that through the neck the cystidium contents escape. His supposed discoveries that the cystidia produce spermatozoa<sup>1</sup> and fall from the gills with the spores<sup>2</sup> were simply further errors suggested by his imagination, and involved with his fanciful theory that the cystidia are antheridia, and that their spermatozoa fertilize the spores, which he regarded as female gametes. The reason why the base of a cystidium is narrowed is readily understood, when one remembers that it is necessary for the developing cystidium to retain its connexion with the small subhymenial cells. The general shape of a cystidium is very much like that of a prop, such as one sees used for preventing the collapse of the sides of deep trenches made in the ground. The narrowed end at the base and the blunt rounded end at the apex are well suited to prevent the cystidia from being pushed into the hymenium of either gill by lateral pressure.

The cystidia are some of the largest cells found in connexion with fungi; they are 0.12 to 0.17 mm. long, and 0.02 to 0.03 mm. wide. Illustrations showing their variations in shape and size are given in Pl. L, Figs. 9, 10, and 11. When a cystidium has become mature, it contains a very large central vacuole, and its protoplasm, except for a slight accumulation at each end of the cell, is reduced to a thin lining layer (Pl. LI, Fig. 12). Owing to the limited time during which the living material was available, I was unfortunately prevented from investigating the nature of the cell-sap.

The distribution of the cystidia over the gills is fairly uniform (Pl. L, Fig. 6). It was estimated that about 75 to 100 are situated on each square millimetre of gill-surface. The cystidia are sufficiently numerous to make it impossible for the gills to sag laterally, so that they should come to touch one another with their general surfaces.

The gill-edges are not thickened, and in this are unlike those of *Coprinus comatus*. They are provided with cystidia which before the expansion of the pileus abut upon the stipe (Pl. L, Figs. 5 and 6).

The basidia are of the usual *Coprinus* pattern (Pl. L, Figs. 8 and 11). They project considerably beyond the paraphyses, and are inserted in the hymenium by narrowed bases. The spores are oval in shape, and very dark brown in colour. The mode in which the basidia are spaced, and the manner in which the spores are situated on the sterigmata, are shown in Pl. L, Figs. 6 and 11.

The cells composing the young hymenium are fairly uniform in size, and more or less club-shaped. However, as differentiation proceeds, those cells which are to become basidia elongate in the direction of their long

<sup>1</sup> W. Smith, *Reproduction in Coprinus radiatus*. Grevillea, vol. iv, 1875.

<sup>2</sup> W. Smith, *Ibid.*, p. 60. 'The spores naturally fall to the earth, and with them the cystidia, and it is upon the moist earth that fertilization is generally carried out.'

axes, whilst those which are to become paraphyses expand laterally. Here, as in the Coprini generally, the paraphyses have at least two functions: (1) they act as spacial agents, in that by their presence they keep the basidia so far apart that the spores of neighbouring basidia cannot come into contact, and (2) by their gradual expansion they help in bringing about the expansion of the pileus. The second of these functions was first noticed by Brefeld<sup>1</sup> in his studies of *Coprinus stercorarius*. During the movement of the gills of *Coprinus atramentarius* from the vertical to the horizontal position (cf. Pl. L, Figs. 1-4), not a single cell is added to the hymenium, and probably none to the subhymenium and trama. The elongation of the gill-margins which takes place during this movement seems to be entirely due to the expansion of the cells.

The mode of ripening of the spores and their manner of liberation in *Coprinus atramentarius* is practically the same as that which I have described for *Coprinus comatus*. The spores ripen on each gill from below upwards. As they ripen, they gradually turn brown in colour, so that in the mass they look black. Hence it is that the gills turn black at their bases first, and that the blackening progresses upwards on each gill (cf. Pl. L, Figs. 1-4).

When the pileus has become expanded to the extent shown in Pl. L, Fig. 1, the process of spore-discharge begins. The first spores to be discharged are those which are situated in a narrow zone which extends along both sides of the extreme lower margin of each gill (*s* in Pl. L, Fig. 2). The zone of spore-discharge which has thus come into existence then gradually moves upwards on each gill from the bottom to the top. As soon as a narrow zone (somewhat less than 0.25 mm. wide) along the bottom of each gill has become spore-free owing to spore-discharge, the process of auto-digestion begins. The cells composing the gills in the spore-free zones break down, become fluid, and disappear. This process, which is known to mycologists as 'deliquescence', I have called auto-digestion,<sup>2</sup> for there is every reason to suppose by analogy that the gill-tissues are destroyed by enzymes which are liberated from the cell-sap of the dying cells. The zone of auto-digestion which has come into existence in the manner just explained gradually ascends each gill, which thus becomes destroyed from below upwards. The zone of auto-digestion follows hard after the zone of spore-discharge, but never invades it. It simply involves the zone which has become free from spores. After auto-digestion has begun, five zones can be distinguished in succession from above downwards on the surface of each gill within half a millimetre of, and parallel to, its edge: (1) a zone of basidia with ripe spores, (2) a zone of spore-discharge, (3) a zone of spore-free surface, (4) a zone of auto-digestion, and (5) a dark adhesive liquid film

<sup>1</sup> O. Brefeld, Untersuchungen, Heft 3, 1887, pp. 64 and 65.

<sup>2</sup> A. H. R. Buller, Researches on Fungi, p. 200.



at the gill-edge (Pl. L, Fig. 7). These five zones keep their relative distances apart unaltered. They gradually move upwards, so that in the course of about forty-eight hours they involve the whole of each gill (cf. Pl. L, Figs. 1-4).

The discharge of the spores from the basidia in *Coprinus atramentarius* takes place in a manner similar to that which I have described for other Coprini and for the Hymenomycetes generally. The four spores of each basidium are violently propelled more or less perpendicularly outwards from the hymenium into the adjacent interlamellar space. The four spores of each basidium leave the sterigmata one by one, and are not all discharged together. The discharges can easily be watched if one lays a piece of a gill, like that shown in Pl. L, Fig. 7, in a closed compressor cell (where it is protected from undue loss of moisture), and looks down upon it from above. The spores can then be seen leaving the basidia in the zone of spore-discharge. Some of the basidia will be seen to have four spores upon them, some three, some two, some one, and some none at all.

In my previous researches I<sup>1</sup> have shown that the spores of the Mushroom, of *Polyporus squamosus*, and of *Coprinus plicatilis* are shot forward in almost a straight line into the interlamellar spaces to a distance of about 0.10 mm., and that the horizontal motion is very rapidly brought to an end owing to the resistance of the air. In still air, in consequence of this resistance and of the attraction of gravitation, a spore, when nearing the end of its horizontal flight, describes a sharp curve and falls vertically downwards. I have called the peculiar trajectory of a spore between the gills the sporabola.<sup>2</sup> By using methods which have been described in detail elsewhere, I have proved to myself that the spores of *Coprinus atramentarius* have a trajectory similar to that of *Coprinus plicatilis* and other Coprini, and that the horizontal distance of discharge is of the order of 0.05 mm. Unfortunately an exact determination of this distance could not be made owing to the limited time during which living fruit-bodies were at my disposal. On the assumption that 0.05 is the average distance of discharge of spores shot out perpendicularly from the hymenium, I have indicated the sporabolas of a few spores in the zone of spore-discharge in the semi-diagrammatic drawing in Pl. LI, Fig. 19. It is certain that the spores are shot forward to such a distance from the basidia that they fall downwards somewhere near the middle of the interlamellar spaces. I have already pointed out that the advantage of violent spore-discharge lies in the fact that thereby the adhesive spores are prevented from coming into contact with one another or with the gill-surfaces during their fall.

The cystidia are so large and numerous that, if they persisted until the zone of auto-digestion reached them, they would form a serious hindrance to the escape of the spores from between the gills. They would block up

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, pp. 141 and 142.

<sup>2</sup> *Ibid.*, l. c., p. 185.

so large a proportion of the interlamellar spaces that a great many spores would settle upon them, and in that way be prevented from escaping into the outer air. A beautiful arrangement is provided to make this impossible. *The cystidia do not undergo auto-digestion at the same time as the basidia and paraphyses in their immediate vicinity, but a short time previously thereto.* They destroy themselves in succession from below upwards on each gill, and each one disappears a few minutes before the basidia in its neighbourhood come to be involved within the upwardly progressing zone of spore-discharge. The semi-diagrammatic drawing given in Pl. LI, Fig. 19, shows what was made out by means of sections taken transversely to the gills or parallel to their edges. The section through three gills is supposed to be quite vertical. From above downwards one can distinguish the following seven zones: (*a*) a zone of less ripe spores in which the cystidia are fully turgid, and are acting as props to keep the gills apart; (*b*) a zone of riper spores in which the cystidia are disappearing; (*c*) a zone of ripe spores from which the cystidia have already disappeared; (*d*) a zone of spore-discharge where cystidia are absent; (*e*) a spore-free zone; (*f*) a zone of auto-digestion; and finally, (*g*) a liquid film, the products of auto-digestion, at the gill-edge. It is clear that, owing to their early auto-digestion, the cystidia cannot possibly hinder the fall of the spores and their escape from between the gills.

Since the cystidia of *Coprinus atramentarius* are essential constituents of the fruit-body mechanism in that they alone prevent the very thin, very broad, and very flexible gills from coming into contact with one another, it is not surprising that we should find that they are retained between the gills as long as practicable without their becoming hindrances to the fall of the spores. They are removed only just in time to prevent them ever extending between those parts of the gills where spore-discharge is taking place. The cystidia-freed portions of the gills which hang downwards (Pl. LI, Fig. 19, zones *c*, *d*, *e*, *f*, and *g*) are only about 0.25 mm. in depth. They are kept apart by means of the cystidia above them, so that there is no danger of the interlamellar spaces between them becoming reduced in width. As a matter of fact the interlamellar spaces in the regions of spore-discharge appeared to be distinctly broadened out owing to a slight contraction of those parts of the gills undergoing auto-digestion (cf. Fig. 19).

The fate of the cystidia was found out by studying sections, about 1 mm. thick, which were cut transversely through the gills. Some of the sections were cut perpendicularly to the gill-edges and therefore resembled that shown in Pl. L, Fig. 5, whilst others were cut in such a way that one side of each section was made up of the free auto-digesting edges of several gills. In all cases the sections were placed in a compressor cell in order to prevent undue transpiration. Where a section included the free gill-edges, it was turned upside down so that the edges looked upwards. On looking at the sections with the low power of the microscope, it was possible not

only to see the cystidia stretching across the interlamellar spaces but also to observe their disappearance.

About 0.5 mm. above the extreme edge of each gill where the liquid products of auto-digestion are adhering, the cystidia can be seen in the first stages of their disappearance. This is indicated by a slight diminution in their diameters. Nearer to the auto-digesting gill-edge the cystidia are seen to have become very much reduced in diameter (Pl. LI, Fig. 19, zone *b*). Still nearer to the gill-edge they are seen to have become detached from one gill and to have become partly withdrawn to the other gill, usually to the one from which they originated. Sometimes, however, they can be seen to be broken in two either in the middle or at one end. The cystidia which are just above the zone of spore-discharge are reduced to practically nothing, so that it is difficult or impossible to detect any trace of them.

The disappearance of individual cystidia was observed in a considerable number of instances. Stages in the auto-digestion of six cystidia are shown in Pl. LI, Figs. 13 to 18. The time which elapsed between the initial shrinking of a cystidium and its total disappearance was found to be less than half an hour. It took ten minutes for the cystidium represented in Fig. 15 to pass from the fully turgid condition to the final stage shown, and the break in the cystidium represented in Fig. 16 occurred fourteen minutes after the initial thinning was detected.

One may ask: what becomes of the fluid which is liberated from a cystidium during its auto-digestion? It is possible that part of it simply evaporates, but I think it very probable that much of it is absorbed by the subhymenial cell with which the cystidium is connected and by other cells in its immediate vicinity. At first the cystidium only becomes thinner but retains its form. Probably at first, therefore, the cystidium cell-sap is merely transferred to other cells and the cystidium wall settles down over its diminishing fluid contents. If there were no absorption of the kind I have just suggested, whenever, owing to saturation of the air with water vapour, evaporation was brought to a standstill, the cystidium on its dissolution would form a large drop on the hymenial surface which would surely spread over some of the neighbouring basidia and prevent the liberation of their spores. However, in order to decide quite definitely what becomes of the cystidium products, some further investigations will need to be undertaken.

The disappearance of the cystidia from the gills is so well timed that it seems certain that it is a regulated process. Where the stimulus comes from which acts upon the protoplasm of a cystidium and thus indirectly initiates its auto-digestion, is at present uncertain. Possibly, when a basidium is discharging its spores, it sends out messages to all the cystidia within a certain radius commanding their self-destruction.

The existence of the interlamellar spaces between the gills provides (1) a space in which the basidia of opposing gill-surfaces can develop with-

out touching one another, and (2) sufficient room for the violent discharge of the spores in the region of spore-discharge. As I have pointed out elsewhere, the spores of all Hymenomycetes, when moist, are very adhesive and, when brought into contact with one another, stick together. If the spores on basidia of opposing gills were to touch one another during development, it is probable that during the subsequent expansion of the pileus they would pull one another off the sterigmata, with the result that later on they would not be properly liberated. However, whilst the spores are ripening, opposing hymenial surfaces are entirely prevented from coming into contact with one another owing to the fact that they are propped apart by the numerous cystidia.

The interlamellar spaces seem to be somewhat too wide, if we are to suppose that their one function is to provide space for the free development of the basidia and spores (cf. Pl. LI, Fig. 19). However, from my studies of the mode of spore-discharge in the Coprini and in the Hymenomycetes generally, it has become clear that the interlamellar spaces require to be sufficiently broad to permit of the spores being violently discharged into them without any risk of their striking and adhering to the opposing gills towards which they are propelled. The spores of *Coprinus atramentarius* are shot forward from the basidia for an average distance of the order of 0.05 mm. before they begin to fall vertically downwards. Each interlamellar space, in order to permit of successful spore-discharge, must therefore have a minimum width just exceeding 0.05 mm. The actual width of the spaces just exceeds 0.10 mm. It appears probable, therefore, that with the allowance of a slight margin of safety, the width of the interlamellar spaces is reduced to a minimum with due regard to the liberation of the spores. Since the width of the spaces is determined by the length of the cystidia, we may conclude that the length of the cystidia is correlated with the width of the space between the gills required for the violent discharge of the spores in the region of spore-discharge.

From the facts which I have now recorded it seems clear that the cystidia of *Coprinus atramentarius* are fitted for their prop function by their structure, size, position, number, and early development, whilst the time, order, and mode of their destruction are arrangements which prevent their becoming obstacles to the escape of the spores from the fruit-body. Even if we were to search throughout the whole range of the vegetable kingdom, it would be difficult to find any more beautiful and perfect example of cellular specialization and adaptation of structure to function than is here afforded us.

### III. GENERAL REMARKS ON COPRINUS FRUIT-BODIES.

It was pointed out in the introductory remarks that whereas *Coprinus atramentarius* is provided with numerous cystidia on its general gill-



surfaces, *C. comatus* possesses none in these situations. This marked difference between two fairly closely allied species must now be explained. In *Coprinus comatus* cystidia are not required on the general surfaces of the gills because the interlamellar spaces are provided for by a structural arrangement which renders unicellular props unnecessary. The gill-margins in this species are considerably swollen, with the result that they are nearly twice as thick as the gill-plates, which alone are covered with the hymenium.<sup>1</sup> The swollen gill-margins of adjacent gills are in contact with one another before the pileus begins to open out. Owing to the presence of these marginal thickenings, and also owing to a fitting spacial arrangement of the gills where they are attached to the pileus flesh, the gills are kept a sufficient distance apart to provide the requisite interlamellar spaces for the free development of opposing hymenial layers. In *Coprinus atramentarius*, on the other hand, the gill-margins are no thicker than the other parts of the gills (Pl. L, Fig. 5). It is clear that the cystidia of *C. atramentarius* take the place of the swellings on the gill-margins of *C. comatus*. We may conclude that in the genus *Coprinus* there are at least two methods of providing for the maintenance of interlamellar spaces: (1) the cystidia method, and (2) the gill-margin method. The former is employed not only by *C. atramentarius* but also by *C. narcoticus*, *C. stercorarius*, *C. niveus*, *C. fimetarius*, and a number of other species, and the latter by *C. comatus*, *C. sterquilinus*, and *C. plicatiloides*.<sup>2</sup>

I have examined the fruit-bodies of the following species of *Coprinus*: *C. narcoticus*, *C. stercorarius*, *C. niveus*, *C. lagopus*, *C. ephemerus*, *C. fimetarius*, and two other species which occur at Winnipeg, and are either identical with or very closely related to *C. plicatilis* and *C. Friesii* respectively, and have found that the cystidia are removed from the gills of all of them at the time of spore-discharge by a process of auto-digestion similar to that which occurs in *C. atramentarius*. It seems very probable that self-destruction in connexion with spore-liberation is the rule for cystidia wherever they occur in the Coprini.

In *C. stercorarius*, *C. fimetarius*, and *C. narcoticus* the cystidia have just the same functions as in *C. atramentarius*; they stretch across the interlamellar spaces from gill to gill, and keep the thin gills separated both during spore-development and spore-discharge. In *C. niveus* the cystidia, which are even larger than those of *C. atramentarius*, stretch across the inter-

<sup>1</sup> A. H. R. Buller, *Researches on Fungi*, Pl. I, Fig. 5 and Pl. III, Fig. 14. A thin cross-section, taken through a pileus of *C. comatus* just before it opens out, looks like a wheel: the swollen marginal gill-bands in close apposition form an inner cylinder around the stipe corresponding to the hub, the pileus flesh forms an outer cylinder corresponding to the rim, and the gill-plates held in position between the two cylinders correspond to the spokes.

<sup>2</sup> *C. plicatiloides*, Buller, is apparently a new species. I found it coming up on horse-dung at Winnipeg. *Vide Researches on Fungi*, p. 69. I hope to give a full description of this species in another publication.

lamellar spaces during the early stages of the development of the pileus, and at this time serve to keep the gills apart. However, during the expansion of the pileus just before spore-discharge begins, the gills become widely separated, with a consequent increase in the width of the interlamellar spaces. As a result of this the cystidia become too short to stretch across the interlamellar spaces any longer, and when spore-discharge begins, they simply project from the gills from which they have originated, so that their free ends come to be situated at some distance from the opposing gills. It thus appears that in *Coprinus niveus* the cystidia cease to have any function, and become useless as soon as the pileus begins to open out. In some species, e. g. *C. lagopus*, most of the cystidia are not long enough to stretch right across the interlamellar spaces. However, they probably serve as guards, so that if by accident during development one gill should approach too near another, the general hymenial surfaces of opposing gills will not come into contact. It is possible that in some species some or all of the cystidia may have become vestigial. A more detailed account of the genus *Coprinus* I shall reserve for a future publication.

Massee<sup>1</sup> has stated that 'many species included in *Coprinus*, as *C. plicatilis* and others having dry non-deliquestent gills, have no real affinity with this genus'. Whilst dissenting from Massee's view that *C. plicatilis* and its allies should be removed from the genus *Coprinus*, I<sup>2</sup> accepted his implied statement that these species had gills which were non-deliquestent; and I<sup>3</sup> further said that there is no auto-digestion in *C. plicatiloïdes*. My recent studies have taught me that both Massee and I have been mistaken in supposing that there is no auto-digestion in the small ephemeral species which expand like parasols. I have found that there is a certain amount of auto-digestion at the gill-edges of *C. plicatiloïdes*, *C. plicatilis*,<sup>3</sup> and *C. ephemerus*, which takes place in connexion with spore-liberation in the same general way as in *C. comatus*. In these species the gills split from above downwards, each one becoming Y-shaped in cross-section. The auto-digestion which takes place is limited to the lower portion of the unsplit part of each gill. It does not begin until the time when spores are about to be liberated, and is so slight that it is not surprising that it has hitherto been overlooked. I have now examined the following species of *Coprinus*: *C. comatus*, *C. atramentarius*, *C. sterquilinus*, *C. fimetarius*, *C. micaceus*, *C. narcoticus*, *C. stercorarius*, *C. niveus*, *C. lagopus*, *C. plicatilis*, *C. plicatiloïdes*, *C. ephemerus*, and *C. Friesii*, and have found that auto-digestion to a greater or less extent takes place in all of them. These thirteen species are fairly representative of their genus, and until some species

<sup>1</sup> G. Massee, Textbook of Fungi, London, 1906, p. 364.

<sup>2</sup> A. H. R. Buller, Researches on Fungi, London, p. 209.

<sup>3</sup> Ibid., p. 75.

<sup>3</sup> Auto-digestion of the cystidia and gill-margins certainly takes place in the form of *C. plicatilis* found at Winnipeg, but possibly this is not true of the somewhat dry English form.



are found, if such there are, in which there is no auto-digestion whatever, I think we should regard auto-digestion of the gills as one of the most constant and general of all the characters of *Coprinus* species.

In a former publication I contrasted the Mushroom and the *Coprinus* types of fruit-bodies as represented by *Psalliota campestris* on the one hand and by *Coprinus comatus* on the other. However, since making that contrast, I have realized that it was incomplete. I omitted to mention two quite general and, as it seems to me, fundamental points of difference between the gills of Coprini and of all other Agaricineae, which I have only realized during my more recent and extended studies. In the Coprini the gills are: (1) relatively very thin, and (2) parallel-sided, whereas in fruit-bodies of the Mushroom type the gills are: (1) relatively very thick, and (2) have their sides inclined to one another like the sides of a penknife. It seems to me that the extreme reduction in gill-substance in the Coprini has necessitated that the two sides of each gill should be parallel, and it also seems to me that the ripening and discharge of the spores from below upwards on each gill, together with the gradual auto-digestion of the spore-free portions of the gills from below upwards, are special adaptations which permit of successful spore-liberation from parallel-sided gills. In wedge-shaped gills, on the other hand, such as occur in *Psalliota campestris*, &c., the two gill-sides look slightly downwards. In this type of gill every square millimetre of gill-surface can successfully shed a certain number of spores every minute (or other unit of time) during the whole period of spore-discharge. Hence with wedge-shaped gills there is no need for the spores to ripen and be discharged in succession from below upwards on each gill, and no need for auto-digestion. A fuller statement and analysis of the facts of the comparative morphology of the Agaricineae in relation to the production and liberation of spores I shall reserve for publication in another place.

#### IV. APPENDIX.

Since the above was written, I have had an opportunity of examining some fruit-bodies of *Inocybe asterospora*. It was observed that the cystidia in this species do not undergo auto-digestion, but remain fixed in the hymenium during the whole time of spore-discharge. In a very small unexpanded fruit-body the cystidia were found to be well developed, although only an occasional basidium had begun to produce its spores. The cystidia stretched only about half-way across the interlamellar spaces, and each one had a mucilage drop at its tip. There seemed to be no evidence that the cystidia acted as props to keep the gills apart, to perform which function their peculiar ventricose shape and excretion appear to render them unfitted. Since the cystidia with their mucilage drops only project about 0.02 mm. beyond the spores on the sterigmata, and since the

spores are shot forward into the interlamellar spaces for a distance of about 0.1 mm., it seems clear that the cystidia are here too short to hinder the escape of the spores from between the gills in normally oriented fruit-bodies. The exact function of the cystidia of *Inocybe* still remains to be elucidated.

#### V. SUMMARY OF THE CHIEF RESULTS.

The gills of *Coprinus atramentarius* are of great width and of extreme thinness, and consequently are very flexible. Numerous long cystidia stretch between and connect adjacent gills, the general surfaces of which thus become separated by an interlamellar space about 0.10 mm. wide. The cystidia serve as props, firstly, to keep the gills from touching one another by their general surfaces during the development of the spores, and, secondly, to provide sufficient interlamellar space for the escape of the spores from between the gills during their discharge.

The cystidia do not drop out of the gills when mature. Their disappearance is due to auto-digestion. Excluding the cystidia, the gills undergo auto-digestion in the manner that I have already described for *Coprinus comatus*. Each cystidium begins to undergo auto-digestion as soon as it comes to be situated about 0.5 mm. above the upwardly progressing general zone of auto-digestion and about forty minutes or so before the basidia and paraphyses in its immediate vicinity. During their auto-digestion the cystidia become progressively thinner, their contents are apparently absorbed by neighbouring cells, and they are finally withdrawn in a much reduced state to the gill-sides, where their destruction is completed.

The cystidia, owing to their early auto-digestion, never persist until the upwardly progressing zone of spore-discharge reaches them. Their prop-function, however, is retained up to the last possible moment, and they disappear just in time to prevent the falling spores from striking and adhering to them.

In the genus *Coprinus* there are at least two methods of providing for the maintenance of interlamellar spaces: (1) the cystidia method, and (2) the swollen gill-margin method. The former is employed by *C. atramentarius*, *C. narcoticus*, *C. stercorarius*, *C. fimetarius*, and *C. niveus*, and the latter by *C. comatus*, *C. sterquilinus*, and *C. plicatiloides*. The three last-named species do not have cystidia on their hymenial surfaces.

Auto-digestion of the cystidia has been observed in eight species of *Coprinus*. It seems very probable that self-destruction in connexion with spore-liberation is the rule for cystidia wherever they occur in the Coprini.

Probably, throughout the Coprini, cystidia have a mechanical function in that they serve to prevent opposing hymenial surfaces from touching one another during the development of the basidia and spores. However, if so, this function is carried out in slightly different ways in different species.

A certain amount of auto-digestion occurs in the small coprophilous

species of *Coprinus* which open out like parasols, such as *C. plicatilis*, *C. plicatilloides*, *C. ephemerus*, and *C. Friesii*. It seems probable that auto-digestion to a greater or less extent, beginning at the gill-edges, is a general character of the Coprini.

In the Coprini the gills are (1) relatively very thin, and (2) parallel-sided, whereas in fruit-bodies of the Mushroom type the gills are (1) relatively very thick, and (2) have their sides inclined to one another like the sides of a penknife. In the Coprini the ripening and discharge of the spores from below upwards on each gill, and the gradual auto-digestion of the spore-free portions of the gills from below upwards, are to be regarded as adaptations which permit of successful spore-liberation from parallel-sided gills. In wedge-shaped gills, such as those of *Psalliota campestris*, there is no need for the spores to ripen and be discharged from below upwards and for auto-digestion, since the hymenial surfaces are so arranged in nature that they look slightly downwards.

In *Inocybe asterospora* the cystidia which excrete drops of mucilage from their free ends do not undergo auto-digestion like those of *Coprinus*, but persist during the whole period of spore-discharge. They are, however, too short to form obstacles to the escape of the spores in normally oriented fruit-bodies. Their function has not been determined, but does not appear to be a mechanical one.

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## EXPLANATION OF PLATES L AND LI

Illustrating Prof. Buller's paper on the Cystidia of *Coprinus*.

### PLATE L.

Figs. all of *Coprinus atramentarius*.

Figs. 1-4. Stages in the development of the fruit-body as shown by vertical sections.

Fig. 1. Section of a young fruit-body in which the pileus is opening out. The spores, as is indicated by the shading on the gills, are ripening from below upwards. Spore-discharge and auto-digestion have not yet begun. The flesh is thin and the gills very broad. *c-d*, the direction in which the section shown in Fig. 5 was cut; *r*, the region from which the piece of gill sketched in Fig. 6 was taken. Natural size.

Fig. 2. Section of another and older fruit-body. The spores are still ripening from below upwards on the gills. Spores are being shed and auto-digestion is taking place along the gill-edge at *a*. The broken lines show the shape and extent of the gills at the moment auto-digestion began. *s*, lower edge of the gill where spore-discharge and subsequently auto-digestion first became active. Natural size.

Fig. 3. Section of a still older fruit-body, the pileus of which has become helmet-shaped through expansion. The gills have now become reduced by auto-digestion to about one quarter of their original size. *a*, edge of gill where spore-liberation and auto-digestion are still in progress. Natural size.

Fig. 4. Section of a fruit-body in the last stage of its development when spore-liberation has ceased. The gills have now entirely disappeared. The central part of the pileus flesh still crowns the stipe. Natural size.

Fig. 5. Horizontal section, taken in the direction *c-d* in Fig. 1, through the pileus flesh and four gills. *p*, the pileus flesh; *s*, the edges of the gills towards the stipe; *g*, a gap indicating that the central parts of the gills have been left out for convenience of illustration; *c*, the cystidia which prop the gills apart and thus maintain the interlamellar spaces. Magnified about 57.

Fig. 6. Surface view of a piece of gill taken from the region *r* in Fig. 1. This drawing shows the distribution of the cystidia and basidia just before the beginning of spore-discharge and auto-digestion. *e e*, the oblique free inner edge of the gill bearing marginal cystidia *m*; *c c*, cystidia projecting from the gill; *o o*, places where cystidia from the adjacent gill were in contact with the hymenial surface before the gills were torn apart. Between the cystidia are shown the basidia, each bearing four black spores. Magnified about 103.

Fig. 7. Drawing showing the general appearance of part of a gill-surface, including the gill-edge, after spore-discharge and auto-digestion have begun (cf. Fig. 2). There are five zones running parallel to the oblique gill-edge: (1) *a-a'*, zone of basidia with ripe spores. (2) *b-b'*, zone of basidia discharging spores into an interlamellar space. The spores are shot off their sterigmata successively, so that in this zone some basidia have three spores left upon them, some two, some one, whilst some have lost them all. (3) *c-c'*, zone of basidia which have discharged all their spores. (4) *d-d'*, zone of auto-digestion. The basidia and paraphyses are becoming indistinct and gradually liquefied. (5) *e-e'*, the liquid film on the gill-edge containing the products of auto-digestion. *p*, position from which a cystidium has been removed by auto-digestion. Magnification 460.

Fig. 8. Cross-section showing a cystidium stretching across an interlamellar space between two gills and thus separating two opposing hymenial surfaces. Magnification 440.

Figs. 9, 10, and 11. Single cystidia, showing their variation in size and form and the nature of the subhymenial cells to which they are attached. Magnification 440.

Fig. 11. A drawing showing the relative sizes and position of a cystidium, a basidium bearing four spores, some paraphyses, and some subhymenial cells. Magnification 440.

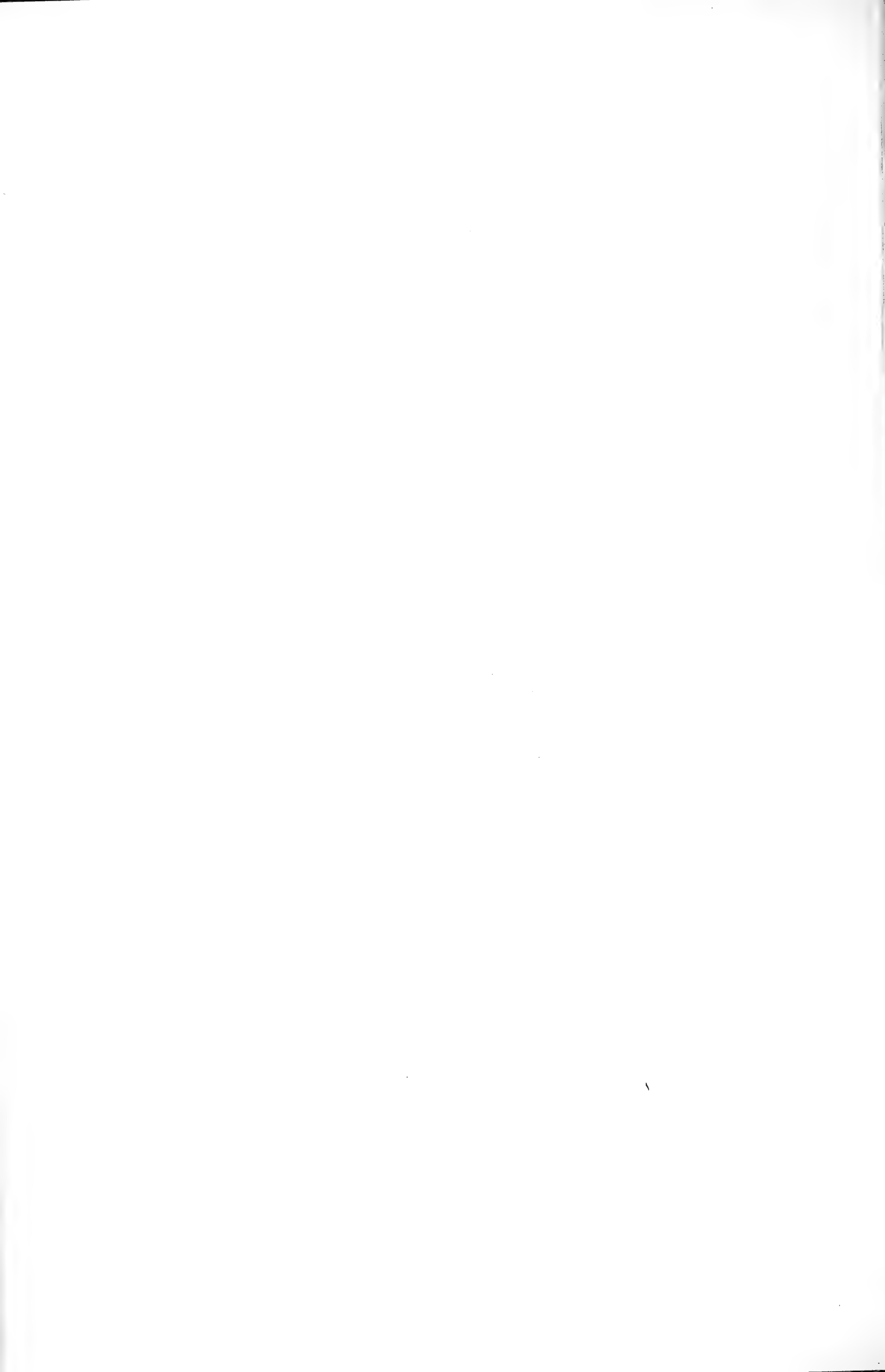
## PLATE LI.

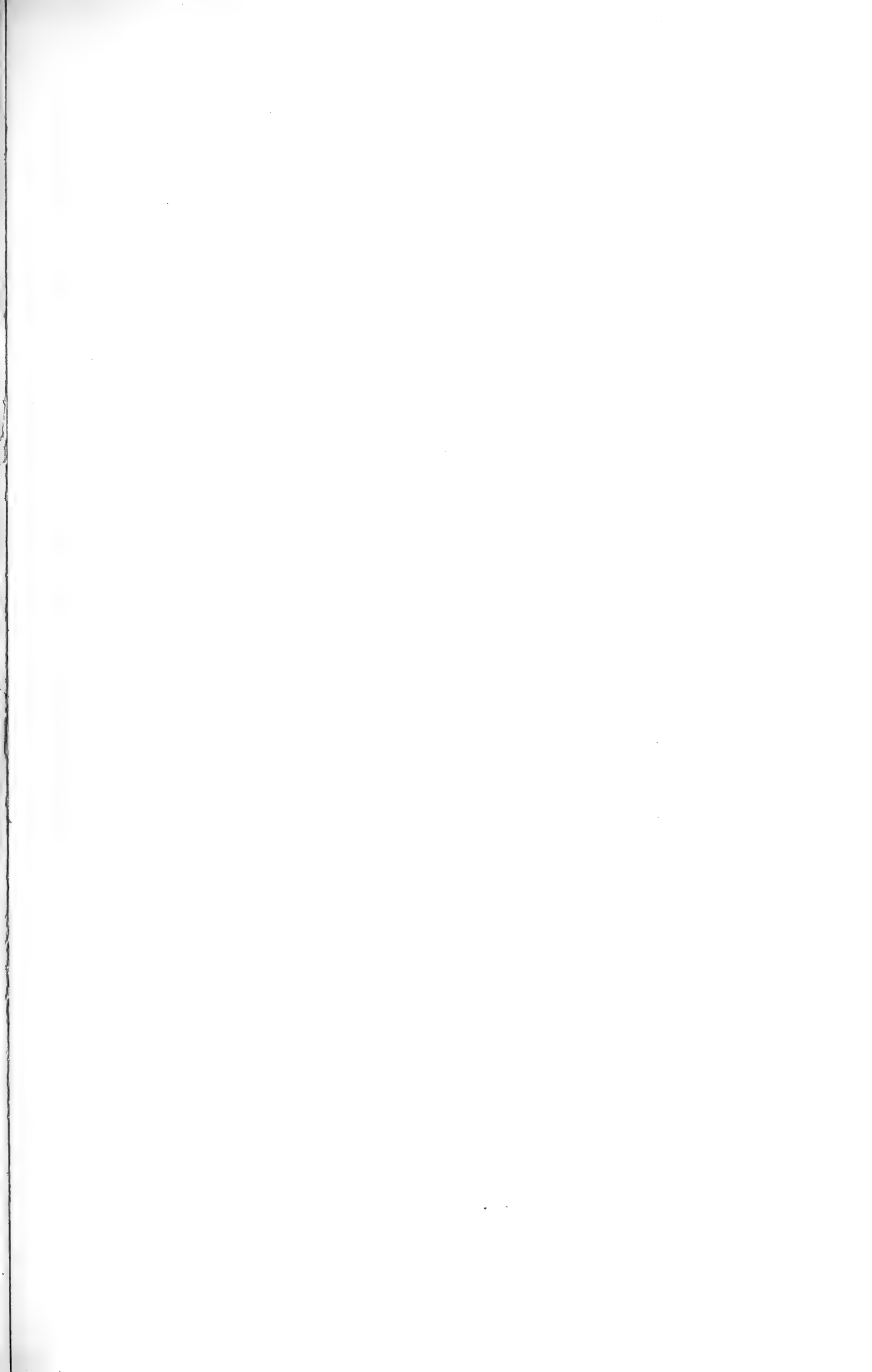
Figs. all of *Coprinus atramentarius*.

Fig. 12. An optical section through a cystidium, showing the cell-wall, protoplasm, and vacuoles. Magnified 440.

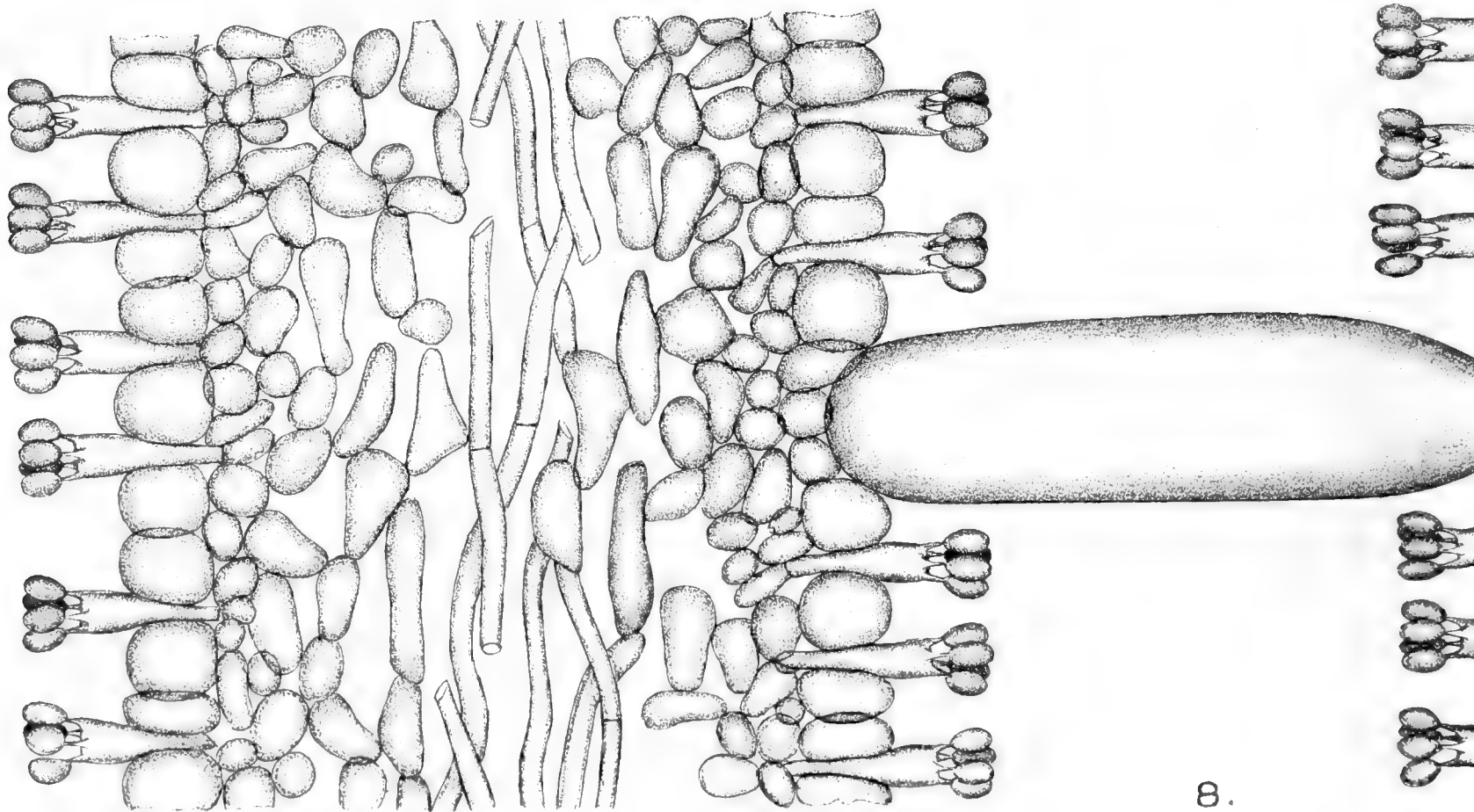
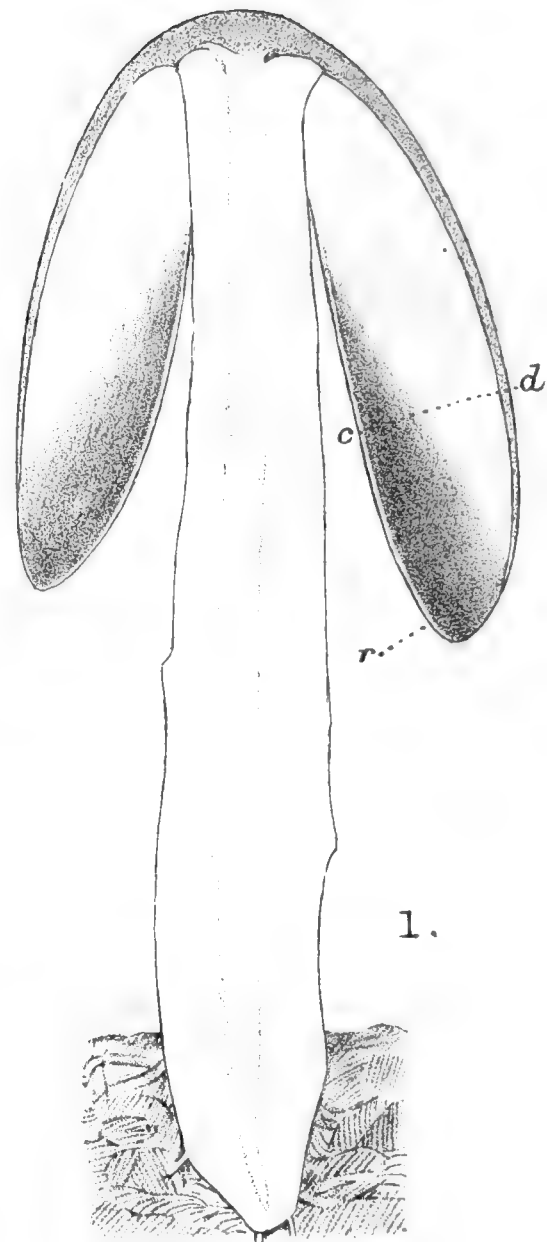
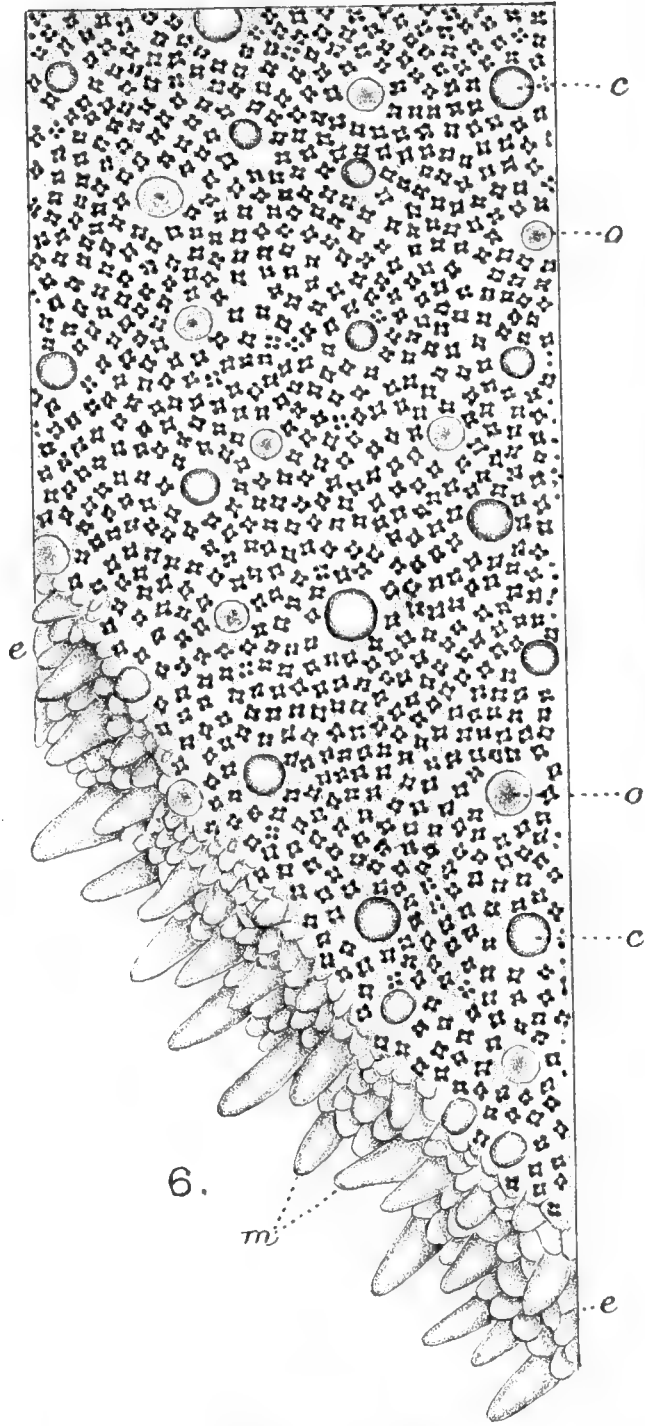
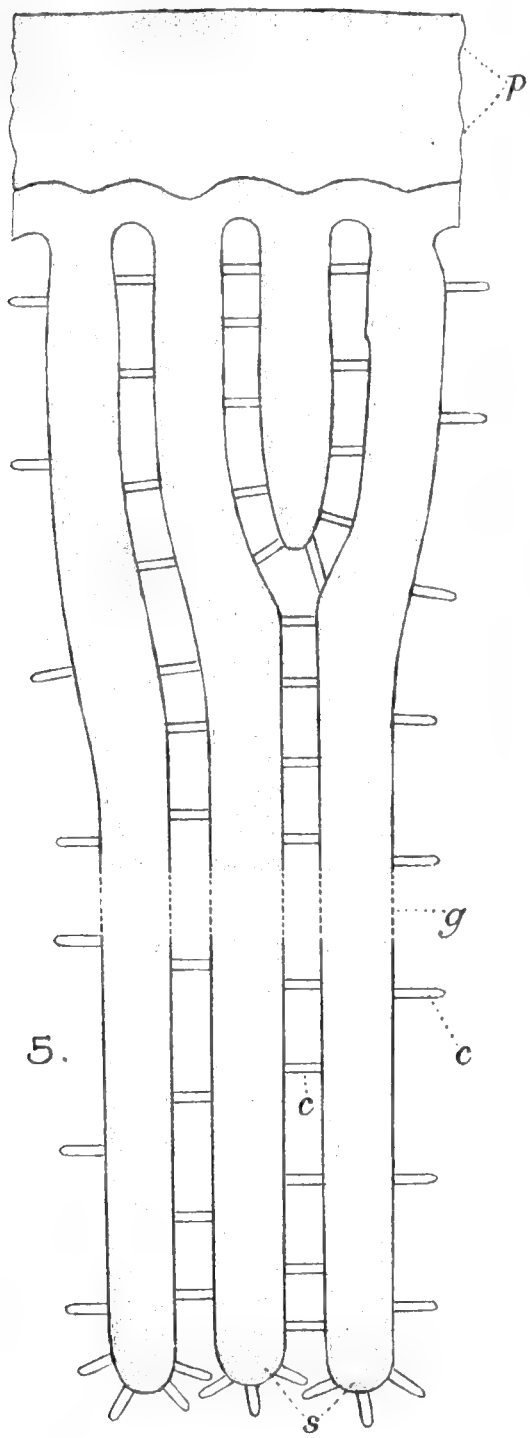
Figs. 13-18 inclusive. The auto-digestion of six cystidia. *a* in each figure is a fully turgid cystidium just before the beginning of its auto-digestion. *b*, *c*, *d*, &c. show stages in the auto-digestion of each cystidium. The cystidia were observed stretching across interlamellar spaces near the auto-digesting edges of the gills. Figs. 13, 14, 15, and 17 show cystidia which became withdrawn to one of the gills. Figs. 16 and 18 show cystidia which, after becoming more and more slender, snapped into two parts; one part became withdrawn to one gill, and the other to the other gill. Fig. 15 shows the auto-digestion of a cystidium which had been separated whilst still turgid from one of the gills with which it was originally in contact with its rounded apical end. Fig. 17 shows the auto-digestion of a cystidium which had been separated whilst still turgid from the gill in which it had originated. Its free pointed basal end is shown at *a*. Magnification about 80.

Fig. 19. Semi-diagrammatic section taken in a perpendicular plane through three gills during spore-discharge and auto-digestion (cf. Fig. 2). Seven zones can be distinguished: *a*, zone in which the cystidia are fully turgid and are propping the gills apart. The basidia bear spores which are nearly ripe. *b*, zone in which the cystidia are disappearing through auto-digestion. The spores are ready for discharge. *c*, zone of ripest spores from which the cystidia have already disappeared. *d*, zone of spore-discharge where cystidia are absent. Spores are being shot out into the interlamellar spaces. Their sporobolae are represented by arrows. *e*, the spore-free zone. *f*, zone of auto-digestion where basidia and paraphyses are being destroyed. *g*, the liquid film at the gill-edge containing the products of auto-digestion. *h*, the hymenial layer made up of basidia, paraphyses, and cystidia; *s*, the subhymenial cells; *t*, the trama. *i i*, cystidia which have been torn from the opposite gills by their rounded apical ends. *j j*, cystidia which have been torn from the opposite gills in which they originated so that they are now attached by their apical ends: their contracted bottle-neck-like basal ends are free. *k k*, cystidia which have become very slender owing to auto-digestion, but which are still attached to both gills. *l l*, cystidia which are being withdrawn to the gill-sides. *m*, a cystidium which has snapped into two parts. *o o*, remains of cystidia. Magnification about 110.

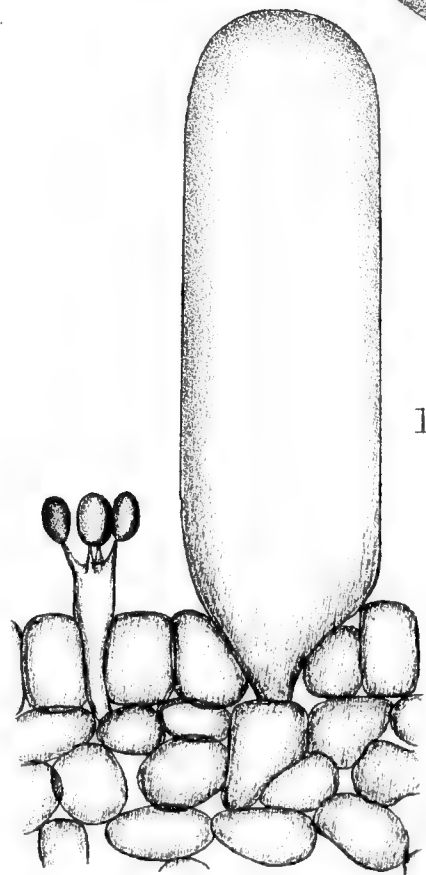
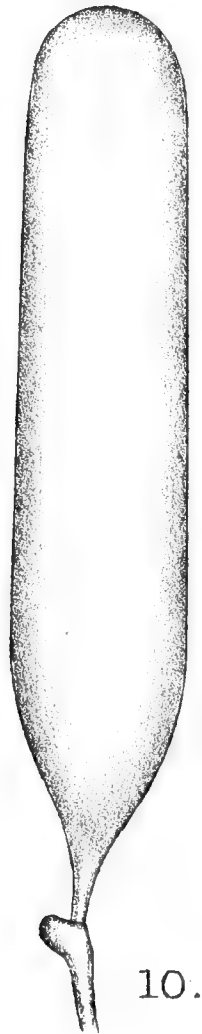
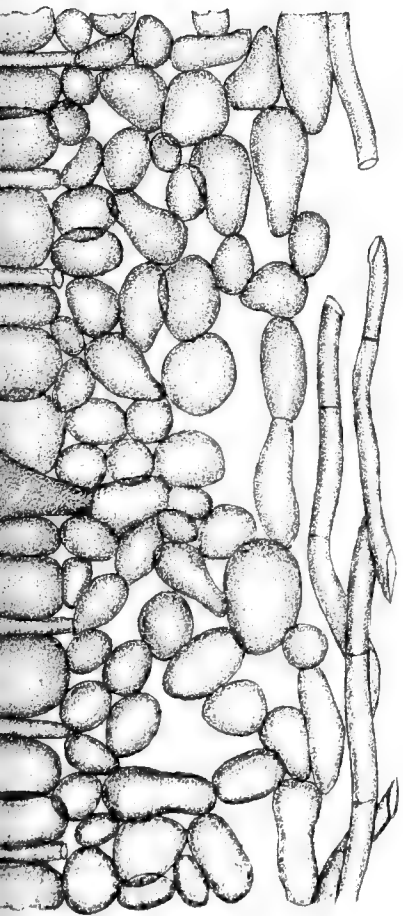
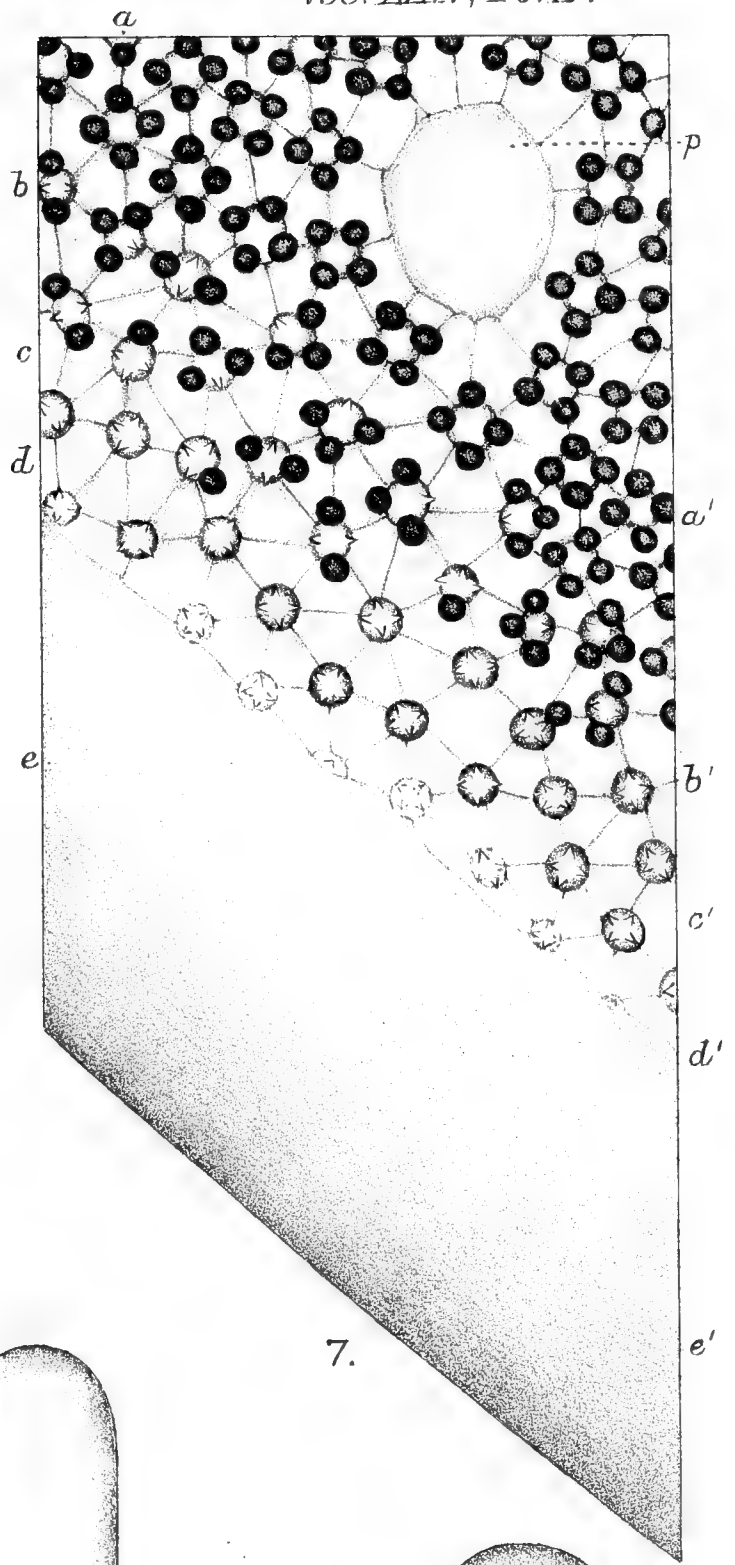
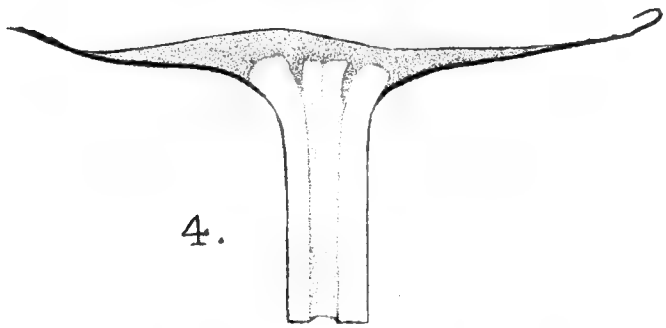
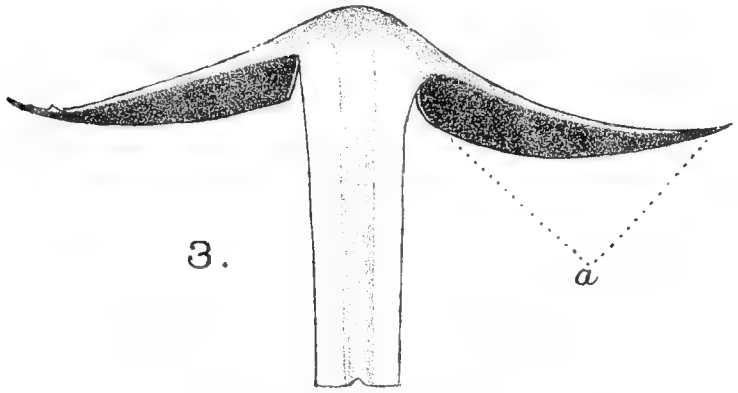
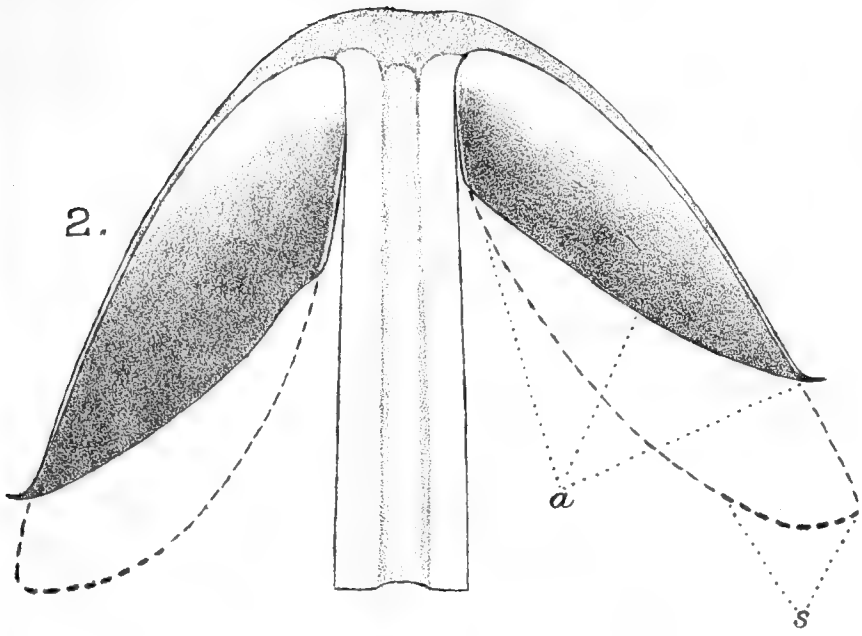








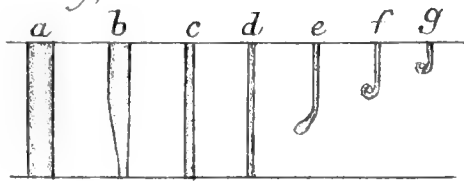
Del. A. H. R. Buller.



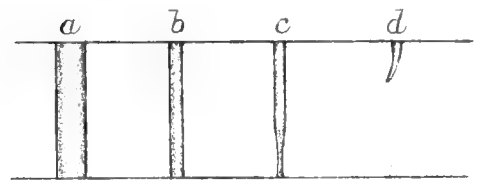




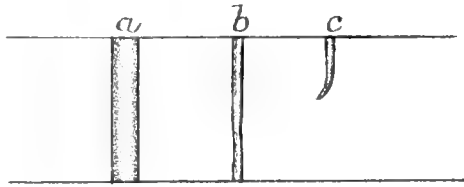
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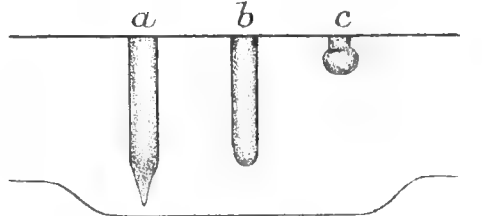
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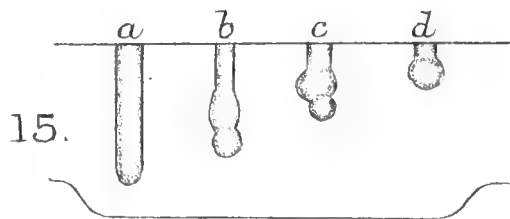
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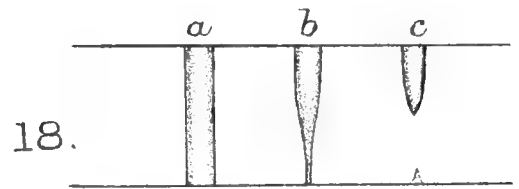
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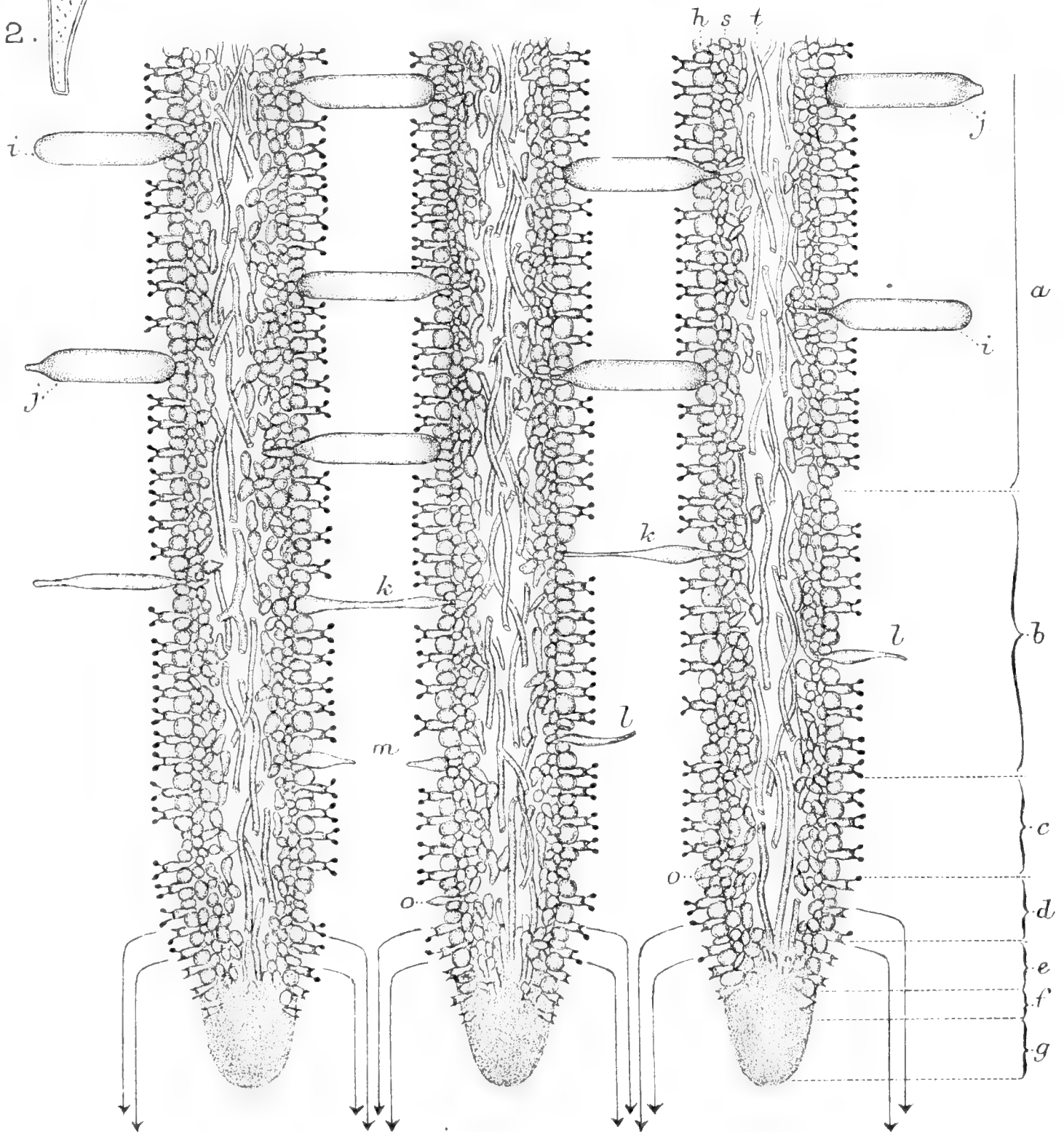
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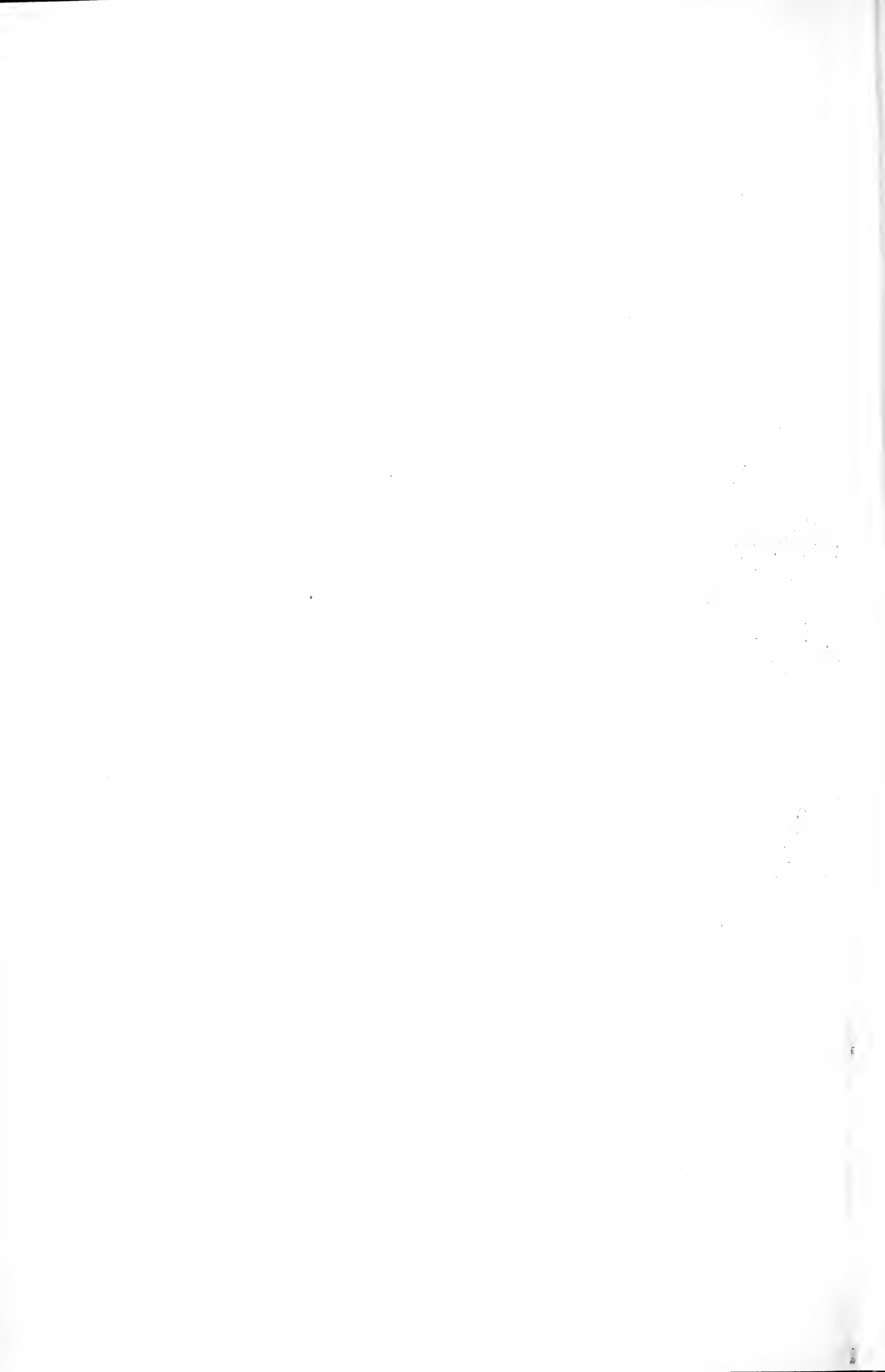
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## Cytological Studies on *Oenothera*. II.

### The Reduction Divisions of *Oenothera biennis*.<sup>1</sup>

BY

BRADLEY MOORE DAVIS.

With Plates LII and LIII.

THE following account of the reduction divisions of *Oenothera biennis*, L., is presented as a part of the writer's plan to study certain characteristic native species of *Oenothera* as a basis for comparison with *O. Lamarckiana* and some of its derivatives, and as preliminary to the investigation of certain hybrids which may readily be obtained. The first paper of this series, 'Pollen Development of *Oenothera grandiflora*' (Davis '09), gives an account of the reduction divisions in the pollen mother-cells of one of the larger American species, which exhibits some interesting peculiarities that are more easily understood after comparison with the conditions in *O. biennis*. Both of these American wild species give opportunity for a comparison of the reduction processes with those of *O. Lamarckiana* and *O. rubrinervis* studied by Geerts and Gates.

As is well known, *Oenothera biennis* is a species which exhibits a considerable range of variation in the size of its flowers, character of its foliage, and habit of growth, so that it is frequently possible to distinguish forms from different regions of America, and it is probable that races or strains may be readily differentiated by pure cultures. The fact that the flowers are self-pollinated before the opening of the bud would undoubtedly greatly assist in holding any differentiated races reasonably true to their types. The strain which furnished the material for this investigation was derived from a rosette transplanted from waste ground at Woods Hole to the author's garden, in 1908. The rosette was selected with some care as an interesting type to hybridize with *O. grandiflora* with certain ends in view. The cross was made during that season and some of the hybrids of the first generation have been briefly described in a paper, 'Notes on the Behavior of Certain Hybrids of *Oenothera* in the First Generation' (Amer. Nat., vol. xliv, p. 108, 1910).

<sup>1</sup> An investigation conducted with aid from the Elizabeth Thompson Science Fund, for which the author desires to express his indebtedness.



Should certain of these hybrids or others of later seasons prove of sufficient interest in the second generations this Woods Hole strain of *biennis*, which was constant in cultures of 1909, will be described in some detail. For present purposes, however, it will be sufficient to state that the strain is representative of the green-stemmed (lacking red dots), broader-leaved types of *biennis* with medium-sized flowers. The main stem grows to a height of about 1 m., and long side branches arise from near the base of the plant. The leaves on the lower portions of the stem are elliptical, the larger about 14 cm. long and 4 cm. broad. The inflorescence is characteristic of *biennis* in having bracts about  $\frac{1}{3}$  as long as the flowers. The petals are about 1.3 cm. long.

#### METHODS.

Considerable attention was given during the summer of 1909 to the problems of fixation, especially to test the effects of Carnoy's alcohol-acetic and chloroform-alcohol-acetic mixtures which Overton ('09) found so satisfactory for certain types. The chloroform-alcohol-acetic mixture caused such shrinkage, even when used for so short a time as ten minutes, that it was worthless for the stages of synapsis and later. The alcohol-acetic mixture was less violent in its action, but nevertheless proved for the same reason to be unsatisfactory for older cells where there is a large amount of cell-sap in cytoplasmic vacuoles. These two fluids of Carnoy have, however, a value for *Oenothera* in the study of presynaptic stages, where the cells are relatively small and the cytoplasm dense, because of the sharp staining quality of the chromatin with haematoxylin.

The most satisfactory fixation was obtained with chrom-osmo-acetic fluids when due precautions were taken to facilitate rapid penetration. To attain this necessary end the anthers, freed from floral envelopes but still attached in a group to the ovary, should be thoroughly brushed with water before immersion in the fixing fluid. Satisfactory results were also obtained when such groups of anthers were dipped for a few seconds in Carnoy's alcohol-acetic mixture before immersion in the chrom-osmo-acetic acid; in this procedure the former fluid appears to prepare the surface of the anthers so that the latter fluid is able to penetrate more quickly. Experience has shown that the formula known as strong Flemming (1 per cent. chromic acid 75 c.c., 2 per cent. osmic acid 20 c.c., glacial acetic acid 5 c.c.) gives somewhat better results than the weaker fluids used in the study of *O. grandiflora* (Davis '09, p. 552). There is another advantage in employing rather strong fluids since material appears to cut better after their use, perhaps because the acids may free the tissue from raphides.

As in the previous study of *Oenothera grandiflora* iron-alum haematoxylin proved to be the most satisfactory stain.

## THE REDUCTION DIVISIONS IN THE POLLEN MOTHER-CELL.

It seems best for the sake of clearness to describe and figure separately the events of reduction in the anther and in the ovule. Although, as would be expected, the cytological features of the processes are essentially the same for both organs, there would necessarily be some confusion in an attempt to present and describe two groups of figures side by side in parallel series. The account of the reduction divisions in the ovule will be made very brief, but the series of stages will be presented in some fullness for comparison with the figures that illustrate the events within the pollen mother-cell.

**Presynapsis.** The structure of the resting nucleus of the pollen mother-cell following the last mitosis in the archesporium of the anther is shown in Plate LII, Fig. 1. As in the case of *Oenothera grandiflora* (Davis '09) there is present besides the large nucleolus a number of deeply staining bodies generally distributed around the periphery of the nucleus, and connected with one another by very delicate strands. The nature and significance of these bodies are of importance in relation to current views that chromosomes are represented in the resting nuclei by chromatic centres or prochromosomes. The bodies as observed in *O. biennis* stain deeply with haematoxylin, and hold their colour tenaciously after the manner of chromatin. They may frequently be counted as fourteen, which is the number of the sporophytic (somatic) chromosomes, or in numbers somewhat under fourteen. Counts of deeply staining bodies in excess of fourteen may also be made in the nuclei, but small granules possibly nucleolar in character are practically indistinguishable from these bodies in the nuclei of *Oenothera*. The writer is inclined to believe that these chromatic bodies are the prochromosomes of Overton ('05, '09), but it should be noted that there is no evidence that they are arranged side by side in pairs on a system of threads that might be interpreted as representing two parallel spiremes. The chromatic bodies of *O. biennis* are distributed irregularly throughout the nuclear cavity, but wherever two lie close together (Figs. 1, 2) the arrangement appears to be end to end upon a delicate strand that runs in the direction of their longer axes. This is possibly a point of significance since the chromosomes differentiated after synapsis are clearly segments of a single spireme and are consequently joined end to end.

The nuclei remain for a long time in the resting condition described above. With the approach of synapsis the delicate strands connecting the chromatic bodies thicken and become much more numerous, forming a clearly defined network. Stages in this process are indicated in Fig. 3, showing a nucleus in a cell adjacent to that illustrated in Fig. 2, and also in Fig. 4, which shows two nuclei separated from one another by a single cell. The nucleus finally becomes quite filled with a dense reticulum (Fig. 5), at

which stage the chromatic bodies can be distinguished only with difficulty, if at all, since they lie in the meshes of the deeply staining strands of the network. A single large nucleolus is present and frequently one or more smaller nucleoli. It is important to note that this linin network develops as a reticulum from its earliest inception; there is at this time no indication of a continuous spireme, much less two parallel spiremes such as have recently been described for a number of forms by Overton ('09), Lundegardh ('09), and Rosenberg ('09 a).

The fate of the chromatic bodies in *Oenothera* as the nucleus approaches synapsis is a problem of great interest, but at present all that can be said is that their substance becomes apparently merged with that of the reticulum, from which they cannot finally be differentiated. It is, however, possible that in this reticulum there are regions, corresponding to the chromatic bodies, which are quite distinct from the linin substance of the network that in earlier stages (Figs. 1-4) is easily distinguished from the chromatic bodies. Such a chromatic framework, if present, may be responsible for the spireme which appears during synapsis.

**Synapsis.** The approach of synapsis is indicated by the contraction of the reticulum away from the nuclear membrane (Fig. 6), and this contraction proceeds until almost all of the threads and meshes in the network are drawn into a close mass (Fig. 7) that lies generally near the large nucleolus. While the contraction is in progress the reticulum takes on the character of a tangle or ball of threads, constituting the synaptic knot, from which a few loops and very delicate strands usually extend into the nuclear cavity (Figs. 7-10). It is impossible to follow the individual threads in the denser portions of the knot, but the free loops at the edge give an opportunity for more detailed study.

Although it is frequently possible to find threads that run closely parallel to one another for some distance the writer is unable to present any evidence that this condition is other than such a close association as would be brought about by the gathering together of a complexly looped thread or system of threads into a tight mass. The loops appear to be quite independent, as though they were parts of a much tangled system rather than portions of a split spireme, or of two independent systems of threads (maternal and paternal spiremes), which might be assumed to be associated with one another side by side. It is, however, impossible to say whether or not the coiled and twisted loops of the synaptic knot are parts of a single continuous thread; if so, it must be a thread of great length.

The seriation of such a group of stages, as is shown in Figs. 7-10, is rendered somewhat easier by the fact that the pollen mother-cells separate from one another during the process of synapsis, each one rounding off and coming to lie freely in the pollen chamber. Fig. 7 is undoubtedly the earliest stage of this group of figures, because the pollen mother-cells were still

angular and united, while Figs. 8–10 are from sections in which the cells had rounded off and separated.

As synapsis proceeds it becomes very clear that the threads thicken (compare Figs. 9 and 10 with Figs. 7 and 8), and there is good evidence that the system becomes shorter. Thus the threads are very much more prominent in the later stages of synapsis (Figs. 8–10) than at the beginning of the contraction (Figs. 6 and 7), and in the later stages the structure begins to take on the appearance of a much-looped and coiled spireme. This history of the differentiation of the threads during synapsis is the same as that for *O. grandiflora* (Davis '09).

It will be noted that the term synapsis is reserved for this period of the first contraction of the chromatic material. A type of contraction that frequently takes place much later, during the formation of the chromosomes, will be described in that connexion.

**The Formation of the Chromosomes.** The period of synapsis, described above, is of relatively long duration, as is shown by the fact that in an inflorescence one to three buds may be found in which all, or almost all, of the nuclei in the pollen mother-cells are in this stage. The succeeding developments, leading to the development of the thick spireme from which the chromosomes are formed, take place more quickly. The chromatic material emerges from the contracted condition of the synaptic knot by a loosening of the coils, with the result that a thickened and very much shorter thread appears, which is evidently a spireme. Such a stage is shown in Fig. 11, which is of especial interest in comparison with Fig. 10, since the two nuclei were from opposite ends of the same pollen chamber. The spireme even in this thickened and much shortened condition (Figs. 11–13) is generally complexly looped and coiled, but the greater part of the thread system can be traced generally without great difficulty, which is impossible in the stages of synapsis.

Shortly after the emergence of the thickened spireme from the synaptic knot there appear indications of a process of segmentation that is to transform the spireme into a chain of fourteen chromosomes. Early stages of this process are shown in Figs. 12 and 13, where the segments are present as long, variously bent rods with thin regions between them, as though separating by constriction. The contraction which has been going on in the spireme continues in these segments until their long diameter is not more than two or three times their width, and by this process of condensation the spireme becomes so much shortened that the complexities of the looped arrangement largely disappear, and it is frequently possible to follow the chain of fourteen chromosome segments from end to end as a single segmented spireme (Fig. 14). Later the spireme may become broken into groups of chromosome segments (Figs. 15 and 16), each group showing the segments arranged end to end; finally during the prophase of the heterotypic



mitosis the segments as fully developed chromosomes separate from one another.

During the process of condensation and shortening of the chromosome segments in the spireme there is frequently found near the nucleolus a massing of the chromatic material (Figs. 17 and 18) which bears a superficial resemblance to the synaptic knot (Figs. 8-10). This is apparently the condition termed the 'second contraction' and considered by some authors as of considerable significance, since it is supposed to bring the chromosomes into close association as pairs in preparation for the stage of diakinesis. The material, however, offered little support to such an explanation, because the chromosomes exhibit only slight tendencies to group in pairs, and it is doubtful if the stage itself is a regular occurrence. It is hard to see for the species under consideration any significance in this 'second contraction' further than an assemblage of the chromosome segments of a spireme so complexly looped that its parts are necessarily drawn close together during the condensation which transforms the fourteen segments into chromosomes.

It is interesting to note the differences in the formation of the chromosomes between this material of *Oenothera biennis* and that of *O. grandiflora*. The spireme of *grandiflora* emerges from the synaptic knot as a much more complexly coiled thread (Davis '09, Figs. 16-18) than that of *biennis*, and the subsequent condensation and shortening of the spireme draw the chromatic material into a closely contracted mass, from which several thick loops extend into the nuclear cavity (Davis '09, Figs. 19-23). These latter conditions might be considered representative stages of a 'second contraction', but in *grandiflora* the changes of structure are so gradual and the variation of form is so great that it is very difficult to distinguish such a phase. Finally the thickened and contracted spireme of *O. grandiflora* breaks up into seven rings, some of the loops being transformed directly into these structures. The later history shows that each ring is bivalent in character, since it separates into semicircular halves (sporophytic chromosomes) during the heterotypic mitosis. There are then fourteen sporophytic chromosomes as in *biennis*, and these are likewise segments of a spireme arranged end to end, but the involved coils of the spireme bring the segments together in loops in such a manner that the fourteen chromosomes become grouped in pairs to form seven rings (bivalent chromosomes). It would be hasty to conclude that the close association of the chromosomes in pairs, characteristic of the material of *grandiflora* studied by the writer, involved any greater affinity between the segments of the spireme than in *biennis*. The striking differences between the two species in the final grouping of the chromosomes are perhaps simply due to the peculiarity of a more complicated looped spireme in the case of *grandiflora*. Furthermore, it will not be surprising if the study of

other strains of the variable *O. grandiflora* showed departures from the conditions in the material described by the writer, and that following synapsis types of chromosome organization will be found which resemble more closely those of *O. biennis*.

The important point to emphasize in this connexion is that in both *grandiflora* and *biennis* the chromosomes are organized from segments of a spireme arranged end to end, and that the history of the reduction divisions in this respect is the same as that described by Gates ('08) for *rubrinervis* and Geerts ('09) for *Lamarckiana*.

**The Heterotypic Mitosis.** The condensation of the chromatin, which takes place during the organization of the spireme and formation of the chromosomes, continues throughout the prophases of the first, or heterotypic, division. As a result the chromosomes at metaphase of this mitosis (Figs. 22 and 23) are very much smaller structures than the segments of the spireme (Figs. 13 and 14) from which they are derived. The mature chromosomes are essentially similar, generally with the form of thickened V's due to the bending of the spireme segments, and are not rounded structures such as have been described and figured by Gates and Geerts.

The process of spindle formation is the same as that of *grandiflora*, and similar to that described for a number of higher plants (e. g. *Equisetum*, *Larix*, *Lilium*, &c.). Fibrillae enter the nuclear cavity with the breaking down of the nuclear membrane, and also push out into the cytoplasm in various directions and thus establish a multipolar spindle (Figs. 19 and 20); the large nucleolus disappears at an early stage in the process. The group of chromosomes is brought by the development of the fibres to the centre of the spindle where the chromosomes lie in irregular clusters, in which, however, some may frequently be found still united end to end in short chains of two to five chromosomes.

The multipolar condition of prophase gradually changes to a typical bipolar spindle characteristic of metaphase (Figs. 21-23) by a rearrangement and gathering of the spindle fibres into two broad sheaves which end in granular areas that merge with the alveolar cytoplasm. During this development the chromosomes separate from one another and usually take on the form of thickened V's by the bending of the ends and the thickening of the chromatin in the middle regions. The arrangement of the chromosomes as they are brought to the equatorial plate during metaphase is quite irregular. Although occasional pairs are found sufficiently closely associated to indicate that they were adjacent segments of the spireme, most of the chromosomes are so separated and scattered (Figs. 21 and 22) that it is impossible to determine with certainty what was their relation to one another in the spireme.

In this respect these conditions in *biennis* are in sharp contrast to those in the material of *grandiflora*, studied by the writer, where the arrangement



of the chromosomes at the equatorial plate was very symmetrical because they remained joined in pairs in the form of seven rings throughout the pro-phases of the heterotypic mitosis (Davis '09, Figs. 31-36). The separated and scattered arrangement of the chromosomes in *biennis* allows far greater opportunity for numerical irregularities in their distribution during the heterotypic mitosis than is possible when they are closely associated in pairs.

As the two sets of chromosomes (seven in each set) move away from one another towards the poles of the spindle the V-shaped form is especially evident (Fig. 23), but during anaphase of the mitosis their structure becomes complicated by a lengthwise fission of each chromosome in the plane of the page upon which the above letter (V) is printed. The seven chromosomes which leave the equatorial plate for each pole of the heterotypic spindle arrive as seven split chromosomes (Fig. 24), this division being a premature fission of each chromosome in preparation for the second, or homotypic, mitosis. The group of seven split chromosomes is best observed in polar views of late anaphase (Fig. 25), and at the time of the reconstruction of the daughter nuclei (Fig. 26).

During the examination of a large amount of material in the stages described above (Figs. 25 and 26) two cases were observed that showed numerical irregularities in the distribution of the chromosomes by the heterotypic mitosis. In both examples eight split chromosomes were found at the poles of the heterotypic spindle where the normal number is seven. Fig. 27 illustrates this exceptional condition, and in this instance five chromosomes were present at the opposite pole of the spindle; six would be the number expected, and it is quite probable that a chromosome had been carried away from the group by the microtome knife. The rarity of these exceptions would make it very difficult to determine whether or not such irregularities might continue throughout the homotypic mitosis and give rise to functional pollen grains with smaller and larger chromosome content than the normal.

The history of the seven split chromosomes is readily followed through the interkinesis between the heterotypic and homotypic mitoses. After the daughter nuclei are organized the halves of each chromosome separate somewhat (Fig. 28), and the ends swing apart until the halves lie in approximately the same plane and resemble somewhat two U's joined together at the middle region (Figs. 29 and 30). It then becomes evident that there are seven pairs of chromosomes in each nucleus, a condition which is, as a rule, most clearly shown in polar views of the structure. The chromosomes increase markedly in size, and remain in this expanded condition throughout the period of interkinesis. The free ends of the chromosomes sometimes exhibit a tendency to branch and become united to form a loose and imperfect network, but generally most of the chromosome pairs of

a nucleus are as clearly defined as in Figs. 29 and 30. The daughter nuclei remain for some time in this resting condition with the seven pairs of chromosomes distributed around the periphery and with one or more nucleoli present. It is clear that the chromosomes of *biennis* maintain their individuality throughout the interkinesis, and their behaviour in this respect is in complete accord with the history during the same phase in *grandiflora* (Davis), *Lamarckiana* (Geerts), and *rubrinervis* (Gates).

The chief conclusions from this study of the heterotypic mitosis of *Oenothera biennis* are (1) that fourteen V-shaped sporophytic (somatic) chromosomes are distributed in two groups of seven each, so that the mitosis is a reduction division; (2) that the chromosomes are assembled irregularly at the equatorial plate, and there is no uniform grouping of the structures in pairs, although occasional pairs may be found; (3) that the chromosomes are essentially similar to one another in form and size; and (4) that during anaphase there is a fission of the chromosomes, so that seven split chromosomes enter each daughter nucleus and are evident as seven pairs during the period of interkinesis.

**The Homotypic Mitosis.** The approach of the homotypic mitosis is indicated, as in the heterotypic division, by the appearance of a delicate web of fibrillae around the resting nucleus of the interkinesis (Fig. 30). With the breaking down of the membrane the fibrillae enter the nuclear cavity (Pl. LIII, Fig. 54, prophase of homotypic mitosis in the ovule) and establish a multipolar spindle which quickly changes into a bipolar structure. The seven pairs of chromosomes are carried towards the centre of the developing spindle (Figs. 31 and 32), where they form an irregular cluster. During these changes the chromosomes by condensation become much smaller than in the expanded condition of the interkinesis, and by the time the homotypic spindle is fully developed (Pl. LIII, Fig. 33) they have returned to about the same size as when they entered the period of interkinesis. The pairs of chromosomes are arranged quite regularly on the equatorial plate of the homotypic spindle, so that as a result the members of each pair are separated by the mitosis. The two spindles may lie side by side or at right angles to one another (Fig. 33).

The form of the chromosomes at metaphase of the homotypic mitosis is that of short and sometimes slightly bent rods, but during anaphase (Fig. 34) one or both ends generally enlarge somewhat, so that the outline becomes irregular. The irregularities of form grow more pronounced after the organization of the daughter nuclei (Fig. 35), in which a chromatic network is finally formed by the elongation, branching, and anastomosis of the chromosomes. The nucleus of the young pollen grain thus passes into a typical resting condition (Fig. 36) possessing one or more nucleoli and a delicate open reticulum in which lie thickened deeply staining regions. The latter are frequently so definite in outline that it seems probable they

are chromatin centres or prochromosomes, but their form is exceedingly variable, and one cannot make constant counts.

The study of the nuclei in older pollen grains and of the mitosis which differentiates the generative from the tube nucleus presents certain technical difficulties that make the investigation of this phase of the gametophyte somewhat difficult, and the writer has not yet taken it up in detail.

The history of the homotypic mitosis in *Oenothera biennis* is identical in all essentials with that of *grandiflora*, *Lamarckiana*, and *rubrinervis*. The mitosis is clearly an equational division distributing the halves of chromosomes whose premature division takes place during the anaphase of the heterotypic mitosis, the halves remaining associated as seven pairs throughout the period of interkinesis.

#### THE REDUCTION DIVISIONS IN THE OVULE.

In some respects the ovule furnishes better material than the anther for studies on the reduction divisions, since when the outer walls of the ovaries are carefully cut away it is frequently possible to obtain a better and more uniform fixation than in the case of the anther. It is, however, at times far more difficult to seriate stages. The study of Geerts ('09) on the ovule of *Oenothera Lamarckiana* gives an opportunity for some comparison of that form with *biennis*.

The reduction divisions in the ovule of *Oenothera* occur just previous to the differentiation of the embryo sac which arises from one of a group of four cells homologous with a pollen tetrad. The embryo sac therefore develops from the homologue of a megaspore and not from the homologue of a megaspore mother-cell as in many types (e. g. *Lilium*, &c.). The four cells of the group (tetrad) are arranged in a line and arise, through a heterotypic mitosis followed by two homotypic divisions, from a single large megaspore mother-cell which is differentiated in the nucellus of the very young ovule at the time of the development of the integuments. Geerts ('09) reports for *Lamarckiana* that the embryo sac always develops from the upper cell of the row of four (tetrad), the other three cells breaking down. This is not true for the present form of *biennis*, in which the embryo sac appears to develop rather more frequently from the lowest cell of the row (i. e. farthest away from the micropylar end), although often arising from the upper cell.

Geerts ('09, Pl. XXII) has pointed out in tabular form that the reduction divisions in the ovule of *Lamarckiana* take place at a stage of the flower's development long after the homologous mitoses in the pollen mother-cell, and the same history is true of *biennis*. Indeed, the pollen tetrads are fully formed before the rudiments of the ovules have begun to develop their integuments.

We shall now outline briefly, and for the sake of comparison with the more detailed account of the pollen mother-cell, the history of synapsis and of the reduction divisions in the ovule.

**Presynapsis.** The resting nuclei of the megaspore mother-cells (Fig. 37) show the presence of chromatic bodies similar to those described for the nucleus of the pollen mother-cell (Figs. 1-4), but here also it is frequently possible to make counts above and below the number fourteen, irregularities for which various explanations might be suggested. The writer is, however, inclined to believe that certain of these structures are prochromosomes. The chromatic bodies are distributed upon a very delicate linin reticulum, and wherever two lie close together (Fig. 37) their arrangement appears to be end to end upon a strand of the network.

On the approach of synapsis the delicate reticulum is gradually replaced by a dense network of deeply staining threads (Figs. 38 and 39) which fill the nucleus (compare with Figs. 4 and 5), and at this stage the chromatic bodies cannot be distinguished with certainty.

**Synapsis.** A contraction of the dense reticulum (Fig. 40) marks the advent of synapsis when the meshes of the network become drawn together into a close mass, the synaptic knot (Fig. 41), generally near to the nucleolus. After remaining for a considerable time in this contracted condition the synaptic knot appears to loosen, when it is evident that the threads of the system have become much thicker (Figs. 42 and 43) and begin to resemble a much looped spireme. This history of synapsis is illustrated for the nucleus of the pollen mother-cell by Figs. 6-10.

**The Formation of the Chromosomes.** Following the loosening of the coils of the synaptic knot there is a rapid development of an evident spireme by the contraction of the thickened threads. The spireme (Fig. 44) is very much shorter and thicker than the system of threads that emerges from synapsis, and is not so complexly looped. A segmentation of the spireme then transforms this structure into a chain of fourteen chromosomes (Figs. 45-47, compare with Figs. 12-17). Occasionally the segmented spireme or the group of chromosome segments derived from the spireme is found so contracted (Fig. 48, compare with Fig. 18) as to suggest conditions of synapsis, but this stage, which some authors would term a 'second contraction', does not appear to be uniformly present, and is not, in the opinion of the writer, of especial significance.

**The Heterotypic Mitosis.** The fourteen chromosomes, which result from the segmentation of the spireme, appear at the equatorial plate of the heterotypic spindle in the form of rod-shaped structures (Fig. 49), some of which even at this late stage of mitosis are frequently found still arranged end to end in short chains. By continued condensation the chromosomes are further shortened, and later take on the form of V's, a condition which is most evident at metaphase (Fig. 50), and also when the two sets of



chromosomes, seven in each group, separate to pass to the respective poles of the spindle (Fig. 51). The chromosomes are not closely or uniformly associated in pairs either during their organization or in their arrangement at the equatorial plate.

The chromosomes divide lengthwise as they pass from the equatorial plate to the poles of the heterotypic spindle, so that each daughter nucleus following the heterotypic mitosis receives seven split chromosomes (Fig. 52). The seven pairs of chromosomes maintain their individuality throughout the period of interkinesis (Fig. 53), during which they increase in size.

Figs. 49–53, outlining the history of the heterotypic mitosis and the period of interkinesis in the ovule, may be compared with Figs. 19–30, which illustrate in greater detail the same events in the pollen mother-cell.

**The Homotypic Mitosis.** The seven pairs of chromosomes present during the interkinesis become by condensation much smaller during the prophase of the homotypic mitosis (Fig. 54), and have the form of short rods by the time that they are arranged on the equatorial plate (Fig. 55, compare with Fig. 33). The members of each pair are distributed by this mitosis into two sets, seven chromosomes in each, the chromosomes assuming a more irregular outline during anaphase (Fig. 56, compare with Fig. 34). With the organization of the daughter nuclei and the development of cell plates across the two homotypic spindles, a row of four cells is organized homologous with the pollen tetrad. The nuclei of these cells pass into a resting condition (Fig. 57, compare with Fig. 36), the chromatin forming a reticulum upon which lie chromatic bodies, variable in number, some of which are probably prochromosomes.

The chief stages in the development of the embryo sac were traced, and the main features of Geerts' account were confirmed. There are only two mitoses, and these result in a group of two synergids and an egg nucleus at the micropylar end of the sac with a single polar nucleus below, antipodals therefore being absent. It is very difficult to obtain good fixation of stages in the development of the embryo sac and during fertilization and in the early segmentation of the fertilized egg, and the writer is not yet ready to take up the cytological features of this phase in the life-history of *Oenothera*.

As is well known the anthers of *Oenothera biennis* discharge their pollen in the bud, so that the base of the stigma is well covered when the flower opens. Sections of the present material showed that the pollen tubes reach the embryo sacs even before the opening of the buds. With such conditions it can readily be understood that ordinarily the production of seeds by *Oenothera biennis* through cross pollination is unlikely. Cross pollination of this form in nature would appear to be probable only when its own pollen is insufficient to provide for the number of ovules matured, a possibility that seems remote.

## VEGETATIVE MITOSIS IN THE OVULE.

The nucellus of the developing ovule presents a tissue especially favourable for the study of the vegetative mitoses which proved to be in all essentials similar to those described in the anther of *O. grandiflora* (Davis '09). The resting nucleus contains a single large nucleolus, frequently accompanied by one or more smaller nucleoli, and a very delicate reticulum in which lie small deeply staining granules of varying sizes (Fig. 58). Some of the granules are sufficiently large to suggest chromatic bodies or prochromosomes, but the writer, after many attempts to establish such relationships, cannot report satisfactory results. The granules are so variable both as to their size and number that uniform counts or groupings could not be made, yet it seems probable that some of the structures are prochromosomes. It is, however, unfortunate for the prochromosome hypothesis that these structures are so difficult of demonstration in the resting nuclei of vegetative tissues, since, if they really stand for permanent structures (chromosomes) of the cell, it should be possible to follow them with a fair degree of accuracy.

The thickening of threads in the network (Fig. 59) indicates the approach of mitosis. These threads have at first a somewhat beaded appearance due to irregular thickenings or to associations of granules, but later the structure becomes more uniform (Fig. 60). Finally there is developed a much-coiled spireme (Fig. 61), which cannot, however, be traced as a single continuous thread since there are occasional points of union of the thread system.

The spireme breaks up into fourteen chromosome segments which have at first the appearance of long, variously bent rods (Figs. 62 and 63), but which by condensation become much shorter and somewhat thickened. The chromosomes, at metaphase of mitosis, may be readily counted in favourable polar views of the equatorial plate (Fig. 64). The form of the mature chromosome is that of a more or less bent rod.

During anaphase of mitosis the V-shaped daughter chromosomes are gathered into two groups with the points of the V's directed towards the poles of the spindle (Fig. 65). Following the reconstruction of the daughter nuclei (Fig. 66) the chromosomes may for a short time be recognized as bands or threads more numerous on the sides of the nuclei furthest from the cell plate, but their outlines are soon lost in the chromatic reticulum which is shortly developed. The reticulum is at first rather coarse and open, but presently becomes very delicate as the nucleus passes into the resting condition shown in Fig. 58. The large nucleolus which disappears during the mitosis is quickly formed again at an early stage in the reconstruction of the daughter nuclei.



## CYTOLOGICAL DISCUSSION.

The foregoing description of the reduction divisions in *Oenothera biennis* agrees with the account of Gates ('08) for *O. rubrinervis*, and Geerts ('09) for *O. Lamarckiana*, in the following chief particulars:—

1. The fourteen sporophytic (somatic) chromosomes that enter the heterotypic mitosis are formed by the segmenting of the spireme which follows synapsis, and are consequently arranged end to end.

2. Although some of these fourteen chromosomes may be grouped in pairs at the equatorial plate of the heterotypic spindle, they are frequently so scattered as to be quite independent of one another. Under these conditions it seems impossible to distinguish two sets of chromosomes (such as might be regarded as of paternal and maternal origin), and there is possible a degree of irregularity in their distribution which is not generally present in the heterotypic mitosis, and was not present in the material of *O. grandiflora* studied by the writer (Davis '09). The heterotypic mitosis is a reduction division.

3. The chromosomes (after the lengthwise splitting in the anaphase of the heterotypic mitosis) may be followed through the interkinesis as seven pairs of chromosomes in each resting nucleus.

4. The members of the seven pairs of chromosomes, present during the interkinesis, are distributed by the homotypic mitosis, which is therefore an equational division.

The periods of the reduction divisions outlined above, that is from the segmentation of the spireme which follows synapsis to the conclusion of the homotypic mitosis, are the least difficult of all the phases to study. Nevertheless this account of *Oenothera biennis* is not in agreement with the descriptions and figures of Gates and Geerts in a number of important particulars. The chief of these differences is in the structure of the chromosomes of the heterotypic mitosis, which in both *biennis* and *grandiflora* have the form of thickened V's and are not subglobular as described and figured by Gates and Geerts. This divergence of results at a very characteristic stage of the reduction processes is but an example of a number of important differences between the writer's accounts and those of the above authors at various phases of the periods under discussion. However, it does not seem best to discuss these different results in detail, since the reader can readily compare our various conclusions as shown by the figures.

The phases of synapsis and presynapsis are very difficult of study, and there are better reasons to expect different accounts and interpretations on the part of investigators than in the stages following the appearance of the spireme as described in the above paragraph. Geerts gives very little information on the periods of synapsis and presynapsis in *Oenothera Lamarckiana*. Gates ('08, Fig. 17) figures for *O. rubrinervis* a close associa-

tion of threads following synapsis, interpreted by him as a splitting of the spireme, which is later closed by the union of the two threads. While it is not difficult in these stages and earlier to find threads that run closely parallel with one another, the structures are so minute, and the coiled arrangements of the threads so intricate, that the writer is not convinced that these relations signify more than such an association as would naturally result from the contraction of a very complicated thread system.

The events of the synaptic contraction and of the presynaptic phases are probably of great significance, but the research so far upon *Oenothera* does not give a satisfactory explanation. All that can be said is, that from a dense reticulum, which develops during the presynaptic stages, there is differentiated a complicated thread system that during the synaptic contraction lies in intricate coils, and that from this thread system, following synapsis, is developed the very much shorter and thicker spireme. It is evident that during synapsis the threads become shorter and thicker, but it is not clear whether or not the synaptic contraction is merely the result of this process or has some deeper significance. Although the threads are reasonably distinct during synapsis, their relation to one another is obscured by the presence of substances either entangled in the thread system or forming with the threads a reticulum.

It seems to be established for *Oenothera* that the chromosomes of the heterotypic mitosis are not formed side by side through the parallel association of two spiremes, but are developed from a single spireme, which by segmentation forms a chain of fourteen chromosomes arranged end to end. This fact makes it unnecessary to assume that the synaptic contraction has for its purpose the bringing together of two distinct spiremes (of maternal and paternal origin), and it is at least possible that the peculiarities of synapsis may have no deeper significance than the contraction and condensation of the chromatin on a very long and complicated system of threads. It is not easy to understand how the chromosomes of the chain could be arranged with such precision that they are alternately of maternal and paternal origin, which would be the logical arrangement if it be assumed that the chromosomes are distributed to give pure germ cells. The ring-shaped pairs of chromosomes characteristic of the heterotypic mitosis of *Oenothera grandiflora* evidently result from the close association of adjacent segments in the loops of the spireme, and similar pairs when present in other species of *Oenothera* have probably the same method of origin.

It is clear that the evidence from this study, in agreement with the conclusions of Gates and Geerts, supports the theory of the end-to-end arrangement, following synapsis, of the full set of sporophytic (somatic) chromosomes through the segmentation of a single spireme, as held by Farmer and Moore ('05), Mottier ('07, '09), Strasburger ('04), and their followers. The fact that many of the chromosomes in *Oenothera biennis*,

*rubrinervis*, and *Lamarckiana* are not closely associated in pairs does not affect the main point, which is that they are arranged end to end in a single spireme during the prophases of the heterotypic mitosis. The very clear association of the chromosomes in pairs shown in the material of *O. grandiflora* studied by the writer is explained by the more complicated looped structure of the spireme in this form, which brings adjacent chromosome segments into such intimate relations that they remain together as ring-shaped pairs (bivalent chromosomes). The writer can see no possibility of explaining the reduction phenomena of *Oenothera* by the theory of a side-by-side pairing of chromosomes through the parallel association of two spiremes as held by Grégoire ('04, '07), Allen ('05), Rosenberg ('05, '09 a), Overton ('05, '09), and others.

With respect to the attractive theory of Rosenberg ('04, '05) and Overton ('05, '09) that chromosomes are represented in the resting nuclei by chromatic centres or prochromosomes, this study offers only indirect evidence. Chromatic bodies may be readily distinguished in resting nuclei, and it seems probable that some of these are prochromosomes, but the counts are too variable to establish their identity with the chromosomes. Furthermore, the chromatic bodies could not be traced to the chromosomes differentiated in the prophases of mitosis since their outlines became lost in the chromatic network which precedes the differentiation of the spireme. The fact that the chromosomes may be followed through the interkinesis between the heterotypic and homotypic mitoses offers strong support to the theory of the individuality of the chromosomes, but it must be borne in mind that these two mitoses (heterotypic and homotypic) are closely bound together as parts of a common process (that of chromosome reduction), the phylogenetic relations of which to the events of vegetative mitosis are not understood. The writer hoped that material fixed in the fluids of Carnoy, as employed by Overton, might give better results for the study of the chromatic bodies in resting nuclei and in the early stages of mitosis and synapsis, but such material proved to be no more favourable than that killed in Flemming's fluids.

#### SUMMARY.

1. The resting nuclei of the pollen mother-cell and megaspore mother-cell contain chromatic bodies (variable as to number), some of which are probably chromosome centres, or prochromosomes. These structures lie in a very delicate reticulum accompanied by one or more nucleoli.

2. Shortly before synapsis the nucleus becomes filled with a close reticulum, and the chromatic bodies become lost in the deeply staining strands of this dense network.

3. The advent of synapsis is marked by a slow contraction of the reticulum away from the nuclear membrane, carrying most of the strands

towards the centre of the nucleus. During the process of contraction numerous threads are differentiated from the reticulum and lie in complicated coils. Although it is frequently possible to find threads that run closely parallel with one another, the writer is unable to present any evidence that this condition is other than such an association as would be brought about by the gathering together of a complexly looped thread, or system of threads, in a tight mass.

4. The synaptic contraction draws the coils of threads into a dense knot close to the nucleolus, which generally lies at one side of the nucleus. A few loops and delicate strands extend into the nuclear cavity from the synaptic knot as a centre. The threads gradually thicken as synapsis proceeds, and the length of the thread system becomes materially shortened.

5. The chromatic material emerges from synapsis by a loosening of the coils of the contracted thread system, when it becomes evident that the threads are very much shorter and thicker, although still complexly looped and coiled. From this condition is developed by further contraction a very much thicker thread which becomes the spireme.

6. The spireme by segmentation gives rise to the fourteen sporophytic (somatic) chromosomes, which are therefore arranged end to end. These chromosome segments continue the process of contraction, which has been going on in the spireme, until their long diameter is not more than two or three times their width, and the spireme becomes so much shortened that the complexities of the looped arrangement largely disappear, and it is possible to follow the chain of fourteen chromosome segments for the entire length of the spireme.

7. The condensation and shortening of the spireme frequently draws the chromosome segments into a close group, giving the condition described as a 'second contraction'. It is, however, doubtful if this stage is a regular occurrence or of especial significance.

8. The chromosome segments of the spireme break apart during the prophase of the heterotypic mitosis, and by further contraction and bending in the middle region become the fourteen V-shaped chromosomes characteristic of this mitosis. The chromosomes are all essentially similar to one another.

9. Although not infrequently found in pairs the chromosomes have generally a scattered distribution at the equatorial plate, which may give an opportunity for numerical irregularities in their distribution by the heterotypic mitosis.

10. The fourteen sporophytic (somatic) chromosomes are distributed as two sets by the heterotypic mitosis, which is therefore a reduction division.

11. A lengthwise fission of each chromosome, in preparation for the homotypic division, becomes apparent during the anaphase of the hetero-

typic division, so that seven split chromosomes enter the resting nucleus of the interkinesis between these two mitoses.

12. The seven split chromosomes may be readily followed as seven pairs throughout the period of interkinesis.

13. The homotypic mitosis is an equational division distributing the members of each pair of chromosomes, seven to each daughter nucleus.

14. The reduction divisions in the ovule result in a row of four cells homologous with the pollen tetrad. The embryo sac appears to develop rather more frequently from the lowest cell of this row (i. e. the cell farthest away from the micropylar end), although often arising from the upper cell.

15. The vegetative mitoses in the nucellus of the ovule are in all essentials similar to those described for the anther of *Oenothera grandiflora* (Davis '09).

CAMBRIDGE, MASSACHUSETTS,  
April, 1910.

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## EXPLANATION OF FIGURES IN PLATES LII AND LIII.

Illustrating Prof. Davis's paper on the reduction divisions of *Oenothera biennis*.

All figures were sketched with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 mm. (num. aper. 1.30) in combination with the compensating ocular No. 12, giving a magnification of 2,000 diameters. Figure 1 from section 4  $\mu$  thick; Figures 2-11, 24, 25, 27-36, and 42, from sections 5  $\mu$  thick; 12-23, 26, 37-41, and 43-66, from sections 6  $\mu$  thick. Sections stained with iron-alum haematoxylin.

### PLATE LII.

*Oenothera biennis*.

Figs. 1-32. Illustrating reduction phenomena in the pollen mother-cell.

Fig. 1. Resting nucleus in pollen mother-cell, showing a large and a small nucleolus, chromatic bodies, and delicate reticulum.

Fig. 2. Similar to Fig. 1, but with a denser reticulum.

Fig. 3. Nucleus neighbouring to that shown in Fig. 2, the strands of the reticulum becoming thicker.

Fig. 4. Two nuclei separated by a single cell: *a*, with a delicate reticulum; *b*, with a much coarser network.

Fig. 5. Nucleus filled with a dense reticulum shortly before the advent of synapsis, chromatic bodies no longer distinguishable.

Fig. 6. The beginning of the synaptic contraction.

Fig. 7. Synaptic contraction well under way, the threads and meshes of the reticulum drawn into a close mass.

Fig. 8. A closely contracted synaptic knot.

Fig. 9. A synaptic knot with loops and threads extending into the nuclear cavity.

Figs. 10 and 11. Nuclei from opposite ends of the same pollen chamber. Fig. 10, from lower end, the threads beginning to shorten and thicken to form the spireme. Fig. 11, from upper end, the spireme which emerges from the synaptic contraction following the shortening and thickening of the thread system.

Fig. 12. A spireme with constrictions which show that the process of segmentation has begun.

Fig. 13. A segmented spireme.

Fig. 14. The segments of the spireme by condensation of the chromatin have become much shorter and thicker.

Figs. 15 and 16. The segments of the spireme are more nearly the size of the chromosomes present at metaphase of the heterotypic mitosis (compare with Figs. 20-23); their end-to-end arrangement is still clearly evident.

Figs. 17 and 18. The chromosome segments in about the same stage as shown in Figs. 15 and 16, but gathered in a close mass which bears a superficial resemblance to a synaptic knot. This is apparently the stage termed by some authors a 'second contraction'.

Fig. 19. Prophase of the heterotypic mitosis. Most of the fourteen chromosomes in the centre of the multipolar spindle are united end to end, nucleolus no longer present.

Fig. 20. Spindle taking on a bipolar form, many of the chromosomes still arranged end to end.

Fig. 21. Bipolar spindle, the fourteen chromosomes gathered in an irregular group, certain of them apparently associated in pairs.

Fig. 22. Metaphase of the heterotypic mitosis. The chromosomes, now bent in the form of thickened V's, show no very close association in pairs.

Fig. 23. Anaphase of the heterotypic mitosis. Seven chromosomes are in each of the two sets which pass to the poles of the spindle.



Fig. 24. Telophase of the heterotypic mitosis. The seven chromosomes at each pole of the spindle are split lengthwise.

Fig. 25. A group of seven split chromosomes gathered at the pole of the heterotypic spindle, viewed from above.

Fig. 26. A group of seven split chromosomes just before the organization of the daughter nuclei following the heterotypic mitosis.

Fig. 27. An irregular distribution of chromosomes by the heterotypic mitosis. Eight chromosomes instead of seven have been brought to the pole of the spindle; five chromosomes (one missing) were found at the opposite pole.

Figs. 28 and 29. Resting nuclei of the interkinesis between the heterotypic and homotypic mitoses. Seven pairs of chromosomes are present in each nucleus, mostly in the form of U's joined together in the bent middle region.

Fig. 30. Nucleus of the interkinesis surrounded by a web of fibrillae preparatory to the organization of the homotypic spindle.

Figs. 31 and 32. The seven pairs of chromosomes being gathered at the equatorial plate shortly before the metaphase of the homotypic mitosis.

### PLATE LIII.

#### *Oenothera biennis.*

Figs. 33-36. Illustrating reduction phenomena in the pollen mother-cell.

Fig. 33. Metaphase of the homotypic mitosis; seven pairs of chromosomes at the equatorial plate. The two spindles lie at right angles in the pollen mother-cell, so that the equatorial plate of one spindle is viewed from the pole.

Fig. 34. Anaphase of the homotypic mitosis; the chromosomes of the two sets, seven in each group, show irregularities of form.

Fig. 35. Telophase of the homotypic mitosis; the seven chromosomes in each nucleus still distinct.

Fig. 36. Nucleus of a young pollen grain, showing the open reticulum upon which lie deeply staining chromatic bodies (prochromosomes?).

Figs. 37-57. Illustrating reduction phenomena in the ovule.

Fig. 37. Resting nucleus in megaspore mother-cell, showing large and small nucleolus, chromatic bodies, and delicate reticulum.

Fig. 38. Development of a much coarser reticulum from the stage shown above, chromatic bodies no longer distinguishable.

Fig. 39. Nucleus filled with a dense reticulum just before the advent of synapsis.

Fig. 40. The synaptic contraction under way.

Fig. 41. A synaptic knot.

Figs. 42 and 43. The thickened threads which emerge with the loosening of the synaptic knot.

Fig. 44. Further shortening and thickening of the threads to form the spireme.

Fig. 45. A segmented spireme.

Figs. 46 and 47. The chromosome segments, for the most part still arranged end to end.

Fig. 48. Chromosome segments gathered in a close group bearing a superficial resemblance to a synaptic knot, apparently the stage termed by some authors a 'second contraction'.

Fig. 49. Bipolar spindle, the fourteen chromosomes gathered in an irregular group.

Figs. 50 and 51. Metaphase of the heterotypic mitosis; the chromosomes bent mostly in the form of thickened V's.

Fig. 52. Telophase of the heterotypic mitosis; the chromosomes at the poles of the spindle are split lengthwise.

Fig. 53. Resting nucleus of the interkinesis between the heterotypic and homotypic mitoses. Seven pairs of chromosomes are present, mostly in the form of U's joined together in the bent middle region.

Fig. 54. Prophases of the homotypic mitosis in companion cells, showing multipolar spindles and the seven pairs of chromosomes.

Fig. 55. Metaphase of the homotypic mitosis.

Fig. 56. Anaphase of the homotypic mitosis, showing the two sets of daughter chromosomes, seven in each set, that result from the separation of the members of the pairs.

Figs. 57. Nucleus of megaspore, showing reticulum and deeply staining chromatic bodies (prochromosomes?).

Figs. 58-66. Illustrating the vegetative mitoses in the cells of the nucellus.

Fig. 58. Resting nucleus, showing delicate reticulum, minute chromatic bodies, and large nucleolus.

Figs. 59 and 60. The thickening of the strands of the reticulum preparatory to the formation of the spireme.

Fig. 61. The spireme giving evidence of segmentation to form the chromosomes.

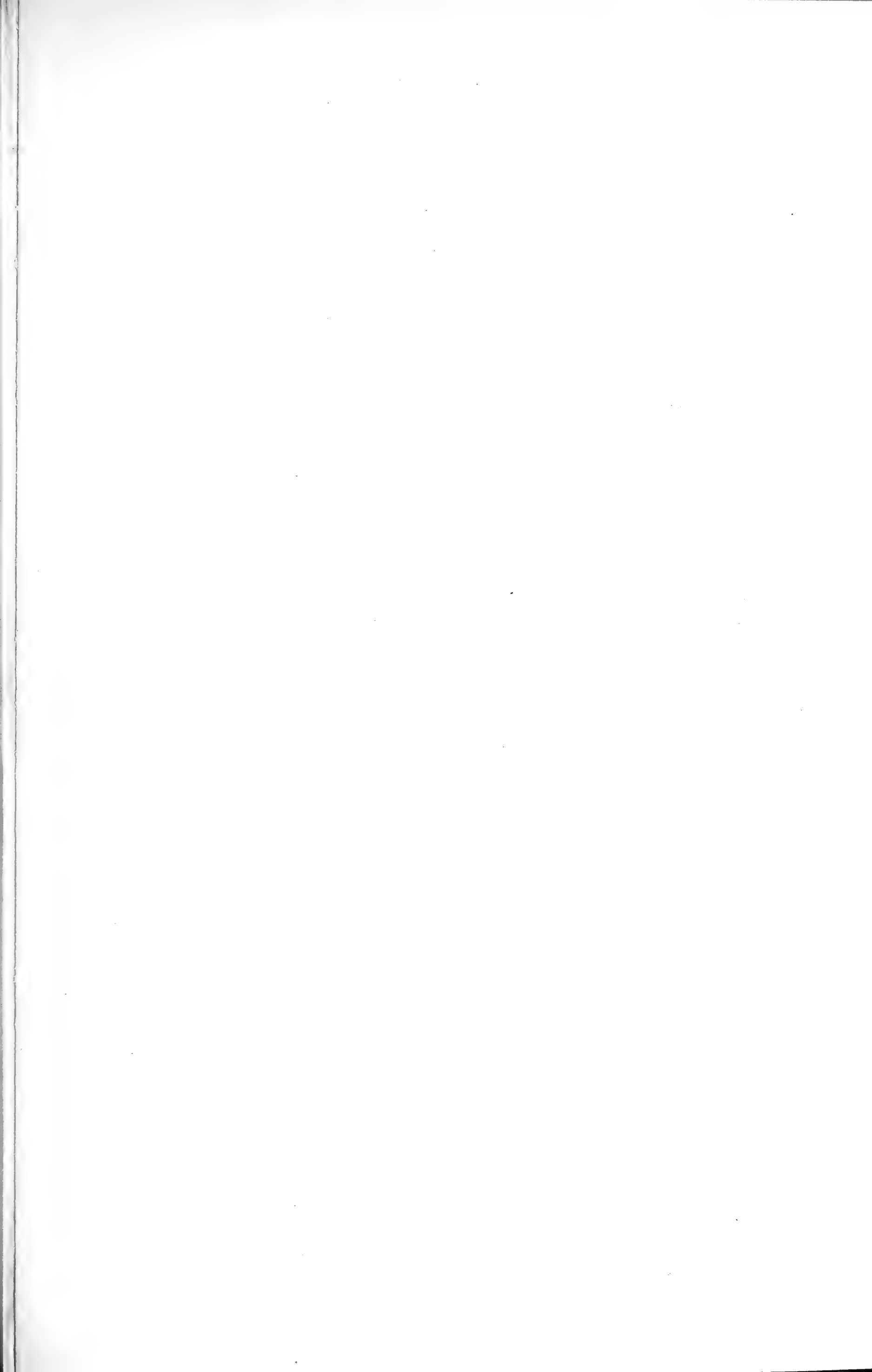
Figs. 62 and 63. Chromosome segments of the spireme.

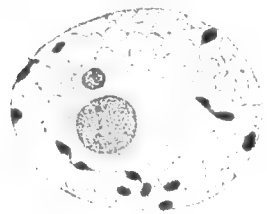
Fig. 64. The group of fourteen chromosomes at the equatorial plate viewed from above.

Fig. 65. Anaphase of a vegetative mitosis; the daughter chromosomes gathered at the poles of the spindle in the form of attenuated V's.

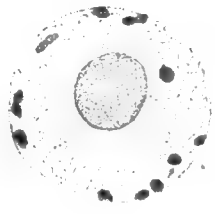
Fig. 66. Daughter nuclei; some of the chromosomes still recognizable as bands or threads, more numerous on the side of the nucleus farthest from the equatorial plate.







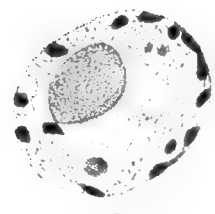
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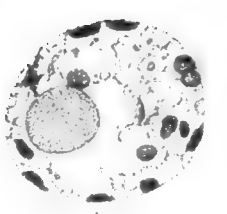
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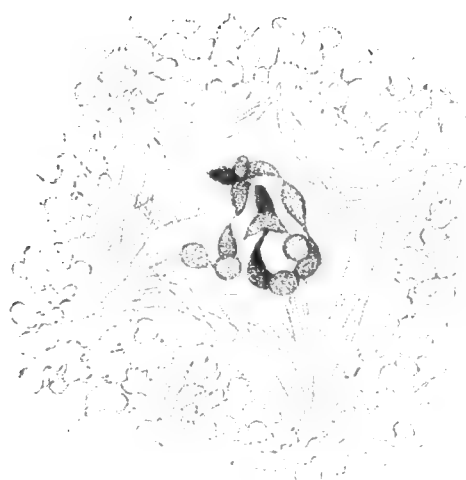
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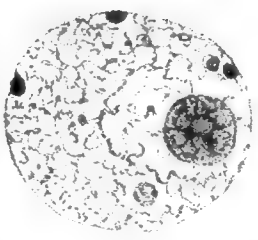
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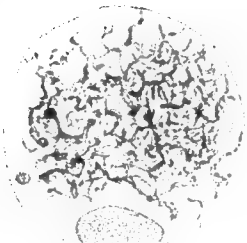
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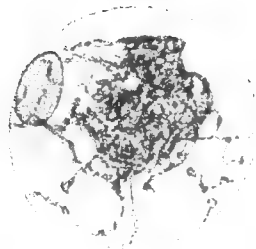
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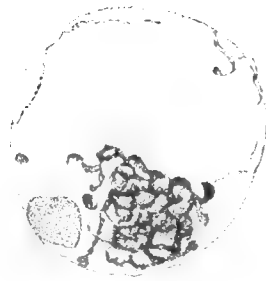
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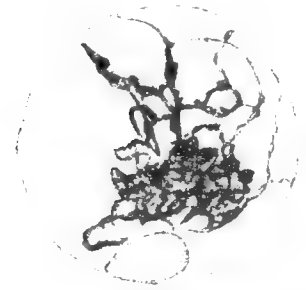
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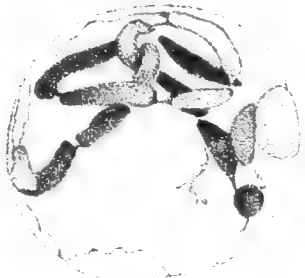
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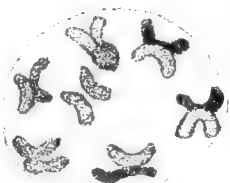
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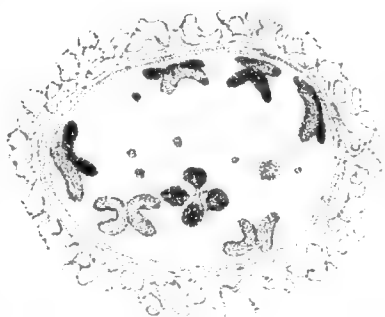
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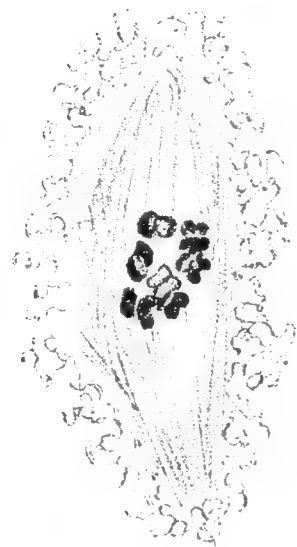
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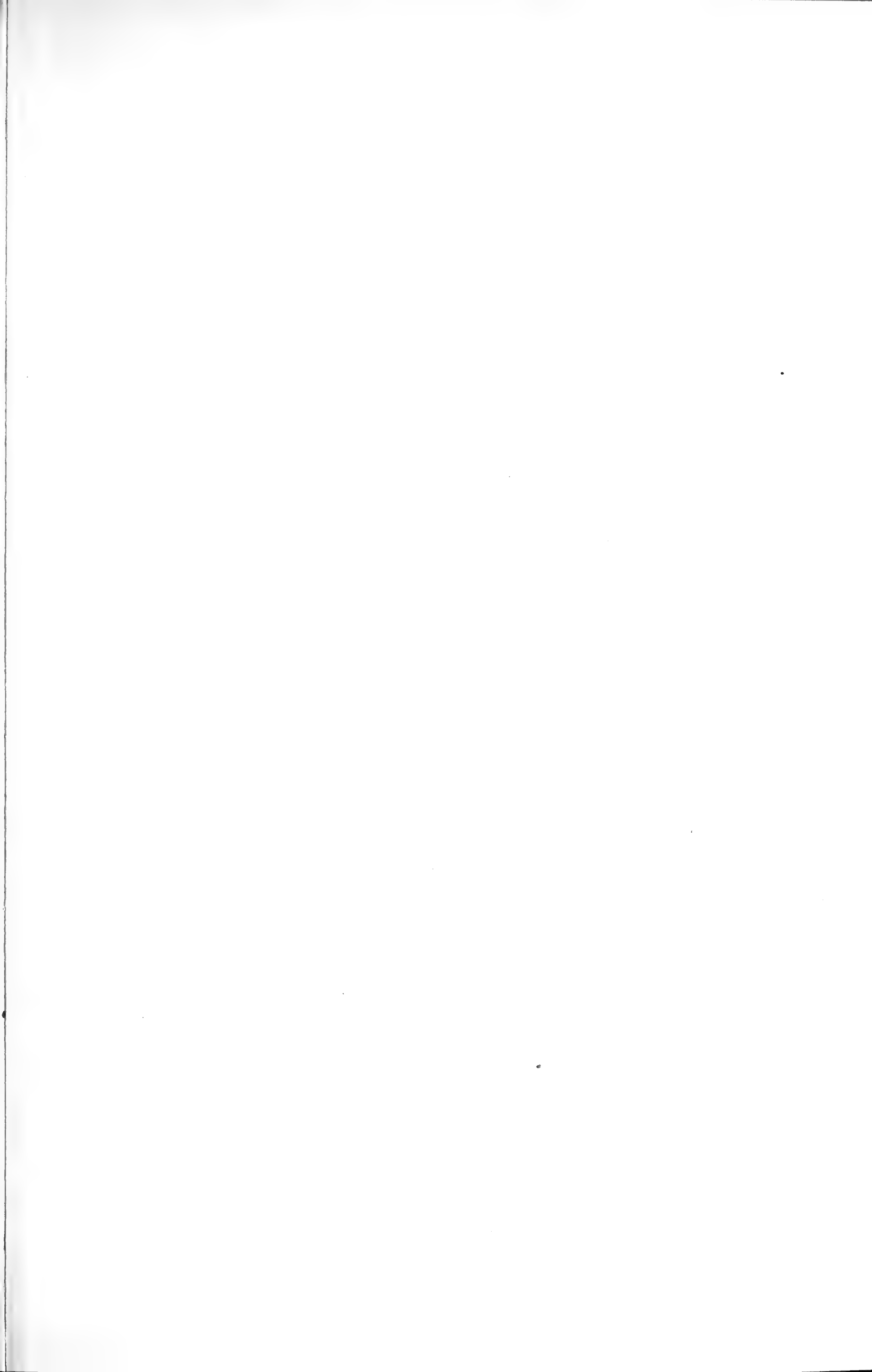
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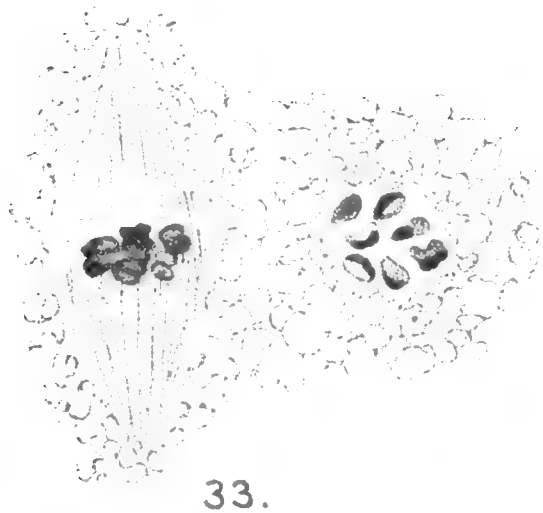


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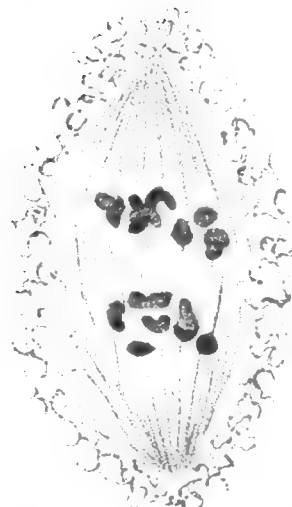




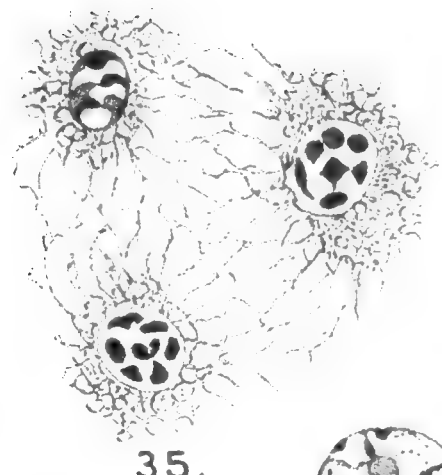




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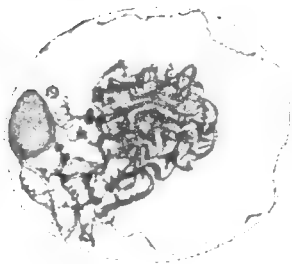
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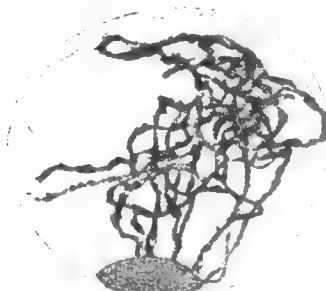
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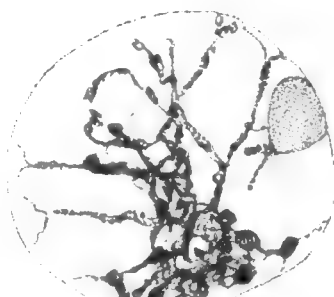
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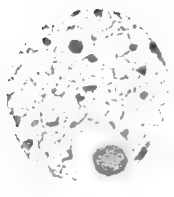
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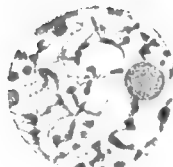
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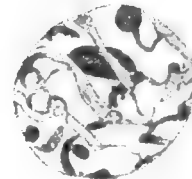
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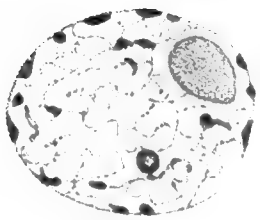


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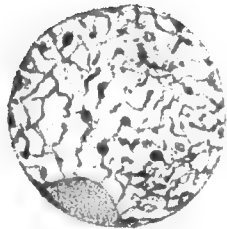


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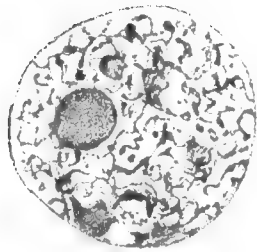
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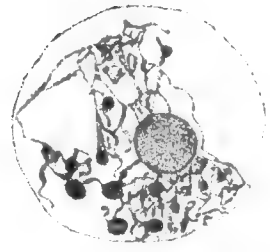
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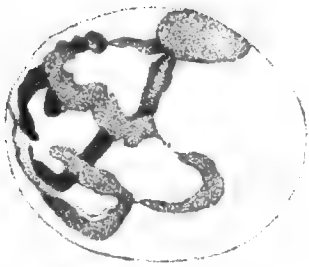
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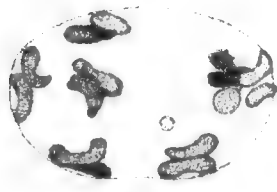
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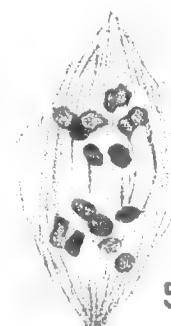
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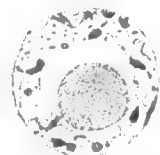
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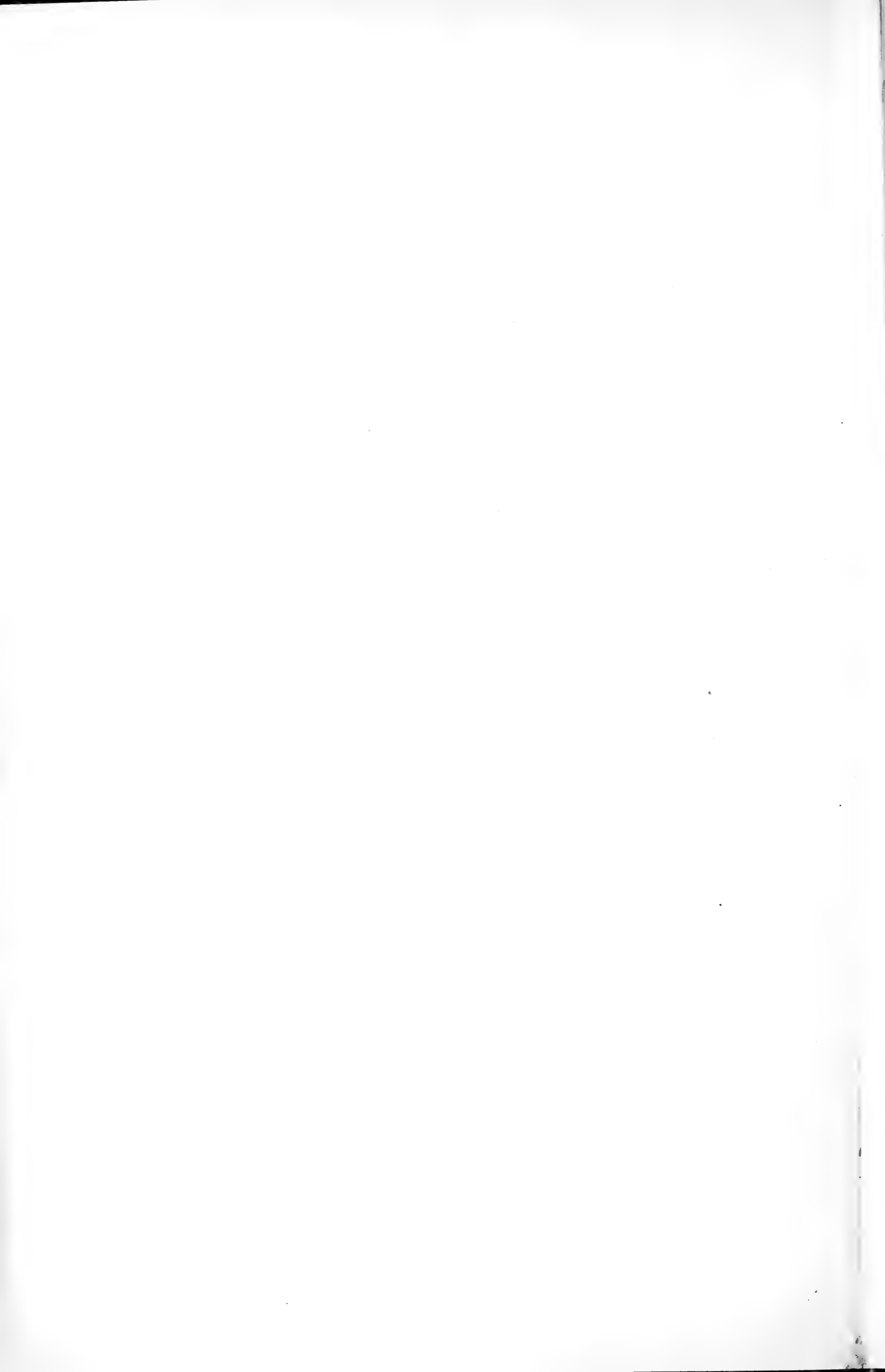
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# The Mechanism of Nuclear Division.

BY

W. LAWRENCE BALLS, M.A.

*Fellow of St. John's College, Cambridge; Botanist to the Khedivial Agricultural Society, Cairo;  
Membre de l'Institut Égyptien.*

With Plate LIV and one Figure in the Text.

## INTRODUCTION.<sup>1</sup>

MORE attention has been given by cytologists to the chromatic substances of the nucleus than to the achromatic substances, partly on account of the difficulty of observing the latter.

Attention has been further attracted to the chromosomes by the discovery of numerical constancy, by their localization as the germ-plasm, and lately by Mendelian developments. The study of the achromatic structures has lagged behind, and our knowledge of their behaviour in the process of karyokinesis is consequently incomplete.

We have no good evidence as to the way in which the spindle fibres are formed, nor of the changes which take place between the splitting of the spireme and their appearance. Many hypotheses have been advanced concerning the spindle fibres, but none of them hold out any hope of generalization. Botanical studies on these structures received a check by the disproof of Guignard's work on the centrosome, and the artifact preparations obtained by other workers have made the cytologist very chary of publication.

<sup>1</sup> Since this paper will presumably be read by professed cytologists, to whose ranks the writer cannot claim to belong, a word regarding the circumstances of its publication seems advisable, by way of apology. The observations arose during an examination of the sexual cytology of Egyptian cotton, made in 1905 as a preliminary to Mendelian research on this plant. They were continued for a year, and the present paper, in its present form, was written during the summer of 1906. The conclusions seemed so heterodox that publication was delayed till more evidence could be accumulated from other plants, such as *Hibiscus spp.*, which might be expected to show similar phenomena to those exhibited by *Gossypium*. Pressure of other work in Genetics and Physiology has prevented the acquisition of such supplementary evidence, and this four-year-old account is now being published in the hope that other botanists, better qualified than the writer, may find something of use in its pages, even if the generalization here advanced may not hold good.

The substance of this paper was communicated to the Cairo Scientific Society early in 1906, and an abstract was read and published at the Dublin meeting of the British Association in 1908.

[Annals of Botany, Vol. XXIV. No. XCVI. October, 1910.]



The large amount of 'achromatin' in the nuclei of the cotton-plant has enabled me to study there some processes which are not readily seen elsewhere. The interpretations which I have placed upon these observations appear to correlate several parts of the problem of the achromatic structures, but the work needs to be tested by other observers.

If the results about to be described are confirmed, it will probably be found that the principle of the matter holds good for the whole organic world.

#### HISTORICAL SUMMARY.<sup>1</sup>

Bütschli, Van Beneden, and Strasburger (in his early researches) believed that the whole mitotic figure was formed from nuclear substance. Fol also considered that the spindle was derived from the nucleus, but regarded the asters as of cytoplasmic origin.

Strasburger changed his opinion at a later period and formulated the conceptions of 'kinoplasm' and 'trophoplasm'. Both of these were cytoplasm in origin; the former being seen as threads which invade the nucleus when the nuclear wall disappears, and acting as the motor mechanism in cell-division.

#### *The Spindle Fibres.*

The mode of origin of the spindle fibres has been explained in almost every conceivable way by those who hold them to be tangible structures. Further confusion is introduced by those who do not see their way to accepting them as real, but regard them as manifestations of some physical stress in the protoplasm.

The popular comparison of the spindle to a field of force between two opposite magnetic poles is only persuasive when the observed organism develops centrosomes and asters. We can conceive of the centrosome as a storm-centre of metabolism which radiates lines of metabolic disturbance, although the centrosome is a very minute organ; but when the astral rays are missing, and the single centre has broken up into a score, or more, must we not then assume that the single line from the inner side of each of these is the sole manifestation of this intense metabolic change? There seems to be no reason why this change should be confined to one side of a granule which is smaller than the wave length of sodium light.

Even if we grant the existence of these lines of metabolic stress, we still lack an explanation of the way in which the chromosome is moved.

Strasburger's suggestion that the chromosomes are chemotactically sensible seems rather unlikely. The chromosomes would have to be self-motile. Now, the chromosome is so closely concerned with the transmission of heritable characters—a function of fundamental importance—that

<sup>1</sup> For references see E. B. Wilson, *The Cell in Development and Inheritance*.

during its phylogeny it has probably lost all other powers and become a passive organ, handed about by other organs which are the blind agents for the distribution of the controlling substance, although they themselves are incapable of directing the fortunes of the cell.

Some such unprejudiced mechanism is required to explain the Mendelian facts of gametic distribution, and the achromatic structures seem to be this mechanism.

The only satisfactory explanation seems to be given by admitting the reality of the spindle fibres, and by assuming the power of contractility in the achromatic structures generally. There is no assumption of vitalism in the latter step, for the fact of fibrillar contractility is fully acknowledged in pseudopodia and cilia. There is, moreover, a probability that future physical research upon surface tension in thin films and threads may be able to give us a physical explanation of the phenomenon. Contractility in the spindle fibres was first advocated by Klein and Van Beneden; Boveri demonstrated that the fibres became shorter and thicker; Hermann showed that the central spindle fibres elongated in mitosis. Hertwig and Wilson were unable to see any increase in the thickness of the fibres, but it must be remembered that these fibres are so delicate that one may be unable to distinguish the diffraction image of a thin one from the real image of a thicker one.

Strasburger has cleared the way for a conception of motor mechanism in cell-division, or 'kinoplasm', but he does not differentiate between the nuclear and cytoplasmic portions, whereas I have reason to consider the two forms as morphologically distinct, though perhaps chemically similar or identical.

#### *The Centrosome.*

A persistent nucleolo-centrosome which divides to form a spindle is found in some unicellular organisms. This body is considered as equivalent to the centrosome. The 'end-plates' of *Actinosphaerium* arise by the division of a nucleolus or plasmosome, and it would seem from the general evidence of these primitive organisms that the achromatic plasmosome and the centrosome have a genetic relationship. This is even more distinct in the case of *Paramoeba*, whose 'Nebenkörper' consist of extruded achromatic substance. From this we pass to Hertwig's work on *Actinosphaerium*, where the centrosomes are proved to arise from the reticulum which is extruded at the poles, and this agrees with the increasing number of cases recorded in which the centrosomes are formed anew.

When asters are found in organic cells they generally seem to be of cytoplasmic origin, and independent of the centrosome. The observations recorded below have led me to the opinion that the asters are heterogeneous, the outer portion being merely stream-lines in the cytoplasm, while the remainder of the rays are true fibres, derived from the achromatic nuclear

structures and connected with the modified portion of those structures which is called the centrosome.

In the higher plants the centrosome is again absent, and the scheme of division (as interpreted below) is similar to that of the *Infusoria*. We may hope that the results to be obtained by study of the fate of the achromatic structures in higher plants will ultimately be translated into terms of the specialized centrosome of the lower plants and the animals.

#### CELL-DIVISION IN COTTON.

While studying the cytology of the flower of Egyptian cotton<sup>1</sup> during the summer of 1905, I noted certain novel and curious structures in cells which were undergoing the reduction division.

The staminal column turns yellow when the flower bud is still young, the anthers having previously been of a pale buff colour. Material taken about two days before this change of colour shows the microspore mother-cells in all stages of development, from synapsis to walled spores, in a single flower.

The synaptic stage (Fig. 2, Pl. LIV) is of the usual kind. The nucleus is comparatively small—about twenty microns in diameter—and contains a large plasmosome, which stains darkly with Heidenhain's haematoxylin. A lightly stained, densely tangled, coiled thread is connected to this nucleolus. The spireme thread appears to be continuous. The spireme thread then begins to open out, and becomes a looser tangle (Fig. 3). In it are embedded rows of darkly-staining granules, each one showing a distinct longitudinal bisection.

The nucleolus next decreases in size, and the granules stain more darkly; the shrinking of the nucleolus appears to be very rapid, for the stage is rare; the darkening of the granules is localized to those portions of the thread which correspond to the future position of the chromosomes. Except in these portions the thread of the spireme is now split.

Each of these clusters of darkened granules becomes a mass of chromatin, or bivalent chromosome. This chromosome is not, however, merely bisected as were the granules, but is also divided transversely to the axis of the spireme thread; four perfectly distinct chromatic areas are thus formed, being the four univalent chromosomes which are to be distributed to the four microspores. These quarters, or daughter chromosomes, are roughly spherical, not elongated. By this time (Fig. 4) the nucleolus is no wider than the spireme thread, with which it is seen to be continuous on both sides, and it scarcely stains at all with haematoxylin. All trace of it is lost shortly afterwards.

<sup>1</sup> Balls, W. Lawrence: *The Sexuality of Cotton*. Khed. Agric. Soc. Yr. Bk., 1905. For systematic position of the variety studied (Affi) see Sir G. Watt's *Wild and Cultivated Cottons*.

The mode of formation of these chromosomes rather suggests a transference of chromatin from the nucleolus to the chromosome areas by progressive chemical change in the original paired granules.

The chromosomes are bunched together at one side of the nucleus. There is no regular peripheral distribution. In this respect cotton differs from most organisms, and from its own vegetative cells. These chromosomes are minute, being only 0.6 micron in diameter, while the univalent chromosomes are correspondingly smaller still. In consequence of this fact the achromatic substance of the nucleus is quite conspicuous, in spite of its slight stain affinity.

The nucleus of the microspore mother-cell thus consists at this stage of twenty chromosomes grouped to one side, and these chromosomes lie in a continuous split spireme thread which is composed of achromatic substance. The nuclear wall has faded away, and the periphery of the nucleus is occupied by a densely granular zone of cytoplasm, which has been visible outside the wall for some time.

The next stage is the most important one, and also the most difficult one to observe. It is completed rapidly—to judge by its comparative rarity—and the achromatic structures form a most complex tangle. The sequence of events appears to be as follows:—The two halves of the split spireme thread move apart from one another at the side where the chromosomes are lying, but this separation does not affect the chromosomes. The chromosomes are isolated by the removal of the spireme halves, but not entirely, for continuity with the latter is *maintained by thin filaments on either side* (Fig. 5). The insertion of these filaments (the young spindle fibres) in the spireme halves causes slight swellings which appear to the eye as black dots.

From this point I propose to refer to the spireme halves as the 'thread-rings', retaining the term 'fibre' for its usual subject.

The two thread-rings continue to separate, the fibres becoming longer and longer, until the part of each thread-ring which bears the dots has moved to a pole of the nucleus. The dots are scattered round some 100° of arc, and the remainder of each thread-ring forms an irregular tangle of loops, which lies between the centrally suspended chromosomes and the granular cytoplasm (Fig. 6). The looping of thread-rings seems to be due to the fact that the circumference of the close spireme is greater than the circumference of the nucleus. This stage constitutes the multipolar spindle.

The next event is the contraction of the dotted portions of the thread-rings, bringing the dots nearer together, and forming the bipolar spindle of metaphase (Fig. 7). The looped thread-rings lying in the clear zone between spindle and granular cytoplasm are quite conspicuous; this was the observation which initiated the present research. It sometimes happens that a stray dot is not drawn up into the cluster at the pole as soon as it

should have been; in this case the spindle fibre which ends in it remains at the side of the spindle, often slack and bent (Fig. 8) instead of being drawn taut.

The polar dots at the ends of the fibres have been noticed by other observers, but I can find no mention of their cross-connexion by the threads.

When separation of the chromosomes takes place in anaphase, the halves retain their continuity by means of fibres; these inter-chromosome fibres appear to be drawn out from the achromatic matrix of the chromosome. The original fibres between chromosome and dot become shorter without any noticeable thickening.

In telophase of this first division the cytoplasm invades the space between the two daughter nuclei, and the inter-chromosome fibres seem to be dissolved in it. There is, however, no disintegration of the thread-rings themselves; it seems quite plain, from the position of their loops in late telophase, and in early prophase of the second division (Fig. 9), that each of the two rings retracts into the daughter nucleus at its own pole, viz. at the pole where it is attached to spindle fibres. In this way the continuity of the thread-ring is *never broken*; the chromosomes are retracted into it, and it proceeds to divide again.

*The second division* follows the first without any noticeable delay. It is even less easy to observe, the spindle being smaller and barrel-shaped and leaving hardly any clear zone, but the threads are again visible, and the fibres terminate on them in dots as before.

The thread-ring divides longitudinally again. This division is visible in the earliest second division prophase (Fig. 10). Indeed, there is some probability (though this is not certain, on account of the complex looping) that it is visible in metaphase of the first division, or even earlier; when the two spireme halves are separating in first prophase there is occasionally a slight indication of a second division of each half. This would produce a fourfold spireme thread, in places, from the very beginning of the reduction division; it should be noted that this quartering is not effected in the same way as the quartering of the bivalent chromosome. The superposition of the loops of the threads in early prophase of the second division is very marked.

The mode of isolation of the chromosomes from the rings has not been clearly seen in second division. If the splitting of the rings precedes this division, then each chromosome is drawn up to the pole by at least two fibres, and the rings have simply to separate. Otherwise, the chromosome is presumably retracted into the ring, and the process of splitting repeated as in the first division.

In the beginning of the process of reconstitution of the four microspore nuclei it can be seen that the chromosomes are connected by threads and fibres, with the 'black dots' at the junction of these latter. It would



seem that the black dots enlarge later, becoming the nodes of the reticulum in some cases. The wall of the nucleus seems to be entirely cytoplasmic in origin. Those spindle fibres which are divided by the wall fade away centripetally up to the wall, while the portion of each fibre inside persists, for a time at least, ending blindly in the wall at one end, and still connected at the other end with the chromosome from which it was formed.

The method of formation of those spindle fibres which run from pole to pole without bearing chromosomes has not yet been mentioned. They do not appear to be common in the nuclei of the cotton-plant; their mode of formation is the same as that of the other fibres, except that they are drawn out from the separating thread-rings at points other than those where the chromosomes are found.

The nuclei of the vegetative cells of the cotton-plant are very minute.<sup>1</sup> A few observations have been made on them, using the root-tip of seedlings, but the ordinary vegetative cells of the flower tissues are far too small for study.

The scheme of division outlined above seems to apply to these cells also, with structural modifications due to the smaller circumference of the spireme relatively to the nucleus. The spireme has about the same diameter as the nucleus itself, and the achromatic threads are thicker; there are, consequently, no tangled loops to confuse the observer, but the larger number of chromosomes compensates for this. The chromosomes are not grouped to one side, but are symmetrically distributed in prophase around the periphery of the nucleus (Fig. 11). The two spireme halves separate directly in parallel planes, instead of rotating over one another as in the reduction division, being evenly tied together by the spindle fibres. The spindle thus produced (Fig. 12) is very fat and broad, while the rings at each end are frequently quite conspicuous. The actual separation of the spireme halves, and the isolation of the chromosomes, is thus more easily recognized (Fig. 11) than it is in the reduction division, although the nucleus is smaller. At the same time, the absence of loose loops of the thread-rings prevents the appearance of any striking structures. The 'dots' are often very distinct, and sometimes of appreciable size, being almost fit for description as plasmosomes.

The large plasmosome, or nucleolus, may either disappear or persist. In the latter case it may present appearances which suggest Wager's figures of the nucleolus in *Phaseolus*,<sup>2</sup> excepting that his figures do not show the plasmosome to be in connexion with the thread-rings by means of thin filaments. These filaments give it the slightly stellate (Fig. 11) appearance. About equally common is another arrangement (Fig. 13), in which the plasmosome is merely a local dilatation of the thread-ring.

<sup>1</sup> About 10  $\mu$  in diameter.

<sup>2</sup> Ann. Bot., 1903.



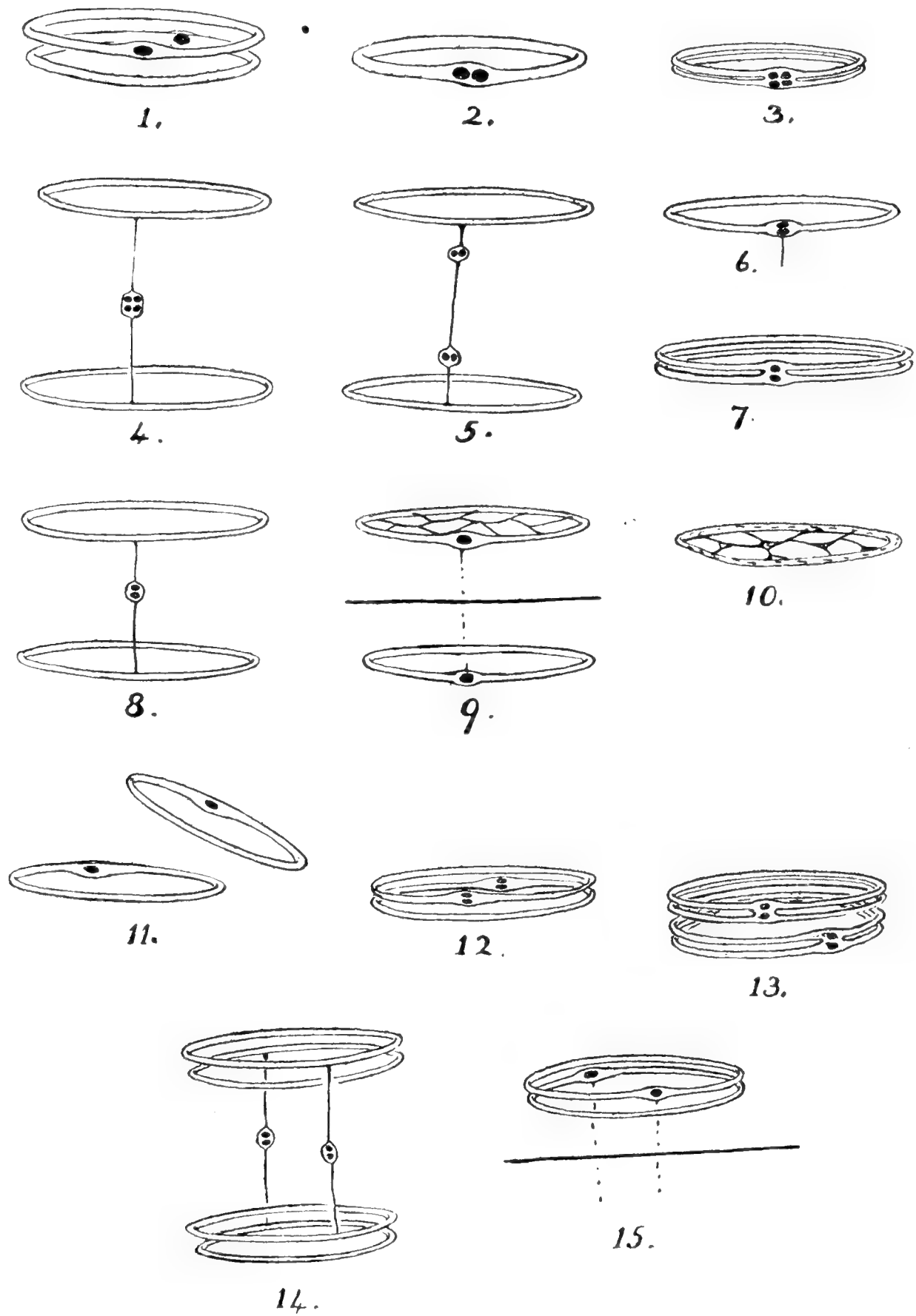


DIAGRAM OF THE NUCLEAR MECHANISM.

Diagrams made on the assumption that the fusion of the gamete nuclei is delayed until synapsis. If this fusion takes place at fertilization we have a simpler scheme. Fig. 1 then resembles Fig. 2, except in the chromosomes, and Figs. 12-15 are simpler.

1. Somatic nucleus with two chromosomes, each in its own thread-ring: i.e. one chromosome and ring from each gamete.

2. Synapsis; fusion of the rings, bivalent chromosomes.

First reduction division:—3. Prophase; 4. Metaphase; 5. Anaphase; and 6. Telophase.

Second reduction division:—7. Prophase; 8. Metaphase; and 9. Telophase, with one represented as having cross-bridles.

10. Gamete nucleus; chromatic substance distributed through thread-ring and bridles.

11. Gamete nuclei approaching in fertilization.

12. First division of zygote: chromosomes dividing.

13.       "       "       prophase  
14.       "       "       metaphase } Any somatic division.  
15.       "       "       telophase

The nucleolus is thus a mere swelling in the achromatic structures, whether in dividing or resting nuclei.

#### DISCUSSION OF OBSERVATIONS ON COTTON.

The two criticisms to which all cytological work has to submit relate to the pre-existence of observed structures in the living cell, and to the accuracy of the observations made upon those structures.

In respect to the first point we have the following facts in support of the reality of the thread-rings. The rings were seen<sup>1</sup> in metaphase from material which had been very badly fixed with acetic-absolute. The majority of the observations were made on material fixed with chrom-acetic, or with strong Flemming; the fixative was injected by an air-pump, transference through alcohols, xylols, and paraffin by twelve-hour stages took ten days, and after sections of five to eight microns thick had been cut, they were stained with Heidenhain's haematoxylin. In such material the fixation was excellent, even the cytoplasm being but little distorted.<sup>2</sup> The appearances were consistent, and similar at each stage, differing only in such details as the number and position of the loops of the thread-rings. The loops are far more conspicuous—individually—than the spindle fibres, and if the reality of the latter is granted, that of the loops and black dots must also be conceded.

The 'artifact criticism' is of the greatest value in exercising a sceptical control over generalization upon insufficient evidence. Nevertheless, when, as in this case, an elaborate structure is developed stage by stage with the development of the cell which contains it, when the same structures can be recognized at every stage, and when nothing inconsistent with expectation makes its appearance, then the burden of proof rests on the artifact critic.

The fact that these structures have not before been found in other plants is only partially negative evidence against them; the dots at the ends of the spindle fibres have been seen by other observers. It must be borne in mind that the twenty chromosomes of cotton are extremely minute, that the nucleus is merely small, and that there is a large proportion of achromatic substance in comparison with the amount of chromatic substance.

The writer is less optimistic with regard to the accuracy of the observations. Still, he believes that subsequent corrections will only affect details, most of which have been indicated above. The absence of proper cytological refinements, microphotographic apparatus, and especially of a mercury vapour lamp, have all been against great accuracy of vision.

The Zeiss IV A stand was used, with Abbe condenser. The lens first employed was only the  $\frac{1}{12}$ -inch achromatic oil immersion; the main facts were ascertained with this. Subsequently the Khedivial Agricultural

<sup>1</sup> See also note on Cannon's observations at the end of this paper.

<sup>2</sup> See Figs. 1 and 2.

Society very kindly procured me the Zeiss 3 mm., 1.40 num. ap., hom. imm., apochromatic objective; the rest of the work was done entirely with this. The eyepieces used were Nos. 2 and 4, with the Zeiss compensating oculars 12 and 18, on a tube of 160 mm.; most of the observations were effected with the 12, using the 18 to differentiate in depth.

Lighting had to be considered very carefully, for the univalent chromosomes are little larger than the wave length of sodium light. It was therefore necessary to reduce the wave length of the light employed. In this respect the use of haemotoxylin had a great advantage, as it stains from black to grey. A water tank filled with a strong filtered solution of copper oxide in ammonia was placed before the Welsbach incandescent lamp, and its concentration was adjusted by the spectroscope until no light passed through below the D line. The full aperture of the condenser was used.

When the Welsbach lamp was in use, an image of the crossing of two wires of the mantle was focused upon the object. Later on, critical illumination was obtained by using a flat-wicked oil lamp.

Microphotographs were made of all the important stages. This method had its limitations, on account of the roughness of the apparatus, but it gave evidence in support of the eye.

The precise position assumed by the bivalent chromosome at the splitting of the spireme, and the number of spindle fibres formed for each chromosome, could not be ascertained by the methods I employed.

#### CONCLUSIONS.

The nucleus is an independent portion of the cell, morphologically discontinuous from the cytoplasm, and consisting solely of chromatin and achromatic substances.

The nuclear sap and membrane belong to the cytoplasm, serving to conduct the chemical interchange between nucleus and cytoplasm.

Assuming that our arbitrary mode of recognition of chromatin and achromatin is approximately just, it would seem that their respective functions might be somewhat as follows:—

##### *Chromatin.*

A mixture of complex bodies, probably the bearers of the hereditary qualities, incapable of automatic motion, constant in composition for any given race, and capable of synthesis only upon a pre-existing basis of their own kind.

The movement of chromatin along the linin thread from nucleolus to chromosome, and conversely, is due to progressive conversion of oxy-chromatin into chromatin, granule by granule. The excretion of chromatin which seems to take place in some cases might be merely extrusion of unnecessary, duplicate 'molecules'. When such extrusion is chemical the residue is oxy-chromatin.

*Achromatic substances.*

Loosely divided into 'oxy-chromatin' and 'linin'. Oxy-chromatin is closely related to chromatin, but its synthesis from, or by, the linin can take place without the pre-existence of the 'molecular basis' (of chromatin) necessary for the formation of chromatin.

The linin is of simpler composition, but very unstable; in consequence of this instability it has the power of movement.

There cannot well be differentiation of the oxy-chromatin granules, so that we may regard the dilated linin of a plasmosome as the reservoir in which the chromatin molecules rest. Their emergence from this retreat is the sign for their segregation or duplication.

Linin can pass back into the cytoplasm, as exemplified in the fading of inter-chromosome spindle fibres after division. It can also be reconstructed from the cytoplasm, as exemplified in the increased size of the synaptic mother-cell. It is closely related to cytoplasmic fibrillae, and may even be chemically identical with them, but it is morphologically distinct and discontinuous from them; the increase in bulk of either is probably due to direct synthesis from simpler, or even non-living, constituents of the cytoplasm.

It is worthy of note that the regularity of distribution of achromatic substance is far greater in the reduction division than in the vegetative divisions. In the latter, a large plasmosome may be very unequally divided.

It should be noted that the broken spindle fibres contain no oxy-chromatin granules, so that the latter are very evenly halved in the reduction division.

The behaviour of the thread-rings in fertilization has yet to be investigated. If the rings do not unite immediately—as seems probable—it will be of interest to determine the stage at which this happens, and to ascertain what takes place in synapsis.

The writer is inclined to regard the spireme stage, after the plasmosome has disappeared, as being the typical form of the nucleus. From this stage it departs towards the resting nucleus with chromatin aggregated in the plasmosome, or towards the differentiated chromosomes of the dividing nucleus. Any stage other than the close spireme might thus be regarded as an adaptation to some special requirement of the cell. The various chromosomes are likely to have definite places in this spireme, and to retain those places at each successive division; such geographical localization is not, however, an inevitable outcome of the present hypothesis. In those cases of vegetative division where the plasmosome is slung in the centre of the thread-ring by linin bridles, we see that complete breaking of the anastomoses of the reticulum is by no means necessary for regular division. In this way the spireme may have been phylogenetically derived from

a plate, or even from a granule of motile linin, in which the chromatin was embedded.

My thanks are due to Mr. R. P. Gregory for his critical assistance and advice, and to the Khedivial Agricultural Society for the purchase of the 3 mm. Zeiss apochromatic.

#### REFERENCE.

CANNON, W. A.: Spermatogenesis in Hybrid Cotton. Bull. Torrey Bot. Club, 1900, p. 161.

The only other paper concerned with cotton cytology. The observations agree with the writer's except in three points.

1. I have not noticed the 'flar plasm' in the peripheral cytoplasm, nor seen any spindle structures proper in this region.

2. The chromosomes in the Egyptian plant appear to be twenty in number, not twenty-eight.

3. The formation of chromosomes is *not* by mechanical condensation of loops. Cannon appears to have fallen into exactly the same error which I made in the first observations on the reduction divisions; I carefully prepared drawings of the loops of the thread-rings under the impression that they were chromosomes which were getting ready to shrink and thicken into the usual 'U' form, and it was not until I tried to follow out this shrinking—and also found mature chromosomes side by side with them—that I began to look for another explanation for these loops.

It is of interest to note that he recorded the conspicuous linin structures, and attributed the formation of part of the spindle to them, although without description or reasons. He also comments on the one-sided arrangement of the chromosomes in prophase.

The irregular divisions which he describes can be found in all cotton-plants if very late flowers or very early ones on rattoons are taken; they are not necessarily due to hybrid constitution, and might very well have been provoked by the greenhouse culture he employed.

I might add that this work was in its present stage before I saw Cannon's paper for the first time.

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#### DESCRIPTION OF PLATE LIV.

Illustrating Mr. Balls's article on the Mechanism of Nuclear Division.

The drawings were made with the Zeiss 3 mm. apochromatic (excepting Figs. 1 and 2) and camera lucida, in blue light.

Figs. 1, 2, 3, 4, and 7 are republished from the Year Book of the Khedivial Agricultural Society, 1905.

Fig. 1. Mature microspore mother-cell, not yet passed into synapsis, although the anther wall with its aborting tapetum and parietals shows it to be much older than Fig. 2, which is taken from

another plant. Fixation good.  $\times 700$ . *ep.*, epidermis; *p*<sup>1</sup>, *p*<sup>2</sup>, parietals; *m.c.n.*, microspore mother-cell nucleus; *tap.*, tapetum.

Fig. 2. Synapsis. Younger anther, parietals and tapetum unaltered, but mother-cell nucleus already in synapsis.  $\times 450$ .

Fig. 3. Portion of spireme and nucleolus of synapsis, slightly later. Nucleolus scarcely shrunken, thread definitely split, chromatic areas fairly evenly spaced, and bisected.  $\times 1,100$ .

Fig. 4. Very late spireme, showing two fully differentiated chromosomes, each of four units, split thread uniting them; nucleolus nearly gone, but still *continuous* with thread.  $\times 1,100$ .

Fig. 5. The cluster of chromosomes at one side of the mother-cell nucleus, in prophase of first division. The halves of the spireme thread are conspicuous objects. The chromosome on the extreme left was seen clearly to be connected on either side to the 'thread-rings', by means of short fibres.  $\times 900$ .

Fig. 6. Late prophase of same division. Spindle fibres connecting chromosomes to thread-rings, but not yet drawn taut in all cases.  $\times 1,300$ .

Fig. 7. Metaphase of first division, separation of chromosomes commencing. Section slightly oblique to spindle, showing insertion of bunched spindle fibres in thread-rings at pole.  $\times 1,100$ .

Fig. 8. The same, in another cell, showing spindle fibres below which have not yet been drawn up into the polar group. Semi-diagrammatic.  $\times 1,500$  about.

Fig. 9. One of the first pair of daughter-nuclei in prophase of second division. The arrow points to the other daughter-nucleus. Note that the loops of the thread-ring are double, approximately superposed (indicating origin by splitting) and unbroken on the side of the arrow. They can be followed throughout their length except on the polar side, where a portion is hidden by the chromosomes.  $\times 1,000$ . (Spindle fibres omitted.)

Fig. 10. Later prophase of same, showing rings very clearly. Semi-diagrammatic.  $\times 1,500$ .

Figs. 11, 12, and 13. Division of vegetative cells in root-tip.

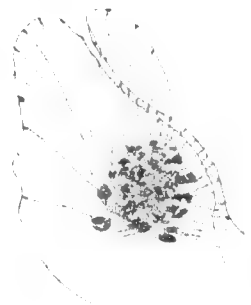
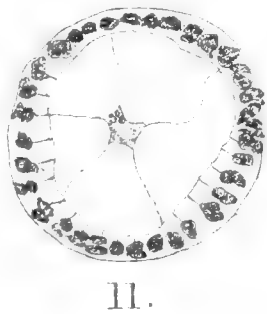
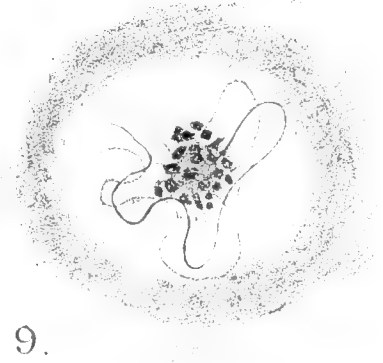
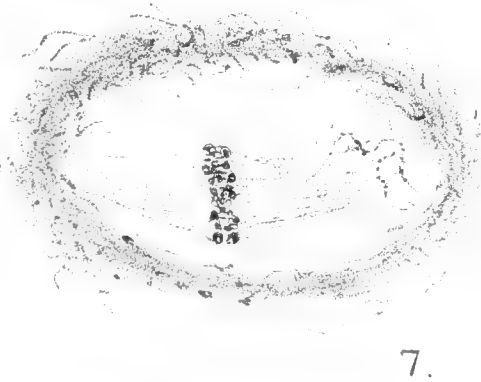
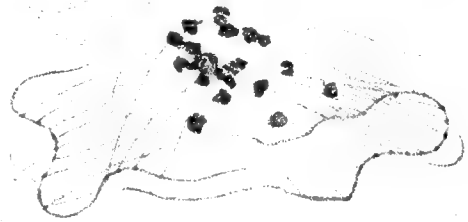
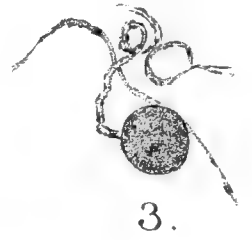
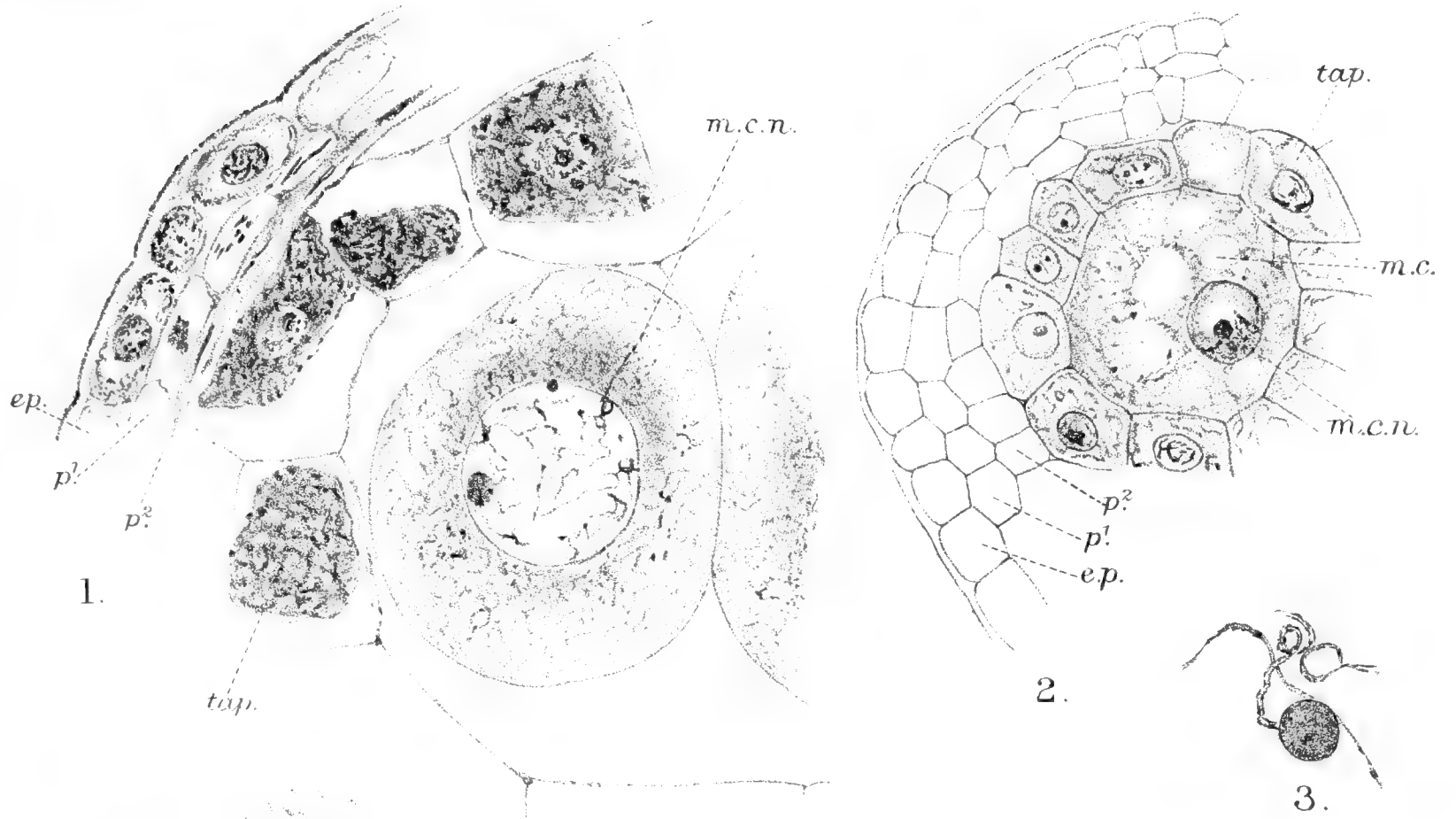
Fig. 11. Polar view of prophase. Nucleolus stellate, slung by bridles of linin to one of the thread-rings. Peripheral chromosomes, connected to both rings by incipient spindle fibres.  $\times 1,500$ .

Fig. 12. Side view of spindle in metaphase, showing rings at either end, with spindle fibres ending upon them.  $\times 1,100$ .

Fig. 13. A single thread-ring in metaphase, showing the plasmosome in this case as a simple dilatation of the ring.  $\times 1,500$ .







BALLS.—NUCLEAR DIVISION.

Huttl lith et imp.



## Root Parasitism in *Exocarpus*

(with comparative Notes on the Haustoria of *Thesium*).

BY

MARGARET BENSON, D.Sc.

With Plate LV and four Figures in the Text.

DURING a visit to Tasmania in 1906 I was much struck by the contrast afforded by the *Exocarpus* shrubs (chiefly *Exocarpus cupressiformis*, Lab.)—the 'Native Cherry' of the Australian colonist—and the surrounding vegetation. The summer was exceptionally dry, and the vividly green switch-like habit of these plants made them very conspicuous against the uniformly grey background of the Eucalyptus 'Bush'. In the autumn of the same year (April, 1906), on visiting New South Wales, I was able by the help of my kind host, Mr. William Benson, of Killara, to examine the roots for the parasitic connexions with other plants which I suspected might exist.

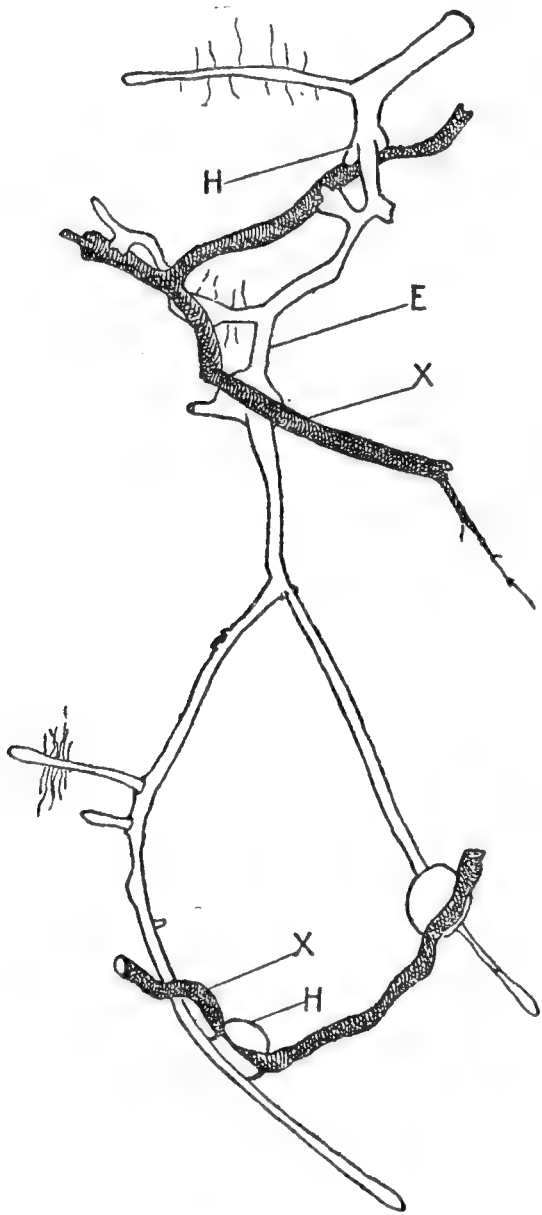
We raised a number of young plants and washed the soil carefully away. By this means we were able to demonstrate innumerable connexions with small foreign roots. Some of these were mounted on the spot in glycerine jelly for examination under a low power of the microscope (Text-fig. 1). Other young plants were preserved in spirit and dispatched home for histological investigation. A small seedling was sent to me later by Mr. Benson, and has been photographed with some fruiting twigs of an adult tree. This shows the *Thesium*-like leaves of the young plant, which are progressively smaller on the lateral branches until they become almost entirely suppressed as in the case on the twigs bearing 'Native Cherry' fruits (Pl. LV, Fig. A).

On my return to England I found that Dr. Barber was engaged in describing the haustoria of several Indian genera of Santalaceae and of allied families.<sup>1</sup>

As *Exocarpus* does not occur in the Indian Flora, it was agreed that I should report on this genus.

<sup>1</sup> Barber: The Haustorium of *Santalum album*. Memoirs of the Dep. of Agriculture in India, vol. i, No. 1, Parts I and II, Jan. 1906 and July 1907. The Haustorium of *Olax Scandens*, *ibid.* vol. ii, No. 4, 1907. Parasitic Trees in Southern India. Proc. of the Cambridge Philosophical Society, vol. xiv, Part III.

The haustoria that had been preserved in spirit were therefore cut, and the series of microtome sections carefully stained by my friends, Miss Chambers and Miss Welsford, to whom my thanks are due for making the best of rather hurriedly preserved material. The slides exhibited features in the conducting tissue of the haustorium which had not previously been



TEXT-FIG. 1. A portion of an *Exocarpus* root, *E*, attacking two foreign roots by means of variously shaped haustoria, *H*.  $\times 8$ .

The foreign roots, *X*, are shaded. The branching of the *Exocarpus* root is apparently abnormal on either side of the pointer, *E*.

recorded for Santalaceous haustoria. Last summer, therefore (August, 1909), I collected a large number of the haustoria of *Thesium* in Canton Valais, Switzerland, for comparison. These were also cut with the microtome and stained in the same way.

The latter preparations have been of great use to me as they have shown clearly that the haustoria of *Exocarpus*, while agreeing in many particulars, differ in others from those of hitherto described Santalaceae. If I enter into some detail in describing these haustoria it is because no previous description based on microtome serial sections of such structures seems to have been published.

Heinricher's detailed papers<sup>1</sup> deal with no member of the Santalaceae. Pierce<sup>2</sup> treats of *Cuscuta* and other genera whose haustoria are by common consent of different origin from those of the Santalaceae. Solms-Laubach's<sup>3</sup> valuable monograph was published so long ago as 1867.

To Dr. Barber's<sup>4</sup> work on other members of Santalaceae frequent reference will be made. His interesting papers should be consulted by any one who wishes to realize the range of problems involved in the investigation of the parasitic habit of the Santa-

laceae. It is much to be regretted that they have been published in memoirs not ordinarily accessible to students. It is only from his own statement that we learn he was hampered in the use of the microtome by the unfavourable climate conditions of Madras.

<sup>1</sup> Heinricher: Cohn's Beiträge z. Biol. d. Pflanzen, B. 7, ii, 1895; Pringsheim's Jahrbuch, vol. xxxii, 1898; Ibid., vol. xxxvi, 1901.

<sup>2</sup> Pierce: Annals of Botany, vii, 1893.

<sup>3</sup> Solms-Laubach: Ueber den Bau und die Entwicklung parasitischer Phanerogamen. Pringsheim's Jahrbuch, vol. vi, 1867.

<sup>4</sup> Barber, l. c.

GENERAL MORPHOLOGY OF THE ROOTS AND HAUSTORIA OF  
EXOCARPUS.

The roots branch very irregularly, and show but an insignificant development of root-hairs (Text-fig. 1). They are generally of a red-brown colour.

The haustoria are minute, and occur chiefly on very slender branches, which makes it exceedingly difficult to demonstrate them except in young plants which it is possible to lift with the soil still attached. Those depicted in Fig. A on Pl. LV are but slightly reduced, but it will be found advisable to use a hand lens in examining them. Those drawn in Text-figure 1 are magnified about 8 diameters. The branches on which the haustoria occur may arise exogenously, and in one case of which I have a serial set of transverse sections the branching was a simple dichotomy. The haustoria are generally, if not always, lateral, two sometimes appearing near the tip of a small root. As recorded by Dr. Barber for other members of Santalaceae, it is generally possible to demonstrate a continuation of the mother root, even though, through a series of many sections, the haustorium appears to be terminal (cp. left-hand haustoria in Text-fig. 1).

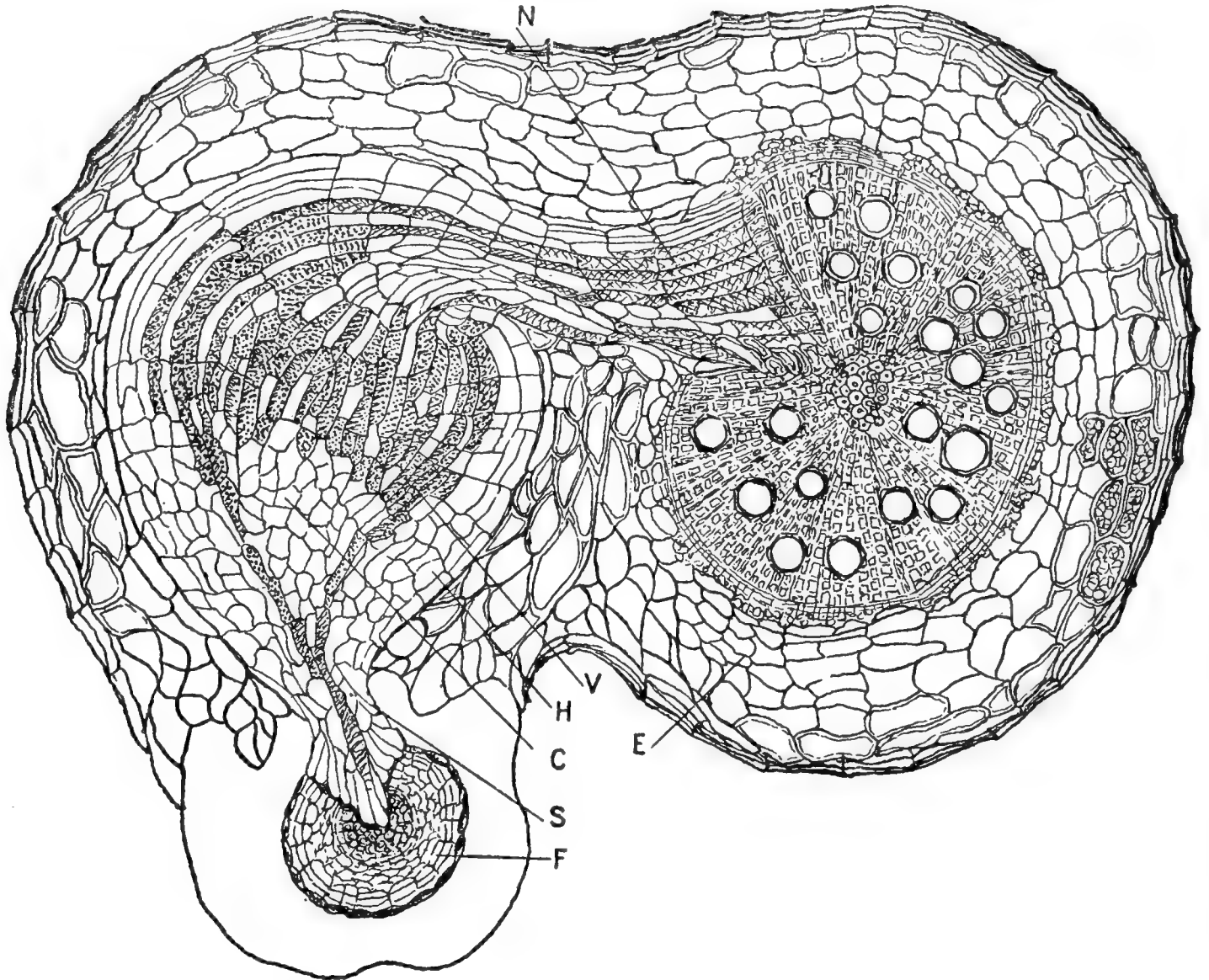
Anatomically the branches bearing haustoria differ from one another. Some contain irregularly disposed, reticulately thickened tracheides like those in the *Thesium* haustorium (Fig. 2, Pl. LV, *n.*). These I will refer to as 'necks', and they will be found invariably to terminate in a haustorium. Others contain a strand of pitted tracheides with phloem, and differ only in the number of protoxylems from the mother root. These always spring from a protoxylem of the mother root, and though often adventitious are obviously best regarded as roots. In the case of equal dichotomy it is impossible to do otherwise. In a third type (that selected for illustration in Text-fig. 2) we see a well-developed haustorium borne on a neck which only differs from an ordinary root in the absence of phloem and in the presence of a terminal haustorium. On the whole it would appear that the roots of *Exocarpus* are (like those of *Thesium*) profoundly influenced in their mode of branching by the new functions superposed on that of absorption from the soil. The direction also of the growth, especially of the lateral roots, is affected more by the proximity of their would-be hosts than by their relation to the vertical position.

## THE HAUSTORIUM.

Before proceeding to discuss the features of interest in these new haustoria it may be well to explain by the help of the diagram (Text-fig. 2) the terms applied to the various parts of the organ. This diagram represents a transverse section of the *Exocarpus* root, *E*, with a haustorium



*H*, cut longitudinally in the median plane. The drawing is constructed from a series of sections stained with safranin and aniline blue. *F* is the foreign root upon which the haustorium is preying. The neck, *N*, of the haustorium contains reticulately thickened tracheides. These are continuous with the conducting cells of the 'nucleus' of the haustorium. The nucleus is composed of two parts: the central core, *C*, of nucleated transparent cells, and the vascular sheath, *V*, which covers the whole surface of the nucleus and is often many cells thick, especially on the proximal surface, where it



TEXT-FIG. 2. An *Exocarpus* root in transverse section bearing a haustorium with a sucker which has reached to the centre of the stele of a foreign root. The cortex cells of the latter have been omitted for clearness. Starch grains were present in all the cortical cells of the parasite, but have been purposely omitted except in a few cases. Further description in the text.  $\times 135$ .

forms a kind of pad. In the distal part it is continuous with the lignified collecting cells in the sucker, *S*. The vascular elements of the haustorium I propose to refer to under the name of phloeotracheides for reasons which are given later. The walls of the tracheides of the neck and of the phloeotracheides differ from those in the xylem of the mother root in the absence of circular bordered pits (compare Text-figs. 3 *a* and 3 *b*). The phloeotracheides contain a thin layer of matrix in which are embedded small approximately spherical bodies which stain a bright blue with aniline. As

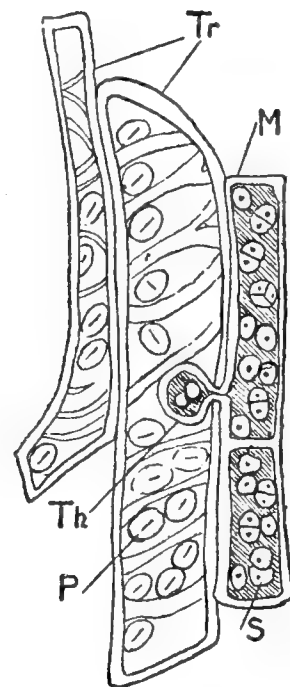
the lignified bands on the walls of the cells in which they lie are stained pink with the safranin, the constituent parts of the haustorium are effectively exhibited in the series of sections from which the diagram is constructed.

### THE PHLOETRACHEIDES.

These cells, the so-called 'vessels' of previous writers on Santalaceous haustoria, are sufficiently unique to merit description.

They appear to be common to all root haustoria, although arranged in several different ways.

In radially symmetrical *Exocarpus* haustoria they form a flask-shaped sheath to the central hyaline core, the neck of the flask abutting upon the host, and being surrounded by nucleated club-shaped cells which probably have a secretory function.



TEXT-FIGS. 3*a* and 3*b*. Enlarged drawings of parts of an *Exocarpus* root with haustorium.

TEXT-FIG. 3*a*. Surface, *S*, and transverse, *T*, sections of the phloetracheides of a haustorium containing granules of various sizes. *N* = a nucleated thin-walled cell accompanying the phloetracheides.  $\times 230$ .

TEXT-FIG. 3*b*. A longitudinal section of part of the xylem of an *Exocarpus* root showing two tracheides, *Tr*, with bordered pits, *P*. Two medullary ray cells (*M*) filled with starch grains abut on the tracheides, and one has thrust in a thylosis, *Th*, which also contains starch.  $\times 230$ .

The phloetracheides, in all but three cases that have come under my observation, contain granules of a carbohydrate.

Although in some cases these phloetracheides, when stained with safranin and aniline, bear a superficial resemblance to the sieve-tubes of some Ferns, there is no ground for thinking the pellicles are callose in nature nor that they are continuous through the thin areolae. The explanation of their presence is, probably, that the residual protoplasm of the partially differentiated tracheide receives such an abundant supply of hydrolysed cellulose that part of it is deposited as amyloextrine.

The important question as to the presence of protoplasm, a nucleus, and plastids in these cells is left an open question by Heinricher (see later, p. 673).

A thin matrix lining the walls is generally demonstrable.

The task of securing evidence as to its nature is a difficult one, but in one series through a *Thesium* haustorium I have satisfied myself that delicate strands of protoplasm can be seen traversing the lumen, and that these are continuous with the matrix in which the granules are embedded (Fig. 3, Pl. LV).

It is much easier to obtain evidence as to the absence of nuclei, for those of the surrounding cells take up a staining reagent which is not taken up by the cytoplasm. Thus it can be shown again and again that the thin-walled, non-lignified cells interspersed among the phloeotracheides contain nuclei, but that nuclei do not occur in the lignified phloeotracheides.

No evidence of the presence of plastids has been obtained. As none are visible at a high magnification, even when the amyloextrine granules are very minute (i. e. when the plastid might be expected to be clearly visible), it is probable that plastids are not present.

The walls of the phloeotracheides much resemble those of the root tracheides, but show no circular bordered pits (Text-figs. 3 *a* and 3 *b*). The areolae are non-lignified, and in *Thesium* appear to consist of nothing but the middle lamella (Fig. 3, Pl. LV). In *Thesium* the lignification of the reticulate bands is continuous from one cell to its neighbour, i. e. the middle lamella can only be demonstrated over the thin areolae, but in *Exocarpus* a middle lamella appears as a bright blue plate between the thickening bands of contiguous phloeotracheides.

The lignified bands are of such thickness that under a high magnification the granules appear to be disposed only over the thin areolae, but with transverse sections it is easy to show that the granules are uniformly distributed over the inner surface of the cell.

By means of a series of longitudinal sections of the haustorium of *Exocarpus* one can demonstrate that the sheath of these cells is much thicker on the proximal surface of the 'nucleus', i. e. there is a continuous mass of lignified cells lying between the transparent central cells of the nucleus and the neck of the haustorium. A series of sections also shows that there is continuity between this pad and the tracheides of the neck, but I have not been able to demonstrate the presence of the blue staining granules in any cells but those of the sheath.

Cells apparently of the same character are found reaching down to the surface of absorption from the host. There is no reason to regard them as secretory. They are found to be surrounded, at the surface of contact with the host, by tubular, thin-walled nucleated cells, and hence appear to be primarily of importance as conducting cells. None the less, the apices, as

has been described by Leclerc du Sablon<sup>1</sup> for the apices of such cells in Rhinanthaceous haustoria, are often non-striated and thin-walled. I have noted what I believe is a nucleus in one apex, and there can be no doubt as to their apical growth.

Fig. 3, Pl. LV, shows the apical part of one of these cells abutting on the starch-containing cells of a medullary ray of the host root.

If we take all these facts into consideration and remember that no true phloem occurs in the haustoria of any so far described root parasite, we realize that these cells have a complex nature.

They collect and act as the pathway for the hydrolysed products of solution of the host cells and deposit in their lumina granules differing from those occurring elsewhere in the root. They are lined with protoplasm, but contain no nucleus, and in these respects resemble sieve-tubes.

They are, however, lignified, and it has been possible in many cases to demonstrate that their end walls are absorbed as in open tracheides.

It will be convenient, therefore, to refer to these cells as phloeotracheides, for they afford an example of the combination of the structure and function of phloem and xylem elements.

#### THE GRANULES IN THE PHLOEOTRACHEIDES.

The only previous record of these granules, as far as I know, is due to Heinricher,<sup>2</sup> who made a careful investigation of the chemical and physical properties of apparently comparable bodies in the conducting cells of the haustoria of *Lathraea*. He came to the conclusion that they were probably of the nature of amyloextrine.

In the footnote of a later paper<sup>3</sup> he again refers to these granules, and states that Wegler believes them to be present in the haustoria of all the Rhinanthaceae. Heinricher uses the expression, 'die Tracheidenreihen häufig mit Amylodextrinstärke erfüllt sind.' He found also the amyloextrine bodies in smaller amount in the cells of the parenchyma of the tracheide head. In *Exocarpus* when granules occur elsewhere than in the phloeotracheides they are starch granules, and are not stained by the aniline. The grains, however, are much smaller than those of the cortical cells.

The amyloextrine bodies abut so closely on the wall that I am inclined to regard them as occasionally deposited upon the inner surface. One of the most remarkable things about them is their early appearance in cells only just cut off from the meristem. They seem to appear simultaneously with the lignification of the wall. Their size bears a rough relation to the width of the bands of thickening in any given cell. Thus

<sup>1</sup> Leclerc du Sablon: Organes d'absorption des plantes parasites. Annales des Sci. Nat., 7th series, vol. vi, Pl. II.

<sup>2</sup> Heinricher, Cohn's Beiträge, Band 7, 1895, p. 344; also Pl. IX, Fig. 7.

<sup>3</sup> Heinricher, Die grünen Halbschmarotzer. Pringsheim's Jahrbuch, 1901, iii, p. 725.



where the bands are fine the pellicles are often numerous but minute, and in a neighbouring cell where the bands are broad the pellicles may be uniseriate and relatively large. This phenomenon rather points against a dependence of their formation on plastids (Text-fig. 3 *a*).

#### CONNEXION OF THE HAUSTORIA WITH THE MOTHER ROOT.

In *Exocarpus* and *Thesium*, one of the most striking features of many of these haustoria is the somewhat abrupt change in character which is noticeable when one passes from the phloeotracheides to the tracheides of the neck. The neck tracheides contain no matrix and no granules. They are often scattered, so that in certain sections of a series there may be no continuity between them and the phloeotracheides.

It may be useful to note that neither in *Exocarpus* nor in *Thesium* have I observed any disintegration of the conducting cells of the haustoria such as Dr. Barber describes<sup>1</sup> as occasionally occurring in *Santalum*. Continuity is established, as Solms-Laubach<sup>2</sup> described for *Thesium*, by reticulately thickened tracheides—'einzelne vielfach zickzackförmige Gefässreihen die Verbindung der Gefässbögen mit dem Bündel der Mutterwurzel bewerkstelligen.' But in *Exocarpus* the phloeotracheides are not limited to two bands ('Gefässbögen') as in *Thesium*. It would be along the tracheides of the neck that the first water-supply must come to the developing haustorium, and it is with the xylem that they are continuous.

#### COMPARISON OF THE HAUSTORIA OF EXOCARPUS WITH THOSE OF THESIUM.

The *Thesium* haustorium, like those of all other members of Santalaceae hitherto described, shows the phloeotracheides in two bands instead of being distributed all over the surface of the nucleus as in *Exocarpus*. Also there is no pad of phloeotracheides at the proximal end of the haustorium. The granules are present in the phloeotracheides (Fig. 3, Pl. LV), but are far smaller and less regular than in *Exocarpus*. In both genera the phloeotracheides may or may not be regularly formed from a meristem.

#### THESIUM AS A HOST.

In three series which had ostensibly been taken through haustoria of *Thesium* it was found that *Thesium* was the host. In one case the attack had been made by another *Thesium* root, and in the other two cases by haustoria of another type (Fig. 1, Pl. LV). I have not been able to identify the form, but it is obviously built on the plan of those of the

<sup>1</sup> Barber, l. c., *Santalum album*, Part II, p. 20 (and also Plates III and IX, 12). Proc. of Camb. Phil. Soc., vol. xiv, Part III, p. 252.

Solms-Laubach, l. c., p. 544.

Rhinanthaceae.<sup>1</sup> The phloetracheides form a head and an axile strand, while the translucent cells which are axile in Santalaceous haustoria surround the axile strand. Thus all the same types of cell are present, but are distributed differently.

Fig. 2, Pl. LV, exhibits a section through a *Thesium* root attacked by another *Thesium* root. The haustorium is borne laterally on a small diarch root and shows the apparently interrupted continuity between the haustorium and the mother root. It is noticeable that the surface of contact between parasite and host is scarcely distinguishable. For this cause the cells of the cortex are not entered in the drawing. Fig. 2 *a* is an enlarged drawing of part of the cells abutting on one another, and no trace of disintegrated cell-wall is visible. This case is in harmony with an observation made by Dr. Barber of a comparable case in *Santalum*. Dr. Barber<sup>2</sup> noted that when *Santalum* haustoria had become fixed to the roots of *Santalum* actual fusion of the tissues seemed to occur, and pointed out that it would appear to be more or less natural that such fusions should occur.

Such cases suggest that when a *Thesium* root attacks one of its own kind a state of equilibrium is reached, and possibly both roots share the supplies. Opposed, however, to this view is the fact that only a trace of starch is to be seen in the medullary rays of the host root, although in other cases the *Thesium* root is charged with starch. Moreover, Barber describes an interesting case in which a *Santalum* root, when undergoing self-attack, had proceeded to occlude the region affected and make some effort to throttle the sucker.

#### DISCUSSION AS TO THE FUNCTION OF THE HAUSTORIA.

In one series of sections the sucker of a *Thesium* haustorium has penetrated and split into two halves the stele of a grass root, and is shown in the act of absorbing the thickening layers on the wall of the further part of the endodermis. This and many other sections suggestive of the same thing show us that the haustoria of these hemiparasites do not limit themselves to an attack on the water-carrying elements. I mention this because it seems rather overlooked in the literature. Thus Haberlandt<sup>3</sup> says of the absorbing cells: 'Sie setzen sich auf kürzestem Wege mit den Gefäßen der Nahrwurzel in Verbindung.'

Solms-Laubach<sup>4</sup> also, while figuring only such cases of root parasitism as do not penetrate beyond the wood, says:—'Der Saugfortsatz ist genöthigt, die sehr feste Schutzscheide und das Holz zu spalten, um seine Gefäße denen der Graswurzel anlegen zu können.'

Such expressions, though true, are liable to be misunderstood, for there

<sup>1</sup> Cf. Solms-Laubach, l. c., Taf. XXXIV; also Leclerc du Sablon, l. c.

<sup>2</sup> Barber, l. c., *Santalum album*, Pt. II, p. 44.

<sup>3</sup> Haberlandt, Pflanzenanatomie, 3. Auflage, 1904, p. 225.

<sup>4</sup> Solms-Laubach, l. c., p. 549; also Tafel XXXII, Fig. 4.



can be no doubt that the haustoria do more than just tap the water-supply of foreign roots. Even animal organisms can be dissolved and absorbed.<sup>1</sup> *Exocarpus*, indeed, more frequently than not wholly dissolves and absorbs the portion of the host root attacked. A vigorous attack on the host is likely to give the most abundant supply, and we find that all kinds of cells, except perhaps cork, yield to the attack.

It is in the light of these observations that possibly we may find the significance of the granules in the phloeotracheides. Heinricher<sup>2</sup> refers to the structures as 'storage tracheides'. But in such a case as *Exocarpus*, which under normal circumstances is exposed to drought conditions combined with much insolation, one cannot but look on these masses of cells interposed between the host and the mother root as serving as a filter. The granules by their precipitation leave the ascending sap less charged with dissolved carbohydrate, which is already present in excess in cortex and medullary rays.

The different habit of *Thesium*, which is abundant on the moist Alps of Switzerland, may account for the insignificant development of granules in its phloeotracheides.

Not only is the period of vegetative activity much shorter, but the host plants would be much richer in watery sap.

#### SUMMARY.

On the roots of various plants of *Exocarpus*, chiefly *Exocarpus cupressiformis*, Lab., growing in the Bush near Killara, N.S.W., were found innumerable haustoria which varied much in size and form.

Their anatomy was examined and compared with that of *Thesium* haustoria gathered in August in Switzerland.

The haustoria contained a large proportion of lignified tissue which was composed of elements for which the name 'phloeotracheides' is suggested. The tissue was not limited to two bands as is the case in the haustoria so far described of other Santalaceae. The function of these cells is discussed, and the probability advanced that they may be of service as a filter.

I should like to take this opportunity of acknowledging my indebtedness to Mr. Maiden, of the Botanical Gardens, Sydney, N.S.W., who was most helpful to me in various ways during my residence in that neighbourhood.

<sup>1</sup> Cf. Barber's Chrysalis, l. c., *Santalum album*, Part I, par. 9, and Figs. 15-17, Pl. III.

<sup>2</sup> Heinricher, l. c., 1901, p. 725, footnote.

## DESCRIPTION OF THE FIGURES IN PLATE LV.

Illustrating Dr. Margaret Benson's paper on *Exocarpus*.

Figures A and 1-3.

Fig. A. A seedling plant of *Exocarpus cupressiformis*, showing the form of the early leaves, which decrease in size on the lateral branches. The roots show many haustoria, which are indicated by the letter *H*. *F* represents a foreign root to which the *Exocarpus* root is attached. To the right of the seedling are some twigs from an adult plant showing the switch-like habit and the 'Native Cherry' fruits. (Slightly reduced.)

Fig. 1. A camera drawing of a Rhinanthaceous haustorium upon a *Thesium* root. *Th* = the *Thesium* root; *H* = the haustorium attached to the neck, *N*. No granules were present in the phloetracheide head, *Ph*. Note the axile row of phloetracheides continuous from the head to the surface of contact with the host. × 87.

Fig. 2. A camera drawing of a *Thesium* haustorium, *H*, attached to a *Thesium* root, *Th*. The neck, *N*, shows continuity by means of short tracheides with the diarch *Thesium* root, *R*. The surface of contact is only faintly distinguishable owing to the almost complete fusion of the two structures. × 87.

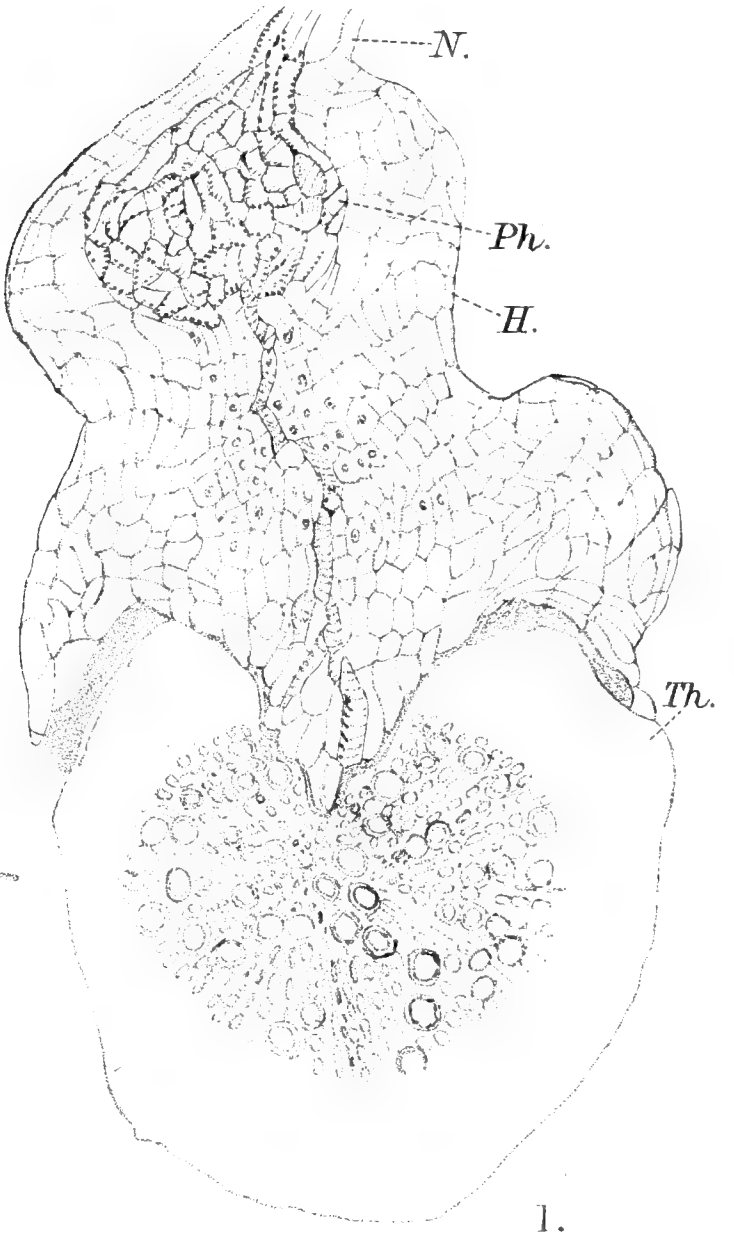
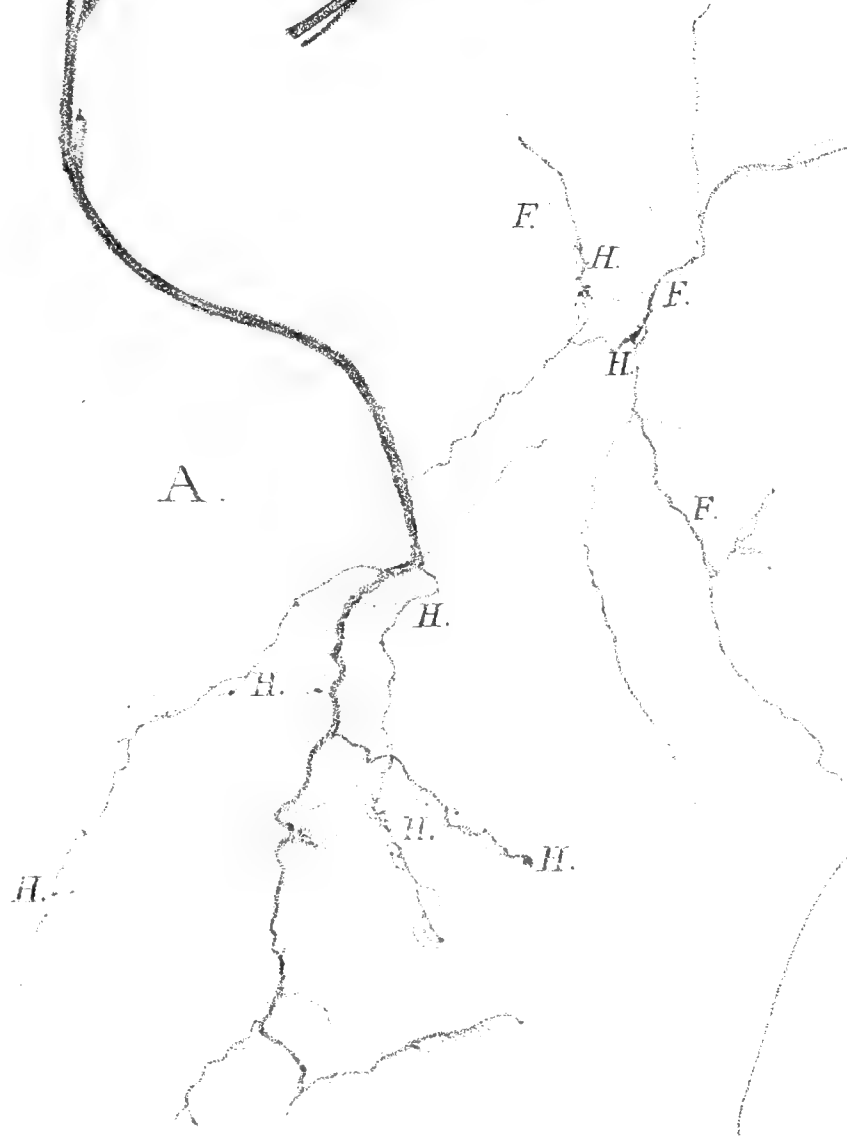
Fig. 2 a. An enlarged drawing of the left-hand phloetracheide of the above figure, showing its insertion into a medullary ray of the host. *M* = the medullary ray cell containing starch grains; *Tr* = a tracheide of the host root; *Ph* = the phloetracheide with its non-striated tip inserted into the ray. × 345.

Fig. 3. An enlarged drawing of an ordinary phloetracheide from a haustorium of *Thesium*. Strands which are thought to be protoplasm are seen in continuity with the living matrix, which contains spherical granules, *G*, of amyloextrine. Two thin-walled, nucleated cells, *N*, abut on the phloetracheide. × 345.

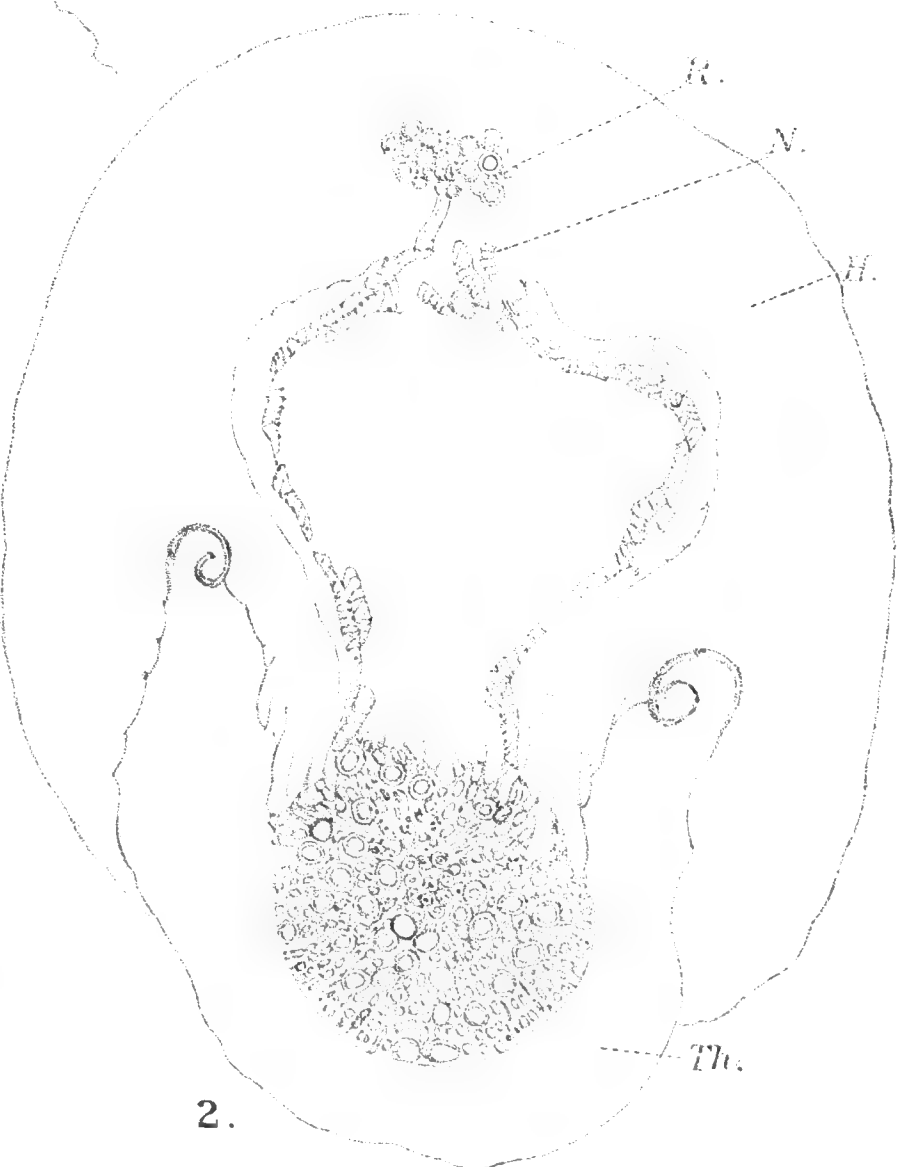




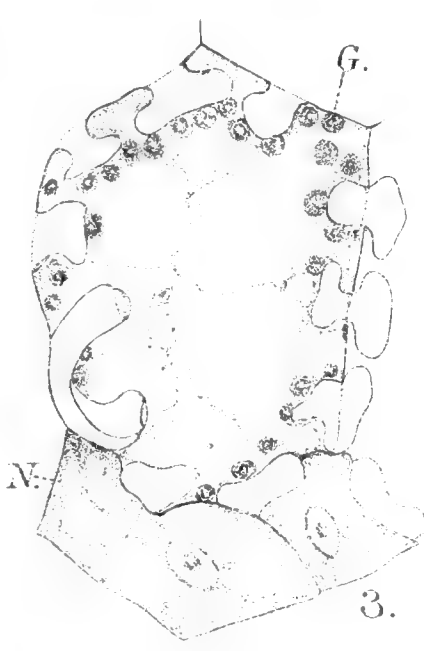
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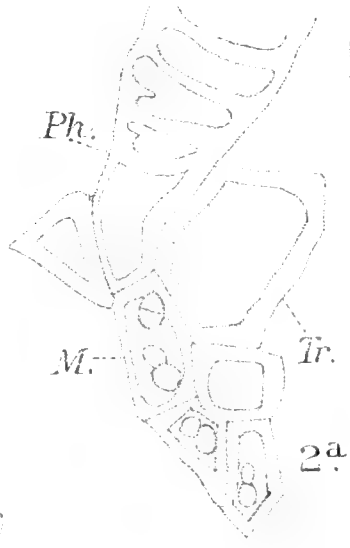
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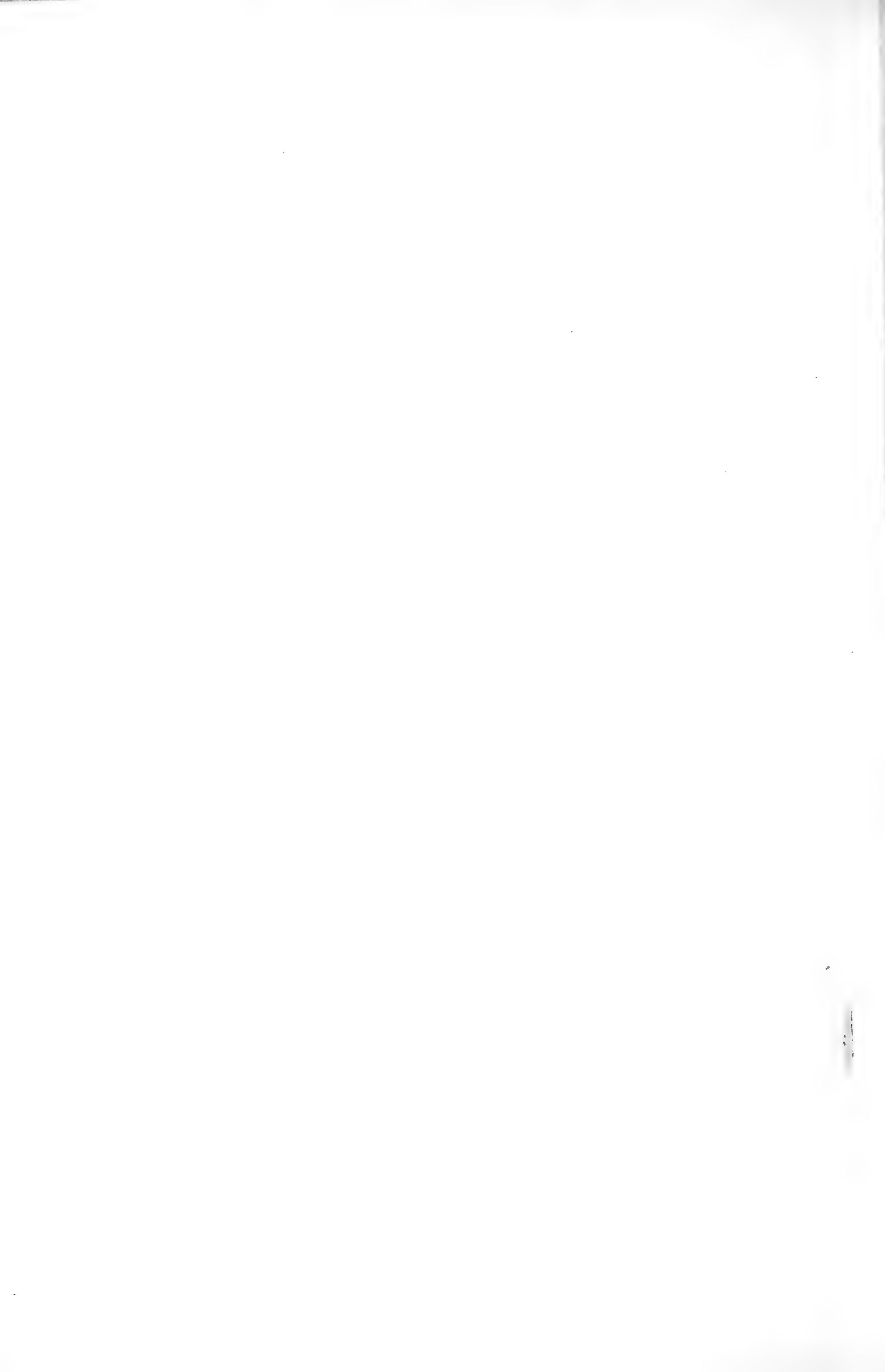


3.



2<sup>a</sup>.

Hatched & imp.



# Further Observations on the Fossil Flower, *Cretovarium*.

BY

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With Plates **LVI** and **LVII**.

**O**BJECT of the paper: To record the discovery of, and shortly to describe, ovules in an ovary of *Cretovarium japonicum*, Stopes and Fujii; and to illustrate the structure of the ovary wall.

Although the original description<sup>1</sup> is based on seven specimens of the ovary of *Cretovarium*, one of them showing the placentae, none of these contained any ovules.

I have recently found an ovary containing several ovules, and now describe it in order to supplement the earlier account of the new fossil—which has for the moment added to its merely academic a somewhat adventitious interest, owing to the fact that it is the only known structural petrification of a true angiospermic flower.

The ovary containing the ovules is shown in Photograph 1, Pl. LVI, in transverse section. It is from a region apparently slightly above the equator of the carpels, where it is free from the perianth. (It will be remembered that the perianth is adherent to the lower region of the carpels.)

In the specimen, the cavities of the loculi are completely filled with black granules of matrix, in which the ovules stand out as shadowy, lighter patches in the photograph. The diagram in Pl. LVII, Fig. 1, gives an outline of the same, and shows the position and extent of the ovules, of which there are so many as five in the complete loculus.

The petrification of the ovules is, unfortunately, far from perfect, but they closely resemble in appearance ovules in preparations of modern plants which have not been well fixed. The enlarged drawing in Fig. 3, Pl. LVII, shows the details of the ovules numbered 2, 3, and 4 in Fig. 1. From this it will be seen that but little definite cell structure is retained,

<sup>1</sup> Stopes and Fujii, Studies on the Structure and Affinities of Cretaceous Plants. Phil. Trans. Royal Soc. B., vol. cc, 1910.



except what, from comparison with recent ovules similarly imperfect, must be taken as the outer integument. This is also seen in Fig. 2, which is an enlargement of ovule No. 6, and shows the cells of the integument comparatively well preserved, but the inner tissue broken down to form a granular, contracted mass. Ovule No. 1 is seen somewhat enlarged in Photograph 2, Pl. LVI.

All the ovules are cut obliquely and tangentially, and none are attached to their placentae. Ovule No. 2, however, suggests that they were anatropous.

As there were five ovules in the one transverse section, it is probable that there would have been about twenty or so in each loculus, since the ovary is not more than 3 mm. in vertical height.

The features shown, or suggested, by these ovules appear entirely similar to those of normal modern anatropous ovules, and are in agreement with the original allocation of the plant, presumably to the Liliaceae.

I take this opportunity further to illustrate the details of the ovary wall, which was shown very imperfectly in the three photographs illustrating the original description; partly owing to their comparatively small scale, and partly to the fact that the preservation of the outer coat of the ovary in those specimens was far from perfect. Round the inner zone of thick-walled fibres there was indeed only a much-decomposed remnant of tissue, and it was then suggested that it was the remains of a soft-celled outer envelope surrounding the fibrous wall, although it was not possible to establish this at the time. In the present specimen this supposition is substantiated, for the soft outer tissues are well preserved, as is seen in the Photographs 1 and 2, and Drawing 3, Pl. LVII, at *ow*.

The cells of this soft zone vary somewhat in size, but are roundish and undifferentiated; a number of them have clear yellowish content, which suggests that they contained mucilage or tannin while alive.

It seems not unlikely that this ovule-bearing ovary was slightly younger at the time of its petrification than those previously described; and that in the others it was not merely bad petrification which left the outer wall so disintegrated, but the natural decomposition of this layer in the ripe carpel.

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DESCRIPTION OF PLATES LVI AND LVII.

Illustrating Dr. Marie C. Stopes's paper on the Fossil Flower, *Cretovarium*.

PLATE LVI.

Phot. 1. Transverse section of the ovary lying in the granular matrix. It shows the three loculi, the double-layered wall, and the ovules in the loculus, which appears black owing to the granules of the matrix. Cf. Fig. 1, Pl. LVII.  $\times 44$  diameters.

Phot. 2. Enlargement of part of the upper loculus, showing the cells of the ovary wall (*ow*) and the inner fibrous layer (*f*). At *a* is the ovule No. 1 in Fig. 1, Pl. LVII; *b*, the ovule No. 2 in Figs. 1 and 3, Pl. LVII; *c*, the small cells of the axis of the carpels.

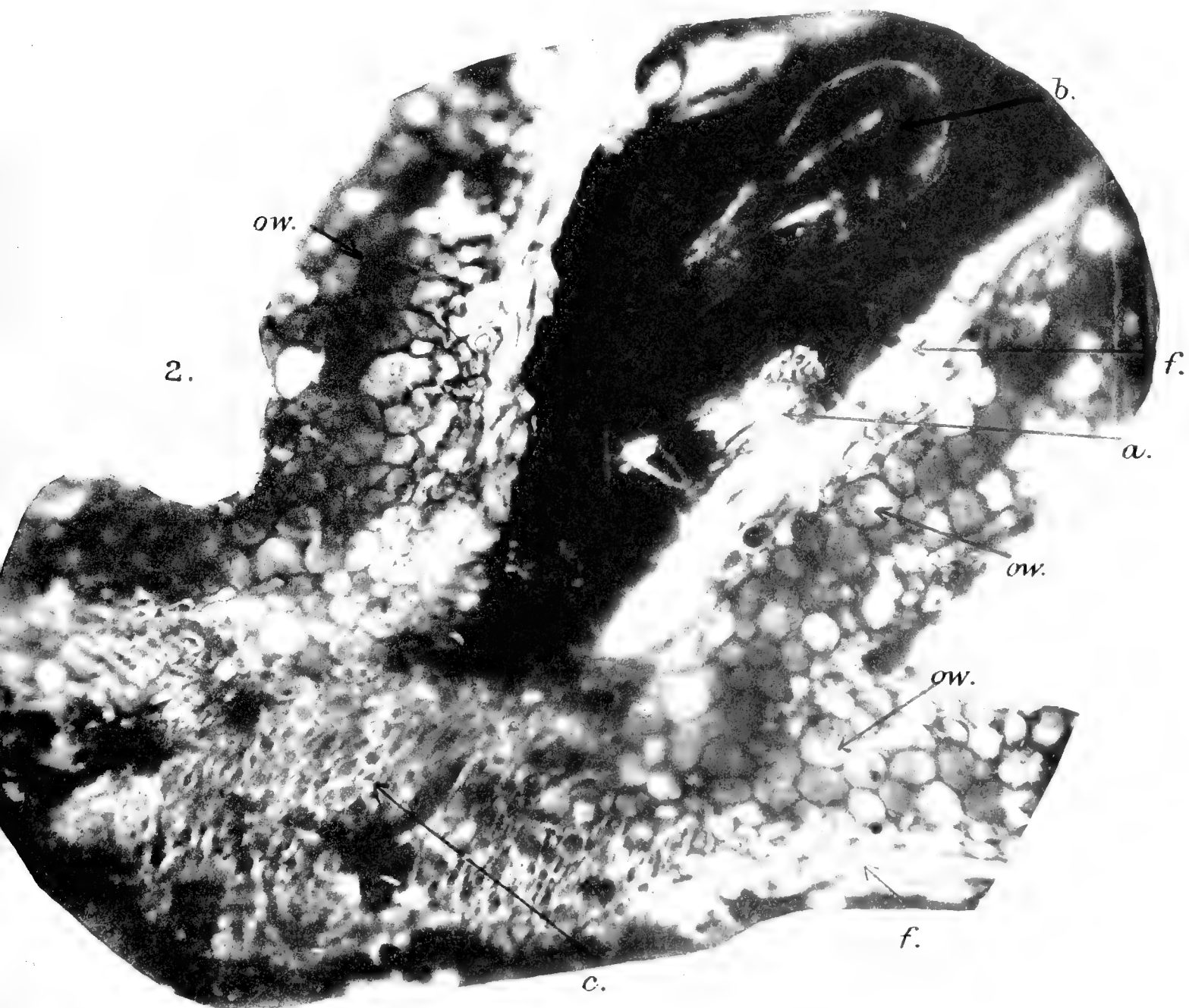
PLATE LVII.

Fig. 1. Diagram showing the outline of the ovary and the ovules contained, numbered 1, 2, &c.

Fig. 2. Drawing of details of ovule No. 6.

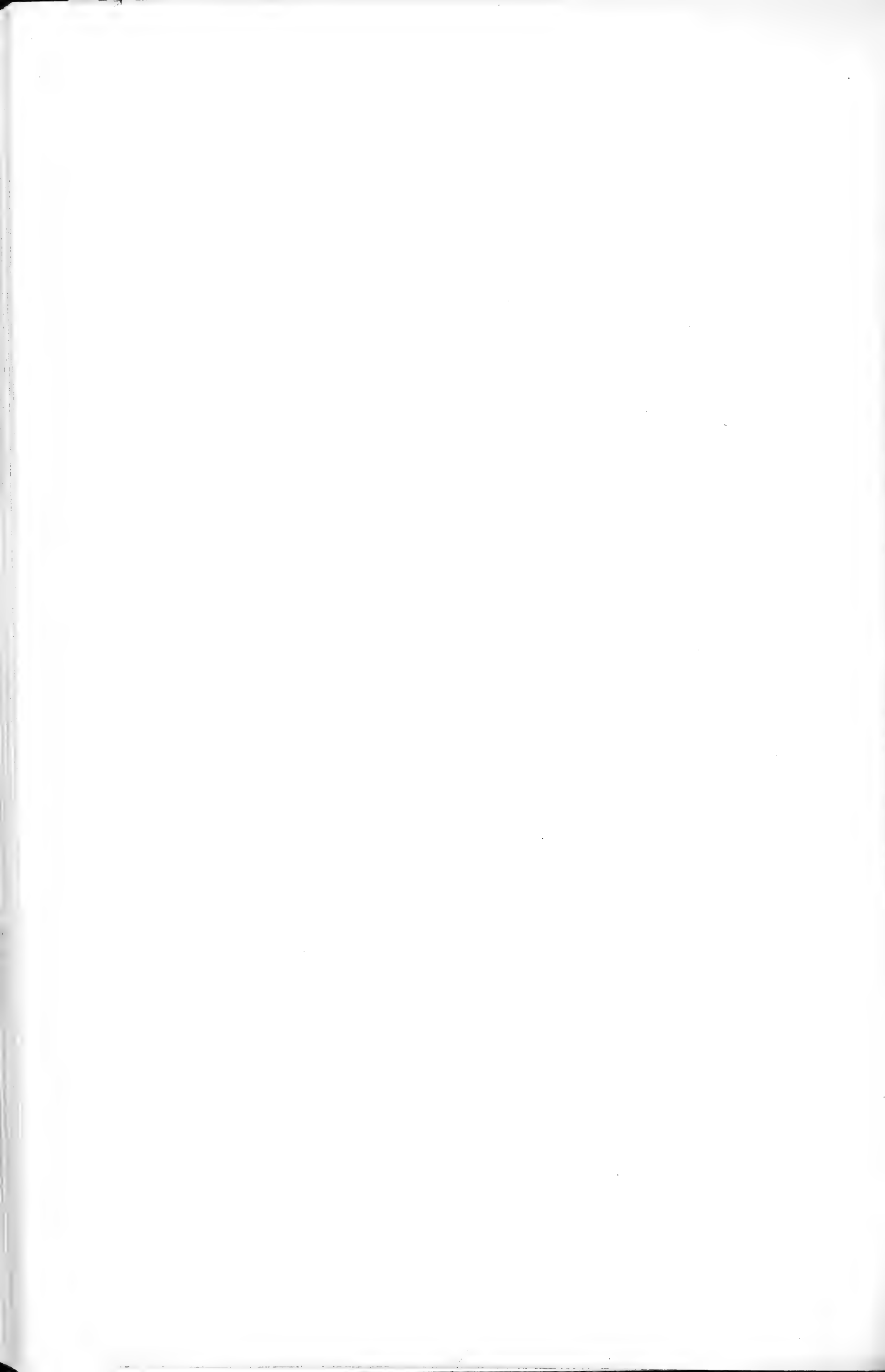
Fig. 3. Drawing of details of ovary wall (*ow*, soft, outer cells; *f*, fibres) and of ovules Nos. 2, 3, and 4.

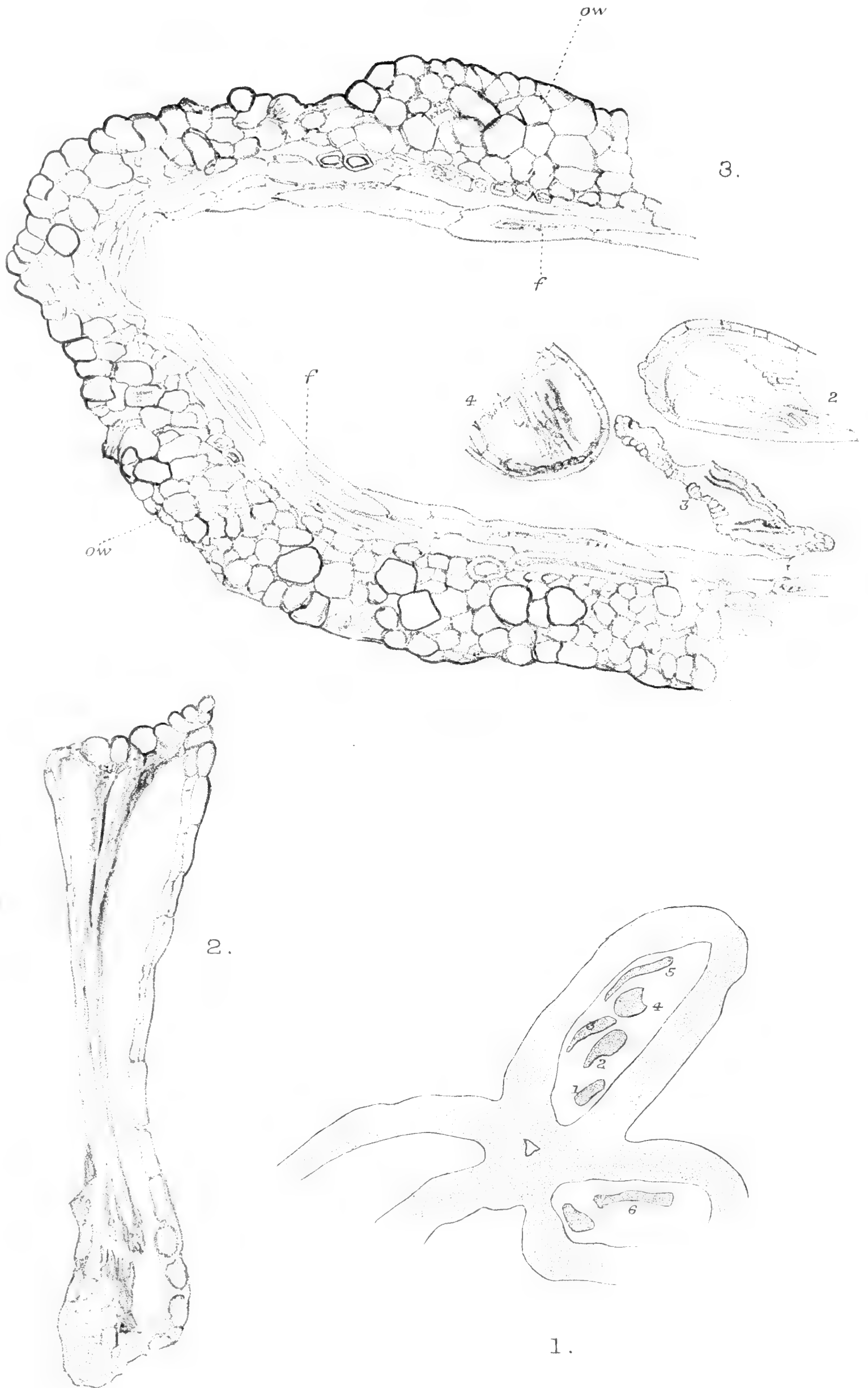




STOPES. ——— CRETOVARIUM.

Huth, coll.





M.C.S. del.

Huth, lith et imp

STOPES — CRETOVARIUM





# A Fossil Solenostelic Fern.

BY

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With Plate LVIII and a Figure in the Text.

AMONG the flora represented in the mineral nodules recently collected by Dr. Marie Stopes from the Cretaceous of Japan, is a fragment of a fern stem which is particularly interesting, since the anatomy shows it to be of the solenostelic type, and hence it seems of sufficient interest to merit description in detail. At present the only recorded example of a fossil fern with this structure is *Psaronius Renaulti*,<sup>1</sup> which seems essentially solenostelic, although in the sections at present known the stele is not completely closed at any one level;<sup>2</sup> thus there is still the possibility that *P. Renaulti* may be a simple dictyostelic stem.

The Japanese stem, although only about 3 cm. long, fortunately includes a node, and shows the departure of a lateral branch with its relation to the leaf-trace. The preservation of the tissues is in parts extremely good, and the structural details can clearly be seen. Although the fossil shows marked resemblances to some of the living groups of ferns, there is not sufficient evidence to identify it with or include it in any of these groups; therefore it seems necessary to form a new genus which might temporarily be used for this and any other such fragmentary portions of fossil solenostelic ferns that may be discovered, and do not show sufficient resemblance to living forms to justify inclusion with them. I therefore suggest the name *Solenostelopteris* for the genus. The species name *japonica* is given to this specimen, as it comes from the Japanese deposits.

## DESCRIPTION OF THE STEM.

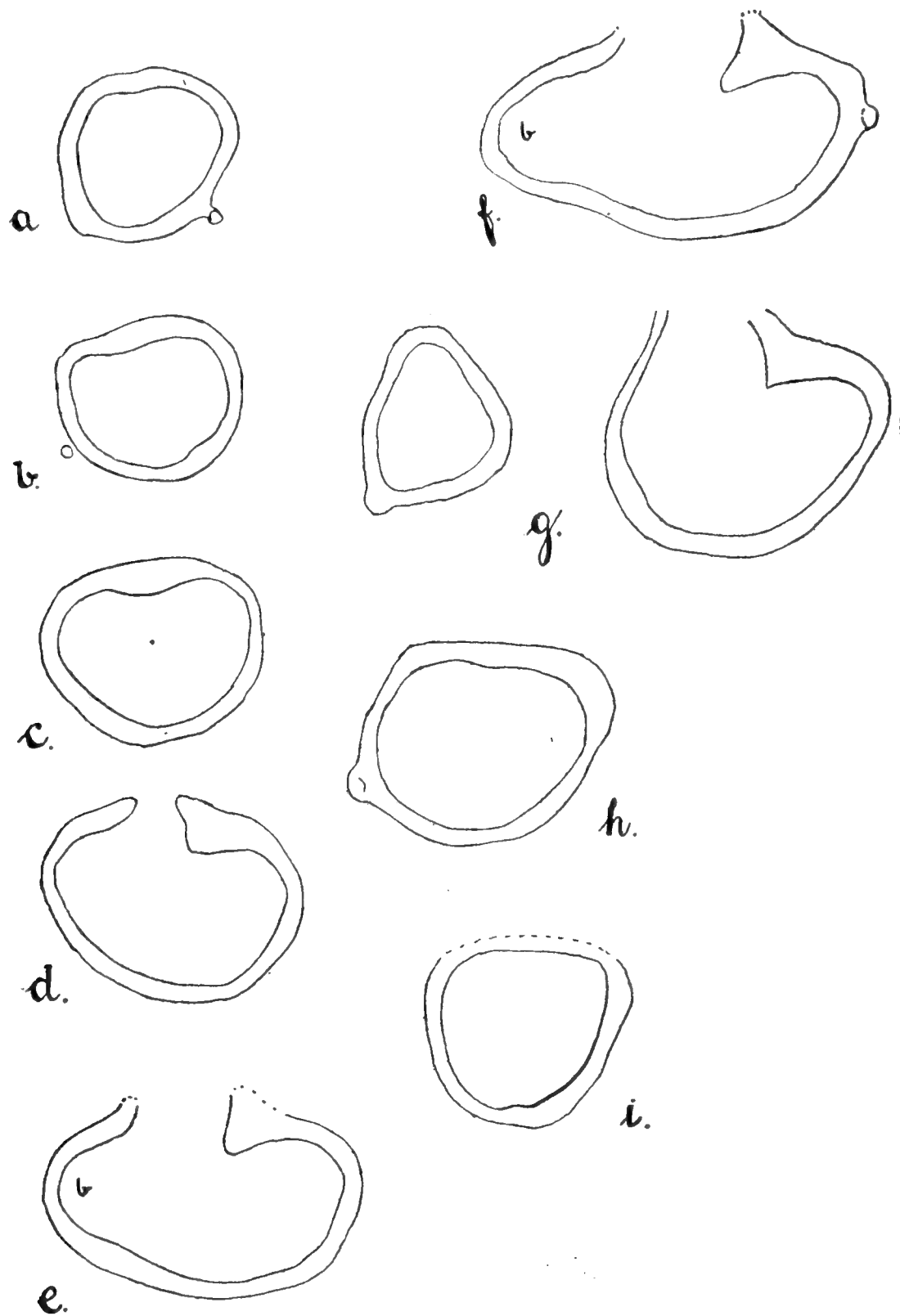
The fragment of the stem was from 3 to 3.5 cm. long, and from this nine transverse sections were prepared.

The Text-fig. shows the general shape of the solenostele in the various sections.

<sup>1</sup> Scott, D. H. ('08), *Studies in Fossil Botany*, 2nd ed., vol. i, p. 301.

<sup>2</sup> I am indebted to Dr. Scott for this information.

Sections *a*, *b*, and *c* are below the node, and the stele is here a complete ring. In section *d* the stele has opened, forming the leaf-gap. Sections *e* and *f*, in addition to the leaf-gap, show a swelling of the stele on one side, which indicates the developing lateral branch (*b*). In *g* the branch has separated from the main stele, in which the leaf-gap is still open. The two



TEXT-FIG. A series of transverse sections through an internode and part of a node of *S. japonica*.  
Description in text.

remaining sections are merely of the lateral branch, the main stele having disappeared.

It seems probable that the rhizome was a dorsiventral one, the leaf-gap being on the upper surface, while the roots arose from the under surface of the rhizome both at the nodes and internodes.

*Anatomy.* The general view of the stem is seen in Fig. 1, Pl. LVIII. Unfortunately in most of the sections the greater part of the cortical ground tissue has disappeared, so it is impossible to determine the size of the complete stem. The stele measured about 1.8 mm. diameter.

*Ground tissue.* The central ground tissue (within the solenostele) is composed of cells 0.05 mm. diameter, with sclerized walls. As may be seen from Fig. 1 at *c. scl.*, the central cells are larger and less sclerized than the peripheral ones. These larger central cells are often filled with rounded bodies which were probably starch grains, such as are found in similar cells in living ferns, e. g. *Microlepia*.

Almost at the edge of the central ground tissue, separated only by one or two layers of sclerenchyma from the solenostele, is a layer of rather irregular, thin-walled cells, which in many parts are very crushed in the fossil (Figs. 1 and 2, *s.*). A similar layer of thin-walled cells amongst the sclerized ones is frequently met with in the central ground tissue of the stem of living solenostelic ferns.

The cortical ground tissue is not seen very favourably in many of the sections, and is in no case complete. Immediately surrounding the stele there is a layer of sclerenchyma about three cells in thickness (Figs. 2, 3, 4, *o. scl.*). Many of these cells, as in the case of the cells of the central ground tissue, are filled with the rounded bodies referred to above as starch grains. In most of the sections the tissues outside this sclerized ring are lost, but in some of the retained portions there may be seen, outside the sclerized ring, several rows of parenchymatous cells usually rather crushed, but which were probably of a soft, spongy nature, and had large air spaces between them. This tissue can be seen as a dark ring just outside the sclerized cortical tissue in Fig. 4, *i. c.*

The outer cortex, which was probably a much stronger tissue, is partially preserved in some of the sections—see Figs. 3 and 4, *o. c.*; but in no case can the epidermal tissues be seen. The cells composing this outer cortex, usually hexagonal in shape and averaging 0.07 mm. diameter, fit together closely, leaving no intercellular spaces.

The stele conforms to the definition of a solenostele given by Gwynne-Vaughan,<sup>1</sup> for 'the vascular tissue is arranged in a single hollow cylinder with phloem and phloeterma on either side . . . the complete continuity of which is interrupted only by the departure of the leaf-traces; the gaps thus produced are closed up in the internode above, before the departure of the next leaf-trace'. There is only one node and a portion of an internode in the fossil fragment, but those parts are in agreement with the above

<sup>1</sup> Gwynne-Vaughan ('01), Observations on the Anatomy of Solenostelic Ferns (Part I). *Annals of Botany*, vol. xiv, March, 1901.

description, and it is reasonable to assume that the whole of the rhizome was of this solenostelic type.

*The xylem* is composed of a ring of tracheides, one or two layers deep, in the ventral portion of the stele (Figs. 1, 2, and 3, *x.*), but becoming five or six layers deep in the dorsal portion in connexion with the outgoing leaf-trace (Figs. 1, 3, and 4, *a*). A few parenchymatous cells occur here and there among the woody elements. Protoxylem elements of the spiral and annular type are not found in the stem, but fairly frequent exarch groups of scalariform elements are found around the ring of metaxylem (*p. x.*, Fig. 2), quite similar to the 'protoxylem elements' described for recent solenostelic ferns.<sup>1</sup> Unfortunately there are no longitudinal sections of the rhizome, but in some of the oblique parts of the transverse sections it may be seen that both the protoxylem and metaxylem are composed of scalariform elements, which possess a single series of pits on each wall. In some of the better-preserved and uncrushed parts of the rhizome distinct pits can be seen (Fig. 5) between the walls of adjacent xylem elements, such as Gwynne-Vaughan<sup>2</sup> has described in the fossil Osmundaceae, and has since shown to exist in the xylem elements of many of the living ferns.

*The phloem* forms a continuous ring on either side of the xylem (Fig. 2, *i. ph.* and *o. ph.*), but unfortunately, as is the case with the other soft tissues in the stem, it is usually so crushed that the structure is not very clearly seen. In the better-preserved parts one can recognize two or three layers of sieve-tubes, but no distinction into protophloem and metaphloem. The phloem layer is separated from the xylem on each side by an irregular layer of parenchymatous cells (Fig. 2, *par.*). Surrounding both the inner and outer bands of phloem is a single layer of pericycle cells (Fig. 2, *i. per.* and *o. per.*), often rather irregularly arranged. The solenostele is limited on each side by a well-marked endodermis, which is clearly shown in all sections, since the cells, unlike the surrounding ones, are usually filled with masses of black, carbonized material. They are slightly elongate tangentially—that diameter being 0.02 mm. (Fig. 2, *o. end.* and *i. end.*). There are no undulations on the radial walls of the cells, but the corners are slightly thickened. This endodermal layer is remarkably well defined and recognizable for a fossil.

#### THE LEAF-TRACE AND BRANCH.

As was mentioned previously and shown in the Text-fig., the continuity of the solenostele is interrupted in some sections by the outgoing leaf-trace. The lateral shoot of the rhizome, as in living solenostelic ferns,

<sup>1</sup> Gwynne-Vaughan ('03), Observations on the Anatomy of Solenostelic Ferns (Part II). *Annals of Botany*, vol. xvii, 1903.

<sup>2</sup> Gwynne-Vaughan ('08), On the real nature of the Tracheae in the Ferns. *Annals of Botany*, vol. xxiii, July, 1908.

is inserted upon the base of the petiole and attached to one margin of its vascular strand. An attempt has been made in Fig. 6 to represent a node of the rhizome with leaf-gap, and to show the relations between it and the leaf-trace and adjacent lateral branch. The restoration is in part hypothetical, for although its position is obvious the actual leaf-trace is not seen in any section, and therefore its shape, direction, &c., are merely suggested, and possibly not the actual ones. A comparison of Fig. 6 with the series represented in the Text-fig. will show the general arrangement of the various parts. At *a*, Text-fig., which is a section below the node, the solenostele is seen to be of uniform thickness; *b* represents a section a little higher and nearer to the node. Fig. 1, Pl. LVIII, is a photograph of this same section. The solenostele is no longer of uniform thickness around its circumference. The dorsal portion which will very soon open to form the leaf-gap is slightly thicker than the remaining part of the stele. Diagram *c*, Text-fig., shows this dorsal thickening more markedly. In the next section, represented at *d*, the solenostele has become open in preparation for the departure of the leaf-trace, and the free margin of the leaf-gap is seen to be considerably thickened, owing to an increase in the number of tracheides at this point. The xylem ring at this point is at least twice as thick as in any other part, so that the free margin of the leaf-gap projects considerably towards the centre of the stem.

This thickening of the margin is of interest, a similar development having been described in several species of *Dicksonia* and *Davallia* by Gwynne-Vaughan.<sup>1</sup> It is regarded by him as a preliminary step in the formation of internal steles. A further step in this direction is seen in *Dipteris conjugata*, described by Seward and Dale.<sup>2</sup> In this fern the thickening of the margin of the leaf-gap extends through the internodes as well as the nodes, and this portion of the xylem has become almost completely separated off from the solenostele.

The anatomy of the fossil shows that *Solenostelopteris* must have occupied a similar position as regards the development of internal steles to *Dicksonia apiifolia* among recent ferns. In both these ferns the development is limited to a nodal thickening of the xylem of the free margin of the leaf-gap, without any indication of a separation of the thickened part of the xylem from the solenostele, as is seen in *Dicksonia adiantoides* and more clearly in *Dipteris conjugata*.

Section *e* shows a further change in the shape of the stele preparatory to the formation of the lateral shoot given off in connexion with the leaf-trace. The stele is seen to be considerably stretched in the horizontal plane, the region marked *b* being destined to form the branch. The limits

<sup>1</sup> Gwynne-Vaughan ('03), l. c.

<sup>2</sup> Structure and Affinities of *Dipteris*, &c. Phil. Trans., Series B, vol. cxciv, p. 499, and Fig. 4, 1901.



of the margins of the leaf-gap in this section cannot be accurately given, since the fossil is broken along the part indicated by dotted lines in *e*. The thickened margin is possibly almost complete, and did not differ much from its form in the previous section, *d*.

The other margin to which the leaf-trace must have been attached is probably more seriously damaged. The next section, *f*, of which Fig. 3, Pl. LVIII, is a photograph, shows the stele still more stretched, and the part *b* which is to form the branch is larger, but the actual leaf-trace is not preserved.

Section *g*, Text-fig. and Fig. 4, Pl. LVIII, shows the lateral branch now free from the main stele, though it is still joined up to the main stem by cortical tissues. The branch evidently became detached from the main stele by a gradual constriction of the xylem ring in quite a similar way to the departure of the branch in *Microlepia*. From an examination of the main stele it is obvious that at this level the leaf-trace had not yet departed, for the leaf-gap shows no signs of closing, and the free margin is still considerably thickened (Fig. 4, Pl. LVIII). It is suggested from a comparison with *Microlepia*, which *Solenostelopteris* resembles in many respects, that the leaf-trace would probably be a hook-like mass of xylem attached at the end of the elongated portion of the stele on the side of the branch, at about the point where the xylem is broken (Text-fig., *e* and *f*). The branch was thus on the same side of the rhizome as the leaf-trace, and became free from the main stele shortly before it. The vascular anatomy of the lateral shoot (Fig. 4 and Text-fig., *g*, *h*, *i*) is exactly like that of the main stem.

#### ROOTLETS.

Rootlet bundles are seen in many of the sections at different points on their way out through the cortex. In all cases they arise from the lower surface of the solenostele, i. e. the side remote from the leaf-gap. They are of the usual fern type—a diarch plate of xylem forming an almost circular mass with phloem on either side, a pericycle and clearly marked endodermis around (Fig. 8, Pl. LVIII).

While passing through the ground tissue of the stem the rootlets possess no ground tissue of their own, a feature noted by Gwynne-Vaughan in recent solenostelic ferns. The rootlet represented in Fig. 8 is the one seen at *r* in Fig. 3, in the sclerized tissue outside the solenostele. The groups of protoxylem (*p. x.*) show clearly at either side, also the development of metaxylem between. Unfortunately, in this particular rootlet, all tissues between the xylem and endodermis, which is quite similar to that surrounding the stele of the stem, are lost. The diarch plate of xylem is placed with its long axis tangential to the circumference of the solenostele, as is usual in recent ferns. The mode of origin of the rootlet is easily seen in

the various sections. A bulging of the xylem on the outer side of the ring is seen, which causes the phloem, pericycle, and endodermis to project outwards considerably. As this excrescence of the xylem grows it gradually becomes separated from the main mass of xylem (Fig. 7), the endodermis of each side of the rootlet bundle joins up, forming a complete ring, and so the rootlet becomes free from the solenostele.

A solenostelic fern very similar to but rather smaller than the one just described appears among the Japanese preparations in Dr. Stopes's possession, but unfortunately in only one slide of a series. The tissues of this stem agree very closely with those of *Solenostelopteris japonica*. As there is no gap in the solenostele, the section must have been taken through the internode of the rhizome. The xylem ring is composed of an irregular row of scalariform elements with scattered groups of smaller elements towards the outside in some parts. On each side of the xylem ring there is a well-marked layer of parenchymatous cells separating the xylem from the ring of phloem, which is made up of several layers of small sieve-tubes. The pericycle and endodermis can be seen on either side of the solenostele, the endodermis showing the same black, carbonized contents as were described in *S. japonica*. At one point a rootlet is seen arising from the stele in the manner described above. It seems probable, from a close comparison of the tissues, that this fern is merely a fragment of a smaller branch of the fern rhizome described.

#### DIAGNOSIS.

##### *Solenostelopteris*, gen. nov.

Rhizomes of fossil ferns, vascular system a solenostele.

##### *S. japonica*, sp. nov.

Rhizome dorsiventral, solenostele 1.8 mm. diameter (without cortex). Xylem elements scalariform, with one series of pits on each wall. Protoxylem exarch, scalariform. Internal and external phloem. Pericycle and endodermis well marked. Central ground tissue sclerized, with a layer of thin-walled cells separating it from the stele. Cortex with sclerized layer a short distance outside the stele, followed by thin-walled parenchymatous cells, probably with air spaces between; the outermost cortex consisting of hexagonal parenchymatous cells without intercellular spaces. Xylem of free margin of leaf-gap considerably thicker than the rest of the xylem ring, lateral branch given off on same side of stele as, and in connexion with, the leaf-trace.

*Horizon.* Upper Cretaceous.

*Locality.* Hokkaido, Northern Japan. Collected by Dr. M. C. Stopes.

*Type.* Stopes's Collection, Nos. I YA. 16-24.

## AFFINITIES.

From the small fragment of the stem available and without any indication of what the external characters were, it is impossible to determine the definite affinities of the fern. The vascular anatomy is the only ground to work from, and the exact value of that as a factor in classification of ferns is yet hardly known; moreover, there is much necessary information concerning the vascular anatomy lacking, for the structure of the petiole and its connexion with the stem are not known. Many of the anatomical features resemble those in the more typical solenostelic forms of the Davalliaceae, in particular *Microlepia*. The marginal thickening of the xylem of the leaf-gap, which seems a point of considerable interest and importance, is developed to a similar extent in *Microlepia hirta*. The distribution of sclerenchyma, arrangement of xylem and phloem, &c., are also very similar in this species. Owing to our ignorance of the soral and sporangial characters, and the incompleteness of this, the only specimen of the plant, this account of *Solenosteleopteris japonica* is necessarily a purely descriptive one. The specimen is interesting as affording an example of a definite stelar type common in recent ferns, but not previously described in detail among fossils. It also adds a new type to the Cretaceous Flora, as described by Stopes and Fujii.<sup>1</sup> We may perhaps venture to go so far as to recognize it as probable that the affinities of the fossil are with the typical solenostelic members of the Davalliaceae.

I wish to express my sincere thanks to Dr. Marie Stopes for so kindly placing her material at my disposal, and also for the helpful interest she has shown. To Dr. Lang, also, I am indebted for many useful suggestions.

## DESCRIPTION OF PLATE LVIII.

Illustrating Miss Kershaw's paper on a Fossil Solenostelic Fern.

Fig. 1. Photograph of the solenostele below the leaf-gap, showing at *a* on the dorsal side of the stele the greater thickness of the xylem. *c. scl.*, central sclerized ground tissue; *s.*, thin-walled cells amongst sclerized ones; *x.*, xylem. (Slide I YA 17.)

Fig. 2. Drawing of a small part of the solenostele, showing details of anatomy. *o. per.*, *i. per.*, outer and inner pericycle; *o. end.*, *i. end.*, outer and inner endodermis; *o. ph.*, *i. ph.*, outer and inner phloem; *p. x.*, protoxylem; *par.*, parenchymatous cells. (Slide I YA 16.)

<sup>1</sup> Stopes and Fujii ('10), Studies on the Structure and Affinities of Cretaceous Plants. Phil. Trans. Roy. Soc., London, Series B, vol. cci.

Fig. 3. Photograph of the rhizome, showing leaf-gap with one margin considerably thickened, and the beginning of the lateral branch. *o. c.*, outer cortex; *i. c.*, inner cortex (crushed); *r.*, rootlet in cortex (enlarged in Fig. 8). Other lettering as in Figs. 1 and 2. (Slide I YA 21).

Fig. 4. Photograph of the rhizome, showing main stele with leaf-gap still open, and the stele of the lateral branch now separate. (Slide I YA 22.)

Fig. 5. Group of xylem elements, showing pits between adjacent vessels.

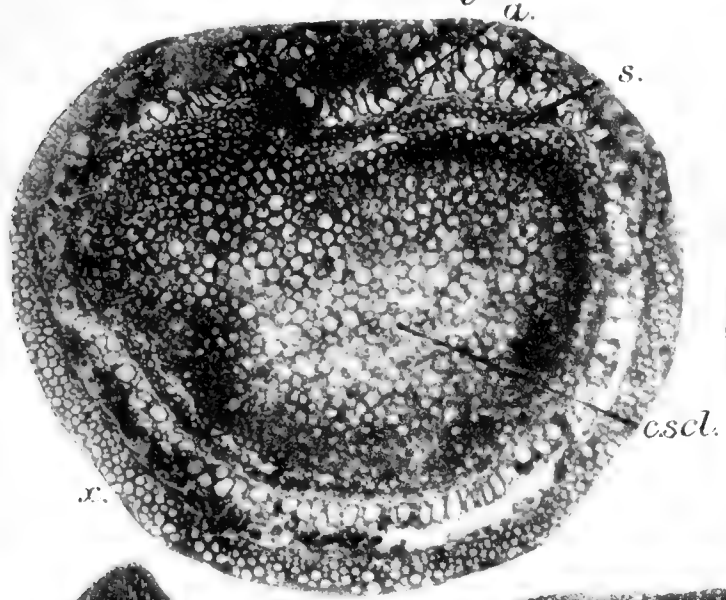
Fig. 6. Diagram of the probable relation of stele, leaf-trace, and lateral branch at a node of *Solenosteleopteris japonica*.

Fig. 7. Drawing of stele of rootlet which is about to become separate from the solenostele.

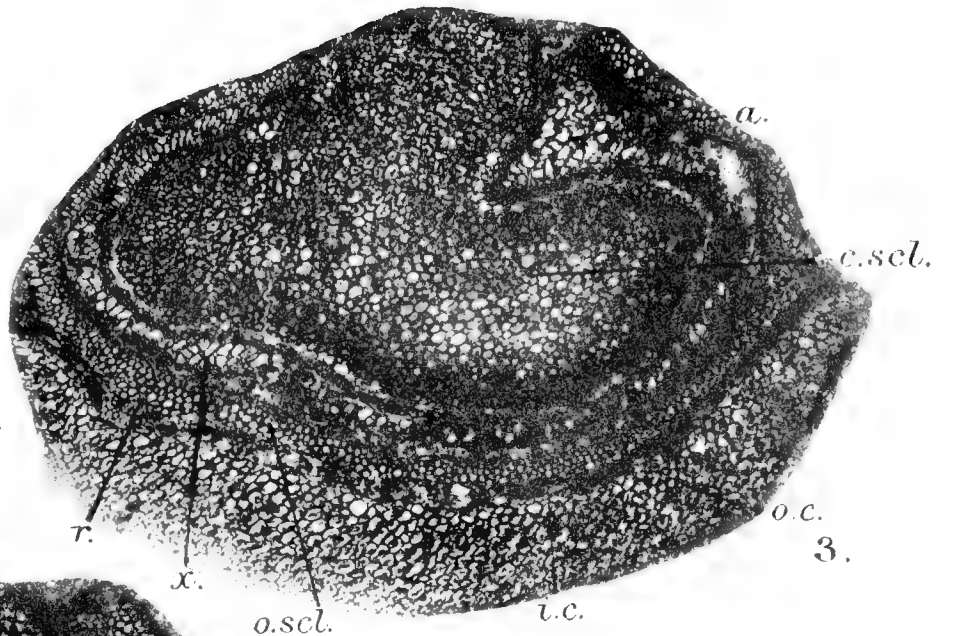
Fig. 8. Rootlet in cortex; the stele surrounded by endodermis but with no cortex of its own.







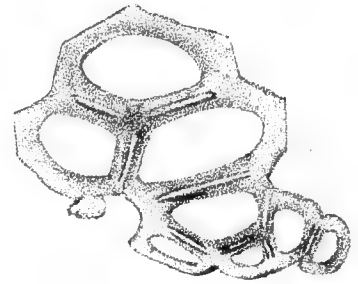
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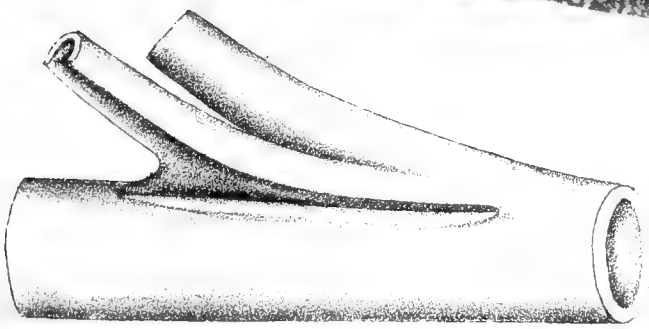
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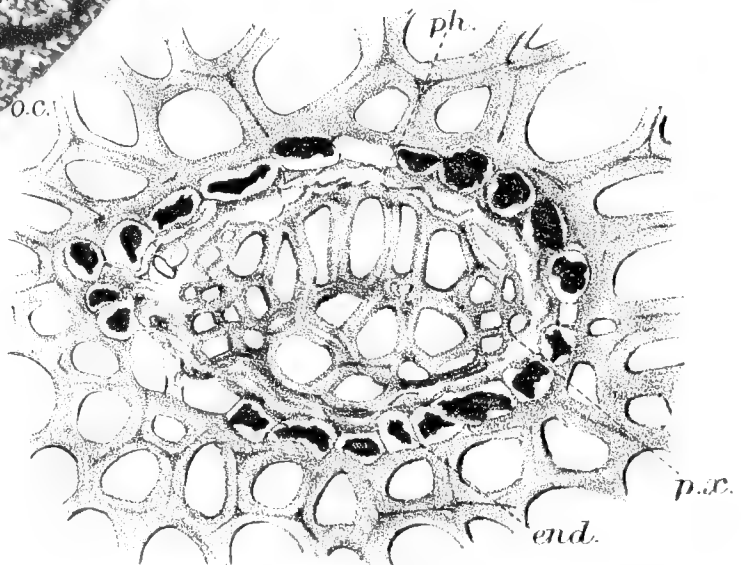
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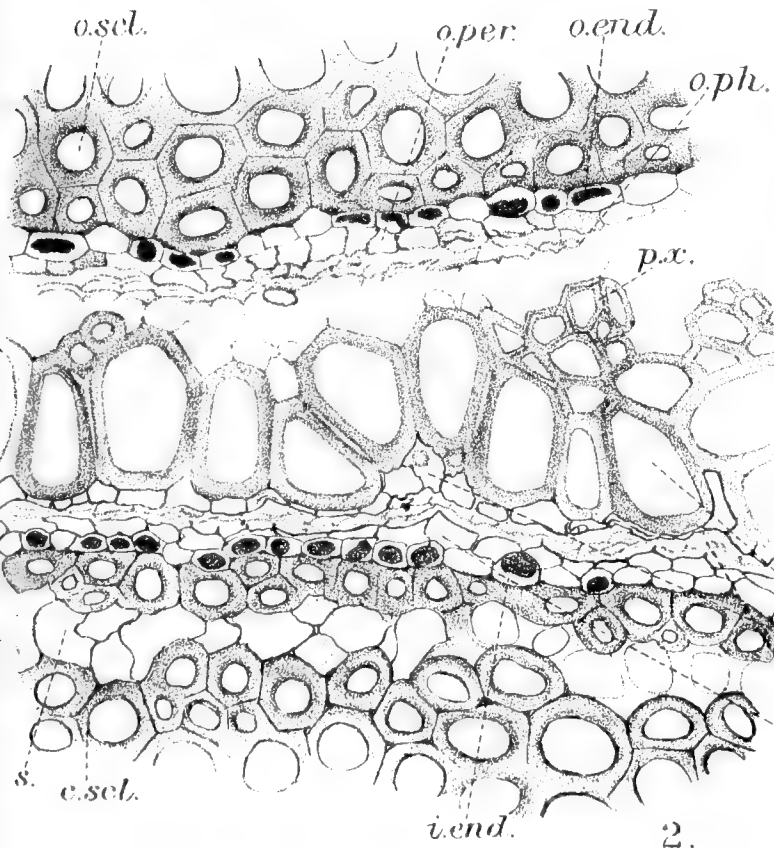
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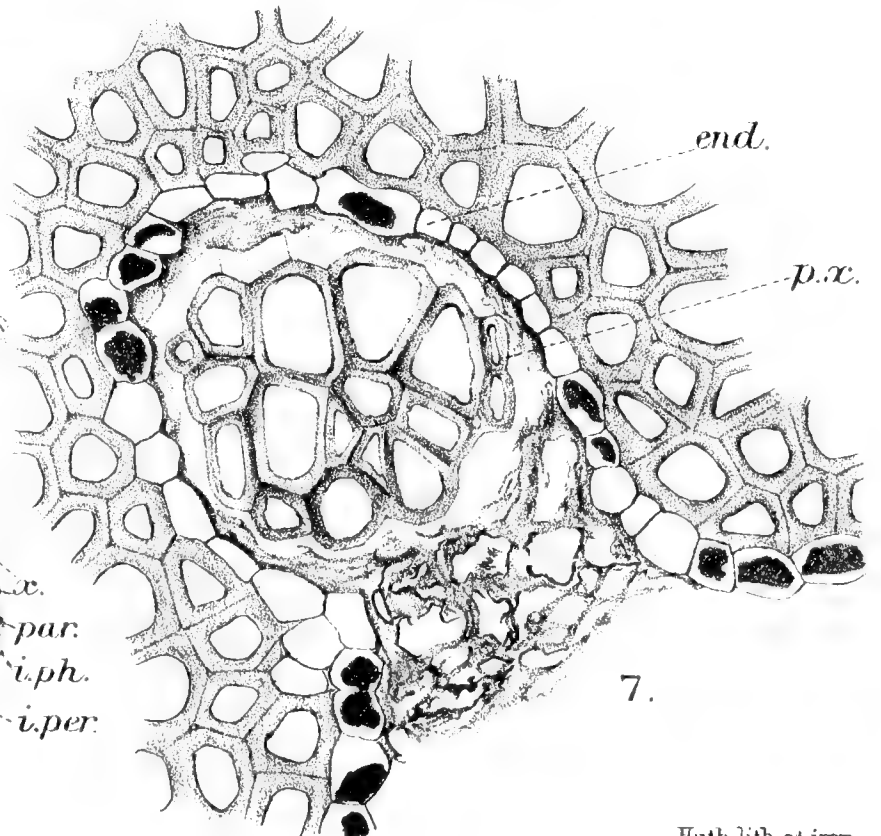
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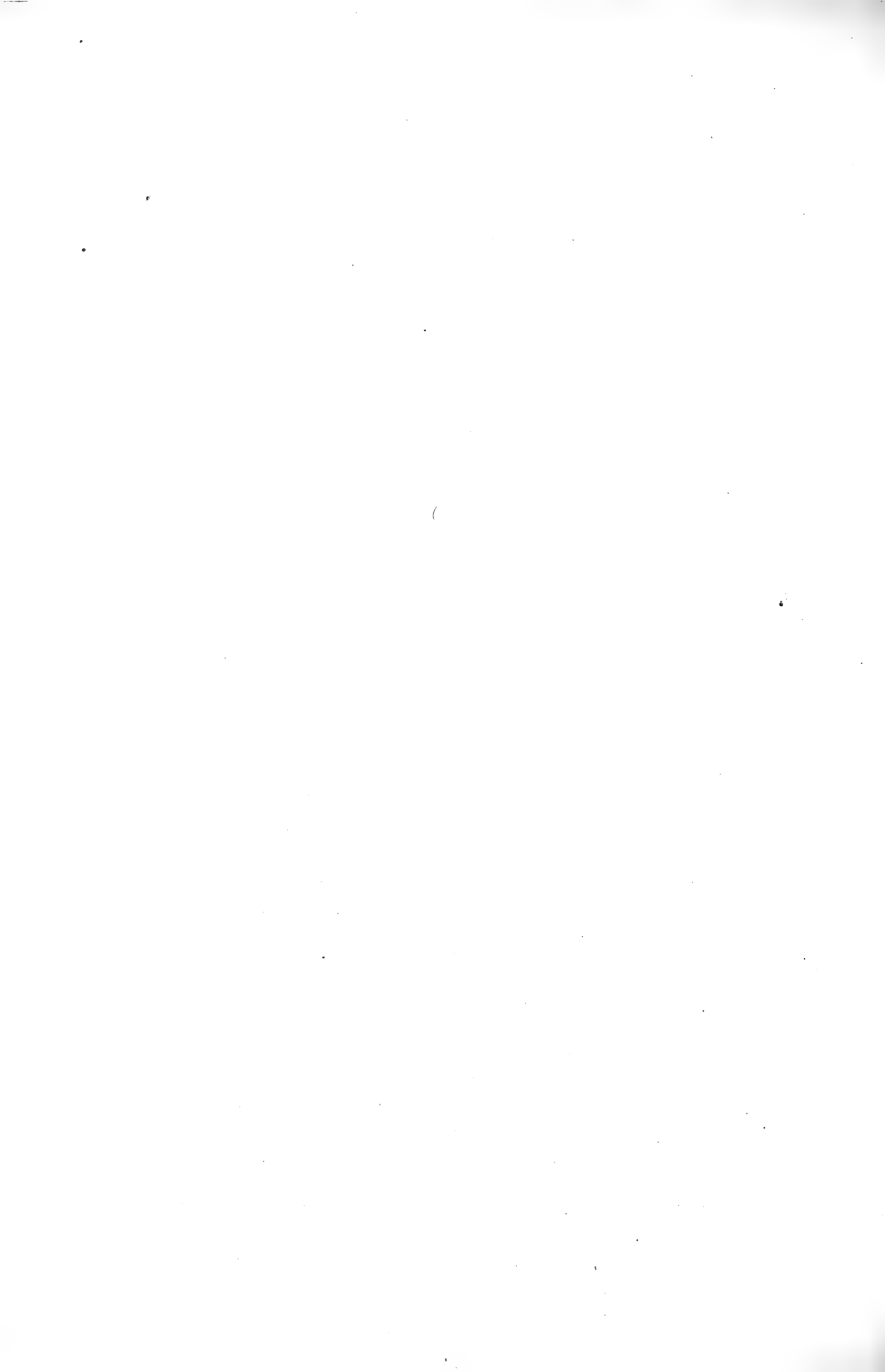
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# A Physiological Study of the Germination of *Helianthus annuus*.

BY

EDWIN C. MILLER.

With twenty-four Figures in the Text.

## HISTORICAL SKETCH.

IN 1842 De Saussure (1) carried on the first investigations in regard to the chemical changes which take place in oily seeds during their germination. He undertook this work in order to test the suggestion of Raspail that the oil in seeds might subserve the same purpose during germination as the starch in the cereal grains. De Saussure investigated the seed and seedlings of the hemp, rape, and madia, and obtained the results shown in the table below:—

	<i>Before Germination.</i>	<i>After Germination.</i>
<i>Rape.</i>		
Oil . . . .	40.7 %	36.9 %
Sugar . . . .	9.8 %	10.3 %
<i>Madia.</i>		
Oil . . . .	26.6 %	24.2 %
Sugar . . . .	5.7 %	8.1 %
<i>Hemp.</i>		
Oil . . . .	28.6 %	26.0 %
Sugar . . . .	3.7 %	4.8 %

During his experiments with these seeds, De Saussure investigated for the first time the respiration of oily seeds during the early period of their germination. He found that the amount of oxygen absorbed by them in a given time was greater than the volume of CO<sub>2</sub> produced. A portion of rapeseed for instance had absorbed, up to the period when their hypocotyls had reached a length of 11 mm., 31.4 c.c. of oxygen, and had produced only 24.39 c.c. of carbon dioxide. He observed this same phenomenon in the other oily seeds with which he experimented.

As a result of these experiments he announced two important conclusions: (1) That during germination the seedlings of oily seeds generate sugar, and that they destroy, in part at least, the oil contained in them; and (2) that

oily seeds differ from those which contain starch in that they absorb during germination a larger volume of oxygen than they produce carbon dioxide.

Letellier (2), while working in the laboratory of Boussingault, found that the oil contained in oily seeds diminished as the germination progressed. His data for the seeds and seedlings of rape and madia are as follows :—

	<i>Madia.</i>	<i>Rape.</i>
Oil in 1 gram of seeds . . . . .	0.41	0.50
Oil in 1 gram, roots 3 cm. in length . . . . .	0.39	0.43
Oil in 1 gram, roots 10-12 cm. in length . . . . .	0.18	0.28

From the work of these two investigators it was thus definitely established that the oil in seeds is a storage product and subserves the same purpose as the reserve carbohydrates, although the chemical processes in the utilization of these classes of substances are different.

Hellriegel (3) in 1855 made the most extensive and thorough investigations on the germination of oily seeds up to his time. He worked only with the seedlings of the rape, but examined his material at five different stages and thus obtained an idea of the changes which take place from the beginning of germination to the time when the seedling had cast off the seed-coat. He found that at first the seedlings increased in weight, but in a short time there was a gradual decrease. He found that the oil decreased from 47 % in the seed to 36 % in the oldest seedlings, and at the same time the sum of the sugar, organic acid, and tannin increased from 7.7 % to 15.4 %. He observed that the seeds contained 3.4 % of cane sugar, and that during the early stage of germination it disappeared and was replaced by glucose. He concluded that during the early stage of germination the cane sugar present in the seed is transformed into glucose, and that this is used at once by the young radicle. He believed at the beginning of germination an absorption of oxygen takes place and thus increases the weight of the seedling. At a later stage the oil undergoes a breaking down. The seedling transforms, on the one hand, part of its oil into carbon dioxide and water by the oxidation of the constituent carbon and hydrogen, and obtains thereby the necessary energy for its growth ; on the other hand, it takes up a further quantity of oxygen which is incorporated into its residual constituents. These two processes combine to produce some compound which is rich in oxygen and from which by further splitting glucose is formed.

Sachs (4, 5) in 1859 and in 1863 made a very detailed study of the products of oily reserves during germination. He investigated in a micro-chemical way the seeds and seedlings of *Ricinus communis*, *Helianthus annuus*, *Cucurbita Pepo*, *Amygdalus communis*, *Allium Cepa*, and numerous others. Sachs concluded from the investigations that the oil in seeds was transformed during germination wholly or partly into starch. According to his view, in some seedlings all of the oil was transformed into starch, while in others only part of the oil was changed into starch and the

remainder was converted directly into sugar. He held that the starch could not be a transition between the oil and sugar because the oil in many seedlings is transformed directly into sugar, while starch appears only in the starch sheath. On the other hand, he argued against the idea that the oil was first changed to sugar and then to starch, because starch often appears in the germination in many places in the seedling where sugar is not present before or at the time of its appearance there.

Peters (6) in 1861, by analyses of the seedlings of *Cucurbita Pepo*, found that during germination the amount of oil decreased from 49.5 % of the dry material to 12.7 % when the seedlings were well developed, and that during this period there was an increase in the amount of carbohydrate, especially starch.

Fleury (7) in 1865, in a series of investigations carried out on the seedlings of the castor-oil bean, sweet almond, rape, and spurge, observed again the fact that as the amount of oil decreased, there was an increase in the amount of sugar. He also observed the fact that the amount of oxygen in early stages of the seedlings was greater than the amount in the resting seeds. He observed that some of the oil had become volatile, and that one or more non-volatile acids had made their appearance. He concluded that during the germination an oxidation of the oil takes place with the formation of sugar. He suggests that this might be due to some catalytic agent.

In 1871 Müntz (8), in a study of the seeds of rape, poppy, and radish, observed for the first time that during germination the oil in the seeds gives rise to free fatty acid. He found that the amount of free fatty acid in the oil increased from 10 % in the resting seed to as high as 98 % in the ten-day seedling. He concluded that during the germination the oil is split up into free fatty acid and glycerine, and that the glycerine at once disappears, since he was not able to detect it at all. He also observed that the free fatty acid during the time of germination undergoes a slow but progressive absorption of oxygen not exceeding 3 % to 4 % of its weight.

Laskovsky (9) (1874) worked with the seeds of the squash and verified the earlier work of Peters. He observed, as did his predecessors, that as the content of oil diminished the amount of cellulose, starch, and sugar increased, and thought that it was probable that the carbohydrates were formed at the expense of the oil which disappeared.

Detmer (10, 11) in 1875 found in the study of the hemp seed that in seven-day seedlings the oil content had decreased from 33 % to 17 %. During that period starch had made its appearance and amounted to 8.9 %. In ten-day seedlings the amount of oil had fallen to 15 %, and the starch to 4.6 %. Since no sugar was detected, he concluded that the starch is the direct product of the oil. In his work in 1880, in discussing the work of Müntz, he suggested that the glycerine formed is changed at once into unknown bodies, and that the free fatty acid might be the source of the

starch formed. He gave the following formula to indicate a possible origin of starch from oleic acid :—



In 1890 Green (12) carried on a detailed study of the changes which take place in the reserve products of *Ricinus communis* during germination. He discovered the enzyme lipase, and showed that the glycerides were split into free fatty acid and glycerine by its action. Green thought that the glycerine thus formed gives rise directly to sugar, and that the free fatty acid gives rise to a crystalline vegetable acid soluble in water, but which he was unable to identify. He concluded that the starch formed in the embryo and young plant is a direct product of the sugar formed from the glycerine, and not a direct product of the oil as Sachs suggested.

In 1891 Schmidt (13) published the results of the most extensive experiments that have ever been carried out on the transportation of the oily reserves in seedlings. He concluded that in many seedlings the oil as such is transported to the different parts, and that only after it has reached its destination is it broken up into the necessary products for the growth of the seedlings. His conclusions were based upon the fact that in seedlings in which oil transportation seems to take place, the amount of free fatty acid present in the place of the storage of the oil is small, and further, that in many parts of the plant remote from the cotyledons considerable quantities of neutral oil appear. He found that the cellulose walls of plant-cells are permeable to oil which contains free fatty acid, and that the greater the amount of acid the more readily permeable are the cell-walls. According to his view the free fatty acid unites with some substance in the cell-wall to form a soap which not only holds a capillary attraction for the oil, but in part emulsifies it and in that manner passes it through the cell-walls.

Schmidt observed also a decrease in value of the iodine number in the unsaturated oils and fatty acids during the progress of germination.

In 1893 Leclerc du Sablon (14) in a note states that he found a decrease in the amount of oil in the endosperm of *Ricinus communis* from 69 % in the resting seed to 11 % when the seedlings had reached a length of 12 cm. Since during that time the reducing sugar increased from 0.4 % to 14 %, he concluded that probably the oil was transformed into glucose.

The same author (15) in the following year worked with the seedlings of the hemp. The seeds contain about 3 % of cane sugar as a reserve. At the beginning of germination this decreases and glucose appears. During the progress of germination the amount of saccharose increased, and when the radicles had attained a length of 5 cm. it amounted to 12 % of the dry material. The glucose in this time had increased to only 5 %. Leclerc du Sablon concluded from this that saccharose is the first product of the oil. The glucose is derived from the saccharose by a process of hydrolysis.

Frankfurt (16) (1894) examined the seed and seedlings of the hemp and the sunflower. In his work on the hemp seedlings he confined himself to the proteid and its products.

In his work on the sunflower he examined the seeds and the seedlings at the age of four weeks. He identified both asparagin and glutamin in the hypocotyls and the roots, but only asparagin in the cotyledons. In the seedlings he was able to identify malic acid. He identified the reducing sugar in the seedling as glucose, and the non-reducing sugar as saccharose.

In 1895 Leclerc du Sablon (17) made extensive analyses of the seedlings of the castor-oil bean, rape, hemp, poppy, and several others. He found in all the seedlings examined practically the same results as in his preliminary work. He found that the amount of free fatty acid as well as the sugar content increased during the progress of germination, while the oily reserve diminished. He was unable to detect glycerine at any time during the germination. He concluded then that an enzyme might be present in the seedling which can liberate the fatty acid in such a way that glycerine is not set free. He believes that the process of the liberation of the acid is a more complex one than simple saponification.

Leclerc du Sablon thought that the glycerine with the acid still combined with it goes directly to form the carbohydrate, while the free fatty acid may be a transitory decomposition product which will give rise to other products capable of being assimilated. He believed that the first carbohydrate formed was either saccharose or a sugar very nearly related to it. In some cases he believed that dextrins appear as transition products between the oil and the sugar formed.

Wallerstein (18) (1896) in his investigations of malt found that the amount of oil diminished with the age of green malt. At the same time the value of the iodine number fell, and the acid value of the oil increased.

Merlis (19) (1897) observed in the germination of *Lupinus angustifolius* that the oily content of 7.4 % in the seeds diminished to 1.6 % in the fifteen-day seedlings, while the cellulose increased from 1.5 % to 8.4 %.

Leclerc du Sablon (20, 21) (1897) investigated the seedlings of the sweet and bitter almonds and of the black walnut in the same manner that he had previously studied the oily seeds, and obtained results substantially the same as those from his former work. In the walnut he also investigated the changes which occur during the formation of the oil as a reserve product. He found that the acid value of the oil when it first begins to appear is greater than when the seed is nearing maturity. In the earlier stages of the formation of the seed, reducing sugar was present to the amount of 7.6 %, but decreased in amount as the oil appeared, and finally could not be detected. The processes of building up the oil for a reserve product seem to be just the opposite from those taking place during germination.

An attempt was made by Hanriot (22) (1898) to isolate and identify the



products produced by the artificial oxidation of oil. He succeeded in getting oil to absorb 15 % of its weight in oxygen. In the product thus obtained he could detect neither tannic nor oxalic acid, but acetic acid and butyric acid were identified. The product obtained gave none of the reactions of starch, cellulose, or sugar.

The germination of *Arachis hypogaea* and *Ricinus communis* was extensively studied by Maquenne (23) in 1898. He selected these two seedlings in order to make a special study of the behaviour of saturated and unsaturated oils during germination. Arachic acid is a saturated compound with the formula  $C_{20}H_{40}O_2$ , while ricinoleic acid is unsaturated and has the formula  $C_{18}H_{34}O_3$ .

Maquenne found in *Arachis* that the cellulose and sugar content at its maximum amount in the seedling had increased 5.6 % of the weight of the dry material. In the seedlings of *Ricinus*, on the other hand, these constituents had at their maximum increased 16 % of the dry weight. The amount of carbohydrate which the glycerine of the oil could furnish for 100 parts of the dry material would be about 5 %. The amount of carbohydrate obtained in the case of *Arachis* is approximately that amount, while in *Ricinus* it is much higher. From this Maquenne concluded that only the glycerine or the saturated oils contribute to the formation of sugar, and that their fatty acids serve only for oxidation. The fatty acids of the unsaturated oils, on the other hand, contribute to the formation of sugar. The production of carbohydrate from an unsaturated fatty acid is due to the presence of the allyl group in its molecule. These groups, rendered free by the oxidation of the two ends of the chain, transform themselves into glycerine, and then by polymerization form the carbohydrates.

Sani (24) in 1900 observed in seedlings of the olive one week old a decrease of the oily content from 42 % to 6.2 %.

Mazé (25) (1900) by the autolysis of the macerated seedlings of *Ricinus communis* obtained an increase in the amount of reducing sugar. After a period of twenty-two hours at a temperature of 53° C. he obtained an increase of 2.6 % reducing sugar. The maximum increase of sugar found was 3.5 % of the material used or 7 % of the oily content. Since the controls during the same time showed no increase of sugar content, Mazé concluded that in the seedling of this plant an enzyme is present which has the power to transform oil into sugar.

Kirkwood and Gies (27) (1902), in a chemical study of the coco-nut, found evidence of the appearance of carbohydrate at the expense of the oil.

Jegorow (27) (1904) investigated the seedlings of *Cucurbita maxima*, which were taken at periods of 6, 10, 20, and 28 days. He found that at the end of the first period the quantity of oil had increased from 45 % to 47 %, and that the total weight of the dry matter had also increased. During the rest of the period there was a gradual decrease of the oily reserve. The

value of the iodine number of the oil decreased from 113.5 to 105. The acid value of the oil increased from less than 1% to 56%. A considerable part of the acid was volatile. The cellulose and sugar content during the progress of germination each showed an increase of nearly 10%.

Von Fürth (28) (1904) examined the oil of *Helianthus annuus* and *Ricinus communis* before germination, and after the radicle of the seedlings had attained a length of 4 to 5 cm. He found that the acetyl value of the oil of *Helianthus* during the period decreased from 87.5 to 50.5, while there was little change in the iodine number. The mean molecular weight of the fatty acid during the period did not change materially. From these results, Fürth concluded that the normal fatty acid does not change into oxy-fatty acid, and that there is no ground for assuming that the unsaturated acid becomes saturated during germination. Since the mean molecular weight of the acid does not change, there is no proof that the fatty acids are broken down into the lower carbon groups. Fürth was unable to detect the vegetable acid mentioned by Green in his experiments with *Ricinus communis*.

In 1905 Green (29) continued his investigation of the germination of *Ricinus*. He found that the amount of lecithin decreased in the early stages of the seedlings, but subsequently a gradual increase of this compound took place. He thought it probable that the oily reserve furnished glycerine and acid groups for this compound. He identified the non-reducing sugar present as saccharose, and the reducing sugar as invert sugar. He concluded that his previous opinion that the sugar present during germination is derived from glycerine is erroneous. The organic acid was again investigated, but its identity was not established. He considers that it is derived from the oil by oxidation, but that it has no connexion either directly or indirectly with the formation of carbohydrate.

## EXPERIMENTAL INVESTIGATIONS.

### CULTURE METHODS.

The sunflower seeds used in this work were of the variety known as the 'Large Russian', and were purchased from a local seedsman in the spring of 1909. The quantity of seeds purchased at that time was sufficient in amount to furnish all the material needed in this investigation.

The seedlings for these experiments were grown in white quartz sand. This sand was washed first with hot and then with cold water before being transferred to the boxes or pots in which the seedlings were grown. The seeds were planted in the sand at a depth of about one half-inch, and were watered with the ordinary city water.<sup>1</sup> The vessels containing the seeds

<sup>1</sup> The total amount of solids in the New Haven city water is very low, only 65-75 milligrams to the litre.

were placed in the greenhouse, where the temperature was kept between  $65^{\circ}$  and  $75^{\circ}$  F.

Most of the investigations on the germination of oily seeds have been limited to the early stages of the seedlings before they have reached the surface of the ground. The few investigations that have been made on plants of this kind at a more advanced stage have been made on seedlings which have been grown in darkness. As it is a well-known fact that plants which are grown in darkness are abnormal, chemically as well as morphologically, one should be careful in accepting the results obtained under such conditions as indicating the processes which take place in the seedling under normal conditions.

Since it was thought advisable to examine the seedlings of the sunflower at several stages after they had reached the surface of the soil, a method was sought whereby the seedlings could have the normal tonic

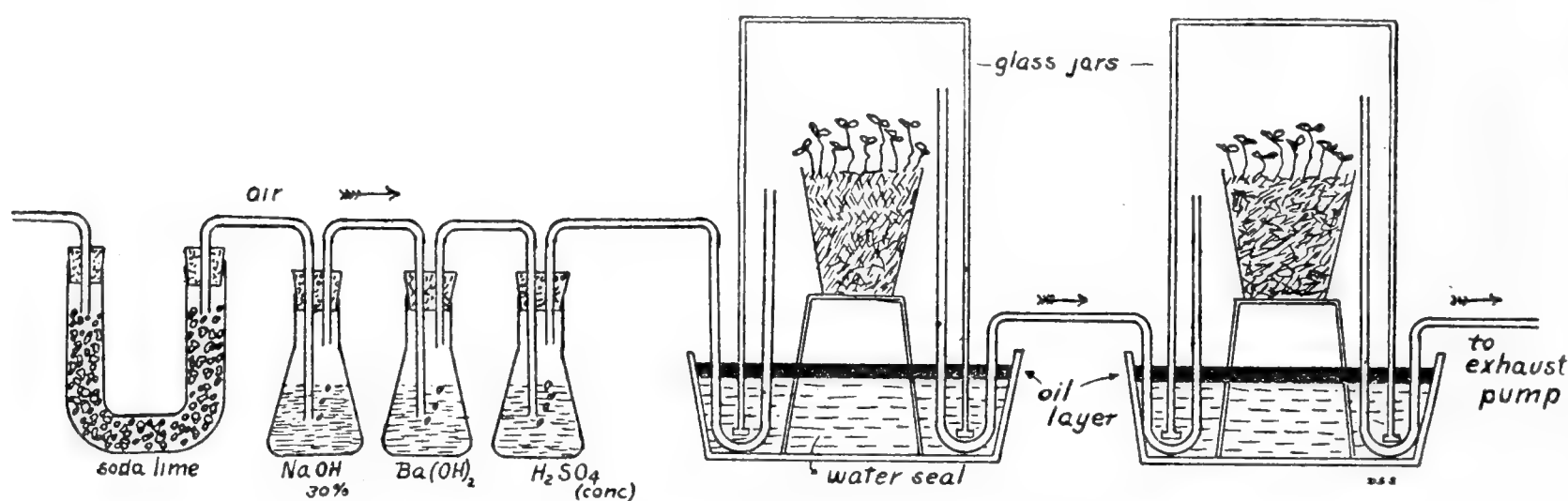


FIG. 1. Apparatus for growing plants in the absence of carbon dioxide.

influence of the sunlight and at the same time be unable to carry on the processes of photosynthesis. For these advanced stages, the seeds were planted in the sand in flower-pots or pans which had been coated on the inside with a layer of paraffin. After the seedlings had reached the surface of the sand, the pans and pots containing them were placed in the apparatus as shown in Fig. 1.

This apparatus consisted of twelve large glass jars, placed in large tin pans and connected with each other by means of glass tubing. The jars were sealed with water and the water then covered with a layer of engine oil about  $\frac{3}{4}$  cm. in thickness. This layer of oil retarded the evaporation of the water into the jars above, and thus prevented abnormal conditions which might arise from an excess of moisture. At intervals in the connexions between the jars, wash-bottles containing strong sulphuric acid were inserted in order to take up the moisture in the air due to the transpiration of the seedlings, and the evaporation from the sand. The series of jars was connected at one end to a filter pump, and a stream of air continually pulled through the apparatus while the seedlings were contained in

it. The air before its entrance into the apparatus first passed through a soda-lime tube, then through a wash-bottle containing 30% sodium hydroxide, then through baryta water, and finally through concentrated sulphuric acid. That the baryta water showed no signs of turbidity was a sufficient indication that the air entering the apparatus was entirely free from carbon dioxide. To avoid the increased pressure due to condensation of the moisture in the bends of the glass tubes connecting the jars, the apparatus was taken apart once or twice during the growth of the advanced stages and the tubes dried. This was always done at night and the apparatus again set to work, so that by daylight the air in the apparatus was free from carbon dioxide. This method of growing the seedlings has the advantage not only of preventing photosynthesis and absorption, but at the same time it supplies the plant with oxygen and provides it with the normal light conditions. The carbon dioxide liberated by the seedling by respiration during the daylight is, under these conditions, probably used in photosynthetic processes before it leaves the plant. There seems to be no possible means of preventing the  $\text{CO}_2$  thus liberated from being utilized by the plant in daylight.

In many of the experiments carried out on seedlings the analyses have been made upon material obtained by grinding up the whole seedling. Without making separate analyses of the hypocotyls and cotyledons in a seedling of the type of the sunflower, it would be impossible to obtain a correct insight into the metabolic changes taking place in the reserve material and the manner of their transportation from place to place. In this work separate analyses were made of the hypocotyls and cotyledons. The seedlings were taken at the different stages, and after carefully washing them to free the sand, the hypocotyls were separated from the cotyledons by means of a razor. The two parts were then ground up separately and dried according to the process described below. In preparing the seeds for analysis the hulls were removed and the tip or rudimentary hypocotyl and root were removed from the cotyledons. The tips and cotyledons were dried down separately in the same manner as below mentioned.

#### STAGES EXAMINED.

*Seeds.* The seeds of *Helianthus annuus* contain as a reserve material between 50 and 56% of ether extract and about 25% of proteid matter. The oil, according to Thorp,<sup>1</sup> consists of the glycerides of oleic, palmitic, arachidic, and linoleic acids. The amount of free acid present in the ether extract in the seeds used in this work amounted to less than one per cent. There is also a non-reducing sugar present to the amount of nearly 4%. This has been identified by Frankfurt<sup>2</sup> as saccharose. No starch is present, and only a trace of reducing sugar.

<sup>1</sup> Thorp, *Outlines of Industrial Chemistry*, p. 326.

<sup>2</sup> l. c.

The proteid is present in the form of granules or grains, and according to Osborne and Campbell (30) consists principally of the globulin edestin. All the cells of the seed, including the rudimentary root and hypocotyl, are closely packed with reserve material.

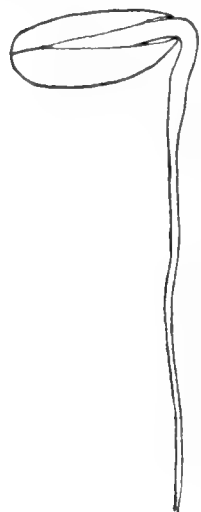


FIG. 2. Stage I of Seedlings.

*Stage I.* At this stage the hypocotyls and roots of the seedlings have reached a length of from 2.5 to 3.5 cm., and have the appearance shown in Fig. 2. The reserve material at the beginning of germination first disappears from the stretching tip. The disintegration and disappearance of material then take place in the lower end of the cotyledon. At this stage the reserve material has disappeared, for the most part, from the hypocotyls and roots, and the proteid grains have broken up in the lower third of the cotyledons. The proteid matter in the upper two-thirds of the cotyledons is apparently yet intact. Starch can now be detected in the starch sheath of the hypocotyl. The root tip is now active as a growing point, and the root is increasing in length in the usual way.

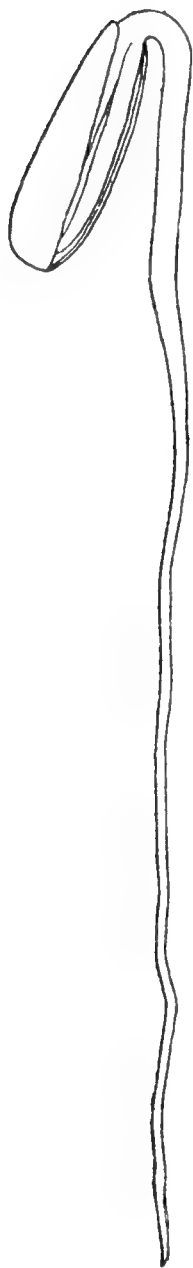


FIG. 3. Stage II of Seedlings.

*Stage II.* The hypocotyls and roots have now reached a length of 7.5 to 11.5 cm. The seedlings were just breaking the ground and the cotyledons were not yet separated from the seed-coats. The cotyledons as yet were only yellowish in colour. The time required for the seedlings to reach this stage was from 4½ to 5 days. The proteid material at this period has disintegrated into small granules in all the cells of the cotyledons. It has almost all disappeared from the lower part of the cotyledons, and the cells in that region are beginning to form vacuoles. The starch sheath is filled with starch as in the preceding stage, and starch also makes its appearance in the parenchyma of the mid veins in the lower part of the cotyledons. See Fig. 3.

*Stage III.* The time required for the seedling to reach this stage was about seven days. As soon as the plants had appeared above the surface of the ground the pots and pans containing them were transferred to the apparatus free from CO<sub>2</sub> and kept there until they had made the desired growth. The seedlings in this stage were kept in the CO<sub>2</sub>-free apparatus one day. At this stage the cotyledons had become a bright green and were spread out perpendicular to the hypocotyl. The hypocotyls had now reached a length of 5 to 6.5 cm. above the ground. The root had reached the same length, while the side roots had attained a length of from 2.5 to 3.5 cm. See Fig. 4. The plumule had as yet not developed. The cells of the starch



sheath are yet filled with starch and it appears in the parenchyma of the veins for the greater part of their length in the cotyledons.

The greater part of the proteid matter had now disappeared from the cells and most of them were beginning to form vacuoles.

*Stage IV.* The time required for the seedlings to reach this stage was about ten days. Six days were required for them to reach the surface

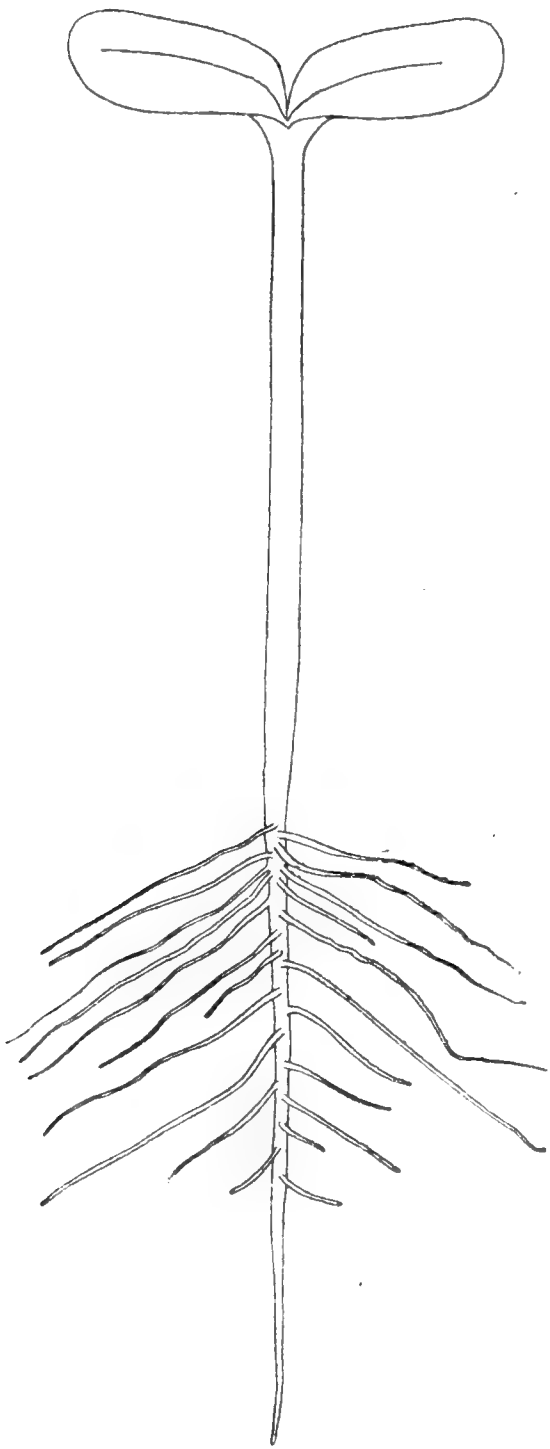


FIG. 4. Stage III of Seedlings.

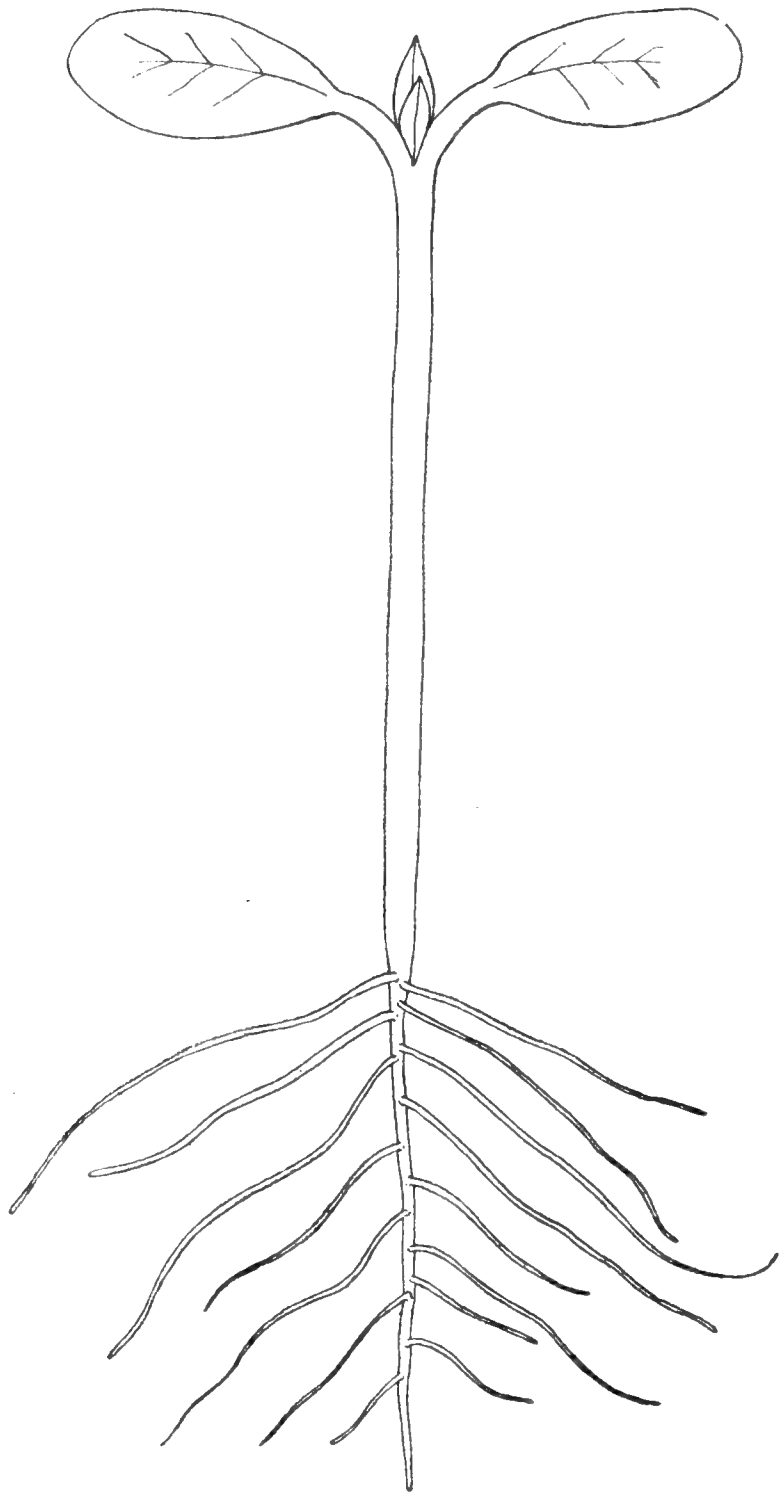


FIG. 5. Stage IV of Seedlings.

of the ground, after which they were placed in the  $\text{CO}_2$ -free apparatus and left four days. The cotyledons had broadened and lengthened by this time and the plumule had become two-leaved. See Fig. 5. The hypocotyl had a length of from 8.5 to 10 cm. from the ground to the cotyledons. The main roots had a length of from 6 to 7.5 cm. and had developed a copious growth of side roots. The starch still remained in the places mentioned in the previous stages, but in a much smaller quantity. The



cells of the cotyledons were now apparently free from all reserve material. They had enlarged and had the appearance of the parenchyma cells of an ordinary vegetative leaf. At this stage the plant has evidently become wholly independent.

*Stage V.* The age of the seedlings used at this stage was thirteen

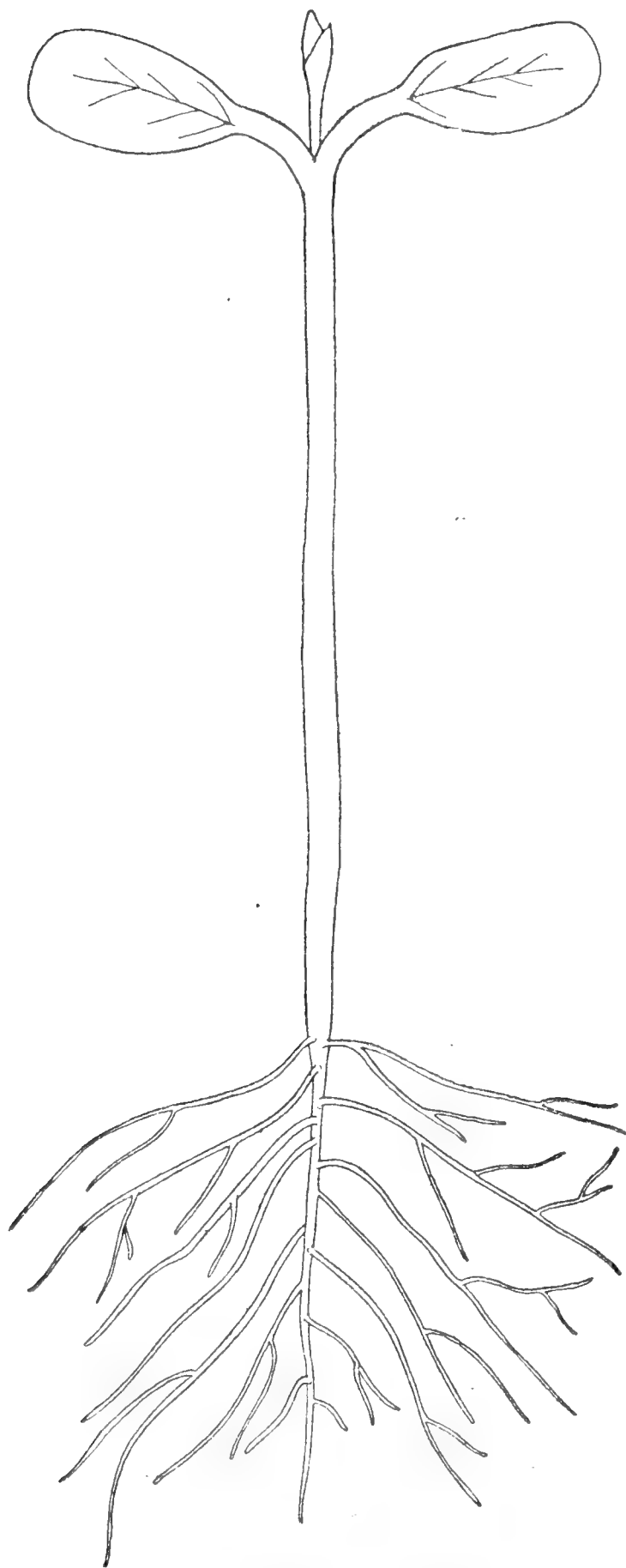


FIG. 6. Stage V of Seedlings.

days, and during the last seven of these the plants were kept in the  $\text{CO}_2$ -free apparatus. The seedlings at this stage differ very little from those of the previous period. The plumule had lengthened and the first internode had a length of 0.6 to 1.2 cm. The hypocotyl had lengthened a little and more copious side roots had developed. The seedlings for several days previous to the end of this period had shown no further growth of their parts. This was to be expected, since the plants, being cut off from the supply of nourishment both from the soil and the air, had no material for growth after the reserved material had been consumed and were slowly starving. See Fig. 6.

#### ANALYTICAL METHODS.

To avoid the oxidation of the oil during the preparation of the material for analysis, the following modification of Lowenstein's (31) method was used. After the wet material had been thoroughly ground and pulverized in a mortar, it was transferred to broad, shallow pans on the water-bath and treated with twice its volume of 95% alcohol. After the alcohol had evaporated the process was repeated a second and a third time and again with absolute alcohol.

After the evaporation of the alcohol the material was placed in the oven at  $100^\circ$ – $105^\circ$  C. for 20 to 30 minutes and then transferred to the desiccator and finally to tightly closed vessels until needed for analysis.

The material obtained in this manner was not oxidized in the least and was a white or yellow powder.

Upon the material thus prepared duplicate analyses were made for the following constituents: ash, moisture, ether extract, total sugar, reducing sugar, cellulose, total nitrogen, and proteid-free nitrogen. The amount of free fatty acid and the iodine number are determined for the ether extract.

The determinations of most of these constituents were made by the ordinary official methods of the American Association of Official Agricultural Chemists. In order to obtain the amount of protein, the protein nitrogen was multiplied by the factor 5.5, a factor which is based upon the work of Osborne and Campbell<sup>1</sup> upon the proteid of the sunflower seed, which showed it to contain 18% of nitrogen.

For the estimation of the proteid-free nitrogen the following method was used:—

A weighed portion of the finely ground material was extracted with ether and then stirred up with a definite volume of cold water and allowed to stand with frequent stirring for one half-hour. At the end of this period the mixture was heated to boiling, a few drops of acetic acid added, and the boiling continued for several minutes. The mixture was then filtered, and to an aliquot portion of the filtrate an equal volume of a solution of 7 grams tannic acid and 2 c.c. of glacial acetic acid per 100 c.c. was added. The tannin precipitate of the protein was then filtered and an aliquot portion of the filtrate taken for the nitrogen determination.

The iodine number was determined by the Hanus method.

The result of these analyses are expressed in Table I. The figures in any one column of that table represent the averages of the duplicate analyses made. In order to be certain that the results obtained in the analyses of the seedlings at any given stage were indicative of the processes normally taking place at that period, a second lot of seedlings was grown, reproducing the conditions as nearly as possible, and duplicate analyses of these made. Thus in Table I the figures in column *a*, Stage I, represent the average of the duplicate analyses on the hypocotyls or cotyledons for the first lot of seedlings. The figures under *b* for the same stage represent the average of the duplicate analyses on the hypocotyls or cotyledons of the second lot of seedlings grown. In Stages I and II a third lot was grown and analysed for certain constituents as shown. Two series of duplicate analyses were made on the cotyledons of the seeds as shown in the table, thus ensuring against possible error in sampling.

It is seen that the results obtained from the two lots of seedlings of any one stage correspond as closely as could be expected. It was found that under approximately like conditions, the time required for the different lots of seedlings to reach the desired stage varied only a few hours.

<sup>1</sup> l. c.

TABLE I.  
PER CENT. OF CONSTITUENTS ON DRY BASIS.

*Cotyledons.*

	Seeds.		Stage I.			Stage II.			Stage III.		Stage IV.		Stage V.	Plumules IV.	Plumules V.
	(a)	(b)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(a)	(b)	(a)		
Moisture <sup>1</sup> . . . . .	4.3	5.2	50.1	58.3	—	69.0	69.5	—	80.3	87.7	93.7	91.1	92.1	—	—
Ash . . . . .	3.3	3.2	3.1	3.3	—	3.3	3.3	3.2	4.2	4.2	7.2	6.7	9.8	—	—
Ether Extract . . . . .	56.2	55.0	58.5 <sup>2</sup>	56.3	55.6	50.9	51.9	51.0	35.3	32.5	17.9	15.5	13.5	17.6	7.2
% Free Acid in Ex.	0.6	0.9	0.9 <sup>2</sup>	1.7	1.5	1.4	1.0 <sup>2</sup>	1.8	11.1	10.4	25.0	22.9	31.4	20.6	34.2
Iodine No. . . . .	136.2	136.2	133.8	134.1	—	129.0	128.0	—	124.1	124.8	88.5	—	67.4	—	—
Total Sugar . . . . .	4.3	3.9	Trace <sup>2</sup>	1.0	1.3	1.9	1.8	1.8	3.5	2.8	3.1	3.2	2.1	None	None
Reducing Sugar . . . . .	Trace	Trace	Trace	None	None	None	None	None	0.3 <sup>2</sup>	1.4	3.0	3.1	1.1	None	None
Cellulose . . . . .	2.9 <sup>2</sup>	2.2	2.2	2.5	—	2.8	2.6	—	4.8	4.7	6.5	6.6	8.8	7.1	7.4
Total Nitrogen . . . . .	4.7	4.8	4.64	4.89	—	4.82	4.82	—	5.54	5.65	6.58	6.1	6.33	—	—
Proteid-free Nitrogen	0.3	0.3	0.72	0.84	—	1.0	0.98	—	1.98	2.04	2.75	2.28	2.58	—	—
Proteid Nitrogen × 5.5	24.2	24.7	21.6	22.3	—	21.0	21.3	—	19.58	19.85	21.06	21.0	20.6	—	—

<sup>1</sup> On wet basis.

<sup>2</sup> Not taken in averaging the per cent. of constituents.

TABLE I (continued).  
*Hypocotyls and Roots.*

	Rudimentary Roots and Hypocotyls of Seeds.			Stage I.			Stage II.			Stage III.		Stage IV.		Stage V.	
	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(c)	(a)	(b)	(a)	(b)	(a)	(b)
Moisture <sup>1</sup> . . . . .	89.7	91.3	—	94.2	94.0	—	95.2	95.8	—	95.9	96.0	95.7	96.0	95.7	96.0
Ash . . . . .	5.5	5.6	—	6.6	6.6	—	6.4	6.4	—	9.5	9.0	11.6	9.0	11.6	11.6
Ether Extract . . . . .	25.4	22.2	25.0	8.9	10.1	9.0	9.6	9.8	—	10.1	9.5	11.4	9.5	11.4	11.4
% Free Acid in Ex. . . . .	15.9	14.5	15.6	21.1	15.2 <sup>2</sup>	25.0	42.4	43.4	—	54.2	61.4 <sup>2</sup>	51.7	61.4 <sup>2</sup>	51.7	51.7
Iodine No. . . . .	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Total Sugar . . . . .	8.7	9.5	—	21.4	20.8	25.2	17.8	19.6	—	11.6	13.6	7.7	13.6	7.7	7.7
Reducing Sugar . . . . .	8.5	9.0	—	19.6	18.7	23.1	16.0	18.7	—	11.5	13.8	7.7	13.8	7.7	7.7
Cellulose . . . . .	6.2	6.0	—	8.0	7.8	—	10.8	11.1	—	14.6	14.1	15.2	14.1	15.2	15.2
Total Nitrogen . . . . .	4.77	4.77	—	3.91	3.99	—	3.45	3.49	—	3.84	3.72	3.85	3.72	3.85	3.85
Proteid-free Nitrogen . . . . .	2.33	2.04	—	2.26	2.2	—	1.82	1.75	—	2.79	2.25	2.66	2.25	2.66	2.66
Proteid Nitrogen × 5.5 . . . . .	13.42	15.07	—	9.07	9.84	—	8.96	9.57	—	5.77	8.08	6.54	8.08	6.54	6.54

<sup>1</sup> On wet basis.<sup>2</sup> Not taken in averaging the per cent. of constituents.

By obtaining approximately the same results on seedlings that have been grown at different times, it seems certain that the results obtained at any stage are a true representation of the normal conditions at that stage.

In Table II the averages of the analytical results for each stage of the seedlings are shown.

The results obtained in experiments with germinating seeds in most cases have been expressed as per cents of the dry material analysed. Results of this character fail to give a complete account of the changes taking place, and in fact may give one an entirely erroneous conception of the processes at work. The original weight of a certain number of seeds after a period of germination may have increased or decreased to a considerable degree. In such a case it is readily seen that the expression of the results in per cent. only throws little light upon the actual depletion or increase of constituents. Take for example the amount of the ether extract of the cotyledons of the sunflower as expressed in Table I. In the cotyledons of the resting seed the amount of ether extract as expressed in per cent. of the dry material is 55% to 56%, while in the cotyledons of Stage I, where the hypocotyls have reached a length of 2.5 to 3.5 cm., the per cent. of the oil on the weight of the dry material is practically the same as that in the cotyledons of the resting seeds. The amount of oil has apparently not decreased in the cotyledons during the three days leading up to Stage I. But when one finds that the total weight of the cotyledons has during that time decreased one-fifth of their original weight, it is readily seen that the amount of oil really present in the cotyledons at the stage mentioned is much less than that in the cotyledons of the resting seed.

Evidently the only way in which one can obtain a clear idea of the increase or decrease of the constituents in seeds during germination, or of the changes that are taking place at any one stage, is to find the actual amount of each constituent in a certain definite number of seedlings at the stage examined. One hundred seeds or seedlings were used as a basis in this work. The weight in grams of one hundred cotyledons at any stage examined was multiplied by the amount of the constituent in per cent. of dry material. This thus gives the amount of that constituent in grams in the cotyledons for that stage. The amount of the different constituents in one hundred hypocotyls at any stage was obtained in the same manner.

To find the actual increase or decrease in weight of a lot of seedlings relative to the seeds from which they originated is a difficult task. The two main difficulties encountered are due to the fact that the rate of germination in seeds varies so much, and that in every case some seeds either fail to germinate, or the young hypocotyl is unable to make its exit from the seed-coats. For that reason the attempts at the beginning of the work of planting one hundred weighed seeds, and of digging up the seedlings when they should have reached the proper stage, were a failure.

TABLE II.  
AVERAGE PER CENT. OF CONSTITUENTS ON DRY BASIS.

	Seeds.		Stage I.		Stage II.		Stage III.		Stage IV.		Stage V.	
	Cot.	Rudimentary Roots and Hypocotyls.	Cot.	Roots and Hyp.	Cot.	Roots and Hyp.	Cot.	Roots and Hyp.	Cot.	Roots and Hyp.	Cot.	Roots and Hyp.
Moisture . . . . .	4.7	4.7	54.2	90.5	69.2	94.1	84.0	95.5	92.4	96.0	92.1	95.7
Ash . . . . .	3.3	3.1	3.2	5.5	3.3	6.6	4.2	6.4	6.9	9.2	9.8	11.6
Ether Extract . . . . .	55.6	47.4	55.9	24.2	51.2	9.3	33.9	9.7	16.7	9.8	13.5	11.4
% Free Acid in Extract . . . . .	0.7	0.8	1.6	15.3	1.6	23.0	10.7	42.9	24.0	54.2	31.4	51.7
Iodine No. . . . .	136.2	131.8	133.9	—	128.5	—	124.4	—	88.5	—	67.4	—
Total Sugar . . . . .	4.1	4.1	1.1	9.1	1.8	22.5	3.1	18.6	3.1	12.6	2.1	7.7
Reducing Sugar . . . . .	Trace	Trace	None	8.7	None	20.5	1.4	17.3	3.1	12.6	1.1	7.7
Cellulose . . . . .	2.2	1.9	2.3	6.1	2.7	7.9	4.8	10.9	6.6	14.3	8.8	15.2
Total Nitrogen . . . . .	4.75	5.0	4.76	4.77	4.82	3.95	5.6	3.47	6.3	3.78	6.3	3.85
Proteid-free Nitrogen . . . . .	0.3	0.4	0.78	2.18	1.0	2.23	2.0	1.78	2.5	2.5	2.58	2.66
Proteid Nitrogen × 5.5 . . . . .	24.4	25.3	22.0	14.2	21.0	9.4	19.8	9.3	21.0	7.0	20.4	6.5



The method of Frankfurt<sup>1</sup> for estimating the weight of the seedlings to the seeds could not be used here. The seedlings of the sunflower examined by him were grown over distilled water. Thus the increase in the amount of ash up to a certain stage of the seedling was considered an index to the decrease in weight of the seedling. This method would have been impossible here, since, owing to the advanced stages at which the seedlings were examined, some compact substratum was necessary. The small amounts of salts that the plants no doubt absorbed from the sand used would have influenced the results.

For this work the following method was finally used. One hundred seeds of the average size and appearance were selected and the hulls removed. These were then divided into lots of ten. Each lot was then ground in a mortar and dried down carefully in the same manner as the material used for analyses. This method showed the variations of small lots of seeds. The total dry weight of the one hundred seeds thus selected was 7.387 grams. Another lot of the same number was treated in the same way. The results obtained showed a weight of 7.36 grams. A third set of seeds was taken and the tips or rudimentary hypocotyls were removed from the cotyledons. The removal was made with a razor, and with care one could remove the tip without at the same time taking along with it parts of the cotyledons. The tips, as is known, lengthen into the hypocotyls and roots during germination. Since the hypocotyls and roots were examined separately from the cotyledons during this work, it was necessary to find the amount of material and kind present in the tips before germination. For that reason they were removed and dried down, as were the cotyledons. The weight of the cotyledons plus the tips amounted to 7.275 grams. The difference in the amount of dry material in the three lots of one hundred seeds each is thus practically negligible. Since the amount of dry material in lots of one hundred seeds showed so little variation, the same method was used for obtaining the relative weights of the hypocotyls and cotyledons of the seedlings at the various stages at which they were analysed. A quantity of seeds of the average size and appearance was selected and planted in the same way and under the same conditions as those used for the analyses. When the seedlings had reached the stage desired they were taken up, and one hundred, representative of the stage, were selected. The seedlings thus selected were carefully washed free from sand, and the cotyledons and hypocotyls separated from each other and dried, separately, after the same manner as the seeds. The weight of 100 cotyledons and hypocotyls at the various stages is shown at the bottom of Table III, and is also shown by Fig. 7. The results obtained for the last three periods is only approximately correct, since it is impossible to recover all the small roots from the sand in these stages. As much of the root-system as possible was taken from the

<sup>1</sup> l. c.

sand and the remnants that broke off in washing were carefully saved. Since it was found impossible to remove all the sand by washing from the roots of the last four stages, correction was made for the ash which was insoluble in nitric acid.

Table III shows the amount of constituents present at the different stages examined, in grams per one hundred seeds. These results were obtained by multiplying the average weight of 100 parts of the seed or seedlings as shown in the lower part of Table III, by the average per cent. of the constituents on the dry weight as expressed in Table II. For example, the amount of ether extract in 100 cotyledons at Stage I, as shown in

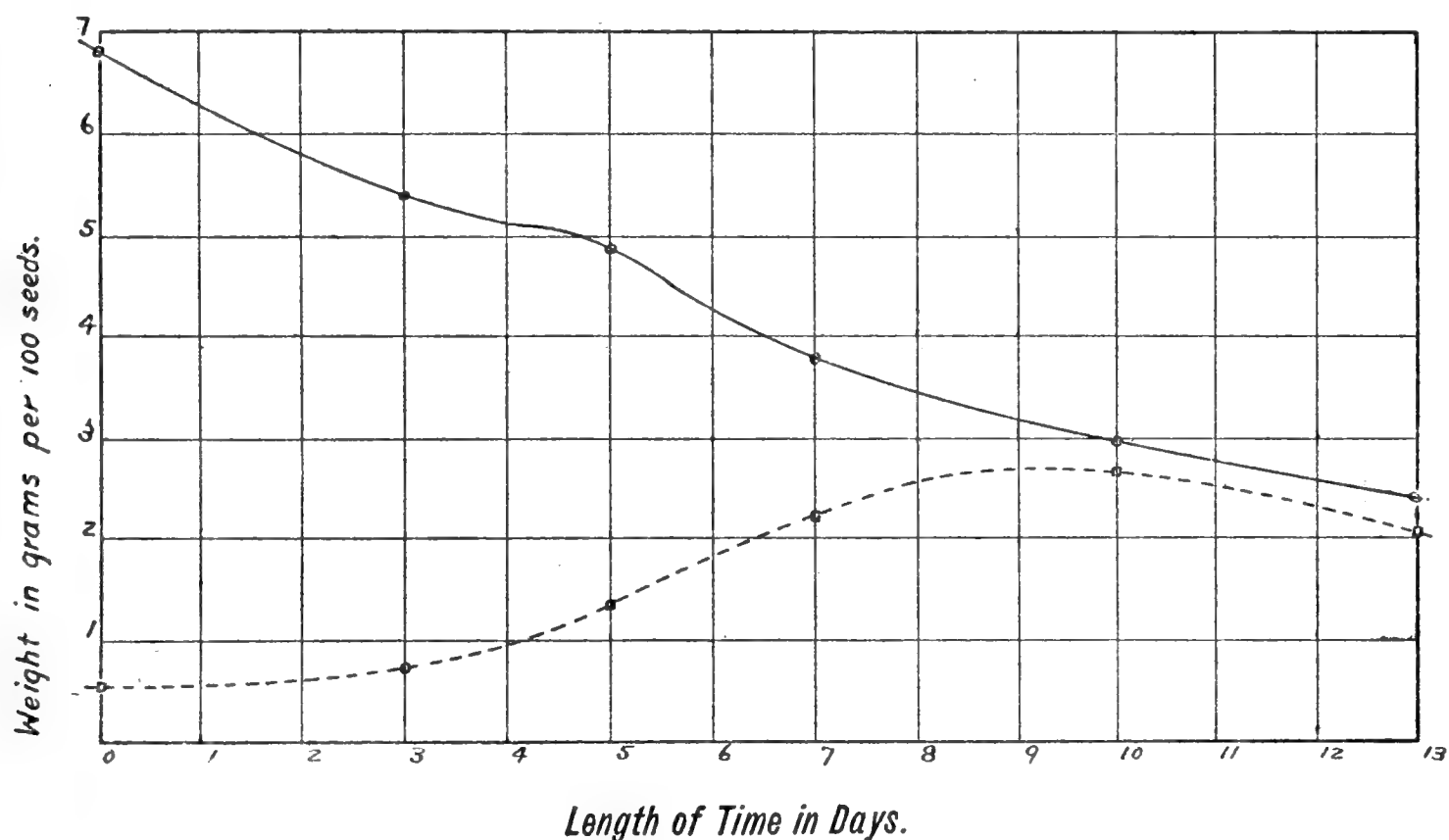


FIG. 7. Relative weight of Hypocotyls and Cotyledons. In this and the following figures the broken lines represent the hypocotyls and the continuous lines represent the cotyledons.

Table III, is 3.0 grams. This was obtained by multiplying 5.368, the weight of 100 cotyledons at that period, by 55.9, the average per cent. of ether extract upon the dry material as shown in Table II.

#### DISCUSSION OF ANALYTICAL RESULTS.

The interpretation of the analytical results in the preceding tables is aided by their diagrammatic expression in the form of curves. Diagrams representing the changes both in per cent. composition and in the total weight of constituents are given in connexion with the following discussion of the variation of the more important materials present in the germinating sunflower.

The changes which take place in the seedling from the tenth to the thirteenth day, as indicated by the tables and curves, are not to be con-

TABLE III.  
GRAMS OF CONSTITUENTS PER 100 SEEDS.

	Seeds.		Stage I.		Stage II.		Stage III.		Stage IV.		Stage V.	
	<i>Cot.</i>	<i>Rudimentary Roots and Hypocotyls.</i>	<i>Cot.</i>	<i>Roots and Hyp.</i>	<i>Cot.</i>	<i>Roots and Hyp.</i>	<i>Cot.</i>	<i>Roots and Hyp.</i>	<i>Cot.</i>	<i>Roots and Hyp.</i>	<i>Cot.</i>	<i>Roots and Hyp.</i>
Moisture . . . . .	0.33	0.021	6.36	7.4	11.1	21.17	20.11	46.94	36.47	66.9	27.5	46.4
Ash . . . . .	0.224	0.014	0.172	0.043	0.163	0.088	0.161	0.141	0.207	0.257	0.23	0.242
Ether Extract . . . . .	3.786	0.219	3.00	0.188	2.53	0.123	1.2987	0.214	0.501	0.273	0.317	0.238
Total Sugar . . . . .	0.279	0.019	0.06	0.071	0.09	0.298	0.12	0.431	0.09	0.351	0.05	0.161
Reducing Sugar . . . . .	Trace	Trace	None	0.068	None	0.271	0.054	0.382	0.09	0.351	0.025	0.161
Cellulose . . . . .	0.15	0.01	0.12	0.047	0.13	0.105	0.184	0.241	0.198	0.398	0.206	0.317
Total Nitrogen . . . . .	0.323	0.023	0.255	0.037	0.238	0.052	0.215	0.076	0.19	0.105	0.148	0.08
Proteid-free Nitrogen . . . . .	0.02	0.001	0.04	0.017	0.05	0.029	0.077	0.039	0.075	0.07	0.061	0.056
Proteid Nitrogen × 5.5 . . . . .	1.66	0.117	1.18	0.110	1.03	0.125	0.76	0.205	0.63	0.195	0.48	0.135
Weight of 100 Parts . . . . .	6.811	0.464	5.368	0.777	4.941	1.327	3.831	2.213	3.0	2.788	2.354	2.086
Total weight of 100 Seed- lings . . . . .	7.275		6.145		6.268		6.044		5.788		4.44	

sidered as normal. The seedlings by that time have used up all the reserve material at their disposal, and under the conditions of the experiment they are deprived of the raw materials for synthetic processes, and are thus

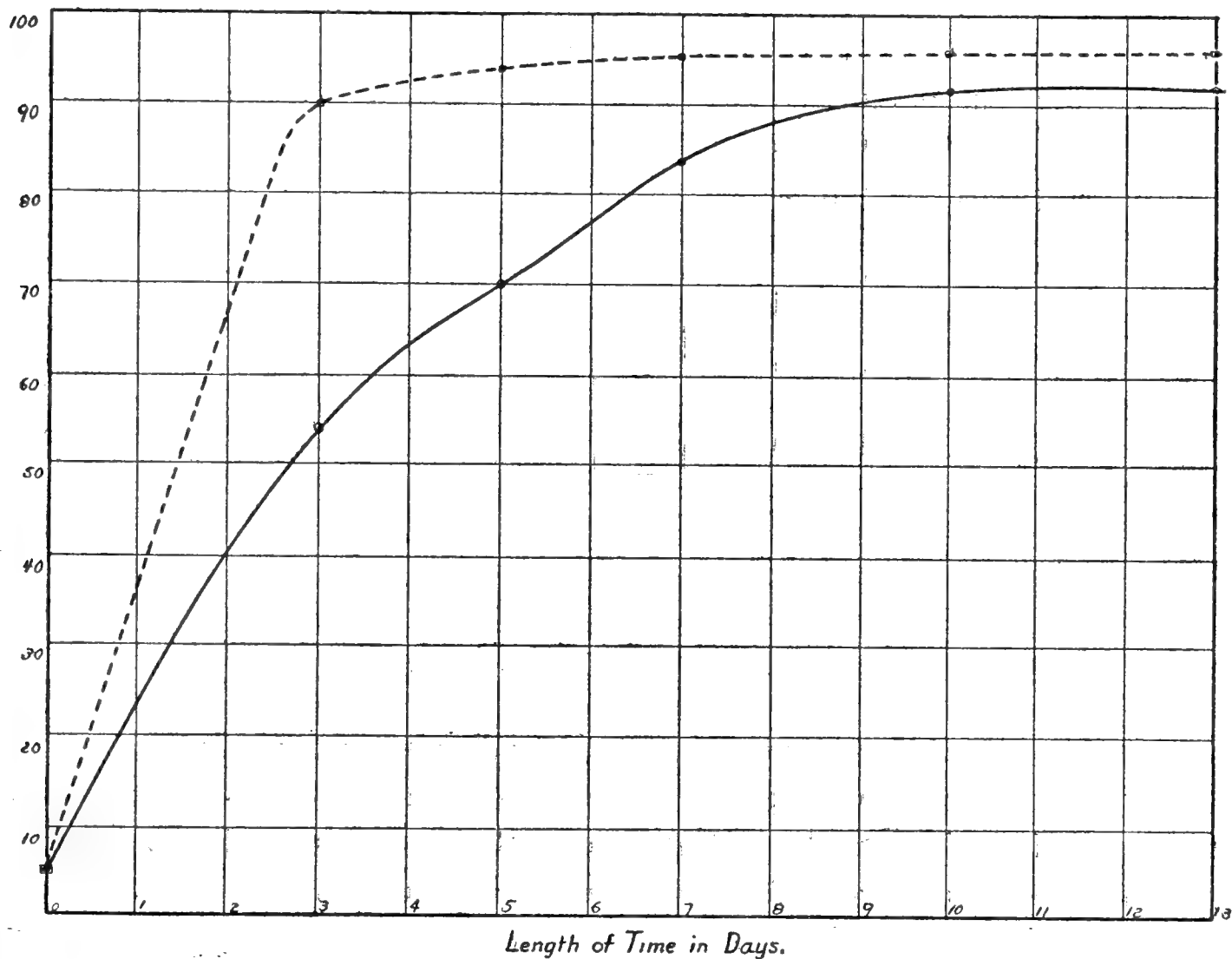


FIG. 8. Water Content, per cent. on wet basis.

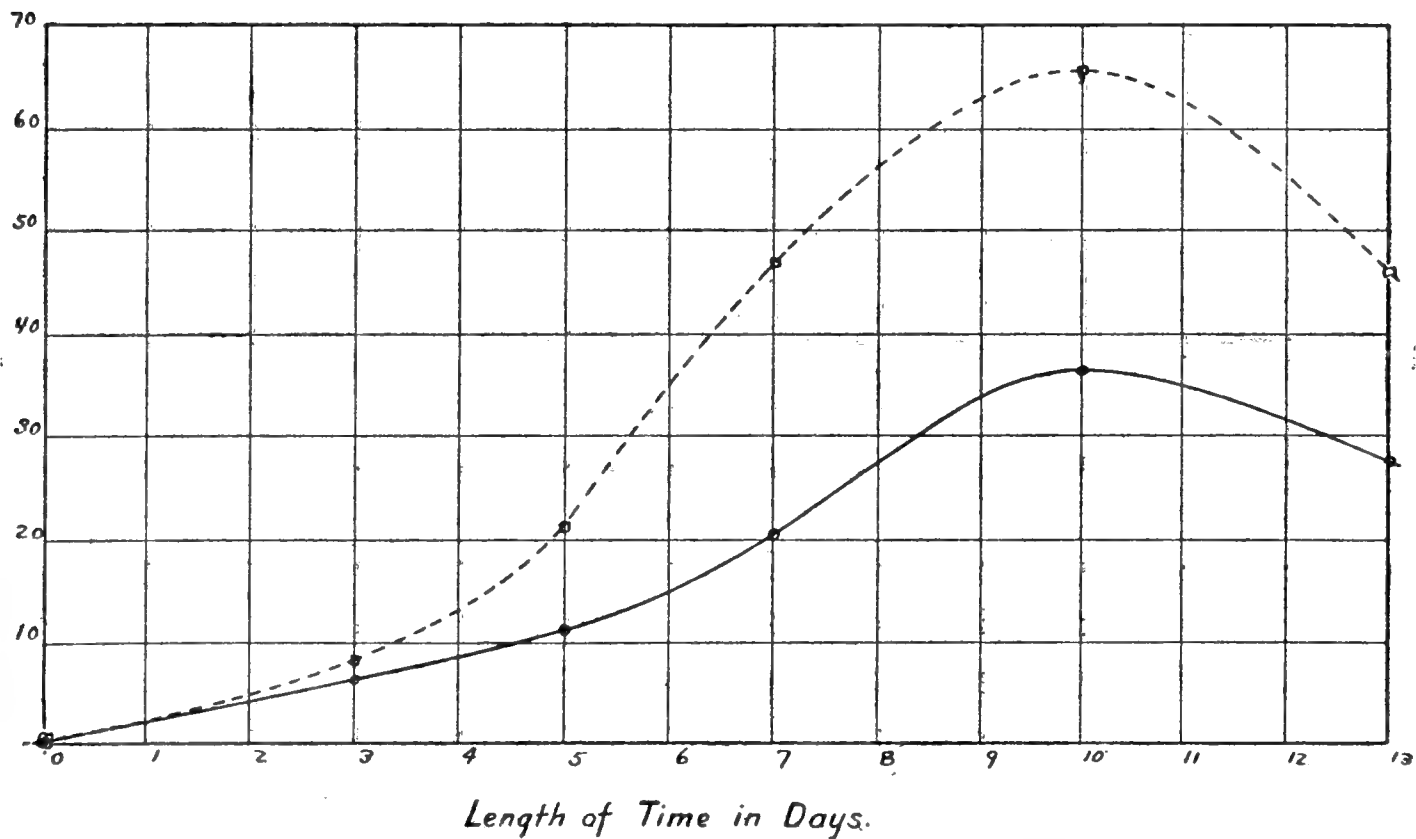
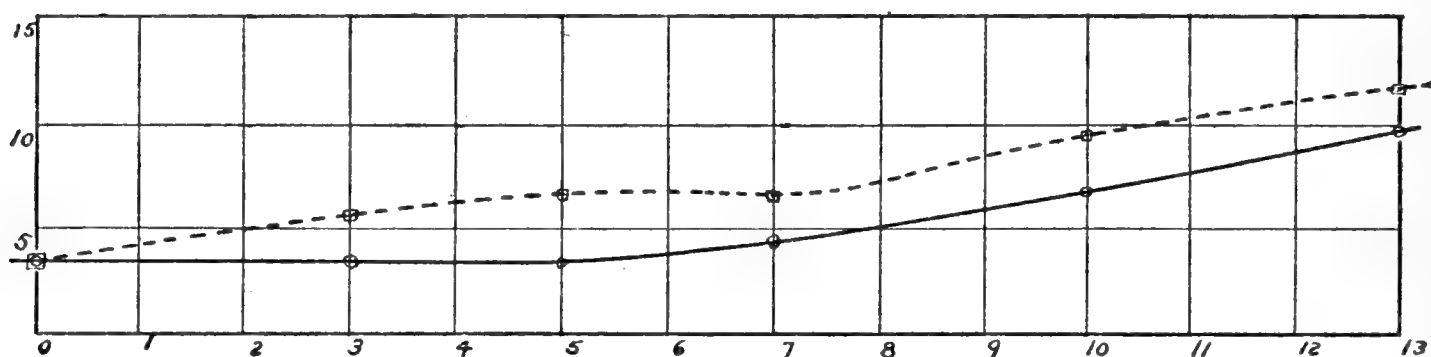


FIG. 9. Water Content, grams per 100 seedlings.

in a state of starvation. The changes taking place must therefore be more or less abnormal, and are so regarded in the discussion of this work.

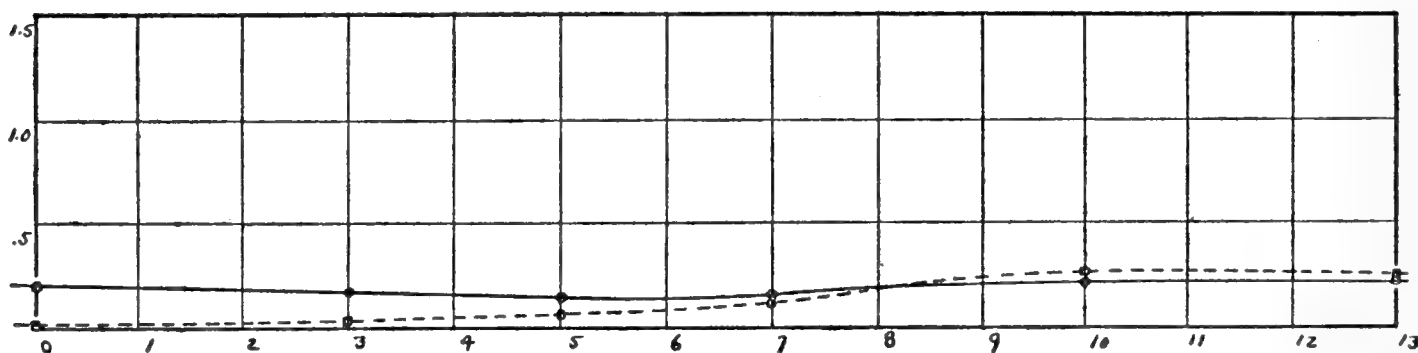
*Water Content.*—The large amount of water which seeds and seedlings absorb during the process of germination is shown by the tables and curves. The per cent. of water in the hypocotyls and roots rises very rapidly, so that at Stage I, only three days after planting, it has almost reached the maximum. After that it rises but little and remains almost constant for all the other stages examined. The per cent. of water in the cotyledons rises more gradually and does not become constant until they are changed into foliage organs. When we consider the great need of water in parts



*Length of Time in days.*

FIG. 10. Ash, per cent. on dry basis.

of the plant where growth and cell-division are taking place, the results obtained are as one would expect. Since water not only has a tonic effect upon the activity of protoplasm but is also the most valuable asset which the growing cells have to enable them to stretch, it is to be expected that the per cent. of water would early become a constant in the hypocotyls and



*Length of Time in Days.*

FIG. 11. Ash, amount in grams per 100 seedlings.

roots. In the cotyledons we would not expect the maximum amount of water to be present until the cells there begin to stretch and the cotyledons to function as foliage organs.

Seeds planted in ordinary soil, and kept under the same conditions as those planted in sand, advance much faster than the latter during the early stages of germination. This is especially true in the length of time necessary for the hypocotyls to begin to show through the seed-coats a stage in the germination reached in twelve hours with seeds planted in rich loam and forty-eight hours with those in sand. This fact is probably

due to the low water-capacity of the sand, so that the large amount of water necessary at the beginning of germination for a maximum advance could not be furnished. This may explain why other investigators obtain certain stages of the seedlings in a shorter time than the same stages were obtained in this work.

*Ash.*—The per cent. of ash in the cotyledons remains at about 3.3 until they are pushing through the ground, after which it gradually increases

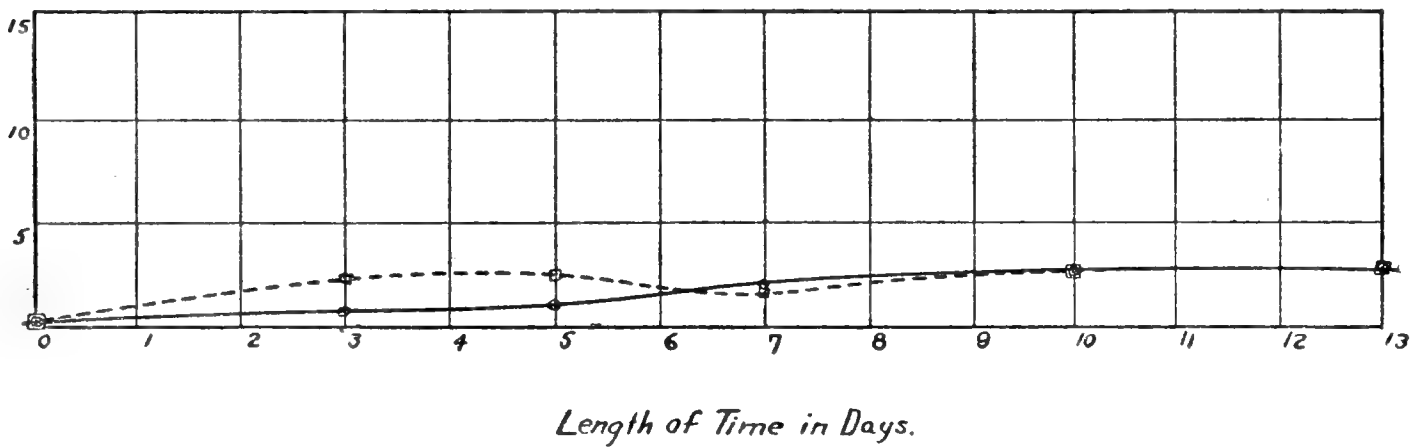


FIG. 12. Proteid-free Nitrogen, per cent. of dry material.

until it reaches almost 10 per cent. in the last stage examined. The per cent. of ash in the hypocotyls rises gradually from the beginning of germination until the seventh day, when its increase is much more rapid.

The weight of ash in the cotyledons decreases gradually until they are beginning to function as foliage leaves, after which it increases in amount. The ash content of the hypocotyls and roots rises gradually from the

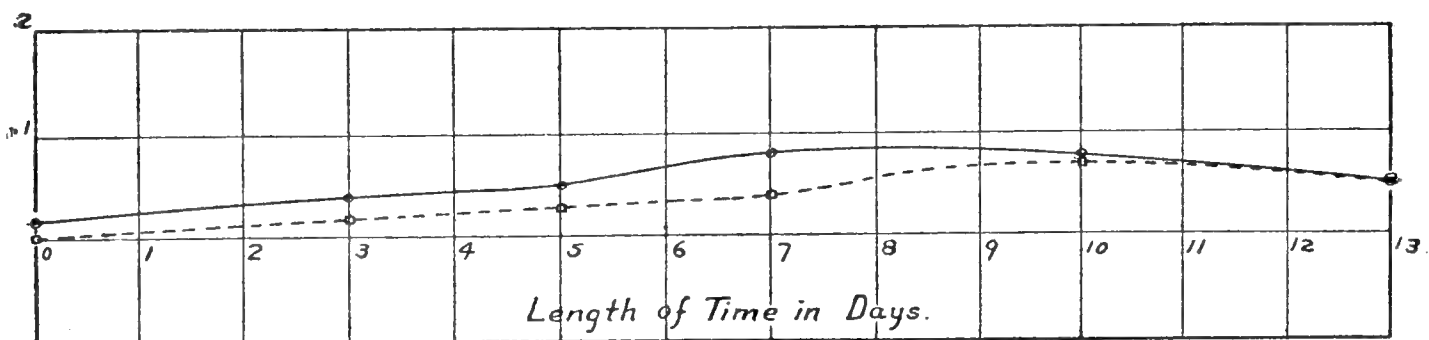


FIG. 13. Proteid-free Nitrogen, grams per 100 seedlings.

beginning of germination to the last stage examined. The amounts are as one would expect when we consider the substratum in which the seedlings were grown. While the cotyledons were functioning as storage organs, and before the hypocotyls and roots could absorb to any degree for themselves, the ash constituents of the cotyledons migrated downward. After the roots became active in absorption, the small amount of mineral matter absorbed was in part transported to the cotyledons, which were now dependent upon the roots for their water supply.



*Protein.*—The per cent. of proteid-free nitrogen in the resting seed is low, amounting to only 0.3. The amount increases slowly at first in the cotyledons, but after they have reached the surface of the ground the non-protein nitrogen amounts to  $\frac{1}{3}$  of all the nitrogen present. The proteid-free nitrogen in the hypocotyls and roots varies from  $\frac{1}{2}$  to  $\frac{3}{4}$  of the total

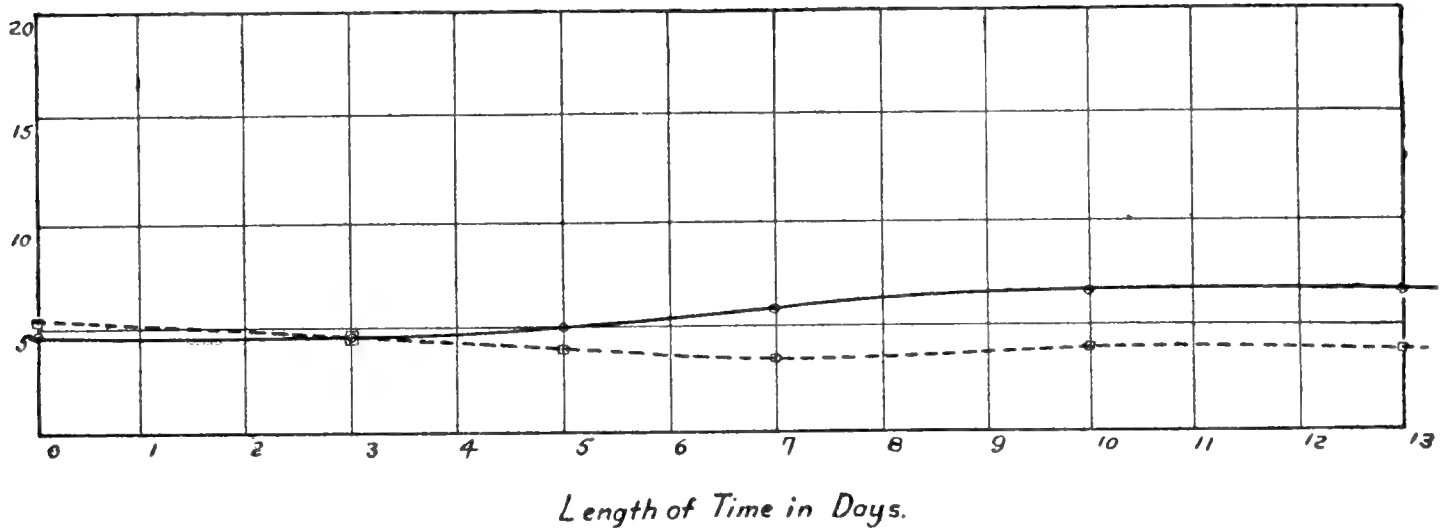


FIG. 14. Total Nitrogen, per cent. of dry material.

nitrogen present in these parts. Frankfurt found that in twelve-day seedlings of the sunflower, grown over distilled water, the glutamin and asparagin present amounted to 4.05% of the dry weight of the seedlings, and that almost  $\frac{1}{3}$  of the total nitrogen present in the seedlings was in the form of these two substances and other amido-compounds.

There is a decrease in the weight of total nitrogen from the beginning

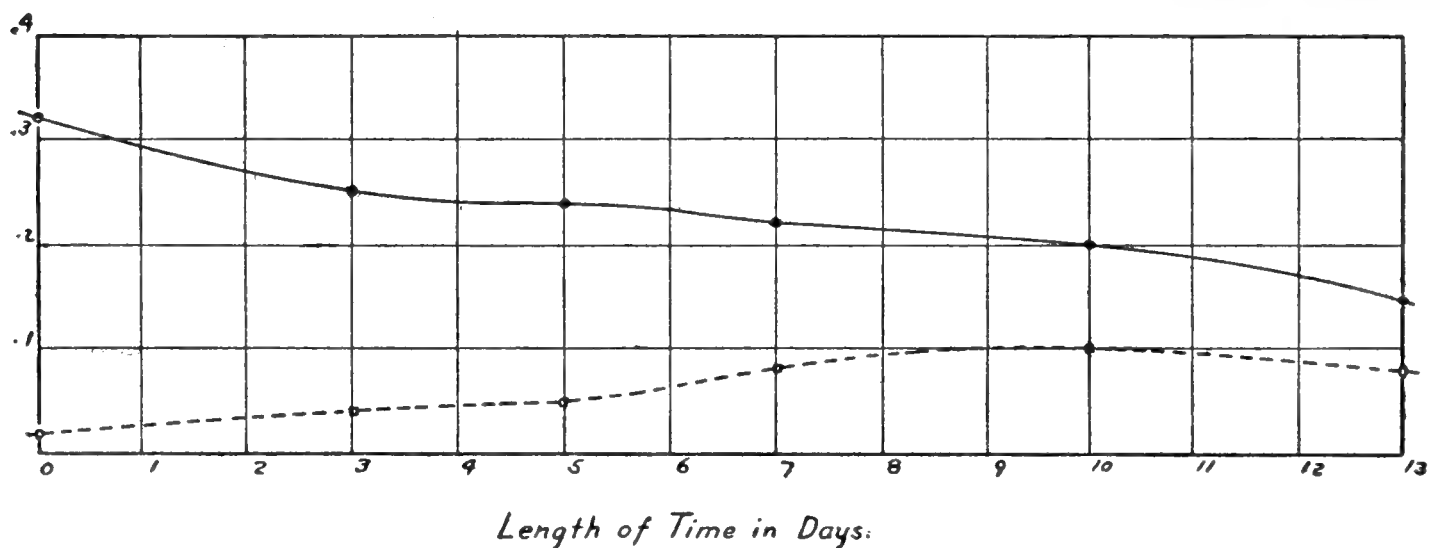


FIG. 15. Total Nitrogen, grams per 100 seedlings.

of germination to Stage I, after which the total nitrogen is a constant for the rest of the stages examined. The loss of nitrogen at the beginning of germination seems to point to the fact that there is probably an oxidation of the proteids taking place during that period.

The changes which take place in the proteid reserve during the germination of this seedling were not examined in detail, since the problem which

here interested us most was the changes in the oily reserve, and because the protein products have been examined in some detail in this seedling, and very thoroughly in many others by Schulze and his pupils.

*Ether Extract.*—The per cent. of ether extract in the cotyledons remains a constant for the first three days of germination. The actual amount of extract has diminished, however, about  $\frac{1}{7}$  of the amount at first present, a fact which shows that during the same period the other reserves of the seed must have been diminished also.

The weight of the ether extract in the cotyledons decreases gradually

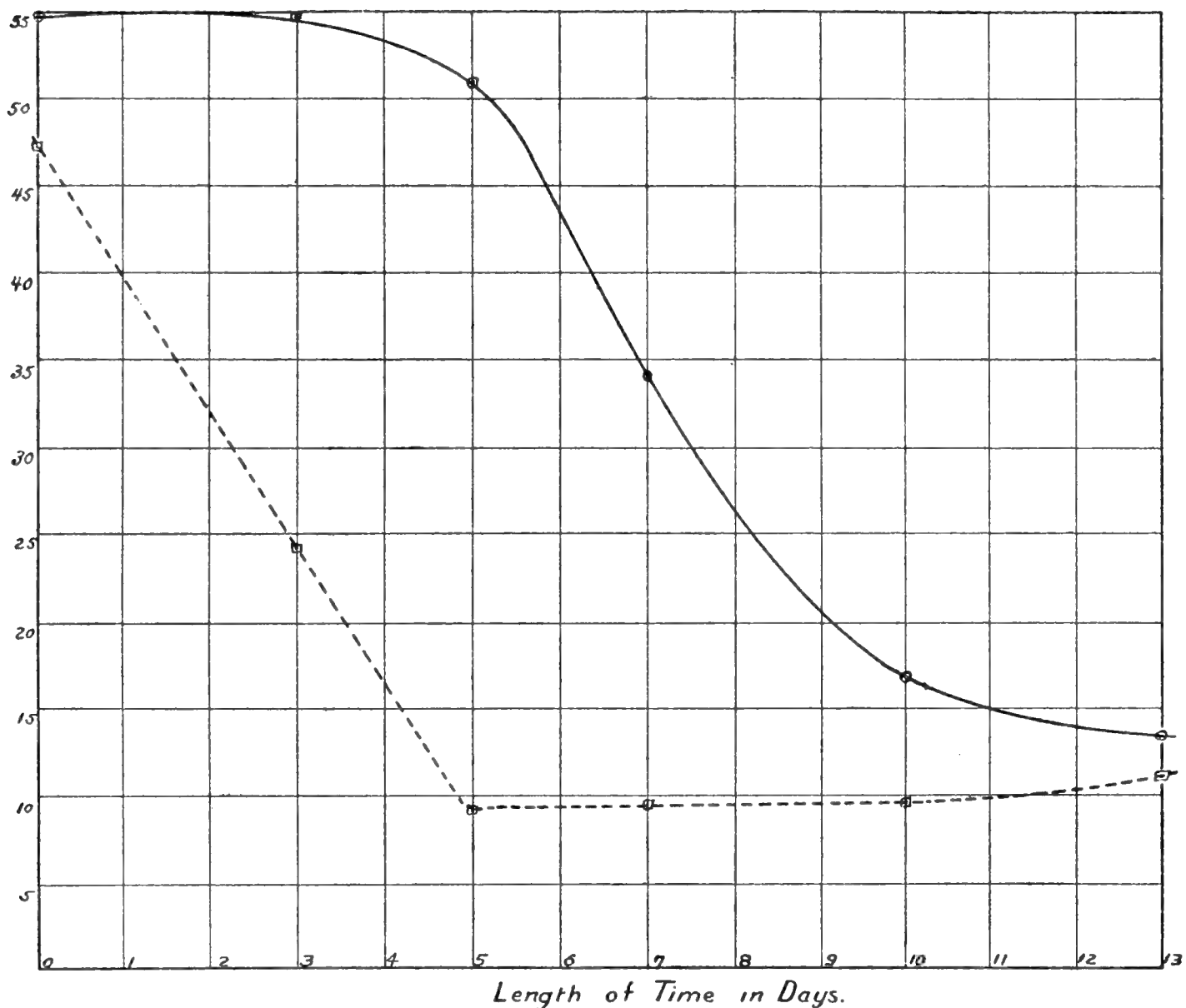


FIG. 16. Ether Extract, per cent. of dry material.

from the very beginning of germination. The most rapid decrease of this reserve takes place between the period when the cotyledons are just beginning to break through the ground and the time when they are fully expanded. The per cent. of ether extract in the hypocotyls and roots falls very rapidly, and after the cotyledons are above ground it remains almost constant. The amount of the ether extract in grams, however, decreases only slightly during the early stages of the seedling, but when the greatest decrease is taking place in the cotyledons, the oil in the hypocotyls and

roots begins to increase in amount and reaches the maximum in the ten day seedling.

The changes which the oily reserves undergo in the progress of the development of the seedling are difficult to determine. This is due to the fact that these reserves in seeds are composed of a mixture of glycerides which have never been separated from each other, and to the lack of knowledge of the exact structure of some of the acid radicles of these glycerides. Nevertheless, certain processes and results can help in a measure to throw some light upon the changes which take place in germination.

The value of the iodine number of the ether extract during different stages of germination gives one an idea whether or not the unsaturated acids are becoming saturated. The iodine number of the ether extract of the

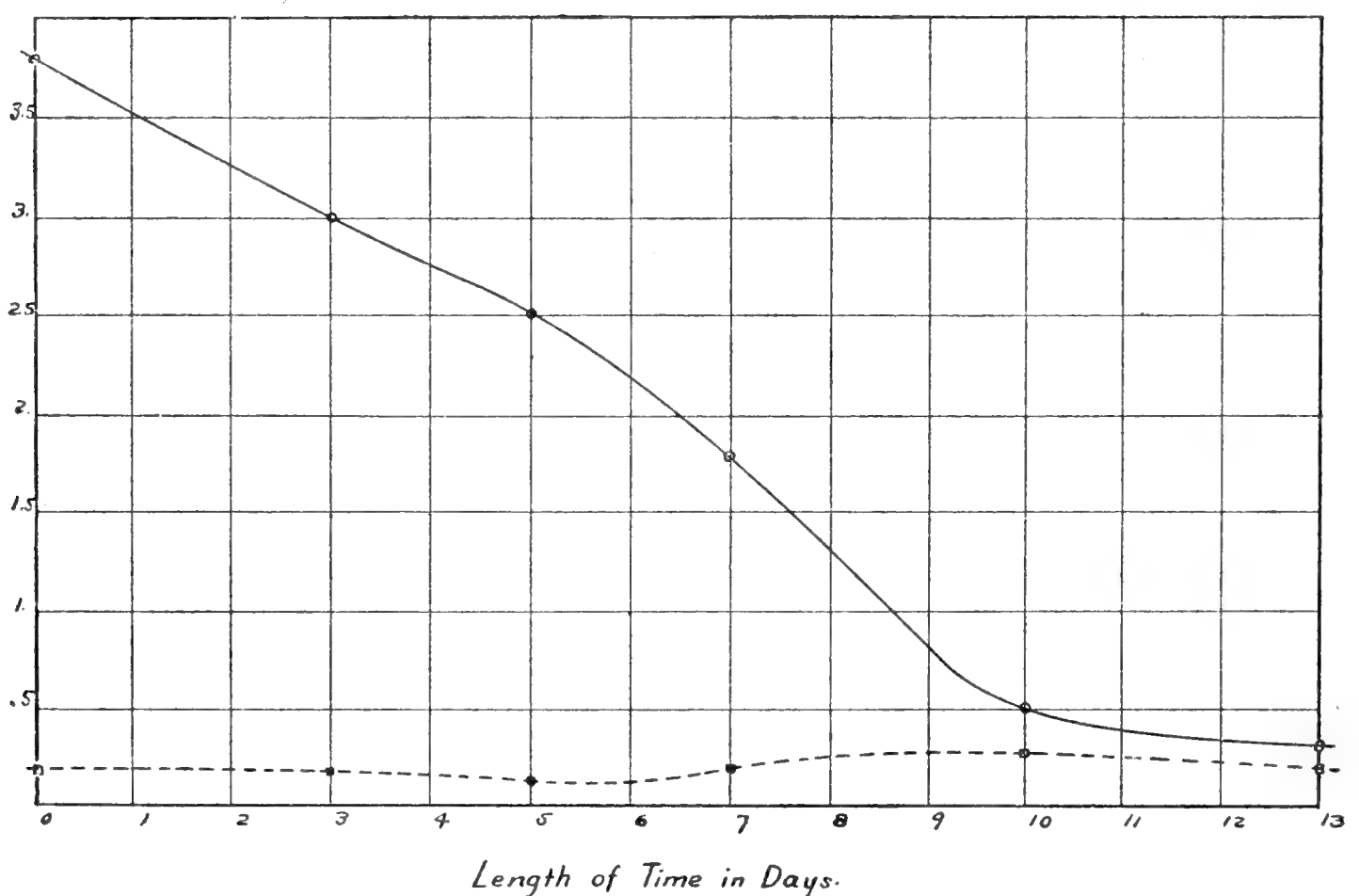


FIG. 17. Ether Extract, grams per 100 seedlings.

cotyledons shows a gradual decrease in value during the progress of development of the seedling. The value decreases from 136.2 for the ether extract of the seed to 67.4 for that of the thirteen-day seedling. The value for the last two stages examined may be a little too low owing to the chlorophyll present in the ether extract, but the value of the other stages is not influenced by this pigment. The decrease of the iodine number seems to indicate that the unsaturated acid radicles of the oil are becoming saturated, probably by the addition of oxygen. Von Fürth found no such evidence of a decrease in the value of the iodine number of the oil of the seed and seedling of the sunflower. His determinations, however, were limited to only one stage of the seedling, and cannot be given the same weight as the extensive work of Schmidt on the fatty acids of the sunflower

seedling, and many other species, showing plainly a decrease in the value of the iodine number as germination progresses.

During certain periods of germination the weight of the seedlings decreases but little, and even increases. For example, the total weight of the seedlings in Stage II, when the hypocotyls have reached a length of 7.5 to 11.5 cm., is a little more than the same number of seedlings two days younger, when the length of the hypocotyls is only 2.5 to 3.5 cm.; the decrease in weight from Stage II to Stage III is very small also. These facts point to an absorption of oxygen by the oil, as suggested by Hellriegel and others, but it can only be definitely proven by an ultimate analysis of the oil.

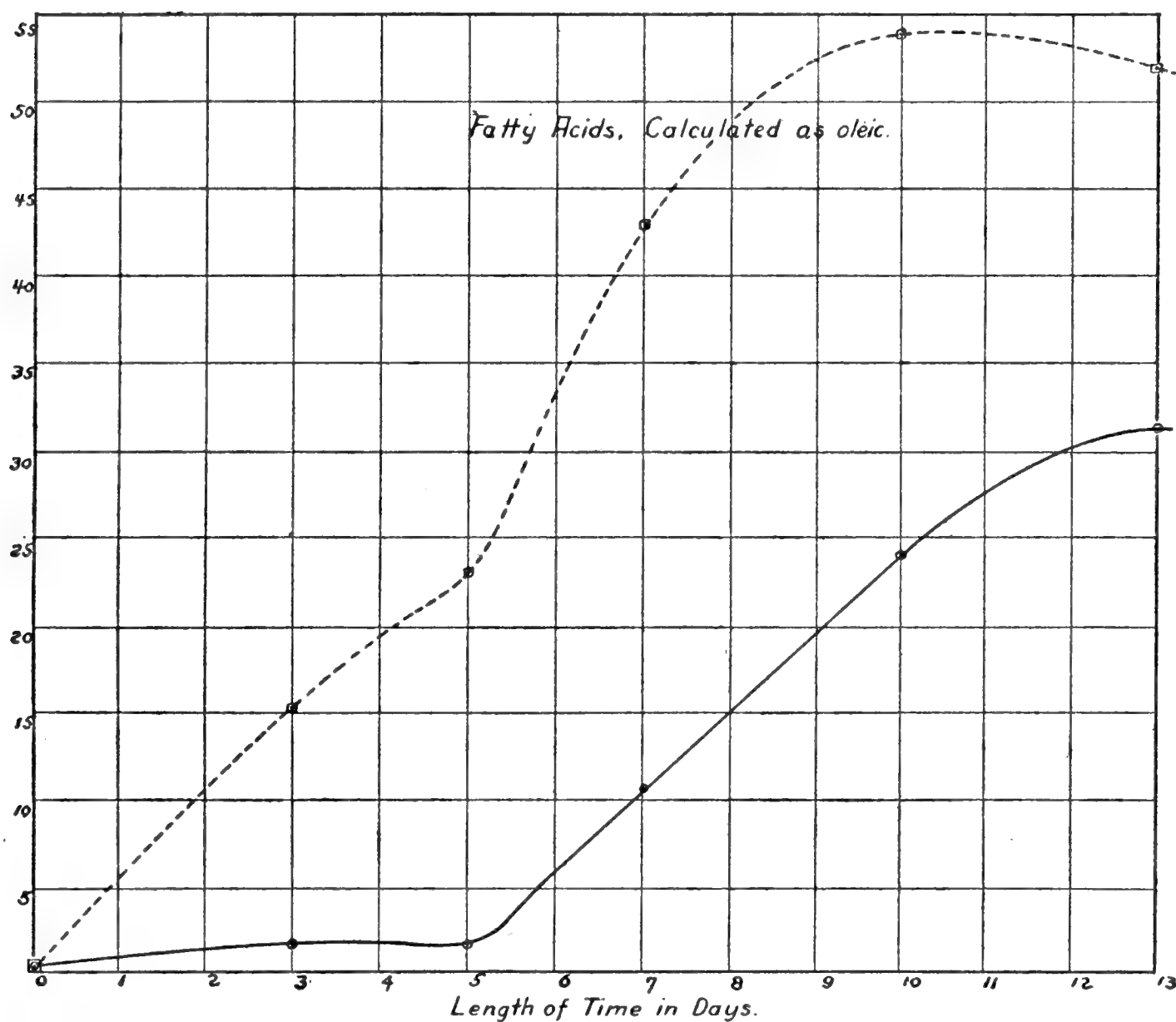


FIG. 18. Free Fatty Acid, per cent. of ether extract.

The free fatty acid in the oil of the cotyledons of the resting seed is less than one per cent. During the early stages of germination this per cent. increases very slowly, and when the seedlings are breaking through the ground it amounts to only 1.6. From that period it rises rapidly, and when the plant has reached the ten-day stage it amounts to  $\frac{1}{3}$  the ether extract.

The acid of the ether extract of the hypocotyls and roots increases rapidly from the beginning, and in the later stages composes over  $\frac{1}{2}$  of the oily content of these parts. These facts indicate that during the course of

germination at least a part of the oil is broken up into glycerine and free fatty acid. Glycerine, however, has never been detected as yet in seedlings, since it seems to be used at once by the plant, or is changed immediately, so that no accumulation of it takes place.

*Carbohydrates.*—The total amount of sugar in the resting seed amounts to 4.1%. Of this amount all except a trace is non-reducing sugar, and has been identified as cane sugar by Frankfurt.<sup>1</sup> This sugar is distributed in

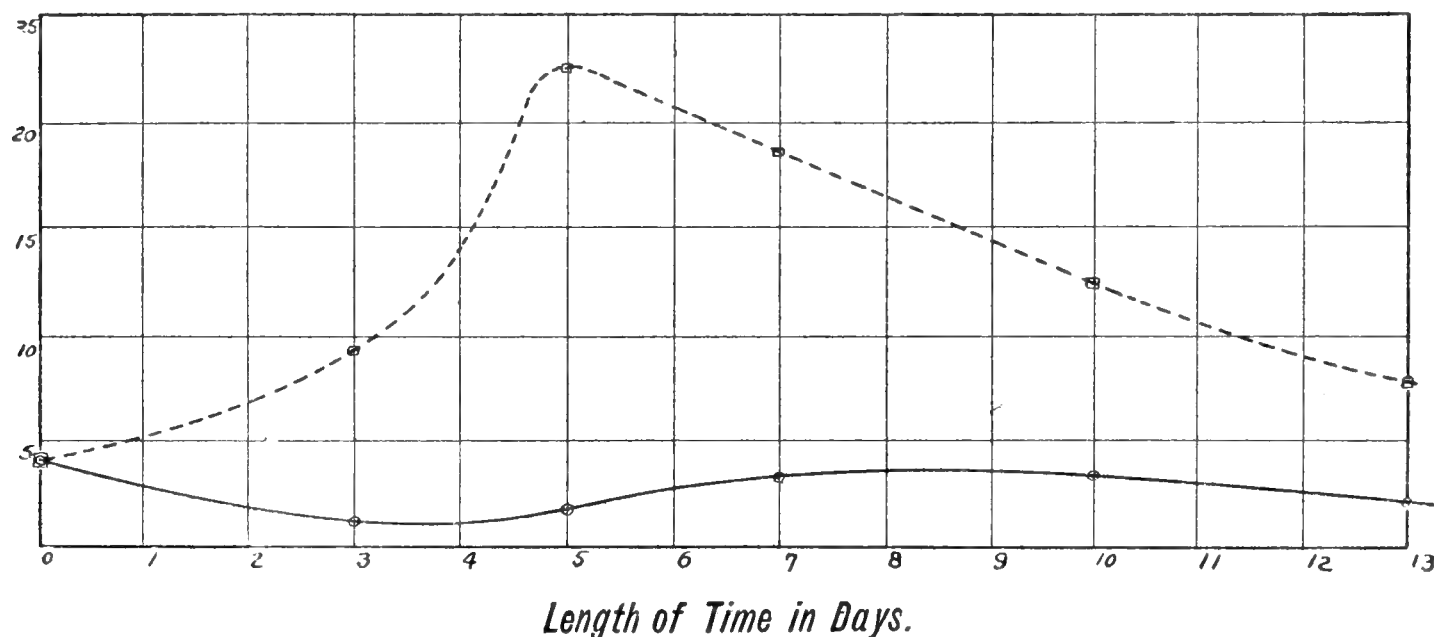


FIG. 19. Total Sugar, per cent. of dry material.

nearly equal proportions in all parts of the seed, as the following analyses show: Tips of seeds 4.1%, middle portion of seed 3.9%, and end of seed distal to the tip 4.2%. The total weight of sugar in the cotyledons of the seedlings at all stages is comparatively small. At the beginning of germination the non-reducing sugar falls rapidly to Stage I, after which it gradually increases until the cotyledons begin to unfold. Up to that time the only

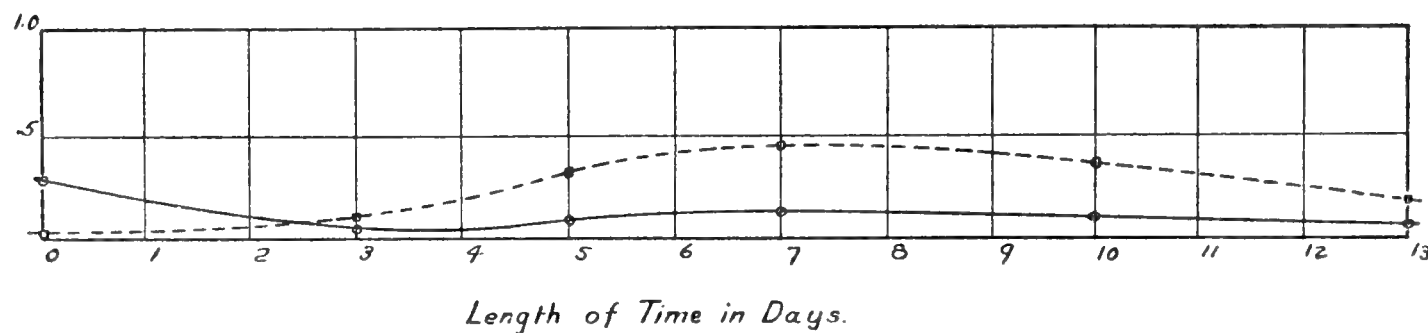


FIG. 20. Total Sugar, amount in grams per 100 seedlings.

sugar present in the cotyledons is of the non-reducing kind, but when the cotyledons assume the foliage function reducing sugar begins to make its appearance, and in the ten-day seedling it is the only sugar present. The per cent. of total sugar rapidly rises in the hypocotyls and roots, and when they have reached a length of 3 to 4½ inches it amounts to 20% of the dry weight. After that period it decreases, and in the ten-day seedling amounts to 12.6%. The actual amount of sugar, however, reaches its

<sup>1</sup> l. c.

maximum at Stage III, when the cotyledons have just spread out, and from that time on it gradually decreases.

The actual amount of cellulose in the cotyledons changes but little during the different stages of the seedlings examined, and the slight varia-

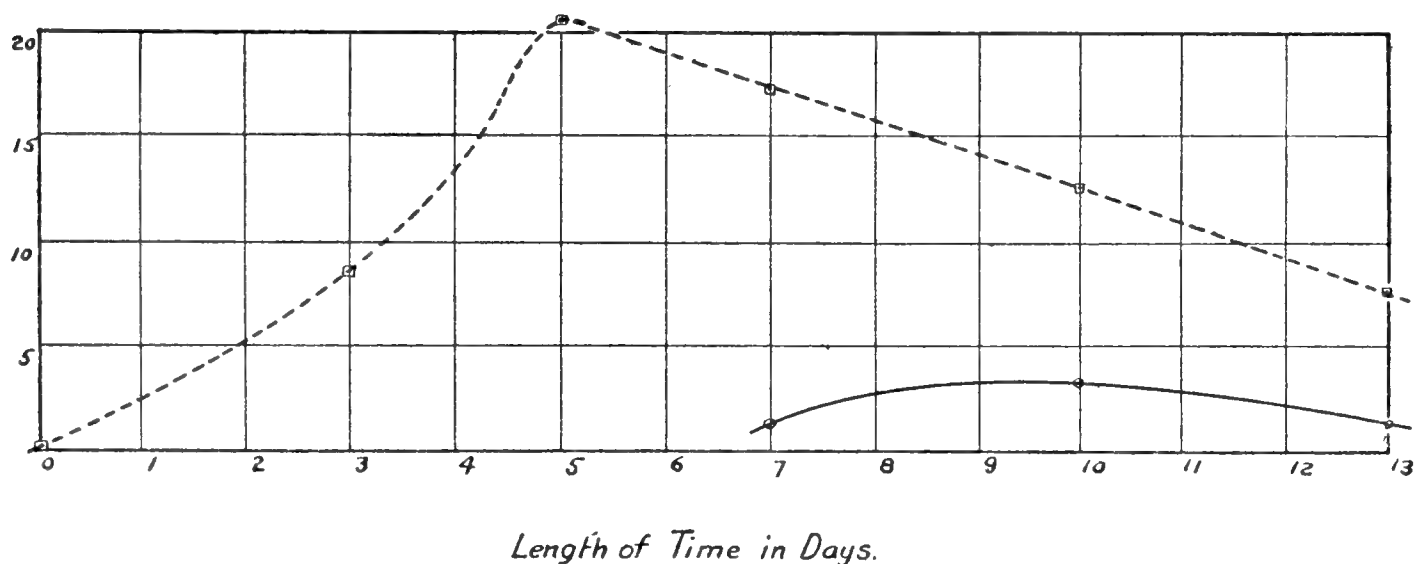


FIG. 21. Reducing Sugar, per cent. of dry material.

tions noticed are within the limit of error of such a determination. The fact that the amount of cellulose does not increase in the cotyledons is to be expected, since, as Sachs first pointed out, there is no formation of new cells in these parts, but only a stretching of the cells already present.

A small amount of starch appears in the starch sheath of the hypocotyls and roots and in the parenchyma cells of the vascular bundles of the

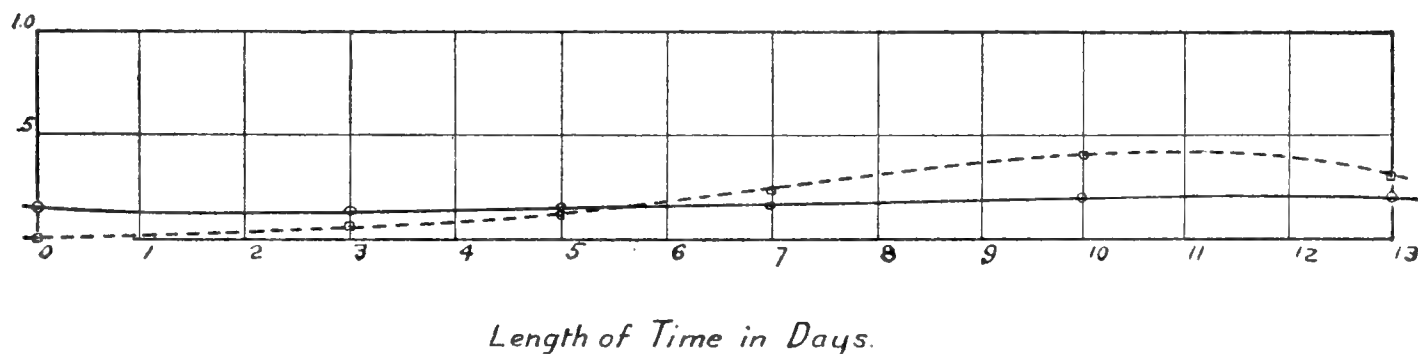


FIG. 22. Reducing Sugar, grams per 100 seedlings.

cotyledons during the progress of germination. The quantity is so small, however, that the ordinary test with iodine upon the ground-up material fails to show any results. Under the microscope, however, the grains are plainly visible when sections are cut and stained with iodine. This fact is worthy of note, since it serves to show the danger of judging the chemical changes which take place in plants by micro-chemical means, unless they are substantiated by analytical determinations. The amount of starch present here is very small compared to the other carbohydrates, yet by micro-chemical methods it is made very conspicuous, and the amount is liable to be over-estimated.

*Initial Losses.*—Judged on the basis of consumption of material the greatest intensity of respiration in the seedling seems to be from the time of



planting to Stage I, when the hypocotyls and roots are 2.5 to 3.5 cm. in length, and the formation of new cells scarcely begun. The seedling up to that time has lost  $\frac{1}{7}$  of the original weight of the seeds. The cotyledons have lost  $\frac{5}{6}$  of the sugar which was present in the resting seed. This is no doubt oxidized at once in the cells where it is found, since it is not probable that it migrates into the hypocotyls at this early stage, for its transportation

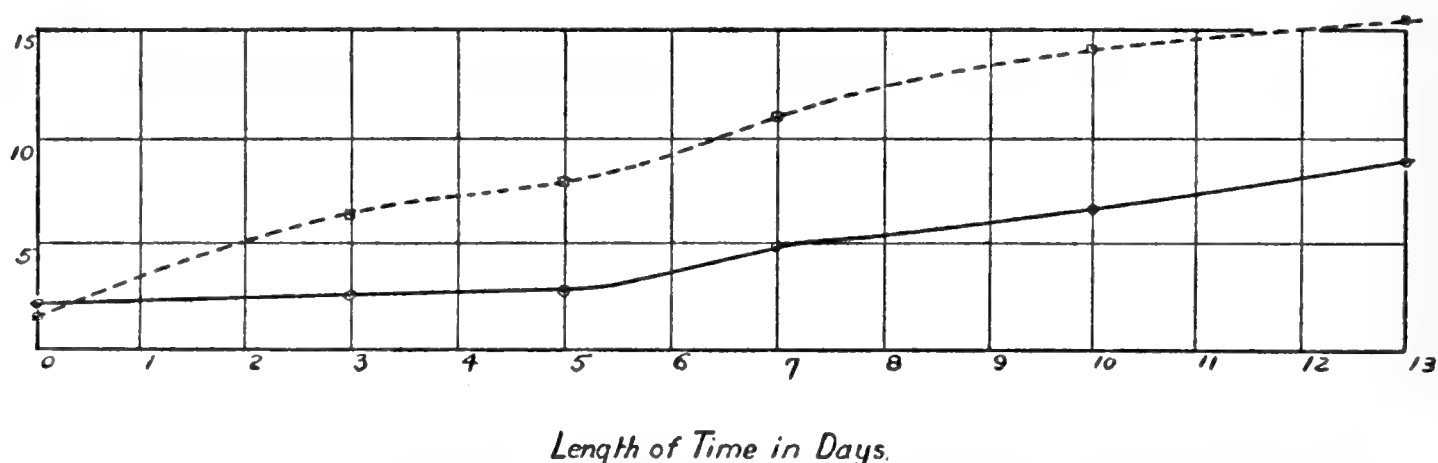


FIG. 23. Cellulose, per cent. of dry material.

from all parts of the cotyledons to those parts would be improbable through cells packed so full of reserve material.

In this period nearly  $\frac{1}{4}$  of the proteid disappears. The loss of the total nitrogen during that period represents approximately that amount. The amount of oil during the same period has diminished  $\frac{1}{7}$  of its original amount. These results give one an idea of the great amount of energy

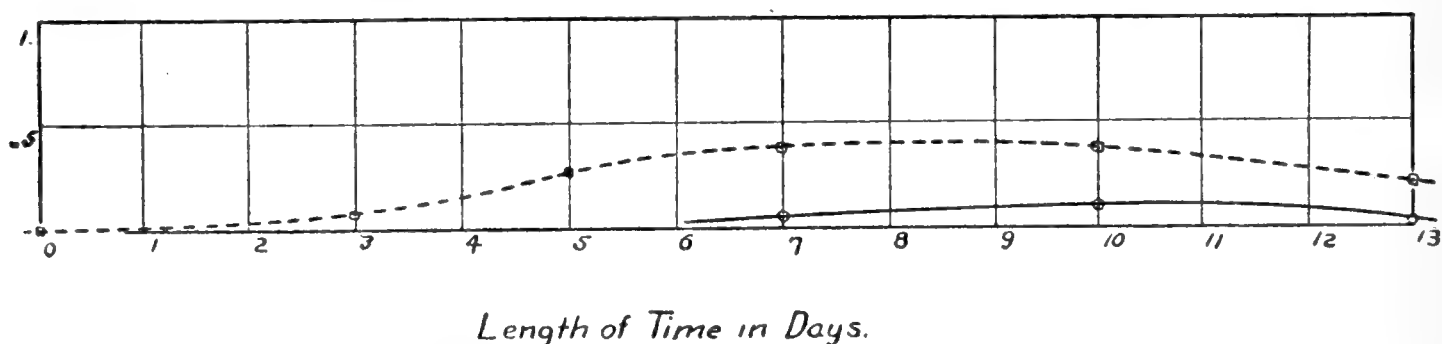


FIG. 24. Cellulose, weight in grams per 100 seedlings.

required at the very beginning of germination to set the life-processes, as it were, in motion. This fact is clearly indicated by the loss of nitrogen in the first stages of germination, a process which is very unusual in the metabolism of plants, for under ordinary conditions the nitrogen of the protein-compounds is never liberated from the plant.

*Problem of Oil-transformation.*—The marked increase in the amount of carbohydrate in the seedling during germination raises the question as to its origin, which in the sunflower could only be the proteid or the oil. It is difficult to understand how the proteid can be the source of any material portion of the carbohydrate which makes its appearance during the growth of the seedling. For example, compare Stages I and III; the total amount of cellulose and sugar in 100 seedlings at Stage I amounts to 0.3 gram, and the proteid nitrogen  $\times 5.5$  amounts to 1.3 grams; at Stage III, when the

amount of sugar is at its maximum, the total sugar and cellulose amount to 0.98 gram, a gain of 0.68 gram, and the proteid nitrogen  $\times 5.5$  amounts to 1 gram, a loss of 0.3 gram. If the protein loss of 0.3 gram had been used entirely for the production of carbohydrate with the formation of carbon-free nitrogen waste products, it would not be sufficient to produce the amount of carbohydrate shown in the 0.68 gram increase. Moreover, we know from the work of Frankfurt<sup>1</sup> that asparagin and glutamin are present to the amount of 4.05% of the dry weight of the seedling, and associated with these two compounds are other carbon-containing nitrogen-compounds so that evidently much of the proteid-free nitrogen is in that form.

The only possible source of the sugar, then, is the oil, but how sugar is formed from this is not known. So far, no intermediate products between the oil and carbohydrate have been isolated. The various theories in regard to the manner in which oil is converted into sugar are mentioned in the historical review. It seems evident that a further knowledge of this problem can only be attained by a more detailed study of the oil and the products which are derived from it during the period of germination.

Investigators have all been of the opinion that oil is converted into carbohydrate during the germination of oily seeds, but the part of the seedling where this takes place is in dispute.

Schmidt,<sup>2</sup> in his extensive experiments on the transportation of oil in plants, worked with the seedling of the sunflower amongst others. He believed that in the germination of this plant the oil was transported as such to the different parts of the seedling and that there it was broken up to form the materials necessary for the plant. He based his conclusions on the facts that the hypocotyls and roots contain considerable quantities of neutral fat, and that the oil present in the seedling has a relatively small amount of acid. If the oil is hydrolysed and transported in the form of fatty acids from the cotyledons to the hypocotyls and roots, its per cent. of acid would be much higher than it is.

Schmidt found that the cell-walls of plants were permeable to oil in the presence of free fatty acid, and that the greater the quantity of acid the more readily did the oil permeate the cell membranes. His theory, then, that oil can be transported as such to these parts is based upon experiments which seem conclusive that such transportation is possible.

Certain authors hold that the oil in the cotyledons is broken up into free fatty acid and glycerine and then transported to the parts where it is needed, either as the free acid or as soap, or that it is broken down into carbohydrate in the cotyledons and transported to the growing parts in that form. The oil present in the hypocotyls and roots they regard as a transformation product of the materials which have been brought there in excess.

The transportation of oil as such to the growing regions and its de-

<sup>1</sup> l. c.

<sup>2</sup> l. c.

composition there would from the standpoint of energy be most advantageous to the plant. This, however, cannot be taken as a criterion for the processes which take place in life, since often they seem to represent, as far as we can see, a useless waste of energy. To be certain, however, that oil-migration does take place, the oil which is found in the growing parts must be shown to be of the same composition as that of the reserve from which it is supposed to have come, and the possibility of oil-migration through plant-membranes must be more firmly established than it is at present. If the oil is not changed in its transportation, it is the only insoluble reserve, so far as is known, which is not. The fact that the amount of free acid in the oil does not reach a high amount at the earlier stages of germination does not necessarily indicate that the oil is being transported as neutral oil from its place of reserve. The oil may be broken down in the cotyledons and the products transported as soon as they are formed, so that no accumulation of them takes place. In fact, in the later stages, the amount of acid rises to  $\frac{1}{3}$  of the oily content of the cotyledons, and to over  $\frac{1}{2}$  of that of the hypocotyls and roots. This appears, too, at a time when the greatest changes are taking place in the seedling, and indicates that an accumulation of acid takes place because of the inability to transport it as rapidly as it is formed.

#### SUMMARY.

During the first three days after the planting of the seeds, the rudimentary roots and hypocotyls reach a length of 2.5 to 3.5 cm.

The cotyledons have absorbed a quantity of water in that time equal to 50 % of their weight, while the per cent. of water in the hypocotyls and roots amounts to 90 % of their weight. During this time the most intensive respiration in the development of the seedling apparently takes place, for at the end of this period the total weight of the seedling amounts to only  $\frac{7}{8}$  that of the resting seed. Five-sixths of the sugar content of the cotyledons,  $\frac{1}{7}$  of the oil, and almost  $\frac{1}{4}$  of the protein has disappeared. The rudimentary hypocotyls and roots at first increase in length by the stretching of the tip, but later in this period the cells of the growing point of the root become active and the increase in length is brought about in the usual way.

As the development of the seedling advances, the depletion of the reserves in the cotyledons advances from the point nearest the hypocotyls to the end remote from it. The most marked change in the reserve products takes place between the time when the cotyledons are breaking through the ground and the period when they are fully developed into foliage organs. This occurred between the fifth and tenth days under the conditions of this experiment.

The protein reserve during the progress of germination is broken up apparently into the ordinary cleavage-products, which are transported into the regions of the roots and hypocotyls, where they are used in the formation of new cell contents.

The oil during the advance of the seedling is, in part at least, broken down into free fatty acid and glycerine. The quantity of free acid in the oil of the cotyledons is comparatively low until the seedling is well developed, while the content of acid in the oil of the hypocotyls and roots rises rapidly at the very beginning of germination and remains high during the different stages examined. Both the neutral oil and the free acid probably take up a quantity of oxygen into their structure, as the decrease in the iodine number indicates.

The marked increase in the amount of sugar during the progress of development of the seedling makes it certain that it has its origin in the oily reserve. The cotyledons at no time contain any appreciable amount of sugar, but it is present in abundance in the hypocotyls at all stages. The amount of cellulose in the cotyledons remains the same, since no new cells are formed there. The sugar produced from the oil is the material used by the plant for the formation of new cell-walls in the growing parts.

Whether the oil as such is transported from the cotyledons to the growing parts and then transformed, or whether the transformation takes place in the cotyledons previous to transportation, cannot be determined from our present knowledge of the subject.

In conclusion, I wish to express my sincere thanks to Professor A. W. Evans and Assistant-Professor A. L. Dean, for their able assistance in all parts of this work.

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# The Somatic, Premeiotic, and Meiotic Nuclear Divisions of *Galtonia candicans*.

BY

L. DIGBY.

With Plates LIX-LXIII.

## INTRODUCTION.

THE cytology of *Galtonia candicans* has been the subject of much study, the large size of the nuclei, the clearness of the figures, and the low number of the chromosomes facilitating examination. Moreover, the tissues are easily 'fixed' and the nuclei take the stains readily. Notwithstanding these favourable features, there is yet much divergence of opinion as regards the sequence, and the interpretation, of the nuclear phases.

In 1904 Strasburger (30) described the heterotype divisions of *Galtonia* and concluded that the univalent chromosomes arose end to end in the spireme (telosynapsis), as previously described by Farmer and Moore (4) in various animals and plants. The following year (1905) Strasburger (31) changed his opinion and stated that the univalent chromosomes were arranged side by side in the spireme (parasynapsis). The meiotic phase was worked out in detail by Miyake (21) in the same year, and he corroborated Strasburger's second view. In 1907 Mottier (22) endorsed Strasburger's original account as to the origin of the chromosomes. Grégoire (10) (1907), while agreeing with the parasynaptic chromosome formation, put a different interpretation on to the 'gamosomes' and 'zygosomes' of Strasburger and Miyake. These he believed were due partly to the action of the fixative and partly to the method of staining. In the course of this paper the different views held by these investigators will be discussed.

As *Galtonia* is easy to manipulate, and lends itself admirably to cytological study, it was suggested to me by Professor Farmer that it would repay further research.

In order to obtain a detailed knowledge of the cytology of *Galtonia*, it has been found expedient to examine the somatic, premeiotic, and meiotic divisions. The somatic divisions have been worked at in the roots. The divisions of the roots are far more easy to elucidate than are the premeiotic divisions of the archesporium, but those of the archesporium are instructive both from a comparative point of view and also for tracing the transition



between the premeiotic and meiotic divisions. The account of the somatic and premeiotic divisions will be followed by that of the meiotic phase.

The methods used have been described in a previous communication (3) and it is superfluous to repeat them here.

### I. SOMATIC AND PREMEIOTIC DIVISIONS.

One of the chief aims of this examination of the somatic divisions has been to ascertain the visible mode of transition from the telophase to the early prophase; that is, to follow the disorganization of the chromosomes and the gradual distribution of their substance throughout the nucleus, and to watch the ensuing reconcentration of their elements and their ultimate reorganization. In order the more clearly to demonstrate this demolition and reconstruction, the more usual plan of entering upon the cycle of nuclear division at the 'resting' stage has been abandoned for that of the metaphase; for at the metaphase one is confronted with tangible entities, whereas at the 'resting' stage one is dealing with a questionable and perhaps hypothetical structure.

#### *Metaphase.*

As the chromosomes go on to the spindle they may show every degree of longitudinal fission (Pl. LIX, Fig. 1). In some the split may have so far extended as to separate the daughter chromosomes widely; whilst in others it is merely to be recognized as a bright line in the substance of the chromosomes, and in others again it may be invisible, the chromosomes apparently being still homogeneous. This homogeneity is especially characteristic of the chromosomes of the archesporium, for in these nuclei the longitudinal fission is often not seen until the chromosomes are about to separate on the equatorial plate. This character may be accounted for by the relatively larger amount of chromatin-staining substance in those cells which are to give rise to the germ cells. On the other hand, in the nuclei of the roots the longitudinal fission is much more precocious, and may be seen in the early spireme (Figs. 8 and 9). The cells of the pericycle show exaggerated fission as compared to those of the inner tissues (Fig. 12). This is probably due to the more rapid penetration of the fixing solution through the outer cells.

The nucleolus is drawn up on to the spindle in the confusion of the chromosome movement. It is deeply staining like the chromosomes, but can be recognized by its circular form. When the chromosomes have arranged themselves on the equatorial plate, the nucleolus is pushed off the spindle, and often appears to be repelled by a chromosome (Fig. 1). It must finally fragment with amazing rapidity, as it is constantly to be seen when the chromosomes have but newly attached themselves to the fibres, but as they complete their equatorial arrangement, and as they separate and

proceed to the poles, the nucleolus vanishes, and leaves no apparent trace of its existence. At the anaphase, the well-known and universally figured refractive brightly staining granules appear round the daughter nuclei, and about the zone of the remains of the spindle. Possibly these may be the remnants of the extruded nucleolus.

Strasburger (31) (1905) and Miyake (21) have shown the number of chromosomes in the somatic divisions to be sixteen. Of these, four are remarkably smaller than the remaining twelve, and in these twelve there is much range in size (Fig. 2). This characteristic difference is extremely well marked, and is always maintained throughout the generations. Unlike *Tradescantia* (5) (1905), whose roots show variable numbers in their chromosomes, *Galtonia* seems to keep persistently to its sixteen. Twelve counts of polar views of equatorial plates have been made, and of these, ten show diagrammatically sixteen chromosomes (Fig. 2); of the remaining two, one shows a possible seventeen and the other a possible fifteen. The larger number is probably due to the nucleolus having come into the field, the smaller number to the close approximation of two chromosomes which were consequently reckoned as one. The 'micro' or small chromosomes in a cross section of a spindle are always seen to occupy a central position (Fig. 2), as Wilson has figured in some Hemiptera (34) (1905, 1).

The chromosomes attach themselves to the spindle by one end and for a short space of time they may lie horizontally, that is to say at right angles to the plane of the spindle (Fig. 3). The end of the chromosome which is attached is often bifurcated, and the split can be seen gradually extending to the free end (Fig. 3). As the split opens out, the chromosomes become drawn in on to the spindle, until they lie at full length on the fibres (Fig. 4). When the daughter chromosomes have nearly separated, each pair resembles a widely extended V, the apex being the hump-like portion formed by the still joined ends of the daughter chromosomes (Fig. 4).

#### *Anaphase.*

As the chromosomes pass to the poles, they are long and rod-like and slightly hooked at their equatorially directed ends. The movements of the two pairs of microchromosomes are always in advance of those of the others; they are the first to separate, and the first to arrive at the poles (Figs. 4 and 5), as in the Hemiptera (34) (1905, 1). The microchromosomes are short and thick with rounded ends. The finest possible hair-like threads constantly connect the chromosomes when they are on the equatorial plate, and these threads can often still be seen when the chromosomes are passing to the poles, not only joining sister chromosomes but even chromosomes which apparently have no relation to one another (Fig. 5). These fine connexions arise from slight projections on the chromosome surface. As will subse-

quently be shown these connexions are present throughout the prophase and originally united the linen (impregnated with chromatin) strands which will ultimately form the chromosomes (Figs. 8, 9, 10, and 13). Grégoire (9) (1906) describes and figures these connexions. Having reached the poles (Fig. 15), the chromosomes approximate closely and form a confused mass (Fig. 16). It is now that the above-mentioned refractive granules make their appearance, scattered in the cytoplasm round the daughter nuclei and around the spindle (Figs. 16, 17, and 18). A cell-plate is laid down midway between the nuclei (Figs. 6 A and 17).

#### *Telophase.*

The chromosomes draw in their free ends and form a compact knot of relatively small size (Fig. 16). Then the knot tends to loosen out, but by this time the chromosomes have begun to lose their individuality (Figs. 17 and 18). In some plants, as shown by Grégoire (9) (1906) and others, the chromosomes in the telophase become skeletons of their former selves. Their centre dissolves, leaving a space bounded on either side by thin threads. In *Galtonia* there is no such diagrammatic vacuolization of all the chromosomes, but it can be seen in parts. The chromosomes as a whole, by transverse division, break up into portions of various sizes, and these are distributed throughout the nucleus and are joined by fine connexions (Pl. LXIX, Figs. 18, 19, and Pl. LX, Fig. 20). At an early stage of chromosome disintegration either one or two 'chromatin' nucleoli are formed, evidently by the flowing together of the substance of portions of the chromosomes (Figs. 17 and 20). Nuclei with one or two nucleoli may be found throughout the prophase, but it has often been observed that those nuclei which start with but one nucleolus possess themselves of two, by direct division of that nucleolus. This happens in early prophase. The nucleolus becomes slightly indented, then hourglass-shaped, and finally breaks into two (Pl. LX, Fig. 29). This mode of duplicating the nucleolus has been previously figured and described by several investigators both in animals and in plants. The nucleoli, as a whole, in the somatic cells of *Galtonia* are not homogeneous, but show refractive spots or granules. As has been shown, the nucleolus persists until the metaphase, when it is cast out into the cytoplasm (Pl. LIX, Fig. 1).

Meanwhile changes have been proceeding in the cytoplasm. The portions of spindle fibres in the vicinity of the nuclei are the first to give place to reticulate cytoplasm, those nearer the equator remain for some time longer. Then the cell plate is gradually replaced by a definite cell-wall (Figs. 6 A, 20, and 21). Thus each daughter nucleus becomes an independent unit in its self-contained cell. Each cell is rich in cytoplasm of a fine reticulate nature. The character of the cytoplasm is modified according to the fixing reagents used. Fixed with strong Flemming

it is soft and cloudy and finely reticulate; with acetic alcohol it is more coarsely reticulate; with Hermann it is stringy and becomes contracted in parts; with strong and medium chrom-acetic it contracts into dense flakes. Preparations fixed with strong Flemming and Hermann show indefinite areas of a dense deposit in the cytoplasm (Fig. 12). In some cases these areas stretch across the walls from one cell to another. Probably they consist of some oily storage material; they are not found in preparations fixed with alcohol and glacial acetic or with strong and medium chrom-acetic.

To return to the nuclear framework.

As the chromosomes break up, their fragments are seen to flatten out into viscous portions of linin (Figs. 6 A, 18, 19, 20, 21), in which the chromatin is carried in a diffuse state. In these nuclei there is no differentiation of linin areas and chromatin discs. The chromatic linin portions assume all manner of shapes and sizes, and strands are joined together by means of finely drawn out threads from the viscous substance. These threads persist throughout the prophases, and finally join the separate chromosomes. If it is remembered that the nuclear framework is of a colloidal nature, it is easy to imagine the variety of forms and shapes that might result. As the chromosome segments flatten out they may become alveolized; the central portion dissolves, leaving the sides as parallel threads (Fig. 21); or large alveolar windows may appear in the linin (Fig. 19); or the linin may break up into small rounded particles which may lie parallel to one another (Figs. 6 A and 21). Whatever form the linin takes it is seen to stain uniformly. From this it may be concluded that it is impregnated with an evenly distributed solution of chromatin. In some nuclei pieces of homogeneous looking chromosomes may still be present, whilst the other chromosomes may have already broken up and be indistinguishable in the nuclear framework (Fig. 21). In the roots of *Allium* Grégoire (9) (1906) has shown that each chromosome resolves itself into two parallel lines, composed of threads or granules. In the nuclei of the roots of *Galtonia* this parallelism is to be seen in parts of the linin, though in not nearly so diagrammatic nor in so regular a manner as in many plants. In the nuclei of the archesporium the linin is in a much more homogeneous and lumpy condition and shows comparatively little trace of parallelism. The linin breaks up into still smaller and smaller pieces, the parallel threads divaricate, and an irregular kind of meshwork results. The linin framework is at first more or less distributed throughout the nucleus, leaving a clear space round the nucleolus (Fig. 21). The space becomes more emphasized as the nucleus approaches the so-called 'resting' stage (Fig. 6 B). Possibly the nucleolus may be exuding substances into the nuclear sap which effectually prevent any of the nuclear contents from encroaching on its vicinity. As the



linin becomes finer and more granular, it is increasingly confined to the periphery. Hence, in order to ascertain the nature of the linin network, it is necessary to examine such sections of nuclei which are so cut as to show either the 'floor' or the 'roof', giving a broad view of the concave or convex surface of the network (Fig. 7). In sections through the centre of a nucleus the threadwork is shown squeezed against the nuclear wall. In such a view there must of necessity appear to be an abundance of parallelism in the threads as the sides of the meshes are seen in perspective. This is an easy source of error.

‘Resting’ stage.

The chromatic linin framework continues to break up until a rough meshwork results. The finely drawn out linin threads now connect small rounded linin granules (Figs. 6 B and 7). The meshes of the network in some places may lie in strands, in others they appear stretched like a net. This is the nearest approach to a ‘resting’ nucleus. There is no real rest in the quickly dividing cells of a young root, or of the archesporium. These so-called ‘resting’ stages are not common in the roots and are still more rare in the archesporium. Strasburger (30) (1904) had difficulty in finding ‘resting’ nuclei in *Galtonia*. Possibly in some nuclei this stage may be omitted and they may pass on directly into the prophase. In the non-dividing portions of the tissues, ‘resting’ nuclei with a far finer, almost cloudy looking, reticulum can be constantly found.

It is necessary here to make a slight digression and to mention curious crystalline looking bodies (Pl. LX, Fig. 28) which are often present in the nuclei of the two or three outer rows of cells of the root, and especially in the outermost row. They are apparently absent from the more quickly dividing cells of the centrally placed tissue. These ‘structures’ seem to be confined almost entirely to those nuclei which are ‘in rest’. Leitgeb (15) found crystals in all parts of *Galtonia candicans* except in the underground portions of the roots and the bulbs. He described a vacuole round the crystals, and this vacuole might increase in size to so great an extent as to burst the nucleus, and the crystals were then projected into the cell lumen. He ascertained that the crystals gave a proteid reaction. Zimmermann (36) has described the presence of crystals in the roots, epidermis, and endosperm, &c., of Ferns and Phanerogams. More recently Walker and Tozer (33) have figured nucleolar budding in the roots of Beans, and have shown that the buds pass out into the cytoplasm. In *Galtonia* these ‘structures’ apparently originate from the nucleolus. There may be one, or more, present in a nucleus. They are often crystalline in appearance and are of various shapes and sizes (Fig. 28). Some are like chips, some like blocks, some are oval. Sometimes they are split so as to appear double (Fig. 28 c) and then closely resemble a small pair of chromosomes. In every case they

take a chromatin stain, but slightly more faintly than does the deeply staining nucleolus. There is always a clear space round each crystalline body, and often one or more bright refractive dots are to be seen in close proximity (Fig. 28, *b* and *d*). In the isolated cases where the outer cells are dividing, the 'bodies' can be recognized throughout the prophase stages and are thrown out at spindle formation, as described by Zimmermann (36). Leitgeb (15) thinks that the crystals are probably reserve material, and that they are connected with the flowering of the plant, for he found that the further situated the tissue was from the bloom, the more sporadic the occurrence of the crystals.

### *Prophase.*

Then follow an inverse series of events concerned in the building up of the chromosomes. The stages immediately following the 'rest' are not easily distinguished from those that precede it. The only recognizable criteria are, that in the prophases the nuclei are slightly larger, and there is a total absence of spindle remains.

The general object and character of the prophases is to compass a gradual and ever-increasing concentration of the linin until the completion of the fully formed chromosomes. The construction of each ultimate chromosome entails both an end-to-end joining of the separate linin fragments which have originated from the cross division of the chromosomes at the preceding telophase, and also a side-to-side approximation of the parallel longitudinal halves of each fragment which have arisen from the alveolization of the same. This early concentration may take diverse forms; the linin may be condensed into bands, or into irregular masses, or into granules (Fig. 22). All these variations may be found in a single nucleus. Again, these bands and masses may be homogeneous or vacuolized, and the granules may be scattered irregularly, or they may lie in parallel rows. In the roots these early stages are beautifully clear (Pls. LIX and LX, Figs. 19, 20, and 21). There is a tendency for the reticulate nature to become modified, and for the meshes to be drawn into more definite strands. Each strand is ladder-like, its sides consisting of parallel rows of beads or granules which are cross connected by fine threads (Pl. LIX, Figs. 8, 9, and 10), and fine threads also join the separate strands to one another (Figs. 8, 9, and 10). The strands become more definite. The fine thread-like connexions persist up to the time when the daughter chromosomes separate from one another on the spindle. Thus, except during anaphase and early telophase, the connexions are present throughout the nuclear cycle.

In *Galtonia* the chromatin does not lie in discs alternating with clear linin areas, but is equally diffused throughout the linin, giving a distinctly homogeneous appearance to the nuclear contents.



At this stage there is still a definite concentration of the linin at the periphery (Fig. 18). As in the telophases, the prophases of the arche-sporium (Pl. LX, Figs. 23, 24, and 25) show a far more decided massing of the linin into solid blocks than do those of the roots (Pl. LIX, Figs. 9 and 10). Concentration proceeds, and the parallel portions of linin, whether they be rows of granules or paired threads, gradually condense to form more homogeneous lengths of spireme (Pl. LX, Figs. 26 and 27). The space which formerly separated the parallel rows, as the sides approached one another, perhaps becomes the longitudinal fission which will eventually split apart the sides of the chromosomes (Pl. LIX, Figs. 8 and 9) as suggested by Merriman (18). These stages in the formation of the somatic chromosomes have been beautifully shown by Strasburger (32) (1907) in the root-tips of the Pea.

It is not easy to form a mental picture of what is actually proceeding in the nucleus during chromosome formation. Two opposite processes are at work. There is the shrinkage of the chromosome upon itself, and at the same time the preparation for the split.

From now onwards, there is a great increase in the stainable material, that is to say in the nuclein substances.

Grégoire in his paper on the somatic divisions of *Allium*, &c. (9) (1906), has shown that the chromosomes may be formed in two ways. Either the chromosome band may keep its alveolar structure until it becomes a definite chromosome, and so retains a certain thickness throughout its formation; or the chromosome band may uncurl into a thin filament which forms a zigzagging spireme owing to the uneven concentration of the alveolar structure. In *Galtonia* these two types of chromosome formation are well seen. Strands of linin may be found whose granules, instead of lying in more or less parallel rows, are arranged in an irregular corkscrew-like way. As concentration proceeds a curling spireme results (Fig. 11), the segments of which only straighten out during their final thickening prior to the evolution of the chromosomes.

#### *Chromosome Formation.*

No continuous spireme exists. As in the early prophases there is a great variety in the character of the linin framework leading to the formation of the chromosomes. In some nuclei the linin may be joined into lengths which are more or less polarized, whilst in others the linin is much fragmented (Pl. LX, Fig. 26), the separate portions being short and rounded, but of all manner of shapes and sizes. In either case the linin fragments, from being flat and ribbon-like, become thickened rods (Fig. 27). As their bulk increases, so does also their staining capacity. During this process of condensation the fragments gradually retreat from the nuclear periphery, and spread themselves across the nuclear cavity (Fig. 27).

Gradually the individual fragments unite end to end until the typical and definite sixteen chromosomes can be identified.

Once more it is necessary to emphasize the fact that in the archesporial nuclei, the chromosome fragments are more or less homogeneous and show little sign of longitudinal fission (Fig. 27), whereas in the chromosome fragments of the nuclei of the roots (Pl. LIX, Fig. 12) the fission is most marked. As the chromosomes form, they lie somewhat polarized towards the nucleolus. The next stage shows them as definite entities, their sides still united by the delicate connexions (Fig. 13) which invariably arise from a slight protuberance. The difference in size of the chromosomes is most noticeable. In the roots the chromosomes often show bifurcated ends and the longitudinal fission throughout their length (Fig. 14), whilst others show only partial fission, and others complete chromatic concentration. The edges of the longitudinal fission are ragged. As has been already mentioned, the cells of the outer layers of the root show an exaggerated fission (Fig. 12). In exceptionally precocious cases the chromosomes may have split apart, forming the widely extended V's, even before the cytoplasm shows any radiations (Fig. 13, v). As a rule the first signs of the radiations, which indicate the line of future stress of the fibres, appear when the chromosomes are distributed throughout the nucleus. The fibres push themselves into the nucleus, the nuclear wall by this time having disappeared, the chromosomes collect at the centre of the nucleus and pass on to the equatorial plate (Fig. 14). The spindle, which is composed of well-marked fibres, terminates bluntly at both ends (Fig. 1). This completes the history of the cycle of the nuclear division.

#### *Conclusion.*

It will be gathered that this investigation supports the views held by Strasburger (30) (1904) and by Grégoire (9) (1906) as regards the story of the somatic divisions. Grégoire has shown that in the alveolization of the chromosomes during the telophase, the chromosome band resolves itself into two parallel threads with an intervening clear space. The spaces become obliterated owing to the separation and interlacing of the fine threads, and thus a network is formed. A nuclear rest ensues. As the nucleus passes into the early prophase, the network is transformed into 'bandes alvéolo-réticulées ou spongieuses', which are identical with the alveolar chromosome bands of the telophase of the preceding division. By progressive concentration the bands become homogeneous chromosomes. There are no chromatic discs.

The somatic divisions of *Galtonia* follow the above summary of Grégoire in its general lines, and his plan can be adopted as the working principle. Nevertheless, these nuclei exhibit great variations, variations

not only between nucleus and nucleus, but within the individual nucleus itself. The somatic division figures of *Galtonia* can neither be adequately illustrated by diagrams nor described 'by a rule of thumb'.

## II. FIRST MEIOTIC DIVISION.

It has already been said that the archesporial divisions have been studied in order to watch the transition of the telophase of the last archesporial division into the early prophase of the pollen mother-cell. In this way a genuine comparison between the premeiotic and meiotic divisions can be obtained.

It is extremely difficult, if not impossible, to assert positively that any one particular telophase is that of the last archesporial division; and as a necessary outcome that its two component nuclei will become pollen mother-cells. The archesporial nuclei in *Galtonia* do not divide synchronously. In a section of an anther before the differentiation of the pollen mother-cells, the archesporial nuclei are at all stages of division. These nuclei pass imperceptibly into the pollen mother-nuclei. There is no rest between the last stages of the archesporium and the differentiated pollen mother-cells, as is also the case in *Hyacinthus orientalis* (13). In the anthers of the very youngest buds that undoubtedly show pollen mother-cells, the majority of the nuclei will be seen to be in the very early prophase, whilst others have apparently only just divided and are still in telophase, and others again may be yet in late anaphase. No dividing line can be drawn between these stages. The nucleus proceeds, without interruption, on its course, and it is only by its general appearance that it becomes evident that it has passed through one phase and has entered upon the next.

Immediately on the differentiation of the pollen mother-cells, the anther increases in size. The cells therefore cover a larger area, and consequently the nuclei become more widely separated. In very young buds the pollen mother-cells of the inner whorl of anthers will be still small and closely packed together, having only just divided, while those of the outer whorl of anthers will already show a great increase in size both in cells and in nuclei. It is from these very young pollen mother-cells that the telophases have been taken as those of the last archesporial divisions.

### *Telophase of the last Archesporial Division.*

Except for the fact that the nucleus is slightly larger in size, the telophase of the last archesporial division resembles in detail that of the somatic divisions (Pl. LX, Fig. 30). The chromosomes fragment transversely into portions of linin. There is much vacuolization in the chromosome portions, and much parallelism in the thin threads resulting from the vacuolization of the chromosomes. As in the somatic divisions fine con-

nexions join the various fragments (Figs. 30 and 31). One or, sometimes, two nucleoli are formed. At first the nucleolus takes a chromatin stain, but it quickly changes this staining reaction and becomes a typical eu-nucleolus of a most colourless nature.

*Early Prophase of First Meiotic Division.*

Rapidly the cells of the anther enlarge, and the nuclei become more widely separated, and at the same time increase in size. A still finer fragmentation of the linin proceeds, resulting in the production of variously sized, rounded, and angled particles (Figs. 31 and 33). Many of these lie parallel to one another. A very common form is that of parallel rows of paired bead-like portions (Figs. 33, 36, and 37). These beads may be in single pairs, or several may be strung together by delicate threads. Each bead is convex on its outer, and straight on its inner, side. In the same nucleus in which some of the linin has thus finely fragmented, there may still be found large alveolized bands of chromosomes consisting of a broad strip of linin showing a clear centre (Figs. 34 and 35). As in the somatic divisions the chromatin appears to be diffused throughout the linin. There is a uniform staining capacity.

Meanwhile the nuclear framework tends to withdraw to the periphery, leaving the nucleolus in a more or less clear space in the centre (Fig. 36). Owing to the increase in size of the nucleus and to its peripherally arranged framework, the nucleus now appears to be emptier than at an earlier stage of prophase. Especially in a thin and superficial section (Fig. 35) of the framework, striking examples of vacuolization in the linin fragments, and of parallelism in the threads, are to be seen. As in the somatic prophases a net-like arrangement of fine connexions joins these fragments to one another. There is no limit to the variety of forms which the linin portions may adopt, but throughout there is a general impression of parallelism in the linin threads, or portions of threads, and of vacuolization in the less segmented chromosome strands. These strands which show vacuolization are portions of chromosomes which have retained their entirety to a comparatively greater extent, and whose sides have not yet separated.

Thus except for the size of the nuclei the somatic and the early heterotype prophases closely resemble one another, and, as will be subsequently shown, the homotype prophases are also similar. The parallel portions in both represent *longitudinal halves of somatic chromosomes*, and are probably the sister halves of the same chromosome, which are now severally coming together and condensing to form the somatic or univalent chromosomes.

From here onwards differences in the two types of division occur. In the somatic divisions the parallel paired portions of the chromosomes become more and more condensed to form each single univalent chromo-

some, but in the heterotype, prior to the formation of the bivalent chromosome, the synaptic stage is intercalated.

The linin becomes more and more confined to the nuclear periphery, and this causes an exaggerated appearance of parallelism as in the case of the somatic divisions (Fig. 37). Gradually the linin becomes contracted into more lumpy portions joined by the fine threads, which often run parallel to one another and crosswise (Figs. 38A and 38B). Once more the linin tends to become distributed throughout the nuclear cavity (Pls. LX and LXI, Figs. 39A and 40). This is the definite beginning of the first contraction. The massing increases, the fine threads becoming obliterated in the general confusion though their double character is still marked (Figs. 39A, 39B, and 40). 'Chromatic' bodies (3) may be extruded at this stage. The linin begins to collect at one side of the nucleus (Pl. LXI, Fig. 41) until gradually the whole nuclear framework is drawn into the knot, which is balled together at one side of the nucleus (Fig. 43). Thus it seems clear that in *Galtonia* the parallel and sometimes fused linin portions which enter the synaptic knot as a general rule represent the concentrating sides of a single somatic chromosome, and not the approximation of two somatic chromosomes as described by Grégoire (10) (1907) and other workers in the heterotype pro-phases of animals and plants. As the parallelism found in the early heterotype pro-phases has the same appearance and the same origin as the parallelism found in the somatic pro-phases, it seems inconsistent to hold that in the heterotype pro-phases each parallel side represents a length of a whole somatic chromosome, and that in the somatic pro-phases each parallel side represents a length of half a somatic chromosome. It is true that there is so much variety in the thickness of the linin portions and so much general irregularity that it would be impossible to determine the significance of any specified parallelism. It is only by taking a broad and comparative view of the heterotype pro-phases in relation to the somatic pro-phases that one is forced to admit that the parallelism of the one is homologous with that of the other. Moreover, if the nuclei of the surrounding tapetal cells (Pl. LX, Fig. 32) are compared with those of the pollen mother-cells there will be seen to be equally striking parallelisms present.

If this interpretation of the parallelisms in the presynaptic stages of *Galtonia* be correct, then the presence of parallel threads in the pro-phases of parthenogenetic eggs would be explained (19) (1907).

The parallelism in *Galtonia* is not so diagrammatically evident as figured in many plants. Though it may be said that the majority of the nuclear pro-phases of the pollen mother-cells do show parallelism to a greater or less degree, nevertheless nuclei can be frequently found in which the linin is of a more concentrated nature and no parallelism is apparent.



*Synapsis.*

The nucleus lies excentrically in the cell (Pl. LXI, Fig. 43), the chromatin mass being always on the side of the narrow strip of cytoplasm (Fig. 43). The spherical nucleolus, more or less hidden by the chromatin, projects into the clear nuclear cavity (Figs. 42 and 43). In some cases two nucleoli are present. There is no definite nuclear wall, the nucleus is bounded by cytoplasmic fibrils, and this continues throughout the subsequent stages, a wall only reappearing at the anaphase.

There is an important difference between this account of the presynaptic and synaptic stages of *Galtonia* and that given by Miyake (21). He describes the massing of the chromatin in the presynaptic stages into 'zygosomes'. The zygosomes are double; the halves each represent a somatic chromosome. They collect in synapsis and retain their individuality. When the knot loosens the chromatin from each zygosome streams into a thread and thus forms the double thread of the spireme. Each thread represents a univalent chromosome. Zygosomes have not been seen in these preparations. Throughout the presynaptic stages there is a doubling in the arrangement of the chromatin, but the chromatin is present in the form of variously sized and shaped portions; there is no concentration into definite centres. With prolonged staining with Heidenhain, followed by much decolorization, darkly staining patches can be distinguished in the synaptic knot, as Grégoire (10) (1907) has described in his observations on *Galtonia*; but, as he points out, these appearances are solely due to excessive differentiation in which all control of the stain has been lost. Sometimes in a favourably stained slide it is possible to distinguish a linin matrix in which irregular chromatin masses are embedded (Fig. 42); but as a rule the knot, at close synapsis, stains practically uniformly and resembles a heap of tightly compressed blocks in which no structure can be seen (Fig. 43). Thus synapsis in *Galtonia* faces one as an impenetrable wall. Great and far-reaching changes and rearrangements are possibly proceeding, but any suggestion as to their nature can be but merely speculative.

The synaptic stage is one of long duration, judging from the frequency with which it is found. At synapsis the pollen mother nuclei of all the anthers of a bud may be found to be at this stage, whereas before synapsis, and especially after synapsis, there is an extensive range in the nuclear phases in the anthers of a single bud. For example, one anther may show its nuclei to be entering on the 'open spireme' stage, whilst the nuclei of another anther may have fully formed chromosomes. Or, again, one anther may have its nuclei in 'diakinesis' preparatory for the heterotype division, whilst the nuclei of another may be completing the homotype division. There is also a considerable difference in the progressive nuclear stages, not



only between the four lobes of a single anther, but also in each individual lobe itself.

During synapsis the 'chromatic bodies' as described by von Derschau (2) are thrown off in abundance (Figs. 42 and 43). There is at this time a great increase in size of the anthers. The contained pollen mother-cells begin to separate from one another. Details concerning the extrusion of the 'chromatic bodies' and the rounding off of the cells have already been given (3).

#### *Hollow Spireme.*

Prior to coming out of synapsis the knot loosens. Shavings of a knot at this stage show it to consist for the most part of irregular masses of chromatin. These masses may occasionally be broken up into smaller granular portions. The knot unravels, and its substance emerges in the form of loops or strands (Fig. 44). Some of these may be more or less ribbon-like (Fig. 44), whilst others may be formed of large beads strung together (Fig. 45); some may show marked longitudinal fission (Fig. 45), whilst others may be homogeneous (Fig. 46). Lengths of spireme may be thick for a certain distance, and then their sides may divaricate widely (Fig. 48) and join other strands; others may be very delicate and anastomose freely and show no order which could be co-ordinated into a scheme. This great irregularity has been emphasized by Maréchal and Saedeleer (17).

In *Galtonia* it is evident from subsequent events that the spireme at this stage is univalent in nature, that is to say that it represents lengths of single somatic chromosomes as described by Farmer and Moore (4), and not lengths of paired somatic chromosomes as described by Grégoire (10) (1907); consequently that the fission in the threads is homologous with the parallelism found in the presynaptic stages and in the somatic pro-phases; further, that this fission will ultimately divide each univalent chromosome into two daughter chromosomes at the second meiotic division, and will not separate two univalent chromosomes at the first meiotic division. As will be shown, in *Galtonia* the homologous lengths of spireme do eventually fuse into thick bivalent strands (Figs. 53 and 54) which split apart, after second contraction, into their two component chromosomes (Pl. LXII, Figs. 58A and 58B, &c.). Although it is only at a later stage that the univalent strands pair, yet when the spireme emerges from synapsis there are already indications of the joining together of lengths of homologous spireme. The thick loops (Pl. LXI, Fig. 44) are striking examples of a telosynaptic union of univalent chromosomes. At the end of the loop there is often a swelling to be seen as if the junction of the two homologous lengths of spireme, each of which will be a univalent chromosome, caused some physiological disturbance. The sides of the loops are sometimes beaded (Figs. 45 and 46), sometimes they are more homogeneous (Fig. 44),

but in either case they closely resemble the univalent chromosomes when they have but newly separated after second contraction, and are still united at one end (Pl. LXII, Fig. 61). The univalent lengths of spireme may show every degree of union with their homologous pair. They may, as has been shown, be arranged end to end (Pl. LXI, Fig. 44), and certainly at a slightly later stage they may be joined side by side (Figs. 52 and 53), and there may be every intervening degree of connexion between these two extremes. Or they may yet be entirely independent one of the other. Although the secondary union of the individual strands is ultimately achieved, each strand is primarily univalent, and it is only by a bending over and fusion of univalent lengths that the bivalent segments are formed.

Thus it appears that the parallel portions of linin which entered the synaptic knot, and represented the condensing longitudinal halves of sections of somatic chromosomes, have, during synapsis, become concentrated and joined end to end to form lengths of whole somatic chromosomes. The concentration is not always complete, for the space between the longitudinal halves reappears in the post-synaptic stages as longitudinal fission. Further, that during synapsis the rearrangement may have been still more extensively elaborated, and that the homologous univalent lengths of spireme may have joined in pairs (Figs. 44 and 45). Overton (24) (1909) has stated that during synapsis there may not only be a conjugation of parental chromosomes, but also 'an actual interchange of influence'.

As the loops and strands come out of synapsis they distribute themselves throughout the nuclear cavity (Figs. 46 and 47). Their anastomosis and coiling is most intricate. Sometimes the spireme lengths appear to be joining end to end (Fig. 51). Sometimes for a distance they are united side by side (Fig. 49). The nucleus then moves to the centre of the cytoplasm which surrounds it (Fig. 51). The nucleolus has generally by this time returned to the nuclear boundary (Figs. 46 and 47). There it may remain throughout the subsequent stages until its final dissolution at diakinesis (Pl. LXII, Fig. 64), or it may be carried out by the spireme as it emerges from synapsis, and then take up a central position in the nucleus (Pl. LXI, Fig. 52). During the loosening of the knot it sometimes stains for a time chromatically (Figs. 46, 47, and 48), but when the 'hollow-spireme' stage is reached, it once more becomes colourless (Figs. 49, 51, etc.).

The nuclei of the 'hollow-spireme' stage again show every degree of variation. In some the loops and strands may consist of beads of chromatin (Fig. 47), in others they may be more ribbon-like (Fig. 49). Possibly this apparent difference in the composition of the spireme may be partly accounted for by the greater or less strain exerted on the nuclear contents. Again, the loops may lie more or less freely in the nuclear cavity (Fig. 47), or there may be an intricate anastomosis (Fig. 48). The anastomosis shows that the combinations necessary for the formation of the bivalent chromo-

somes are still incomplete. The spireme may show every possible degree of longitudinal fission (Figs. 48 and 49) or of homogeneity (Fig. 47). Again the great range of degree of thickness of the various lengths of spireme as shown by a single nucleus must be emphasized (Fig. 49). Grégoire (11) (1910) has written that 'on ne constate que deux épaisseurs de filaments, celle des filaments leptotènes, et celle des anses pachytènes, double de la première'. This statement cannot be said to apply to *Galtonia*. Throughout, one is constantly reminded of the decidedly viscous composition of the nuclear contents, and that the threads may consequently be pulled out to any degree. Thus the comparative thickness of the threads seems to be an unstable foundation on which to build any hypothesis as to their real structure.

The spireme may prepare for second contraction as soon as it has come out of synapsis. In that case its loops leave the periphery and concentrate towards the centre of the nucleus. Such a precocious commencement for second contraction is shown in Fig. 52, where the sides of the loops are being drawn in parallel to one another and in some cases have already joined to form a thick strand. When an interval elapses between the contractions, the spireme goes through a phase of a straightening out of its segments (Figs. 49 and 50). In this case the anastomosing connexions gradually give way and the spireme segments are converted into more or less beaded strands showing longitudinal fission. Some of the spireme segments are generally in contact with the nucleolus, towards which they show a slight orientation (Fig. 50). Delicate hair-like strands connect the different segments and portions of the same segments to one another. Often whilst still in 'hollow spireme' there is a concentration of the chromatic strands in one or more places to form a mass of chromatin which has the appearance of a 'chromatin' nucleolus.

#### *Second Contraction.*

The beginning of the second contraction is marked by a drawing in of the loops and strands towards the centre of the nucleus (Fig. 54). At the same time there is a concentration of the univalent lengths to form the thick bivalent segments (Fig. 52). This is accompanied by a loss of their beaded appearance and a considerable increase in their staining capacity (Fig. 53). Although the chromatin segments become confused and indistinguishable in the conglomeration, yet there is no such absolute obliteration of the course of events as in the first contraction. Usually some portions of loops, or strands, escape from the central mass, showing the parallel univalent strands (Pl. LXII, Fig. 55). The nucleus at this stage has decreased in size.

It seems possible that, during synapsis, the lengths of univalent chromosomes are sorted out, and that during the second contraction the pairing of these homologous lengths of spireme is completed.

*Chromosome Formation.*

In *Galtonia* the evolution of the eight bivalent chromosomes from the apparent chaos of the second contraction is very clearly demonstrated. Sometimes, as the second contraction loosens, the limits of the eight bivalent chromosomes can be identified each in the act of splitting into its two univalent chromosomes (Fig. 57). This split separates the univalent strands which became united during the preceding stages. It must not be confused with the true longitudinal fission in the substance of each univalent chromosome which is the homologue of the somatic fission. The real longitudinal fission is to be seen in the loops of Fig. 56, each side of which represents a univalent chromosome, the two chromosomes being independent of one another except at their extremity. In many cases the second contraction mass of chromatin separates out into large oval or rounded blocks, often joined end to end like a string of beads (Fig. 58 A), in which the limits of the individual chromosomes are at first not discernible. Then a splitting of these bivalent homogeneous blocks takes place (Fig. 58 B). Whatever the shape and length of the segments, whether they are joined together by fine threads, or whether they are more isolated, a fission is to be seen gradually splitting them apart into somewhat flattened ribbon-like pieces with ragged edges (Fig. 58 B). These at first stain faintly, and are visibly of a different constitution to the unsplit segments. Thus by degrees the outline of each pair of univalent chromosomes is identified. Very quickly they become concentrated, assume an entire outline, and stain deeply and homogeneously. So soon does the concentration follow on the split, that the part that has already split is noticeably more deeply staining than the portions that are in the act of splitting (Figs. 59 A and 59 B). Where the segments are joined as a string of beads, they split independently of one another, and then the split portions of either side close up end to end (Figs. 57 and 59 B), recalling the origin of the somatic chromosomes.

All stages of this splitting apart can be seen, but apparently it is a phase that is quickly passed through. Whilst the nuclei of one anther lobe may show the actual splitting phenomena, the nuclei of the adjacent lobe may have fully formed, thick, contracted chromosomes. As the split proceeds, the sides divaricate (Figs. 60 and 61), and each represents a univalent somatic chromosome.

Immediately on the separation of the two parts, representing univalent chromosomes, the original longitudinal fission may be recognized in the substance of each one (Figs. 59 B and 60). This fission was prepared for in the presynaptic stages by the condensation of parallel threads which represented sister sides of a single somatic chromosome (Pl. LX, Fig. 35, etc.). Though lost to sight during synapsis, the fission which reappeared in the lengths of univalent spireme as it emerged (Pl. LXI, Fig. 45) is believed to



bear the same significance as the fission present before synapsis. The fission could again be recognized in the loops as they escaped from the second contraction (Pl. LXII, Fig. 56). It will finally divide each univalent chromosome into the two daughter chromosomes in the homotype division (Pl. LXIII, Fig. 75). The fission is only to be seen for the extremely short interval when the newly differentiated univalent chromosomes are still flattened and uncondensed (Fig. 59 B). As soon as they are concentrated and rounded, the split is once more obscured to reappear in *Galtonia* as the chromosomes approach the poles (Pl. LXIII, Fig. 68).

The two individuals which constitute each bivalent chromosome may break apart at once, or they may remain for a time joined at one end, so as to form a loop (Pl. LXII, Figs. 60 and 61). Often, in such loops, one limb is seen to be composed of homogeneous chromatin, whilst the other limb consists of large chromatin beads (Fig. 61) alternating with linin areas, the chromatin not having yet concentrated. This is strikingly like the figures of King (14) in *Bufo*, except that in *Bufo* each chromatin portion represents a single univalent chromosome, and the intervening faintly staining areas the points of future transverse cleavage between the chromosomes.

When the segments have but newly split, it is possible to identify the eight pairs of chromosomes (Figs. 57 and 60). Apparently the large pairs of chromosomes are joined to form long loops (Figs. 60, 61, and 62). The individual chromosomes are attenuated (Fig. 60) as compared to their appearance in the later stages (Fig. 62). The two pairs of small chromosomes are always in advance of the others. They are the first to be isolated from the second contraction (Figs. 55 and 56), the first to split apart (Pl. LXI, Fig. 53), and the first to become concentrated (Pl. LXII, Fig. 60). When differentiated they are often in the form of a single sausage-shaped mass, which splits into a pair of somewhat bean-shaped chromosomes (Figs. 56, 59 B, and 60). One or other of the small pairs is nearly always in contact with the eu-nucleolus (Figs. 62 and 63). Wilson (34) (1905, 1) has shown that the larger idiochromosome in *Lygaeus bivalens* and the accessory chromosome in the Hemiptera (35) (1905, 2) are in the same way attached to the plasmosome. Stevens (29) in *Trirhabda* has found a similar association in the case of the irregularly paired heterochromosomes.

On the differentiation of the eight pairs of chromosomes there is a rapid concentration and thickening of the individuals (Figs. 62 and 63). The members of each pair always remain close to one another. Strands of linin traverse the nucleus. Sometimes one or more of the pairs may be connected to form a chain (Fig. 63) as in *Tradescantia* (22) and *Oenothera* (1). Before diakinesis the chromosomes become much contracted and are very thick, and stain densely. The small chromosomes are usually round, the larger ones are more rod-like (Fig. 64). Then the nucleolus fragments

(Fig. 64), the chromosomes contract to their extreme limit, move to the periphery of the nucleus, and enter the well-known diakinesis stage. At the same time radiations appear in the cytoplasm (Fig. 64). As already described by Miyake (21) the spindle has a multipolar origin and gradually becomes bipolar.

This description of the origin of the univalent chromosomes of the first meiotic division agrees fundamentally with that of Strasburger in 1904 (30) and of Mottier (22). In 1905 Strasburger (31) and Miyake (21) both concluded that the spireme as it comes out of synapsis is bivalent, and that the two univalent chromosomes are formed by a separation of the two longitudinal halves of the spireme. In this investigation it has been concluded that the spireme is univalent, and that the univalent strands only join together secondarily to form the bivalent lengths which finally split apart into the univalent chromosomes.

#### *Spindle Figures.*

The fibres push their way into the nucleus, and the chromosomes collect on the spindle (Fig. 65). The entire univalent chromosomes of each pair move off one to either pole (Fig. 67). A polar view of the equatorial plate shows the great variety in size of the chromosomes; the two small ones usually take up a central position (Fig. 66). As the chromosomes approach the poles the longitudinal fission is once more to be seen (Pl. LXIII, Fig. 68). The halves may separate widely so as to form V-shaped figures (Fig. 68). The polar view of an aster shows the eight chromosomes longitudinally split (Fig. 69). Again the small ones are centrally placed (Fig. 69).

#### *Anaphase.*

Arrived at the poles, as in the somatic divisions, the chromosomes at first form a condensed mass (Fig. 70). Then they separate out, fragment, and the fragments become alveolized, showing 'windows' and parallel threads (Fig. 71). One or two nucleoli appear, and a nuclear wall surrounds the daughter nuclei. At the same time a cell-plate is laid down at the equator of the spindle (Fig. 72). The refractive granules in the cytoplasm round the nuclei and about the spindle are obvious (Fig. 72). Thus imperceptibly the anaphase leads into the telophase (Figs. 70, 71, and 72).

#### *Telophase.*

The telophase of the first meiotic division is precisely like that of the somatic divisions (Figs. 71 and 72). There is the same fragmentation, and the same alveolization of the fragments resulting in parallel threads (Fig. 73). The linin, as a whole, does not break up into such fine portions as in the somatic divisions, but remains in rather large fragments, as usual united by fine threads. There is no resting stage. Thus the telophase of the heterotype passes into the prophase of the homotype (Fig. 74).



## III. SECOND MEIOTIC DIVISION.

*Prophase.*

The first indications that the nuclei have entered upon the reconstructive prophase stages are the disappearance of the spindle fibres and the reconcentration of the linin portions (Fig. 74). The nucleolus, which when first formed in the telophase of the heterotype was of a chromatic staining nature, has by now become cytoplasmic in staining reaction. No nucleolar budding or 'body' formation has been observed in the homotype divisions. The nucleolus remains spherical and colourless, and is apparently ejected into the cytoplasm as the chromosomes collect on the spindle.

Once more the halves of the concentrating portions of the linin come together, and there are striking cases of concentration to be seen, just as in the somatic and presynaptic prophases (Figs. 75 and 76). Meanwhile the cell-plate has given place to a clear wall which divides the two daughter nuclei, and which is continuous at either end with the circular enveloping wall (Figs. 75 and 77). The nuclei elongate at right angles to the plane of the heterotype spindle (Figs. 75 and 77). At the same time definite spindle fibres appear in the cytoplasm (Figs. 75 and 77). They focus more or less to a point at either end, and widen out as they approach the extremities of the nucleus. Concentration of the segments proceeds. There are still small, flat, angled portions of linin, but they tend to become more and more individualized (Figs. 75 and 76). They are connected by fine threads. Gradually the segments join on one to the other (Fig. 77). Sometimes longitudinal fission can be seen in them, but generally they are ribbon-like and more or less homogeneous (Fig. 77). At a later spireme stage the chromatin contents are drawn out into long strands, which lie in the plane of the elongated narrow nucleus. These strands may lie massed together and be interwoven so as to form an almost undecipherable tangle. The spindle fibres enter the nucleus and penetrate the tangle (Fig. 78). The nuclear wall has meanwhile gradually disappeared.

*Metaphase.*

Then the chromosomes become differentiated, and take up their position on the equatorial plate (Fig. 79). At first they are still long and, lie somewhat in loops and curves (Fig. 78), but when they have completed their equatorial arrangement they contract considerably, and consequently shorten and straighten (Fig. 79). Their outer ends become bifurcated, and, contrary to the direction in the somatic divisions, the split apparently proceeds inwards. The spindle fibres usually focus to a sharp point at either end (Fig. 79). The two nuclei generally (if not always) divide parallel to

one another, at right angles to the plane of the heterotype division (Figs. 79 and 80). As the chromosomes separate they again contract (Fig. 80), but become elongated as they near the poles (Fig. 81).

#### *Diaster.*

The chromosomes of the four daughter nuclei often arrive simultaneously at the poles (Fig. 81), but sometimes one nucleus may be in the diaster stage, whilst the other is still in the metaphase.

#### *Telophase and Anaphase.*

The telophase resembles that already described in the somatic and first meiotic divisions. There is the same alveolization of the chromosomes, followed by a breaking up of the longitudinal halves into more or less beaded portions which are distributed throughout the nuclear cavity and are connected with one another by fine strands (Fig. 82). Each nucleus of the tetrad gradually rounds itself off and becomes independent of the others. The anaphase passes into the 'resting' stage (Fig. 83).

#### *'Resting' Nucleus and Pollen Grain.*

The cytoplasm round the 'resting' nucleus becomes vacuolated. The linin contents may be in the form of granules showing often a double linear arrangement (Fig. 83), or they may be in larger lumps exhibiting definite traces of parallelism representing the remains of the alveolized portions of the chromosomes of the telophase.

Thus the 'resting' nucleus of the tetrad upholds the principle of irregularity so strikingly characteristic of all the divisions of *Galtonia*. The chromatin is here scattered throughout the nucleus, and thus is exactly opposed in arrangement to that described by Overton (24) (1909) in *Thalictrum purpurascens*, and by Rosenberg (26) (1909) in *Crepis virens*, where the chromatin in the resting pollen-grain nucleus is definitely aggregated into prochromosomes corresponding to the reduced number.

Then each nucleus with its cytoplasm passes through those changes relative to pollen-grain formation.

#### GENERAL CONSIDERATIONS.

One of the controversial questions which the study of *Galtonia* reopens is the significance of the parallel threads and linin masses in the heterotype prophases. In *Galtonia* it is believed that the parallelisms in the somatic, premeiotic, and meiotic nuclear prophases are all homologous, and in each case that they represent the approximation and concentration of the two longitudinal halves of portions of somatic chromosomes. This conforms to Strasburger's (30) (1904) theory as to the origin of the somatic

chromosomes in *Galtonia*, but his (31) (1905) and Miyake's (21) interpretation of the parallelisms in the heterotype prophase is very different. They hold that the chromatin in the reticulum of the early heterotype prophase becomes concentrated into masses, 'gamosomes', which correspond in number to the somatic chromosomes. The 'gamosomes' before, and during, synapsis pair to form 'zygosomes'. From each 'zygosome' arises a pair of threads, the 'gamomites'. The 'gamomites' join and become 'zygomites'. The 'zygomite' splits into its two component 'gamomites', each 'gamomite' being a univalent chromosome. In this investigation the 'zygosomes' have been interpreted as the concentrations of the longitudinal halves of the somatic chromosomes. Moreover no conclusion could be drawn as to the constancy in number of these concentrations. They appear to be quite irregular in form and in arrangement. Mottier (22) has found no correlation between the chromatic aggregations and the unreduced number of chromosomes.

Nearly all cytologists agree as to the presence of parallel threads and linin masses in somatic prophases. Strasburger (30, 32) (1904, 1907) has described them in the somatic cells of *Galtonia* and in roots.

Grégoire (9) (1906) has shown that in the telophases of the roots the chromosomes become alveolized; that the two sides of each chromosome separate widely and cross one another, forming a rough network; that when the nucleus enters upon the prophase, and the chromosomes have to be reconstituted, the network is transformed into 'bandes alvéolo-réticulées ou spongieuses', which are identical with those of the telophase; and that finally by concentration the bands become transformed into definite chromosomes.

Farmer and Moore (4) (1905) have figured and described parallelisms in the somatic cells of *Periplaneta*. 'At first the cells which are preparing for division present an almost even granulation of the chromatin within their nuclei, and this in its consistency strongly suggests a foam structure of the ordinary type; but after a time the "chromatic confusion", as it were, sorts itself out into obvious condensations or cloudy areas, and it is apparently unquestionable that each of these primitive chromatic clouds is individually the forerunner of one of the future chromosomes.'

Many writers go a step further and believe that each of these condensations represent a somatic chromosome, and hence call them 'prochromosomes'. Rosenberg (27) (1909) has found in *Nuphar luteum*, *Helianthus peploides*, and several other plants, that prochromosomes are present in the resting somatic cells in the somatic chromosome number.

Overton (23, 24) (1905 and 1909) has stated that in the somatic cells of *Thalictrum purpurascens* there are forty-eight paired prochromosomes, and in the young pollen mother-cells there are twenty-four.

Sufficient evidence has been quoted to show that there are generally

considered to be parallel threads and parallel aggregations of linin in the somatic cells. Further, that these parallelisms are the condensations of the sides of somatic chromosomes, whether of portions only, or of whole chromosomes. Parallelisms are also found in the heterotype prophases by most investigators, but these parallelisms are interpreted in three different ways.

(1) Although the parallelisms in the heterotype prophases may have identically the same appearance as the parallelisms of the somatic divisions, yet that in the heterotype division each side of the parallelism has a different origin to the other side, and each represents a whole somatic chromosome.

(2) That the parallelism in the heterotype prophases must be considered homologous with the parallelism of the somatic prophases, and consequently that in the heterotype prophases, as well as in the somatic prophases, each side of the parallelism has the same origin, that is to say that it arises from the same longitudinally split chromosome.

(3) That the parallelism found in the heterotype prophases has no real significance but is a mere coincidence.

The views of those who hold that the parallelism in the heterotype prophases is the pairing of homologous chromosomes will be taken first.

(1) Rosenberg (25) (1907) figured chromatin masses in the unreduced number in the heterotype prophases of *Tanacetum vulgare* which resembled those aggregations in the somatic divisions. These masses united in pairs in synapsis and each member of the 'Gamosomen-Paar' became a univalent chromosome. In *Crepis virens*, where the unreduced number of chromosomes is six and the reduced number is three, there are six prochromosomes in the somatic divisions; these six reappear in the early heterotype prophase and then unite in pairs, which, according to Rosenberg, must be regarded as the conjugation of two whole somatic chromosomes.

Overton (24) (1909) states: 'I am very strongly convinced that the arrangement of the prochromosomes in somatic and young germ cells is the same, that is, they are parallel in pairs,' and that 'the homologous parental elements are therefore associated in pairs when they enter the reconstructive stages of the germ nuclei'.

The Schreiners (28) have published a beautiful figure (Pl. 23, Fig. 10) of the 'Conjugation' of the chromosomes in the early heterotype prophase of *Salamandra maculosa* which exactly simulates the condensation of the chromosome in the somatic nuclei of the roots of *Galtonia* (Pls. LIX and LX, Figs. 19 and 20). There is the same irregular and partly beaded approximating sides, joined to the skeleton chromosomes on either side by delicate connexions.

Lundegardh's (16) drawings of the early heterotype prophases of *Calendula officinalis* closely resemble those of *Galtonia*, but he considers that the parallelisms represent the pairing of homologous chromosomes.



In *Trollius europaeus* he has shown the alveolization of the chromosomes in the telophase of the last archesporial division, but unfortunately there is a rest between the telophase and the early heterotype prophase, so that it is impossible to trace the relationship between the portions of alveolized chromosomes of the telophase and the 'Gamosomen' of the prophase.

Grégoire (11) (1910) thus defines the difference between the somatic and heterotype prophase: 'Tandis que, dans une cinèse somatique, on voit le réseau quiescent se décomposer typiquement en des bandes alvéolaires ou en des tractus qui, par un mouvement de concentration, se transforment en chromosomes homogènes définitifs, ici (stades préspirémiques) au contraire, il se forme aux dépens du réseau . . . un ensemble de filaments minces généralement assez long.' Further, that in the pollen mother-cells of *Allium* 'le passage du stade réseau au stade leptotène se fait par l'intermédiaire de bandes chromosomiques alvéolisées analogues à celles qui marquent la première origine des chromosomes somatiques, et que c'est de la transformation de ces bandes que résultent les filaments minces par un processus analogue à celui que l'on retrouve dans les cinèses somatiques. . . . Il n'y a donc pas de doute, à notre avis, que chaque filament mince ne soit l'homologue d'un chromosome.' From these quotations it is clear that Grégoire, in the somatic prophases, considers each 'filament mince' to be the longitudinal half of a somatic chromosome, whilst in the heterotype prophases he considers each 'filament mince' to be a whole somatic chromosome. Grégoire perhaps makes insufficient allowance for the concentration of each univalent chromosome in the heterotype prophase which plays such a prominent part in the formation of the chromosomes in the somatic divisions.<sup>1</sup>

(2) One of the chief upholders of the opposed view that the parallelism in the heterotype prophases may possibly be the longitudinally split halves of the same chromosome is Häcker (12). He says: 'Ob nun freilich diese paarige Anordnung der präsynaptischen Chromosomenanlagen tatsächlich auf eine Aneinanderlegung ursprünglich selbstständiger Elemente zurückzuführen ist, das scheint mir angesichts der grossen Schwierigkeiten, welche die folgende Synapsis-Phase der Analyse darbietet, noch nicht mit Sicherheit entschieden zu sein. Ich möchte vielmehr auf alle Fälle die Möglichkeit offen lassen, dass z. B. in den von Overton gegebenen Bildern, nicht eine Chromosomenpaarung im Sinne Strasburger's, sondern die Anlage eines frühzeitig gespaltenen Spirems im ursprünglichen, von Flemming (5) angenommenen Sinne vorliegt.'

Meves (19, 20) (1907, 1908) finds that the parallel threads of the somatic and heterotype prophases are so closely similar that they must bear the same interpretation. Moreover, he considers that the threads are too thick

<sup>1</sup> Since this communication has gone to the printers another paper on prophases has been published, entitled: Kerndeeling en Synapsis bij *Spinacia oleracea*, L., by Theodor J. Stomps, Amsterdam, 1910, pp. 1-162.

and numerous, and the space of the nucleus too limited, to allow of the threads running parallel to one another throughout their length. He maintains that this is a physical impossibility, and that this impossibility is increased by the anastomosis amongst the threads themselves. If the parallelisms do represent the pairing of homologous parental chromosomes, how is the parallelism in parthenogenetic eggs to be explained? (19) (1907).

Goldschmidt (7, 8) (1905, 1908) has shown that in the heterotype pro-phases of *Zoogonus mirus* the chromatin becomes collected into a 'zwei-reihige Lagerung der Körnchen zum Teil schon eine Andeutung der spätern Längsspaltung zeigend'. These behave like ordinary somatic chromosomes and go on to the spindle showing their longitudinal fission. The reduction takes place at the homotype division; the somatic number of chromosomes, which is ten, segregates into two groups of five.

Such is the evidence of those who hold that the parallelisms of the heterotype pro-phases are in no way different from those of the somatic divisions, that is to say that the parallelisms of the heterotype divisions arise from the longitudinal fission of the same somatic chromosome and not the approximation of two different somatic chromosomes. If this view is the true one then the approximation of the chromosomes, which is a necessary act on account of the reduction, must either take place, as Overton (24) (1909) suggests, in the telophase of the last archesporial division, or, as many cytologists have thought, during synapsis, or during the hollow-spireme and second-contraction stages.

(3) Finally, there are those workers who do not believe in the existence of parallelism, as such, in the heterotype pro-phases, and when it does occur they consider it to be a mere chance or coincidence. They base their arguments on the fact that there are often not only two threads running parallel to one another, but sometimes three or more. This anomaly may partly be explained by the fact that concentration for each somatic chromosome is often in the form of the drawing together of a netlike arrangement, as diagrammatically shown in the pro-phases of the nuclei of the root. Or it may be that where several threads run close to one another, concentration for the formation of the somatic chromosomes is proceeding simultaneously with the pairing of homologous chromosomes.

It seems only possible to settle this difficult question as to the homology of the parallelisms of the heterotype pro-phases by tracing their origin from the telophase of the preceding last archesporial division, and in order to do that it is essential to secure material in which there is no 'rest' between the premeiotic and meiotic divisions. There is much evidence to show that the parallelisms of the somatic and of the heterotype pro-phases have the same outward appearance, so that it is only by studying their origin that the truth as to their significance can be arrived at.



Another subject of discussion amongst cytologists is the question as to the origin of the univalent chromosomes which make up each bivalent pair in the heterotype prophase. One school contends that the chromosomes are arranged side by side in the spireme (11) (1910), the other that they are joined end to end (4) (1905).

In *Galtonia* the univalent chromosomes finally arise by a splitting apart of the thick bivalent segments, but these bivalent segments are composed of two primary distinct lengths of univalent spireme which conceivably may have joined during synapsis, although there is no direct evidence of this, but certainly become definitely approximated during the hollow-spireme and second-contraction stages.

As there is so much variety in the spireme as it comes out of synapsis, a variety apparent both in the thickness and in the arrangement of its loops and strands, and as the presence of anastomosis certifies that the arrangement and pairing of the homologous chromosomes cannot yet be in order, it seems possible that as long as the homologous chromosomes do pair eventually, it does not signify when they pair or in what manner they are joined. The important point which *Galtonia* demonstrates is that its spireme is univalent. Whether these univalent strands join with their homologous pairs telosynaptically or parasynaptically, or by any other intermediate method between these two extremes, resolves itself merely into a question of non-essential detail.

Lastly, the varied character and the great inequality shown by the nuclei of *Galtonia* throughout all the division figures must once more be emphasized. There is no 'cut and dried' definite arrangement; it cannot possibly be stated *a priori* that the formation of each individual chromosome is arrived at by some one particular method. The elaborations and intricacies are endless, and all that can be said is that the course of events appear to trend in some one direction, and that finally the same goal is reached, though the method actually pursued may be subject to great variation.

#### SUMMARY.

1. The chromosomes in the somatic and premeiotic divisions are formed from the telophase of the preceding division by an alveolization of the chromosomes and partial separation of the two sides, followed by a reconcentration of the same.

2. The parallel threads and portions of linin present in the early heterotype prophase are homologous with those in the somatic and premeiotic prophase. They are the remains of the alveolized portions of the chromosomes of the telophase of the last premeiotic division. There is no rest between the premeiotic and meiotic divisions. It is believed that during synapsis the parallelisms concentrate to form whole, or portions of whole, somatic chromosomes. The spireme as it comes out of synapsis is

univalent in character, the longitudinal fission in its substance being homologous with that of the presynaptic stages, and consequently with that of the somatic prophases. The univalent homologous lengths of spireme may have joined end to end, or be partially united during synapsis, but it is not until the hollow spireme and second contraction that the pairing and fusion of the univalent chromosomes to form the bivalent segments are completed. As the bivalent chromosome segments come out of second contraction, they split apart into the two univalent chromosomes. At the homotype division these univalent chromosomes split longitudinally.

3. 'Crystalline' structures are present in the nuclei of the outer two or three rows of cells of the roots.

4. 'Chromatic' bodies are given off from the nucleus during the presynaptic, synaptic, and hollow-spireme stages.

In conclusion I wish to express my grateful thanks to Professor J. Bretland Farmer, F.R.S., for the constant and valuable help, advice, and criticism that he has given me throughout the course of this work.

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EXPLANATION OF PLATES LIX—LXIII.

Illustrating Miss Digby's paper on the Nuclear Divisions of *Galtonia candicans*.

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. x2250.

- Figs. 1-14. Somatic divisions in the root.
- Figs. 15 and 16. Divisions in the wall of the ovary.
- Figs. 17-27. Premeiotic divisions of archesporium.
- Figs. 28 and 29. 'Crystalline' structures, and nucleolar division in root.
- Figs. 30 and 31. Telophase of last archesporial division.
- Fig. 32. Prophase in tapetum.
- Figs. 33-73. Heterotype divisions.
- Figs. 74-83. Homotype divisions.

PLATE LIX.

- Fig. 1. Root. Chromosomes collecting on the equatorial plate. Note the different degrees of longitudinal fission. The nucleolus has been thrown out.
- Fig. 2. Polar view of equatorial plate. The small chromosomes are arranged in the centre.
- Fig. 3. The chromosomes are beginning to split on the equatorial plate.
- Fig. 4. The chromosomes have nearly separated. The small chromosomes are in advance of the others.
- Fig. 5. The chromosomes have separated. Note the fine connexions that join them.
- Fig. 6 A. Telophase. Showing the alveolization of the chromosomes and the consequent parallelism of their sides.
- Fig. 6 B. Late telophase which has passed into the 'resting' stage. The linin has broken up into small rounded particles.
- Fig. 7. 'Resting' stage. The linin network is confined to the periphery of the nucleus.
- Fig. 8. Early prophase showing the parallel rows of linin granules. Condensing to form concentrated portions of 'chromosome bands'.
- Fig. 9. Still further approximation of the paired linin strands. Fine connexions join the strands to one another.
- Fig. 10. Slightly later stage in the concentration of the strands. Note the alveolization in the strands, and the longitudinal fission which arose as the space between the condensing strands.
- Fig. 11. Nucleus showing uneven concentration of the linin resulting in a corkscrew-like spireme.
- Fig. 12. Late prophase showing spireme segmented into chromosomes with most marked longitudinal fission.
- Fig. 13. Definite chromosomes. The chromosome (V) has already split, forming a widely extended V. Note the fine connexions.
- Fig. 14. Longitudinally split chromosomes collecting in the centre of the nucleus. Cytoplasmic radiations have appeared prior to the formation of the spindle.
- Fig. 15. Wall of ovary. Diaster.
- Fig. 16. Late diaster; the chromosomes have lost their individuality.
- Fig. 17. Archesporium. Telophase. A cell-plate is forming, the chromatin mass is beginning to loosen out.
- Fig. 18. Polar view of a nucleus in telophase, showing the breaking up of the chromosomes, and the fragments united by fine connexions.
- Fig. 19. Superficial section of a nucleus in telophase, showing the alveolization of the chromosomes.

PLATE LX.

- Fig. 20. Later telophase. The alveolized portions of chromosomes are breaking up into parallel rows of granules.

Fig. 21. Still later telophase, in which the remains of some of the 'chromosome bands' are to be seen, whilst the others have broken up and have lost their identity. There is a clear space round the nucleolus.

Fig. 22. Very early prophase, showing the parallel arrangement of the linin as strands of beads, some strands having already concentrated into chromosome segments.

Figs. 23 and 24. Progressive stages in the concentration of the linin to form the chromosome segments.

Fig. 25. Still further concentration; the linin is now in the form of thickened masses, showing parallelisms in their sides.

Fig. 26. Late prophase, showing segmented spireme.

Fig. 27. Slightly later stage in which the spireme segments have thickened considerably. The segments are homogeneous.

Fig. 28. Root. 'Crystalline' structures in the nucleus. *n.*, nucleus; *c. b.*, crystalline bodies; *r.*, refractive dots.

Fig. 29. Division of nucleoli by constriction.

Fig. 30. Telophase of last archesporial division. There is the same alveolization of the chromosomes resulting in the formation of paired threads as in the telophases of somatic divisions.

Fig. 31. Polar view of a nucleus in telophase of the last archesporial division.

Fig. 32. Tapetal nucleus in early prophase, showing parallelisms in its nuclear contents.

Fig. 33. Heterotype prophase. The telophase of the last archesporial division passes imperceptibly into the prophase of the heterotype division. The linin shows great irregularity in the size of its particles. Note the parallelism resulting from the alveolization of the chromosome segments.

Fig. 34. Nucleus in which portions of the chromosomes show alveolization before the sides have separated.

Fig. 35. Superficial section of a nucleus in the same stage as Fig. 34.

Figs. 36 and 37. Show a still finer breaking up of the chromosomes, the sides of the alveolized chromosomes remaining in places as parallel beads or strands.

Fig. 38 A. The linin is becoming concentrated into larger masses preparatory to going into synapsis.

Fig. 38 B. Superficial section of the same stage, showing that, notwithstanding the contraction, the parallelism is still present.

Fig. 39 A. The massing of the linin increases.

Fig. 39 B. Superficial section of a nucleus at the same stage as Fig. 39 A, showing the longitudinal fission.

#### PLATE LXI.

Fig. 40. Further massing of the nuclear contents, but parallelisms are still to be seen.

Fig. 41. The nuclear contents are collecting at one side of the nucleus.

Fig. 42. Synapsis showing chromatin areas embedded in the linin substance. 'Chromatic' bodies are being extruded.

Fig. 43. Typical close synapsis, with extrusion of 'bodies'.

Fig. 44. Loosening of the synaptic knot; its substance is emerging in the form of thick loops.

Fig. 45. Some of the strands are beaded, and show longitudinal fission.

Fig. 46. Further loosening of the knot. The strands are beaded.

Fig. 47. Hollow spireme stage, in which the spireme is arranged in loops lying freely in the nuclear cavity.

Fig. 48. The loops are beginning to concentrate towards the centre of the nucleus. Note the conspicuous longitudinal fission, the varied thickness of the strands, and the anastomosis between the strands.

Fig. 49. Nucleus which is undergoing a more prolonged 'hollow-spireme' stage. The threads have straightened, they are irregular in thickness, and there is anastomosis between them.

Fig. 50. Further straightening of the spireme, which has now segmented.

Fig. 51. Commencement of the second contraction. The segments are collecting towards the centre; note the two lengths of spireme which are apparently joining end to end.

Fig. 52. Beginning of second contraction. In this case the nucleus is going into second contraction on emerging from synapsis. The sides of the loops are being drawn in parallel to one another, and in places they have joined side by side.



Fig. 53. Further preparation for second contraction. The univalent portions uniting to form the bivalent strands.

Fig. 54. The segments are going into second contraction.

PLATE LXII.

Fig. 55. Second contraction. Those segments which are escaping show the separation of the two univalent chromosomes.

Fig. 56. Loosening of the second contraction. The loops exhibit longitudinal fission. A small pair of chromosomes have already separated from the contraction.

Fig. 57. The eight pairs of univalent chromosomes are seen to be splitting apart as they come out of second contraction.

Figs. 58 A and 58 B. Two sections through the same nucleus. In Fig. 58 A the segments are still in masses, whilst in Fig. 58 B they have begun to split apart into the univalent chromosomes.

Figs. 59 A and 59 B. Sections through the same nucleus, showing the splitting of the bivalent limbs. The eight pairs of chromosomes can be identified.

Fig. 60. Slightly later stage, in which the chromosomes are becoming more concentrated. Note the longitudinal fission in the separate univalent chromosomes.

Fig. 61. Shows a looped bivalent chromosome, of which one side is concentrated, whilst the other is still in a beaded condition.

Fig. 62. Later stage in the concentration of the chromosomes.

Fig. 63. Nucleus with its chromosomes joined end to end like the links of a chain.

Fig. 64. Diakinesis. The nucleolus has fragmented.

Fig. 65. Chromosomes going on to the equatorial plate.

Fig. 66. Polar view of an equatorial plate.

Fig. 67. Chromosomes passing to the poles.

PLATE LXIII.

Fig. 68. Anaphase of the heterotype division. Each chromosome is split.

Fig. 69. Polar view of anaphase, showing the eight split chromosomes.

Fig. 70. Telophase in which the chromosomes have formed themselves into an indistinguishable mass and the alveolization is commencing.

Fig. 71. Polar view of a nucleus in telophase, showing breaking up of the chromosomes and the origin of the paired threads and parallelisms.

Fig. 72. Telophase of first meiotic division.

Fig. 73. Further fragmentation of the chromosomes in the telophase. The remains of the spindle fibres are still visible.

Fig. 74. Homotype prophase, showing a reconcentration of the linin portions.

Fig. 75. The two nuclei elongate, and spindle fibres make their appearance. The nuclear contents become more and more concentrated to form the chromosome segments.

Fig. 76. Polar view of a nucleus in the same stage as Fig. 75.

Fig. 77. Further concentration of the segments to form the chromosomes.

Fig. 78. Long loop-like chromosomes going on to the spindle.

Fig. 79. Equatorial plate of homotype division.

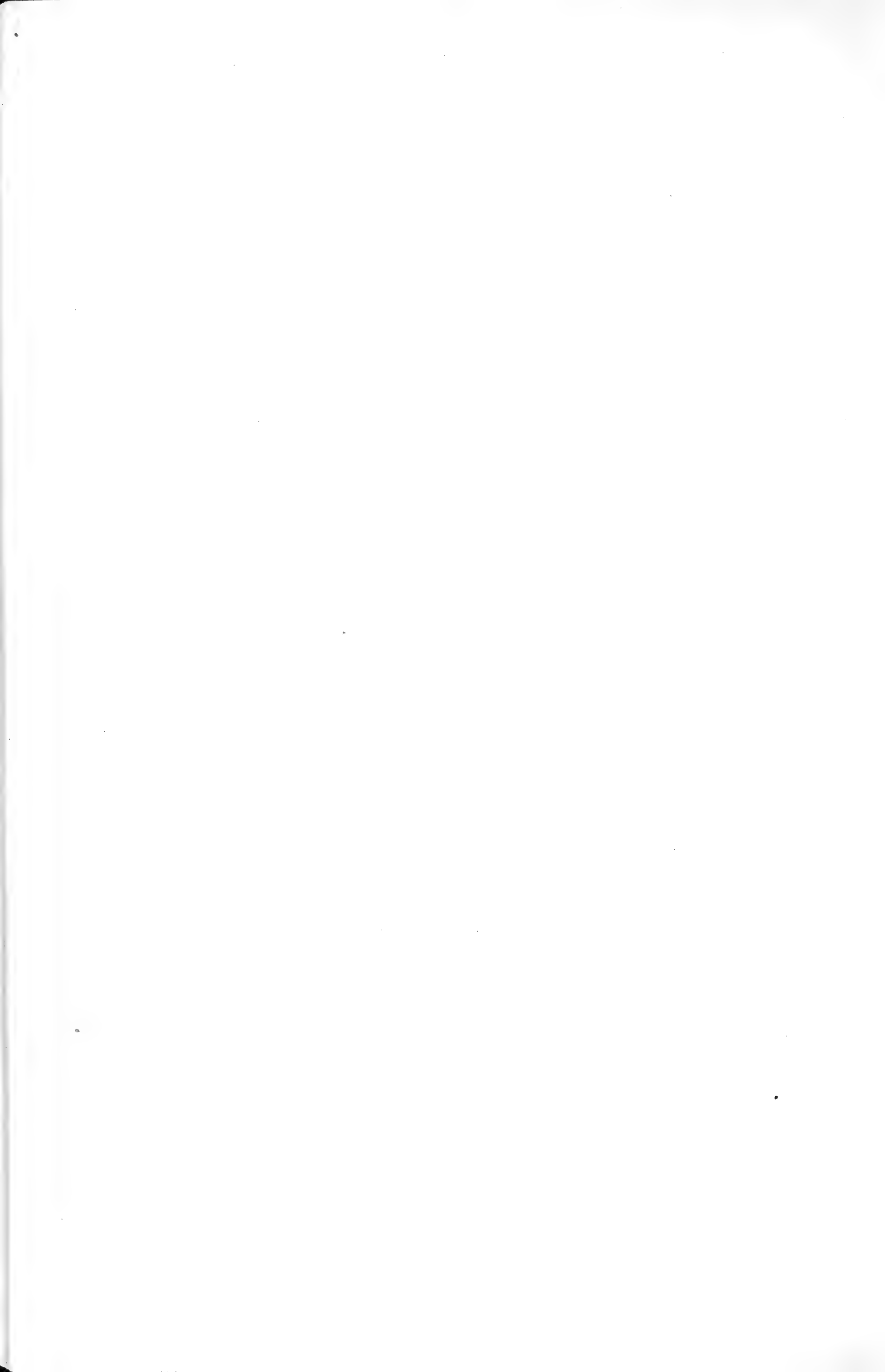
Fig. 80. The chromosomes are moving off to the poles.

Fig. 81. Diaster of homotype division.

Fig. 82. Telophase of tetrad nucleus, showing the breaking up of the chromosomes into parallel masses.

Fig. 83. 'Resting' nucleus of the future pollen grain. The chromatin contents are in the form of granules, and parallelism is still visible.

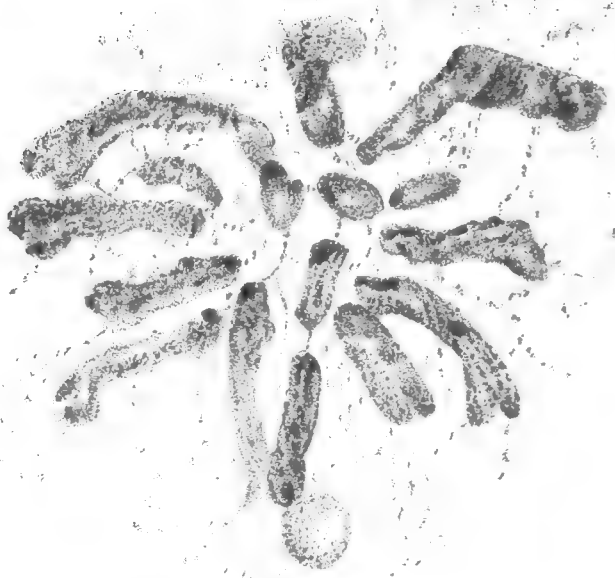








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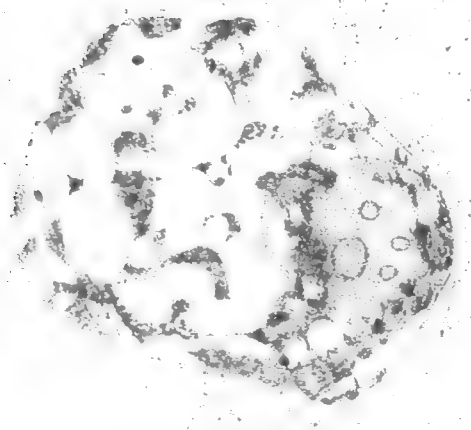
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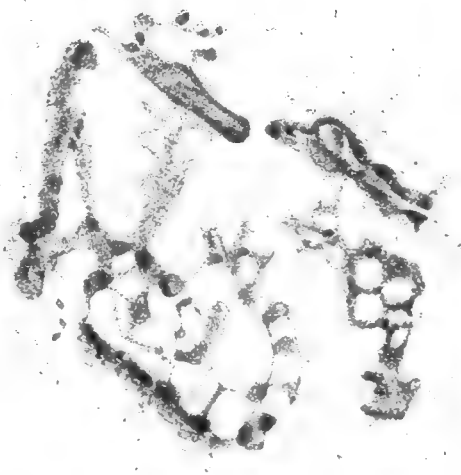
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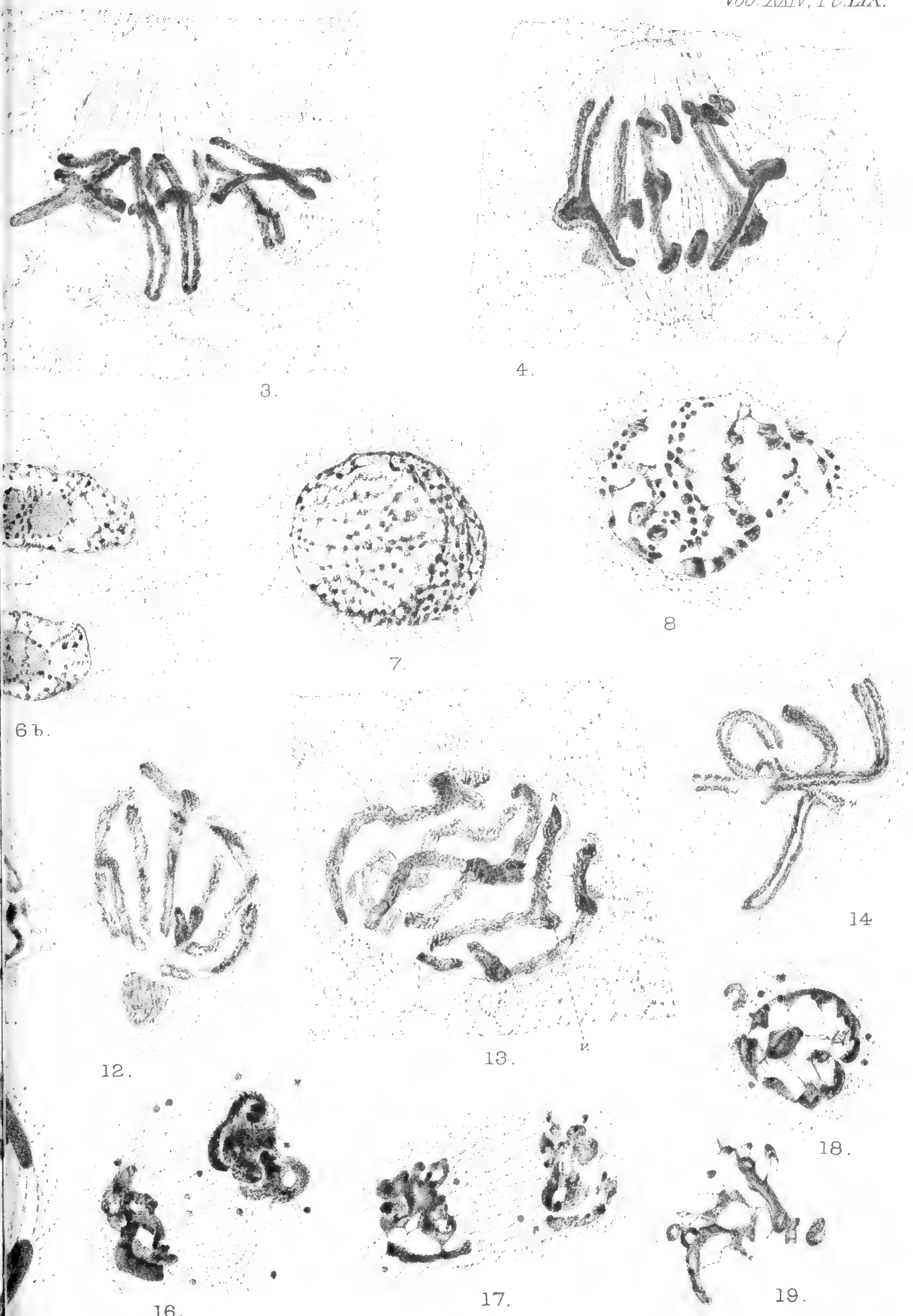


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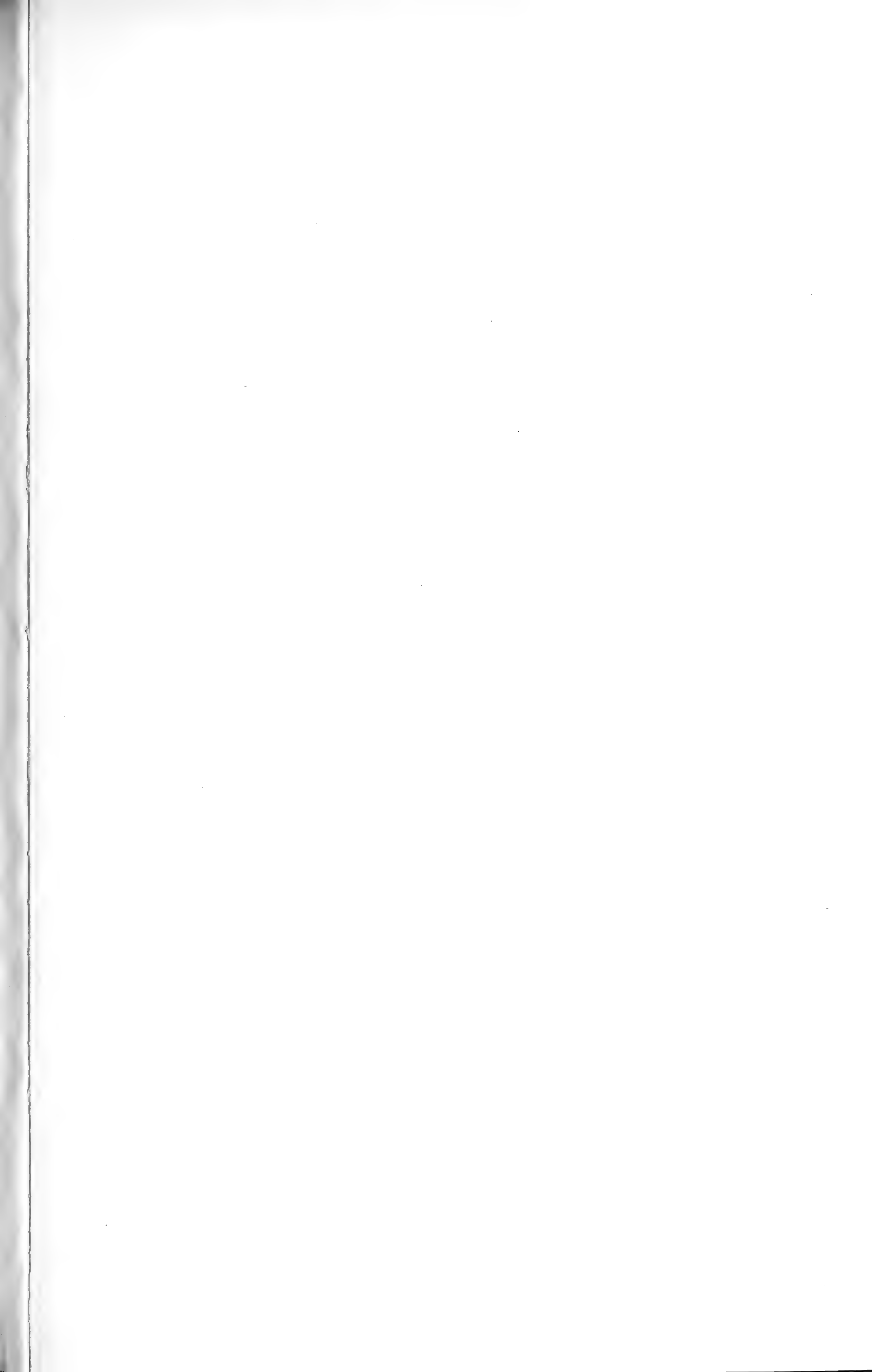


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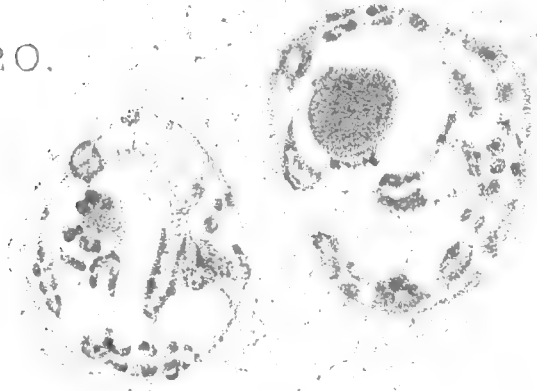




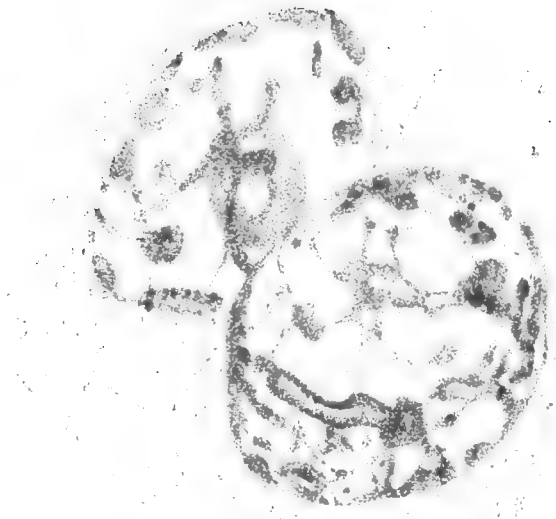




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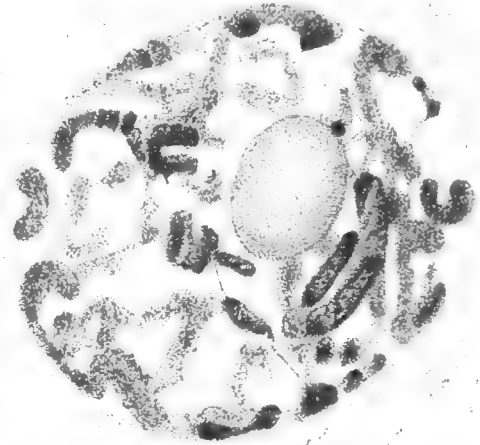
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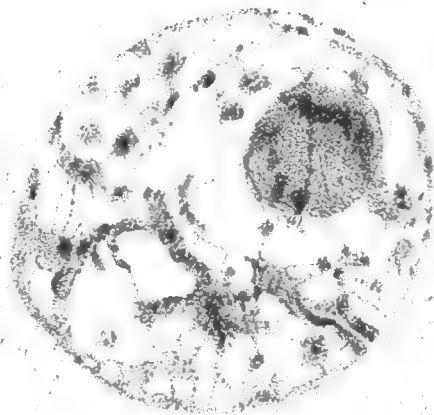
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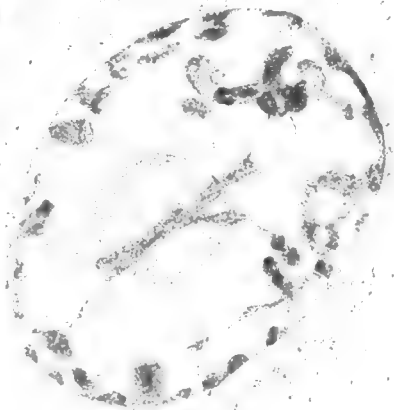
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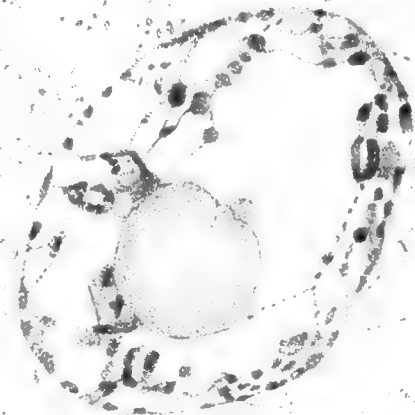
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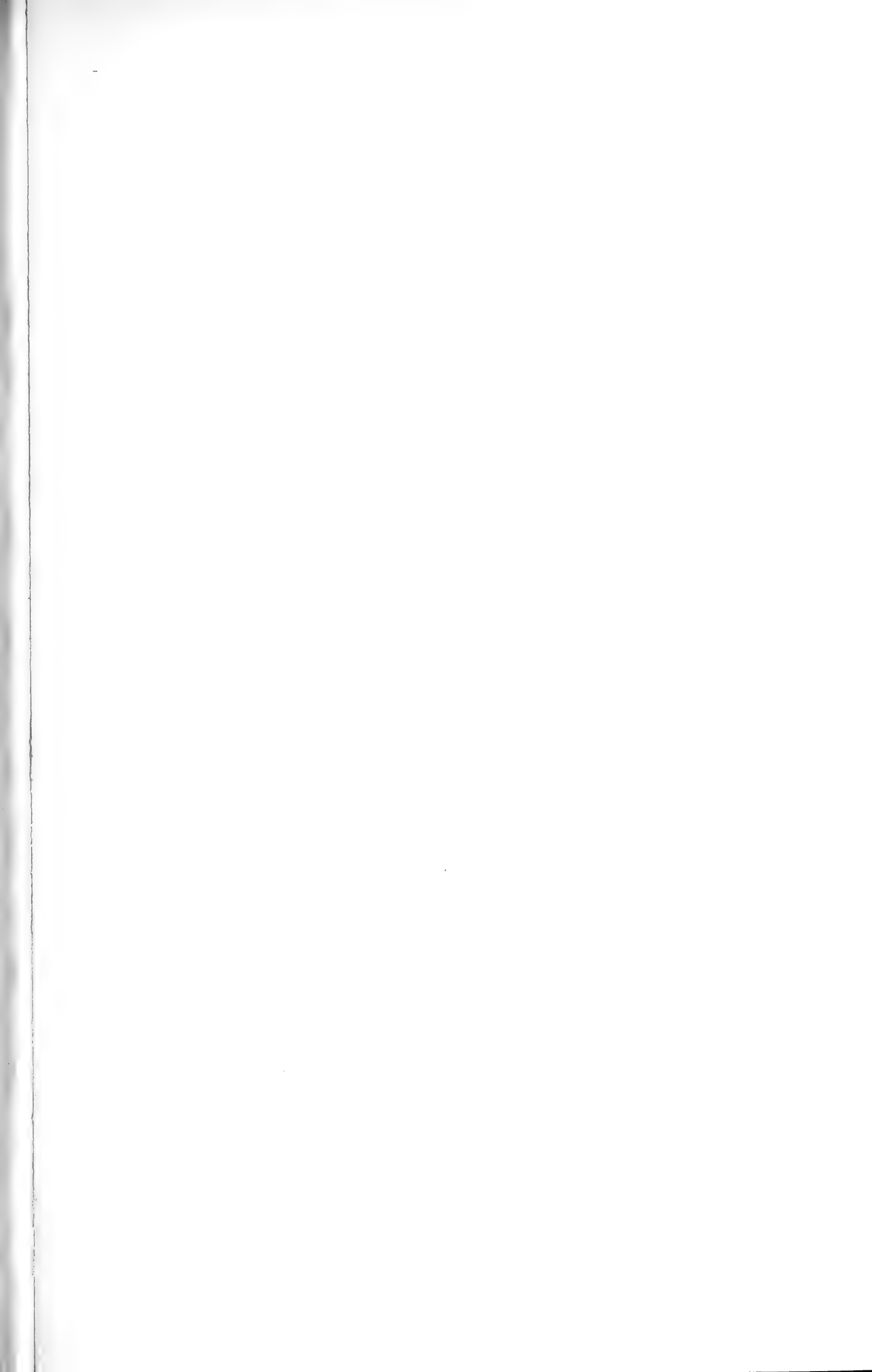
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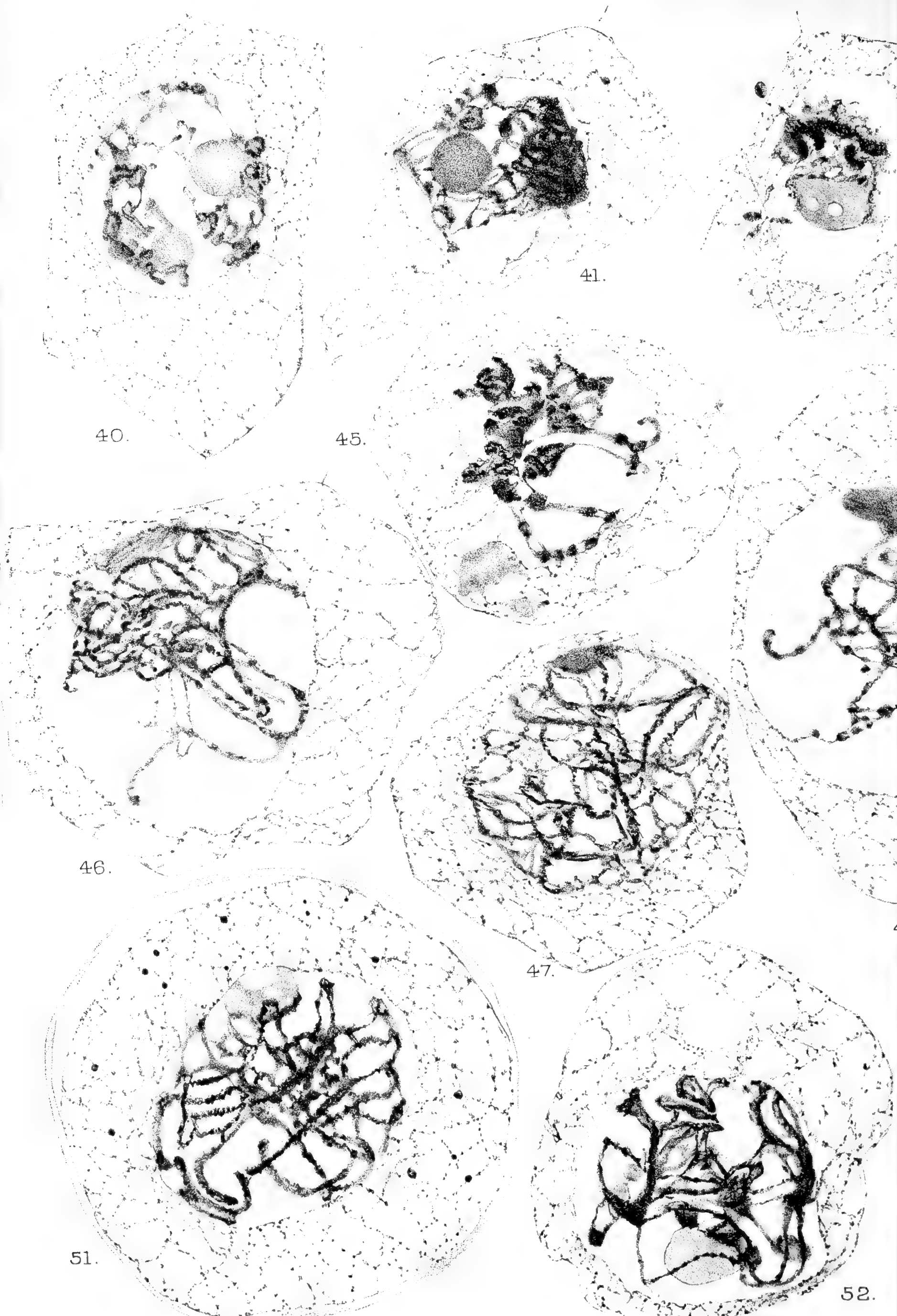
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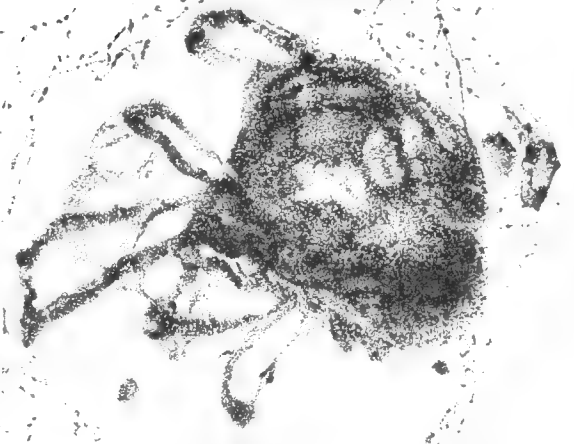
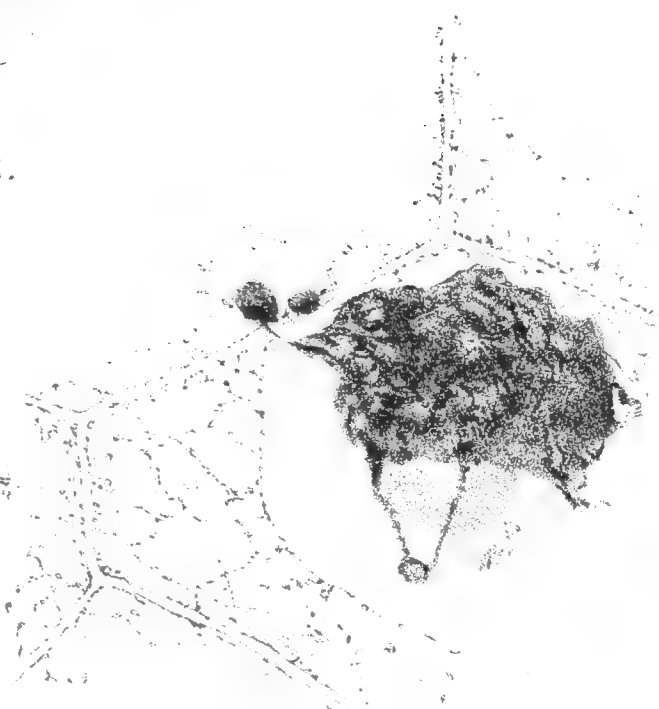




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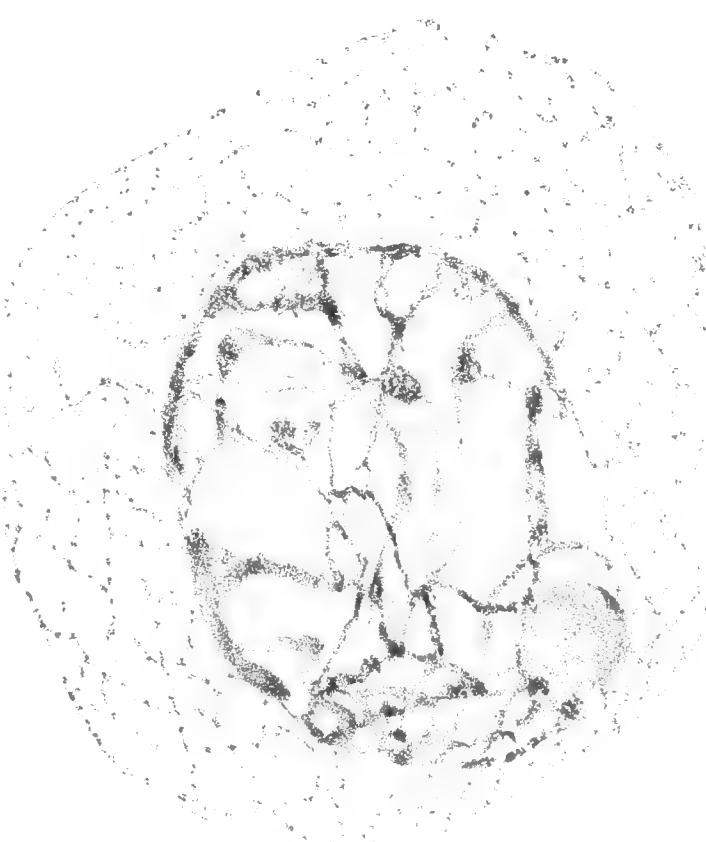
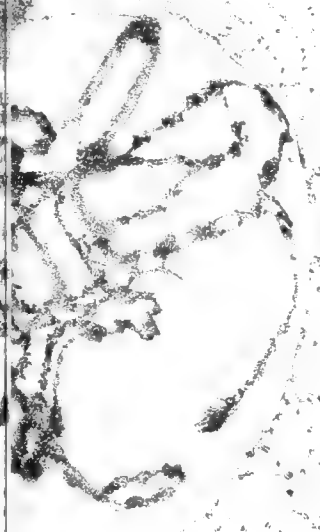
DIGBY.—NUCLEAR DIVISIONS IN GALTONIA.

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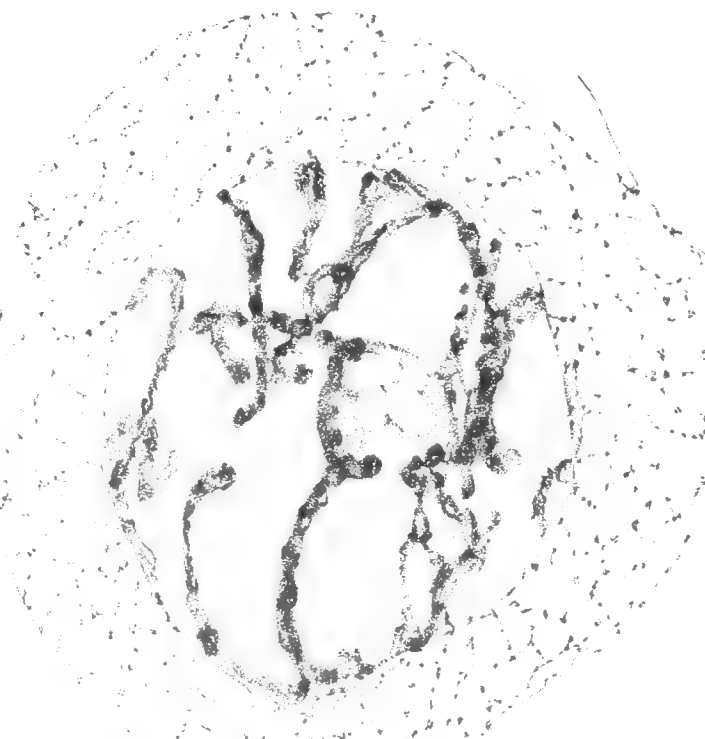


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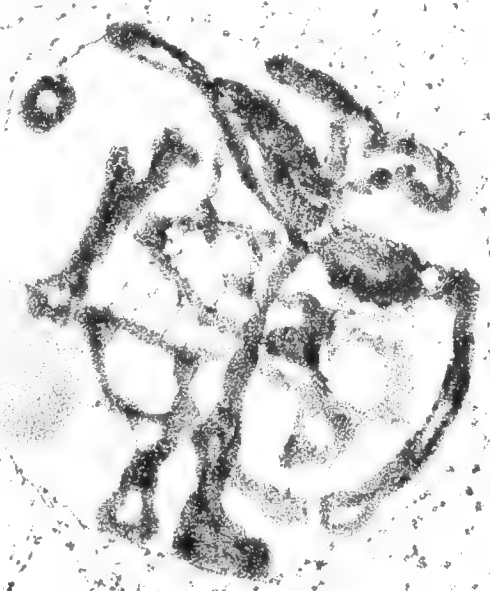
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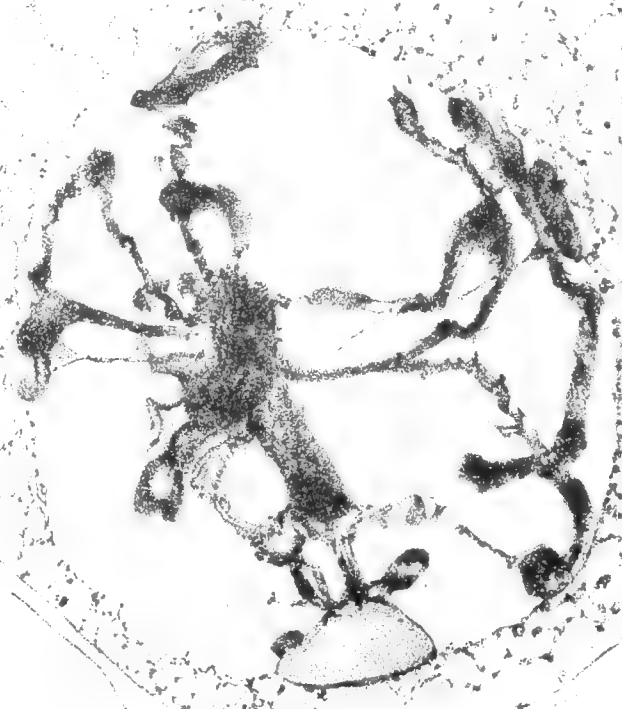
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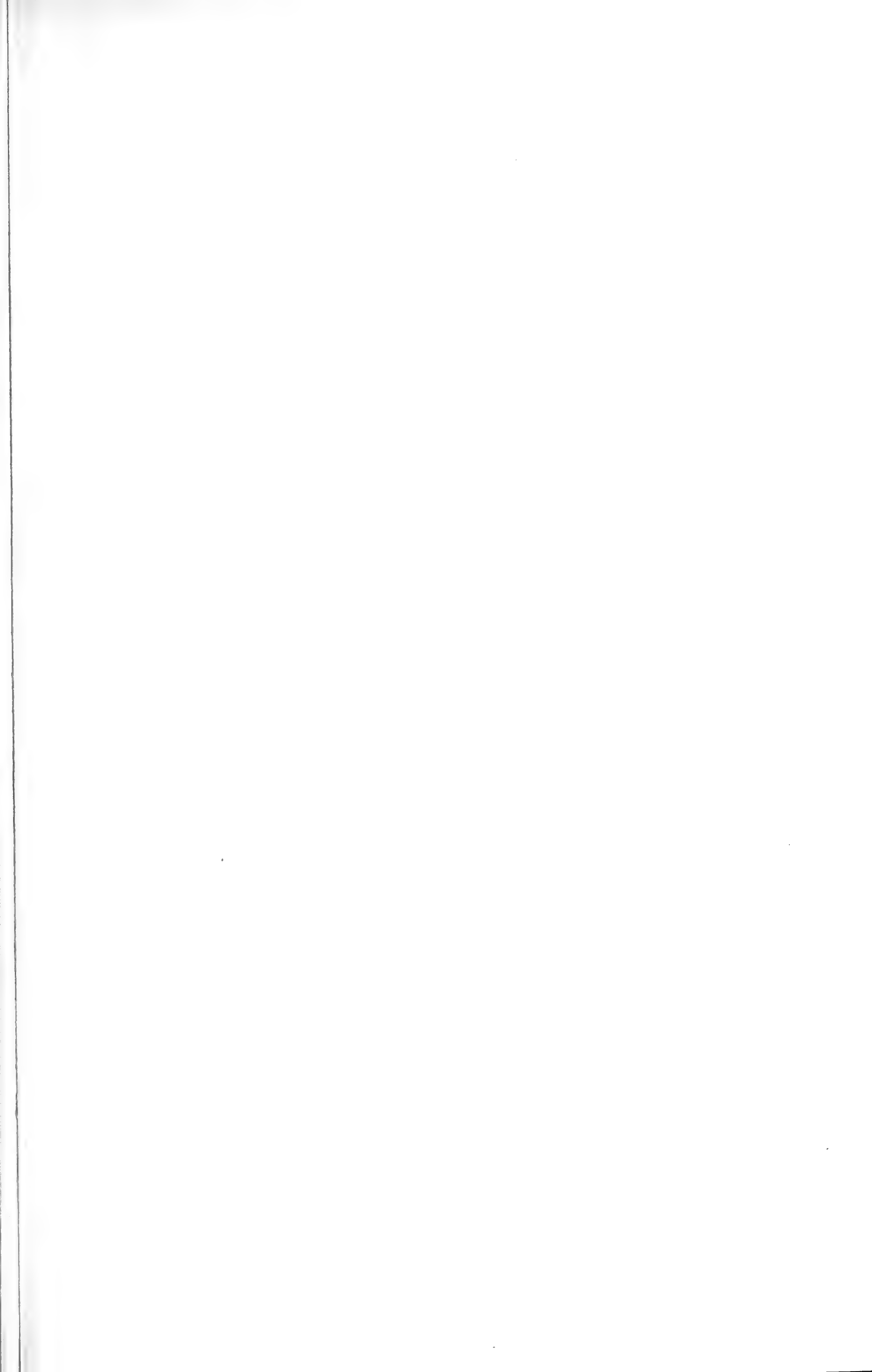
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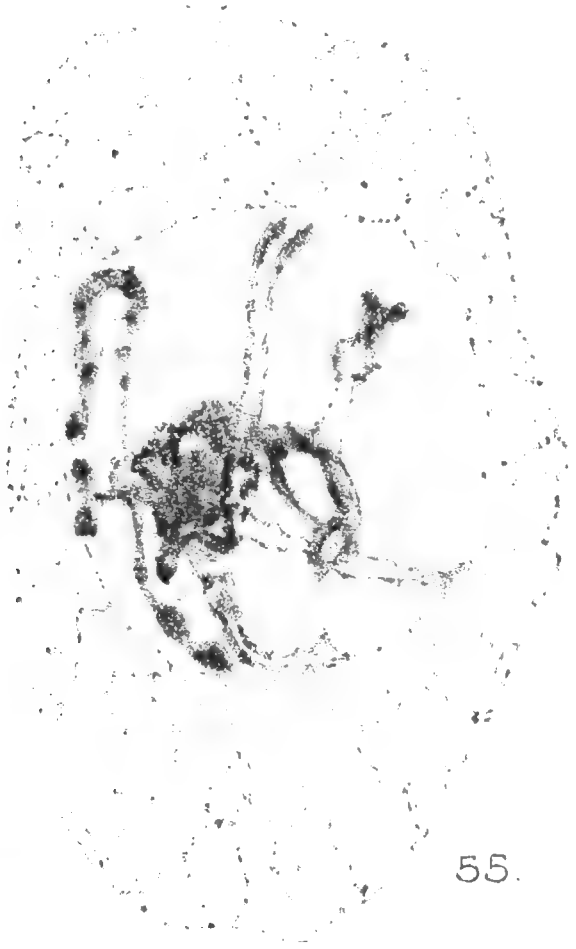


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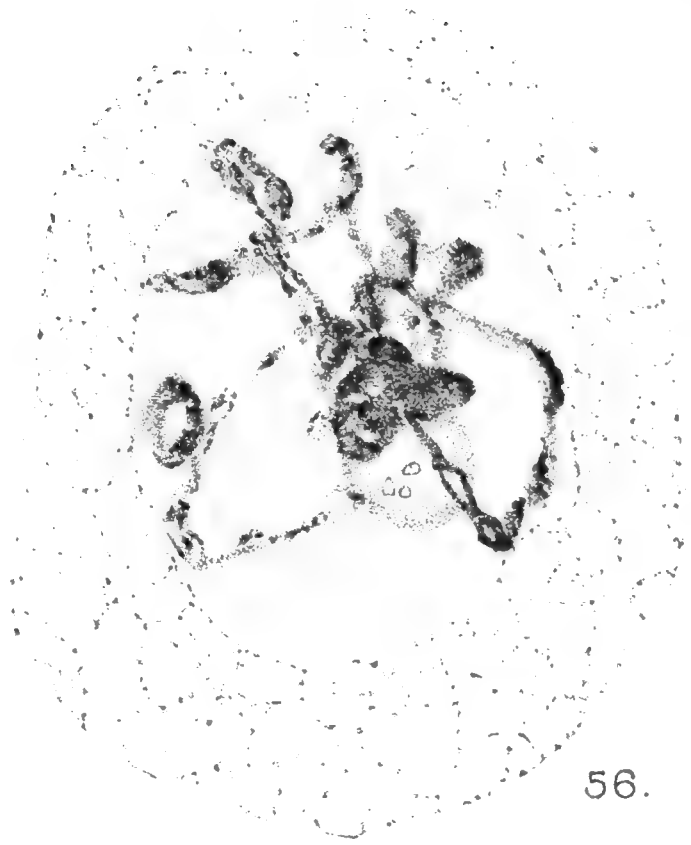




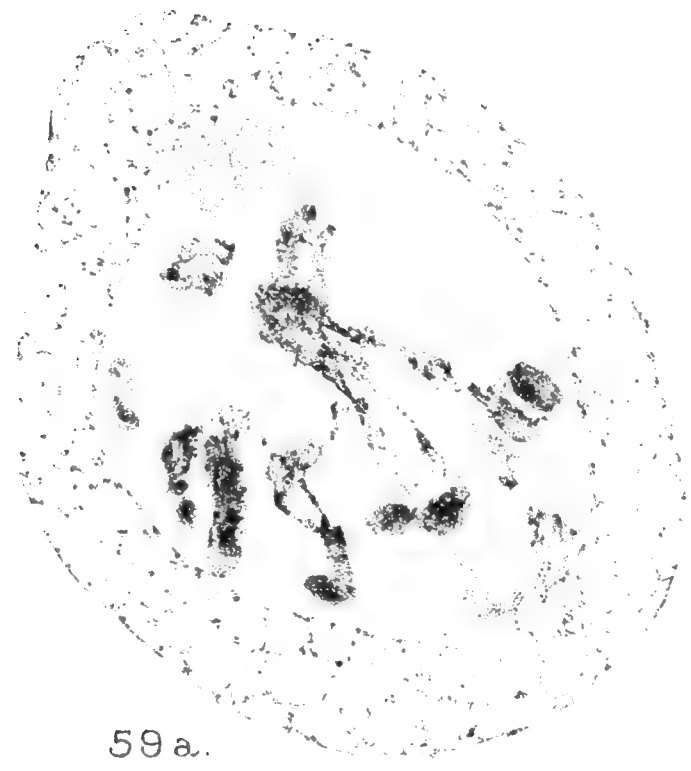
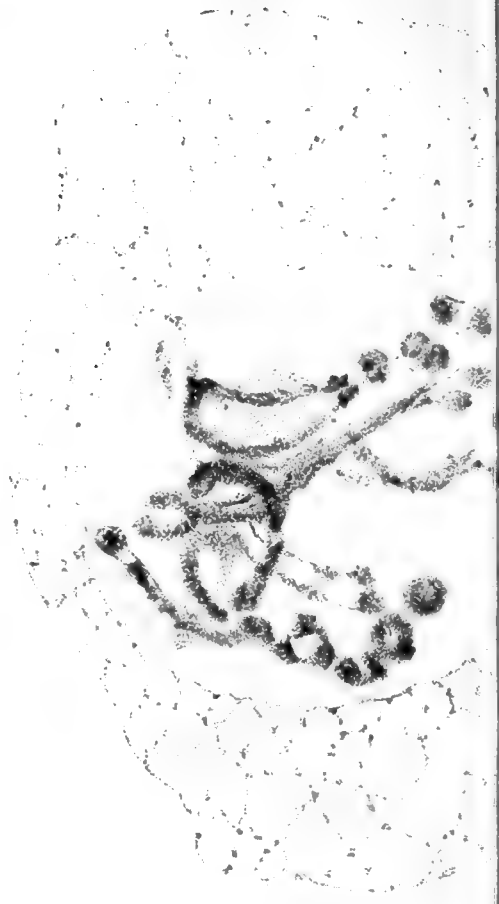




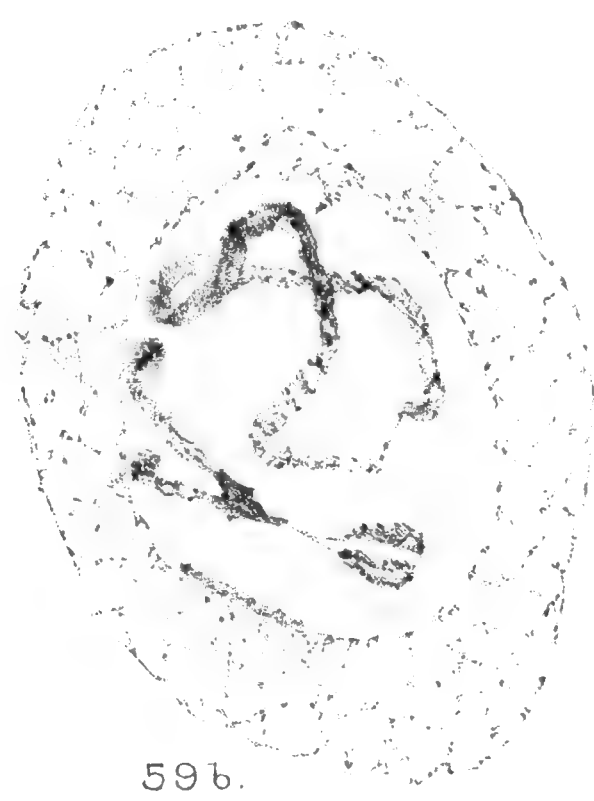
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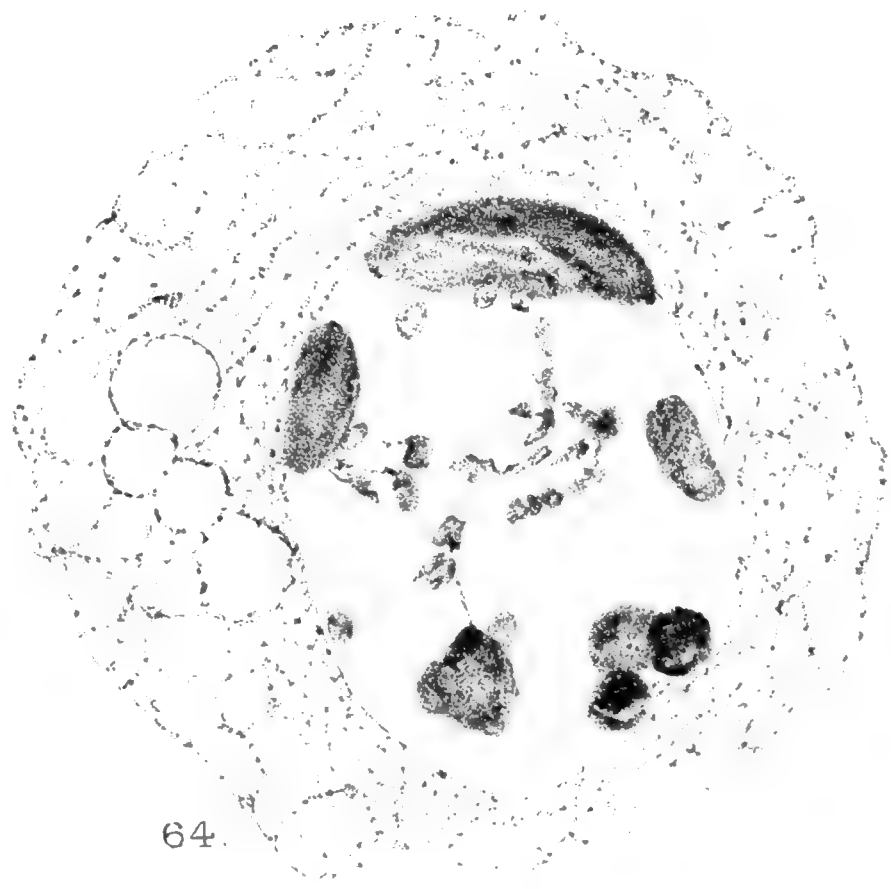


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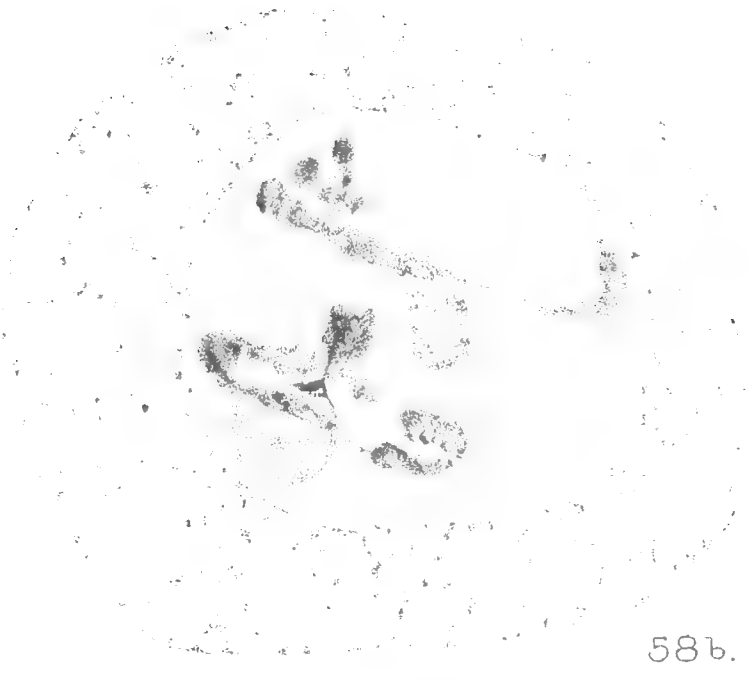
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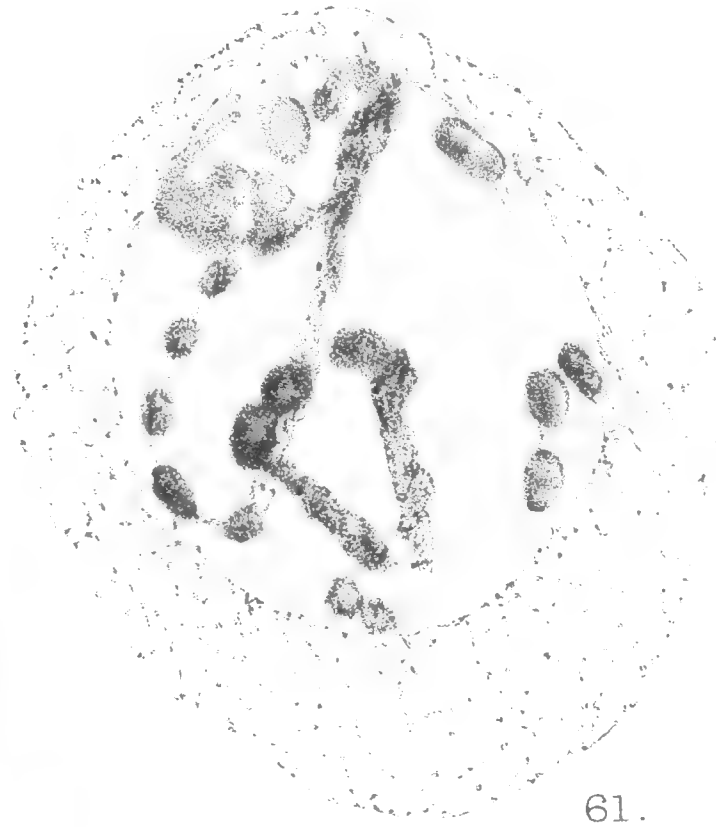


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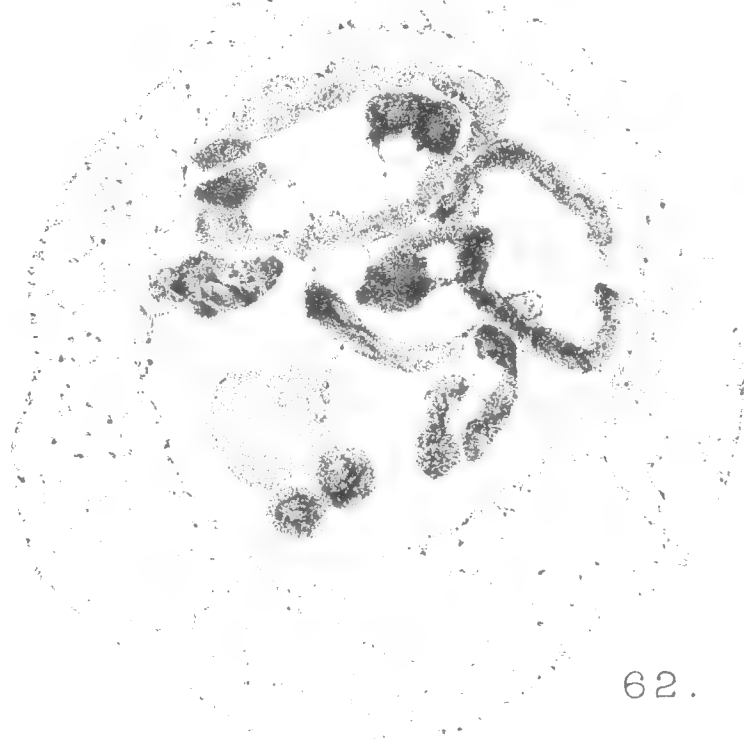


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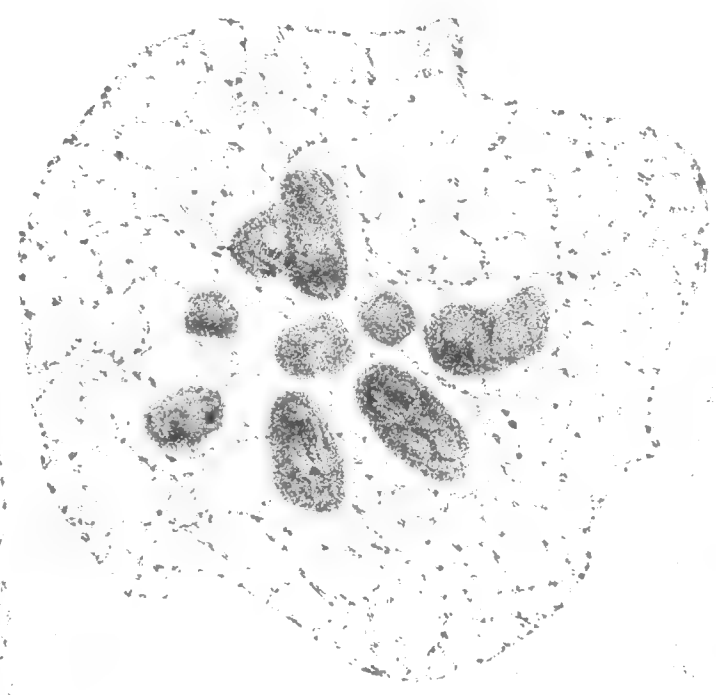
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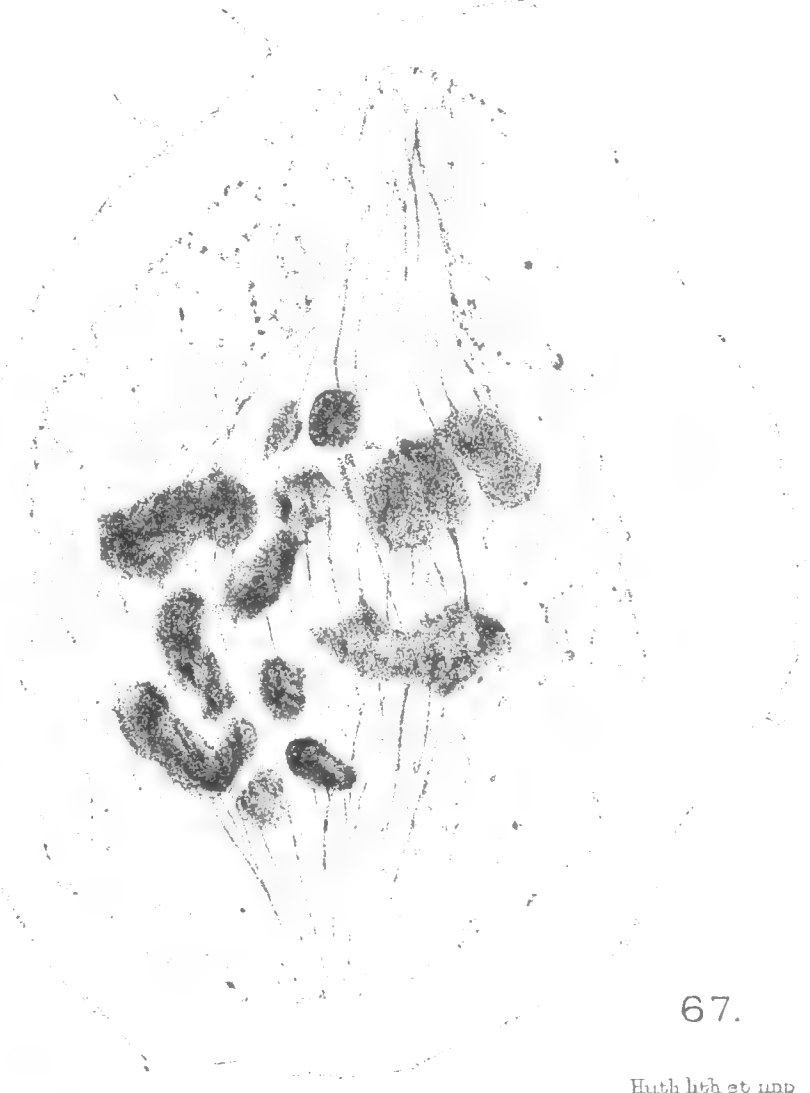
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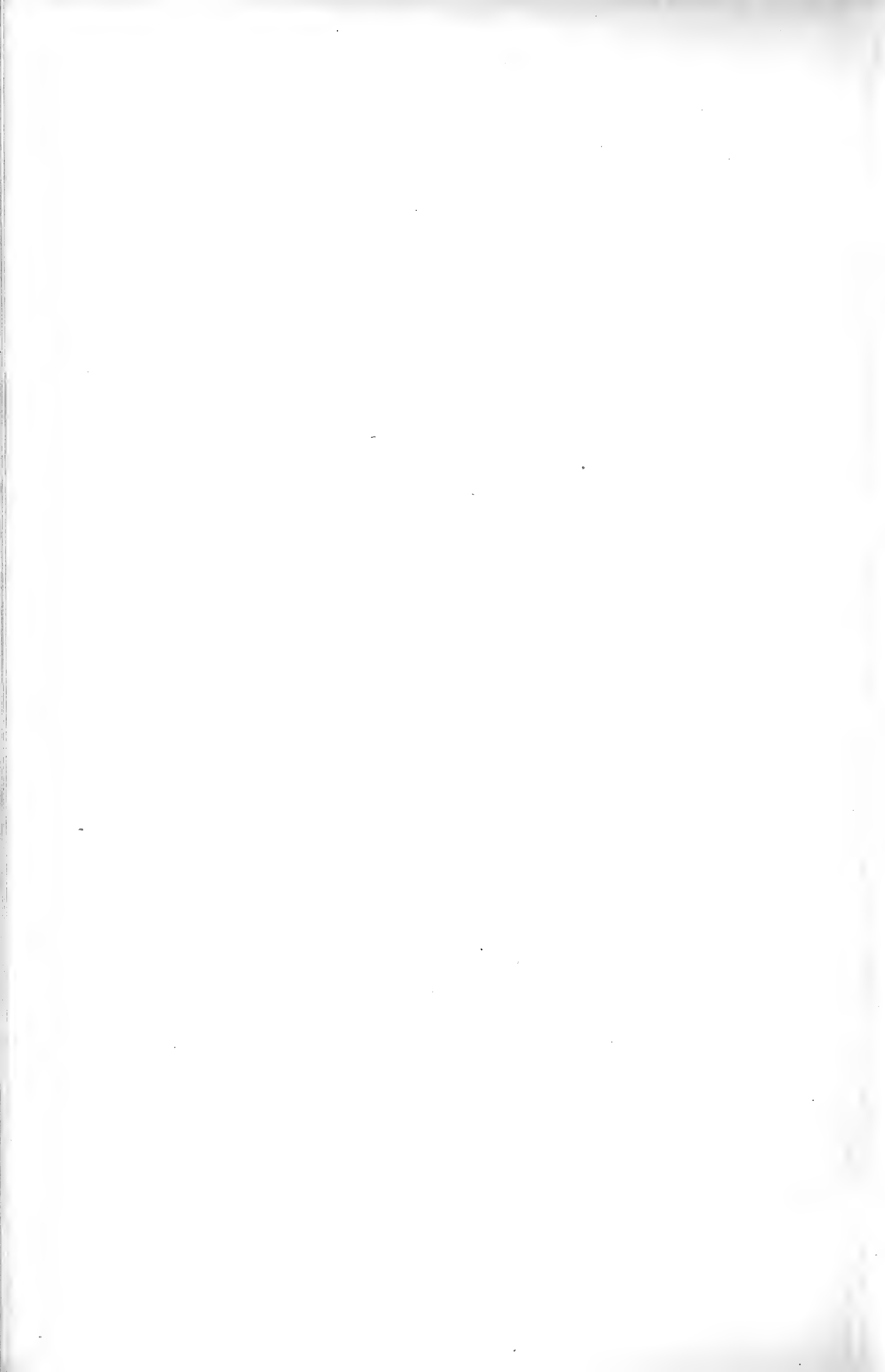
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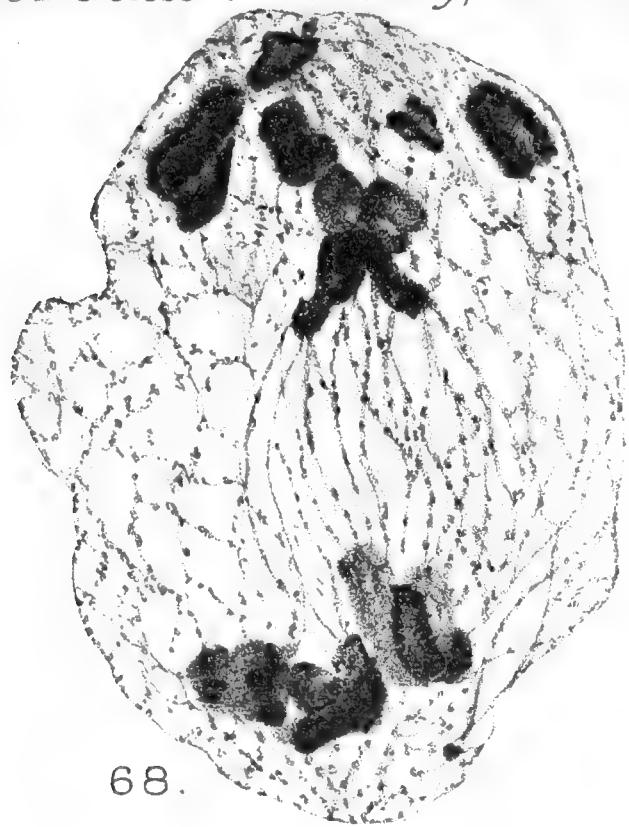


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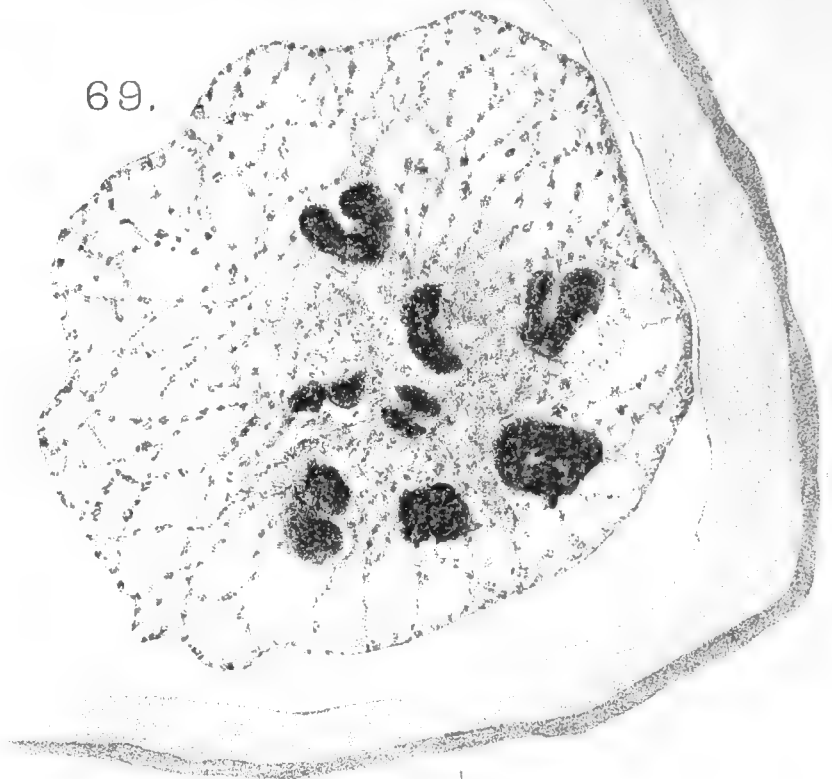








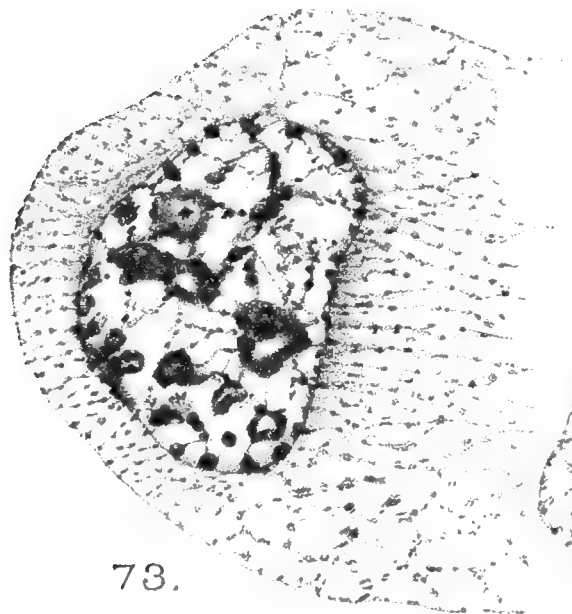
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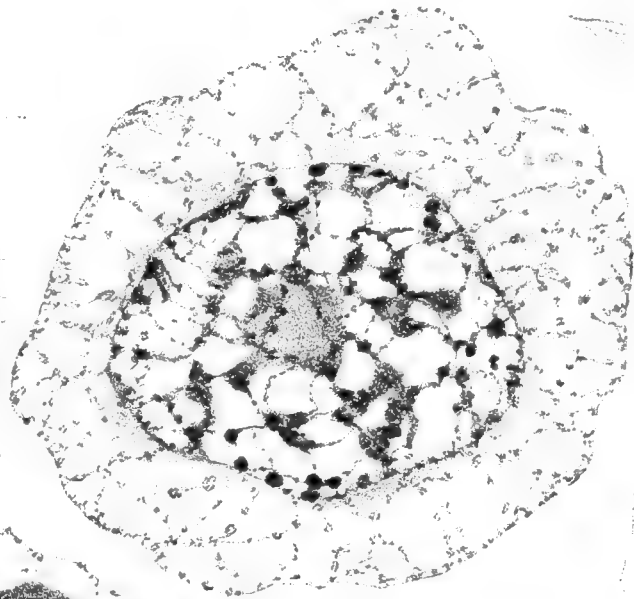
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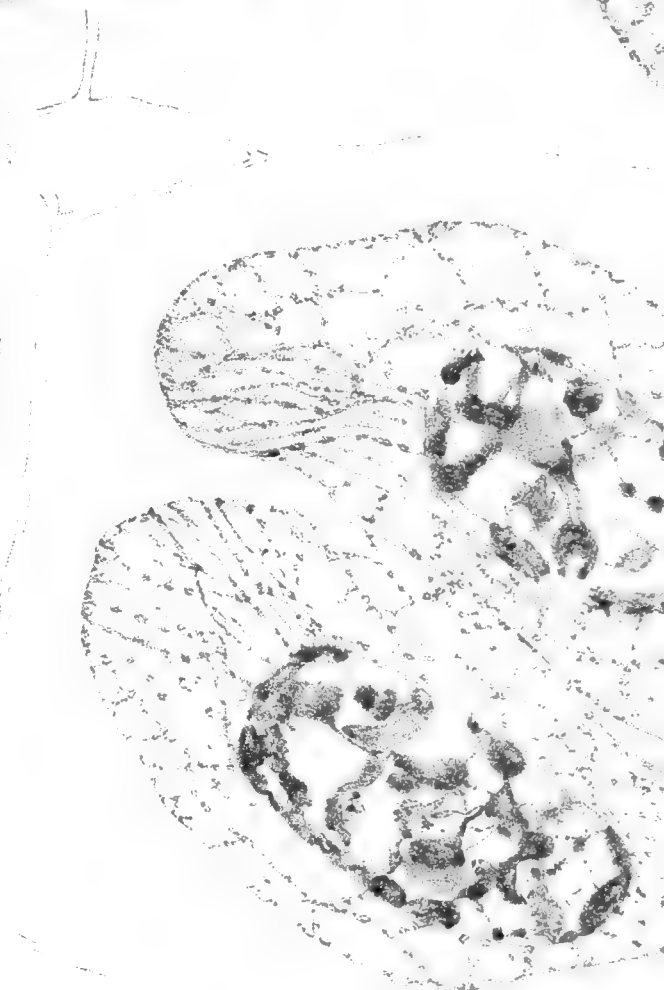
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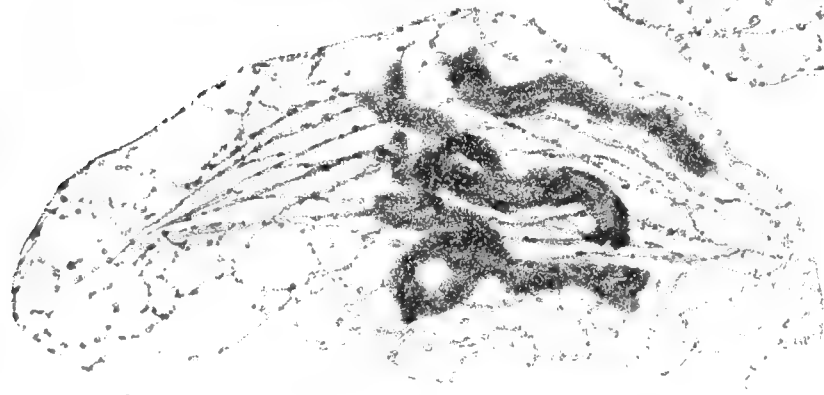
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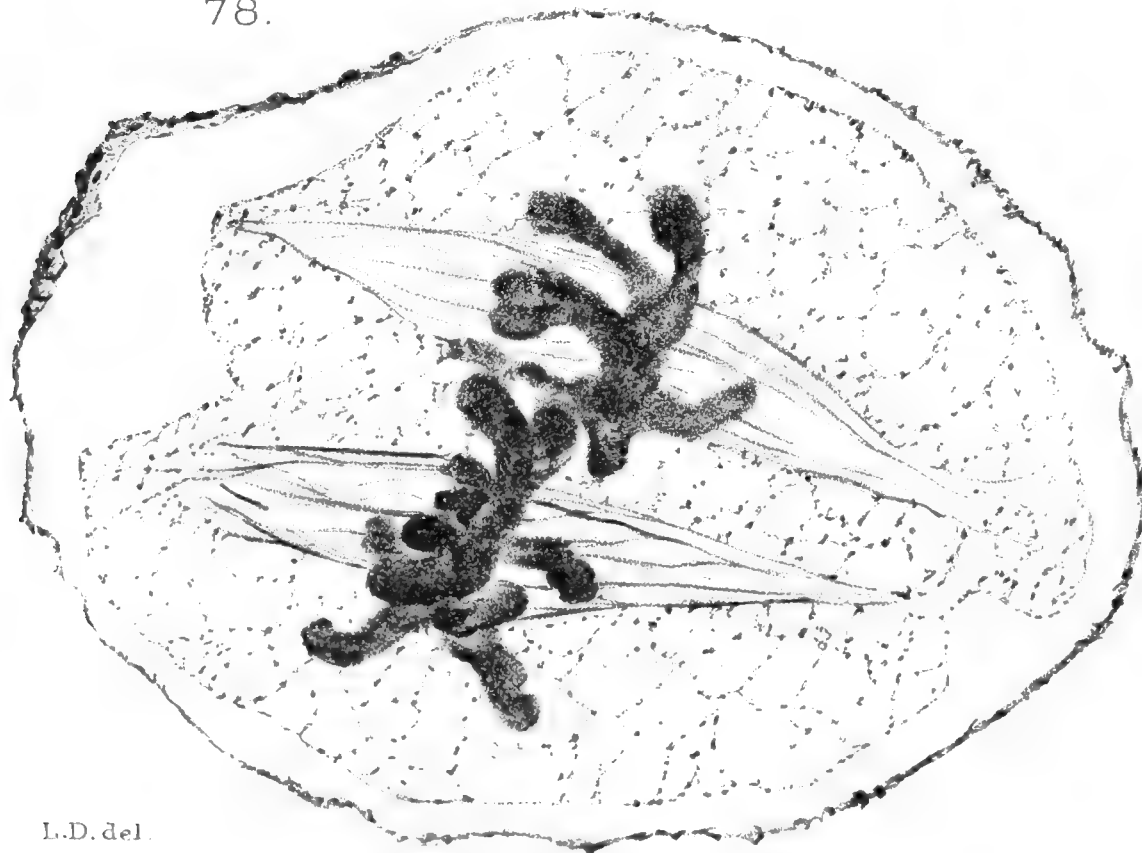
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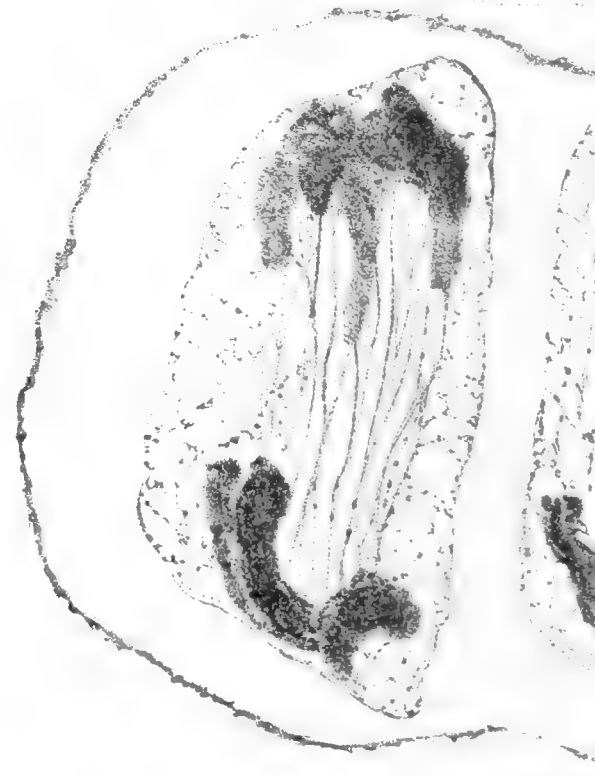
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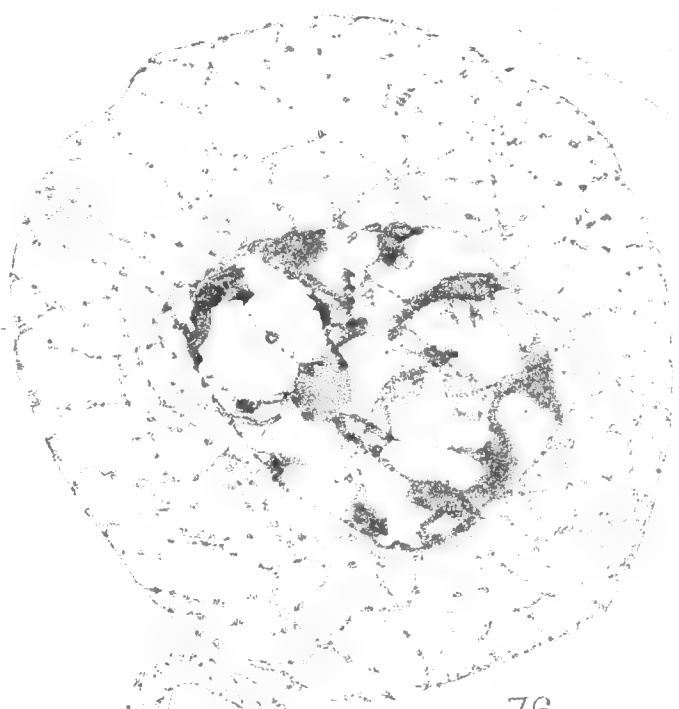
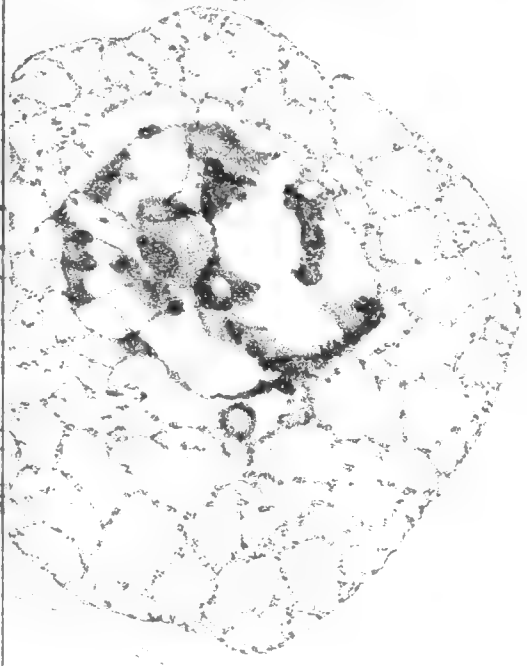
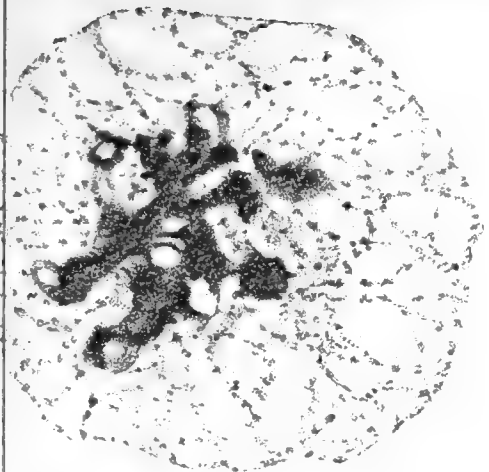
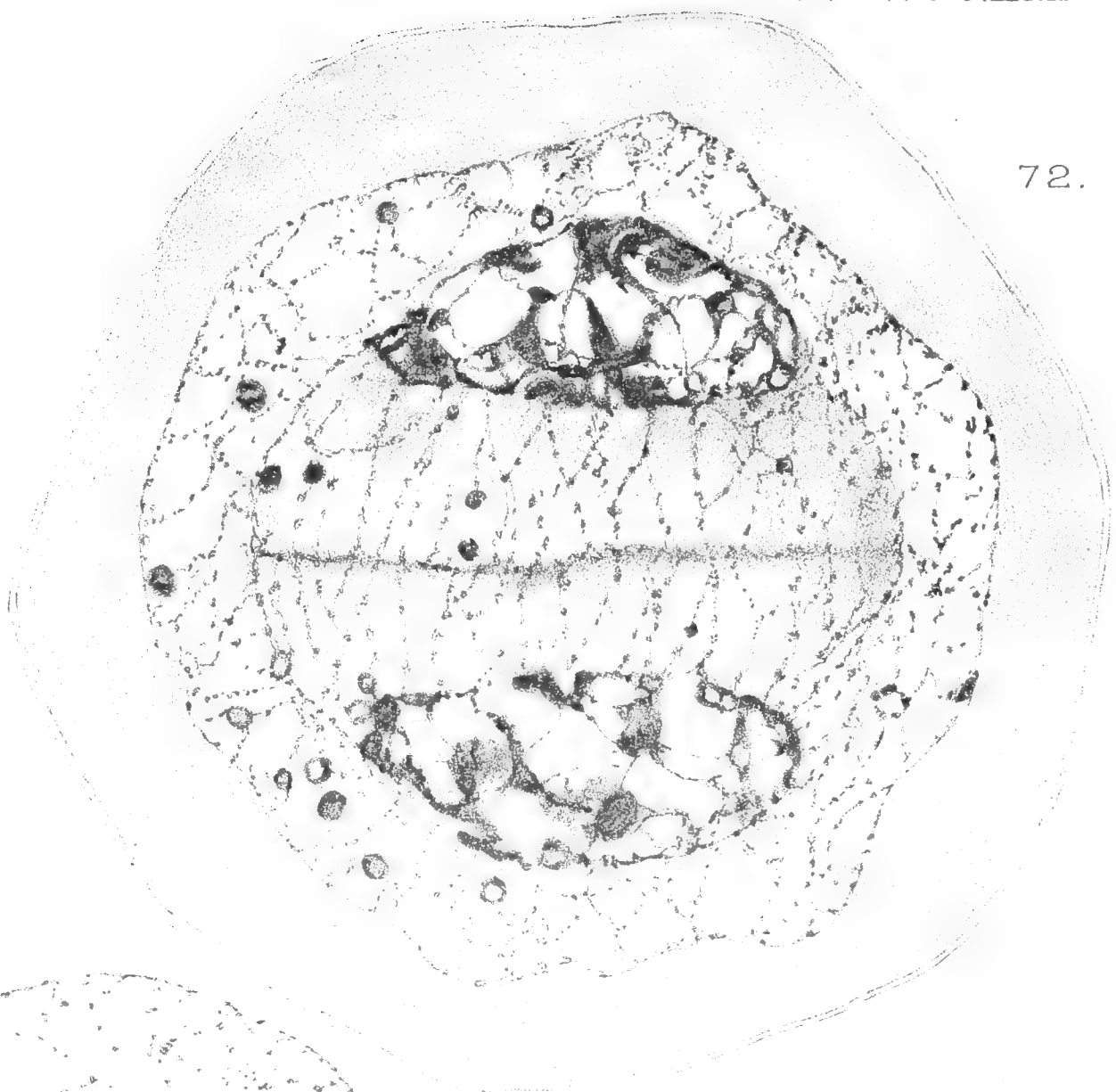
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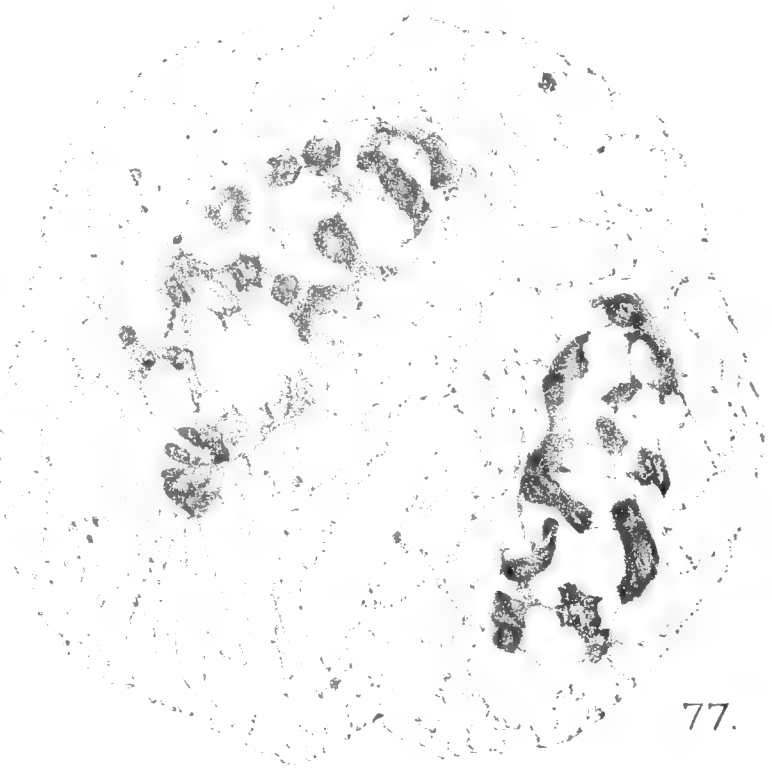
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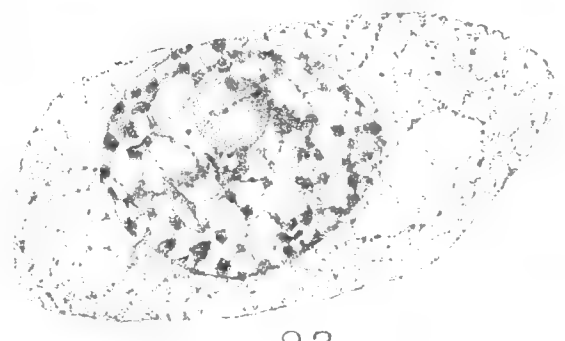
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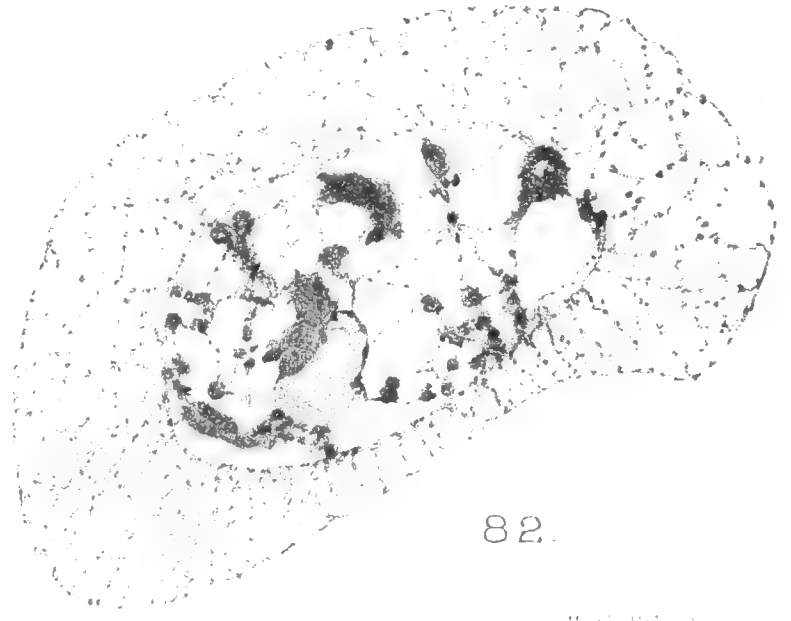
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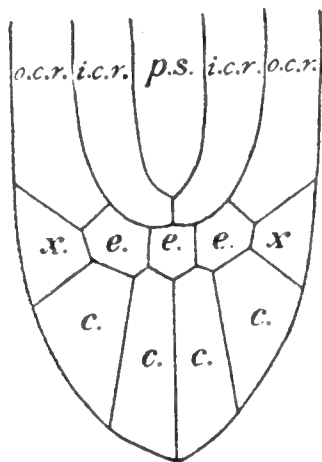
# On the Embryo of *Welwitschia*.<sup>1</sup>

BY

H. H. W. PEARSON, Sc.D., F.L.S.

With Plate LXIV and two Figures in the Text.

AN account of the development of the proembryo of *Welwitschia* up to the stage represented in the diagram (Text-fig. 1) has been given in a former paper.<sup>2</sup> In this condition the proembryo consists of a suspensor and a terminal cell-group. From the latter have been formed twenty-four of the twenty-five cells of which the suspensor consists; it also gives rise to



TEXT-FIG. 1. Diagram of an almost median longitudinal section through the distal end of the pro-embryo of *Welwitschia* at the stage figured in Pearson, 1909 A, Pl. 30, Fig. 85.

$\left. \begin{array}{l} p. s. \\ i. c. r. \\ o. c. r. \end{array} \right\} = \left\{ \begin{array}{l} \text{primary} \\ \text{inner cortical ring} \\ \text{outer cortical ring} \end{array} \right\} \text{ cells of the suspensor (cf. Pearson, l. c., Fig. 87).}$

$e. = 3$  of the 8 cells of the 'presumed embryonic plate' (cf. Pearson, l. c., Fig. 86 B).

$x. = 2$  of the 16 cells of the 'lower ring' (cf. Pearson, l. c., Fig. 86 B).

$c. = 4$  of the 'cap' cells (cf. Pearson, l. c., Fig. 86 A).

the embryo itself. The terminal group at this stage contains (1) eight or more 'cap' cells (Text-fig. 1, *c.*); (2) a ring of sixteen peripheral cells just below the suspensor—the 'lower ring' (*x.*); this surrounds (3) an internal plate of eight cells, the 'presumed embryonic plate' (*e.*).

This was the latest stage found in material collected in Damaraland between January 21 and February 1, 1907. As it was seen many times it seemed possible that further development was preceded by a resting period.

<sup>1</sup> Percy Sladen Memorial Expedition in South-west Africa, 1908–1909, Report No. 3.

<sup>2</sup> Pearson, 1909 A, pp. 364–368.

This suggested the further possibility that the completion of intraseminal growth might be deferred until after the fall of the seed; in certain species of *Gnetum*, as is well known, the embryo does not appear while the ovule is still in position, and this is probably characteristic for the genus. In the absence of any indications as to the later history of the terminal group of thirty-two cells it was provisionally compared with the corresponding region of the proembryo of *Araucaria brasiliana*, to which it bears a curious resemblance.<sup>1</sup> And although it was known that the 'cap' cells of *Welwitschia* are not thrown off in the stages immediately following,<sup>2</sup> as is the case in *Araucaria*, it nevertheless seemed probable that the embryo was formed from similarly situated regions in the two cases, viz. from the so-called 'embryonic plate' (Text-fig. 1, *e.*). It was further assumed that the cells of the lower ring in due course contributed additions to the suspensor.<sup>3</sup>

It was arranged that the Percy Sladen Memorial Expedition in South-west Africa should visit Welwitsch in Damaraland after the end of February in order to collect material of the later stages. The results now recorded have been obtained from ovules gathered there between March 2 and 9, 1909. An expedition to Welwitsch's own locality near Mossamedes in South Angola, in the following month, has yielded no additional information, as the older cones obtained there were diseased.<sup>4</sup>

This investigation has settled all the doubtful points referred to above. The development of the embryo, as far as the stage of Fig. 4, proceeds, apparently without any interruption, while the cone is still in position and intact; and since cones collected by Baines at Haikamchab on May 10, 1861, contained ripe seeds,<sup>5</sup> it is probable that embryo-development is continuous until the seed is mature. The period intervening between the occurrence of fertilization and the completion of the intraseminal growth of the embryo is thus less than four months. In this respect *Welwitschia* shows an agreement with *Ephedra*<sup>6</sup> rather than with *Gnetum*; but in view of the extreme xerophytic conditions to which *Welwitschia* has become adapted this cannot be regarded as of phylogenetic significance. Strasburger's statement that the 'cap' cells are not thrown off is confirmed; in the stage shown in Text-fig. 1 they are still the initial cells of the proembryo; their descendants occupying a similar position retain this character until the superficial layer becomes dermatogen and a cell-mass underlying it at the apex is organized as a meristematic group; the identification of the internal cell-plate (*e.*) as the 'embryonic plate' was therefore erroneous. The suggestion that the cells of the lower ring (*x.*) produce further additions to the suspensor is confirmed.

<sup>1</sup> Strasburger, 1879, Taf. xx.

<sup>2</sup> Cf. Hooker, 1863, Pl. 10, Fig. 25.

<sup>3</sup> Hooker, 1863, p. 3.

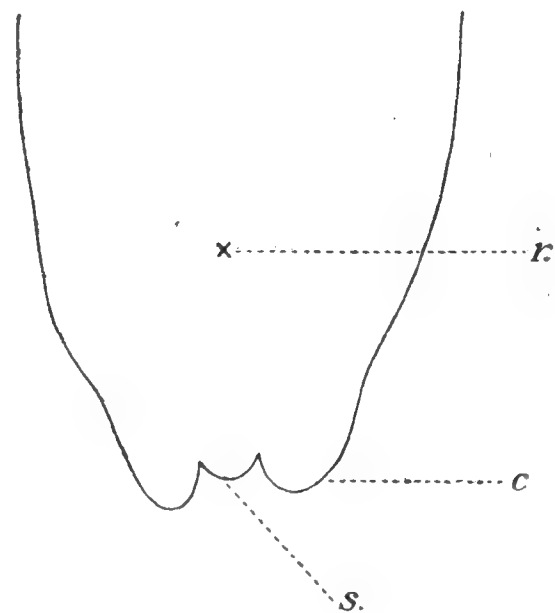
<sup>4</sup> l. c., p. 155.

<sup>5</sup> Pearson, 1909 B.

<sup>6</sup> Land, 1907, p. 281.



After the condition indicated in Text-fig. 1, the terminal initial cells ('cap' cells) undergo both periclinal and anticlinal divisions with the results that new cells are added beneath those of the plate *e.*, and similar to them, and the number of cells in the superficial layer increases (Pl. LXIV, Figs. 1, 6, 7). The cells of the 'embryonic plate' (*e.*) may divide further; they or their descendants lie at the extreme tip of the primary root-cap of which they form a part. Even as late as the stage of Fig. 3, all the superficial cells have not ceased to divide by periclinal walls, though many of them have no doubt become constituents of a dermatogen, and in somewhat more advanced stages they have all acquired this character. After the stage of Fig. 3, cell-division becomes localized in a more or less definite subapical growing region. The activity of this meristematic group results in the formation, on the side towards the suspensor, of a massive tissue whose cells are for the most part arranged in longitudinal rows. This is already indicated in Fig. 3, and becomes much more strongly marked in the axial core of later stages ('Periblemsäule')—see Figs. 4 and 5. For some time, therefore, the root-cap constitutes the greater part of the bulk of the embryo, in which character, as well as in the details of its formation, *Welwitschia* remains true to the Gymnosperm type. In the embryo shown in Fig. 4 the original meristematic group has become divided into two by permanent tissue; separate growing points for stem (*St.*) and root (*R.*) are thus established. The stem apex quickly becomes externally distinguishable from the rest of the embryo by reason of its smaller diameter and dome-like surface (Fig. 4). Its elongation is very slow compared with that of the root; in the embryo of the mature seed (Fig. 5), in which the two regions are still easily distinguished by the difference of breadth, the stem is hardly more than one-fifth of the length of the root. Strasburger showed that the cotyledons arise early from the stem-dome (Text-fig. 2); they grow fairly rapidly and usually somewhat unequally (Fig. 5). In the embryo of the mature seed, thin median sections disclose two small exogenous outgrowths from the stem apex, occupying axillary positions with respect to the cotyledons. Stages immediately following this are not available for comparison, but there can be no doubt that these are the 'lateral cones'.<sup>1</sup> Their appearance thus early in acropetal succession to the cotyledons and



TEXT-FIG. 2. Outline of longitudinal section through distal end of the embryo of *Welwitschia* (after Strasburger, 1879, Taf. xxii, Fig. 91).

*s.* = stem-apex; *c.* = cotyledons;  
*r.* = growing-point of root.

<sup>1</sup> Cf. Bower, 1881, pp. 579, 580, Fig. vii.



in their axils tends to confirm the view advanced by Bower<sup>1</sup> and more recently supported by Henriques, that they are axillary buds.

Meantime the mass of the suspensor has greatly increased. The growth of the cells of the 'lower ring' ( $x$ ) is seen in Fig. 1, while in Fig. 2 they already form part of the suspensor. The peripheral cells immediately beneath these (Fig. 2,  $y$ ) in their turn behave in the same way, and, as the embryonic mass elongates, acropetally developed additions to the suspensor are produced until this structure has attained its final form. These later formed suspensor cells frequently show a considerable degree of independence of growth, as was observed by Hooker.<sup>2</sup> Cases are seen in Fig. 1 and more markedly in Fig. 3. In both, suspensor-cells with free ends occur; in the latter some are growing parallel with the main body of the suspensor ( $a$ ), others appear to be spreading laterally ( $d$ ), while two ( $b$ ) have turned downwards and are advancing into the endosperm in the same direction as the embryo. This may perhaps be of the nature of a structural adaptation to the growing demands of the embryo for food-supplies. In earlier stages, while the suspensor is still slender and compact and the embryonic cell-mass small, absorption by the cells of the peripheral layers of both regions is sufficient. But as they become more massive and the depletion of the endosperm advances, the difficulty of maintaining an increasing rate of absorption must become greater. The common behaviour of the younger suspensor-cells suggests that they have to some extent taken over the functions of root-hairs; morphologically, of course, they are outgrowths from the root-cap.<sup>3</sup> At length the suspensor has assumed its maximum thickness, and henceforward the surface of the root-cap remains smooth (Fig. 4). An interesting and suggestive relation appears to exist between the life-conditions of the embryo in certain parts of its course and the structure of its suspensor. In early stages,<sup>4</sup> when the young embryonic group is growing towards the endosperm, frequently by a route to some extent predetermined by the embryo-sac tube, the suspensor is simply the elongated hypobasal cell. As soon as it enters the endosperm where it meets competing embryos in a narrow area which quickly becomes depleted of food supplies, the suspensor thickens.<sup>5</sup> There is very little doubt that the embryo which first reaches the starch-bearing region of the endosperm<sup>6</sup> normally becomes the embryo of the seed, and, broadly speaking, the more quickly this occurs the greater is the chance that a fertile seed will result. A suspensor which has acquired some degree of rigidity by reason of the addition to its bulk of several series of peripheral cells, and whose more or less spiral form gives to it a certain elasticity, will play an important part in assisting the embryo to penetrate the underlying cell-

<sup>1</sup> Bower, l. c.; Henriques, 1910.

<sup>3</sup> Cf. Land, 1907, p. 280.

<sup>5</sup> l. c.  $p_3, p_4$ .

<sup>2</sup> Hooker, 1863, Pl. x, Fig. 25.

<sup>4</sup> Pearson, 1909, Text-figure,  $p_1, p_2$ .

<sup>6</sup> l. c., *st.*

mass. While the mechanical force developed by the whole system is without doubt of second-rate importance compared with the results of enzyme-action in front of the flattened, bluntly-pointed tip of the embryo, the crushed cells which commonly lie in contact with it bear witness to the exertion of a definite pushing force.

No ovule in which the endosperm was not obviously withered has been found to be without an embryo. This tends to confirm the observation previously recorded<sup>1</sup> that the second phase of the growth of the trophophyte is dependent upon the occurrence of fertilization—either directly or through the embryonic activity which follows it. One case only has been seen in which the one remaining embryo in an ovule was clearly breaking down. The proportion of fertile seeds in normal cones appears to be fairly high,<sup>2</sup> and the efficiency of seed-production in Damaraland is in striking contrast to the rarity of the occurrence of successful germination in that region.<sup>3</sup>

In early stages, as has been already recorded,<sup>4</sup> the ovule usually contains a number of proembryos; but sooner or later all but one disintegrate. It very rarely happens that an advanced ovule is polyembryonic owing to the persistent development of more than one proembryo. In one such case the larger of two embryos (about the stage of Fig. 3) lies deep in the axis of the endosperm, while a second (younger than Fig. 1), having penetrated almost to the depth of the former, has turned abruptly through an angle of  $180^\circ$  and now lies near the boundary of the disorganized region of the endosperm<sup>5</sup> with its apex directed towards the nucellus. This appears to be another instance of the readiness with which the *Welwitschia* embryo changes its direction of growth in response to a nutritive stimulus.<sup>6</sup> While no case of the branching of the suspensor has been observed, polyembryony due to the branching of the embryo itself is fairly common. This frequently occurs in the manner indicated in Fig. 6, in which an approximate bifurcation has taken place. Examples of lateral branching, in which an embryo produces a second one smaller than itself, are by no means rare (Fig. 7). While the former may be compared with the fission of the embryonic group in *Pinus* and other Conifers, the latter recalls the remarkable proliferation of the suspensor in *Gnetum Gnemon*,<sup>7</sup> in which there is little doubt that the outer cells of the massive suspensor are epibasal in origin as they are in *Welwitschia*. More than two embryos in one ovule have not been certainly seen; in one doubtful case there are perhaps three. That the polyembryony of *Welwitschia* is very much more limited than in *Gnetum* is in keeping with the higher degree of specialization to which

<sup>1</sup> Pearson, 1909, pp. 352, 370.

<sup>2</sup> Mr. Lynch informs me that of a considerable number of selected seeds sent to Cambridge from Damaraland in 1907, about 80 per cent. germinated.

<sup>3</sup> Pearson, 1907, p. 536.

<sup>4</sup> Pearson, 1909 A, p. 368.

<sup>5</sup> Pearson, 1909 A, Text-fig. c.

<sup>6</sup> l. c., p. 364.

<sup>7</sup> Bower, 1882, p. 284. A similar proliferation occurs in *G. scandens*.

the former has attained. There appears to be no record of the presence of more than one embryo in the mature seed of *Welwitschia*, and no doubt here, as in *Gnetum Gnemon*, all but one sooner or later are crowded out of existence.

The endosperm from which the stage shown in Fig. 3 was taken has almost exhausted the tissue in the remains of which it is enclosed. As Hooker<sup>1</sup> observed, most of this tissue is not part of the original nucellus, but is mainly formed by intercalary growth below the insertion of the integument. The endosperm is quite free from the surrounding tissue, and has a smooth firm surface composed of a well-defined and regular cell-layer. Its shape is little changed from that outlined in Fig. 89 *k* of my former paper.<sup>2</sup> The upper third is, however, broader in proportion, and the lower region slopes more gently to the broad tip. The cavity marked *c* in the figure last cited has extended with the further penetration of the embryo. Between the embryo and the lower end of the endosperm the cells of an axial cylinder are looser than those nearer the periphery; they contain for the most part very little visible starch and they are usually binucleate; occasionally they contain three or more nuclei. This condition is of common occurrence in the endosperm-cells of Gymnosperms in the neighbourhood of growing embryos and archegonia.<sup>3</sup> In *Welwitschia* the multinucleate state is the result of direct nuclear division.

It is generally assumed that the seeds of *Welwitschia* retain their power of germinating for a long period. Some evidence bearing on this question is now forthcoming. A number of seeds collected in Damaraland in January, 1907, were stored in this laboratory. They were exposed to the atmosphere, but otherwise were kept as dry as possible. Four were sown in the last week of February, 1910. Of these, two have not germinated; the cotyledons of one seedling appeared above the ground on April 4 and those of a second followed after an interval of about fourteen days. The latter is living (May 21), but shows signs of damping off; the older of the two appears to be still thoroughly healthy and vigorous. These seeds were shed certainly not later than June or July, 1906. They have therefore retained their vitality for forty-three months, during which they have survived the almost continuously damp atmosphere of Cape Town and the low temperatures of three Cape winters—conditions which it might be expected would be particularly trying to the seeds of tropical desert plants. It is therefore probable that the most resistant seeds are capable of lying dormant for a much longer period under natural conditions.

<sup>1</sup> Hooker, 1863, p. 32. See also Pearson, 1906, p. 287.

<sup>2</sup> Pearson, 1909 A.

<sup>3</sup> Pearson, l. c., p. 356.

## SUMMARY.

1. The intraseminal development of the embryo is continuous, and is apparently completed before the seed falls. It occupies not more than four months from the time of fertilization.

2. A few terminal initial cells (Figs. 1, 2, 6, 7) are replaced by a single massive meristematic group (Fig. 3) which in due course gives rise to distinct root and stem growing points (Fig. 4).

3. The 'lateral cones' are visible as small protuberances in the axils of the cotyledons in the embryo of the seed.

4. The suspensor becomes greatly increased in thickness by centrifugal additions formed, as in *Ephedra*, in acropetal succession from superficial cells of the root-cap. The later formed of these usually show a degree of independence of the rest of the suspensor in the direction of their growth, and it may be that they are specially adapted to the function of absorption.

5. In intermediate stages the ovules are frequently polyembryonic, owing to the branching of the embryonic mass.

6. So far as is known the mature seed never contains more than one embryo.

7. Two seeds collected in Damaraland in January, 1907, germinated readily in April, 1910.

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## EXPLANATION OF PLATE LXIV.

Illustrating Prof. Pearson's paper on the Embryo of *Welwitschia*.

All the figures are prepared from longitudinal and approximately median sections.

Figs. 4 and 5 are microphotographs.

Figs. 1-3, 6 and 7 are drawn under Zeiss D, ocular 2, with the aid of the camera lucida.  $\times 305$ .

Fig. 1. A stage somewhat more advanced than that of Text-fig. 1.  $x$  = cells of 'lower ring';  $c$  = apical initial cells.

Fig. 2. A slightly more advanced stage.  $x$  = additions to the suspensor derived from the cells of the 'lower ring';  $y$  = the cells which will form the next additions to the suspensor;  $c$  = apical initial cells.

Fig. 3. A more advanced stage.  $a, b, d$  = suspensor cells; *end.* = endosperm cells.

Fig. 4. A more advanced stage in which the suspensor (S) has completed its growth in thickness. *St.* = stem dome; *R* = root meristem. This precedes the stage shown in Text-fig. 2.

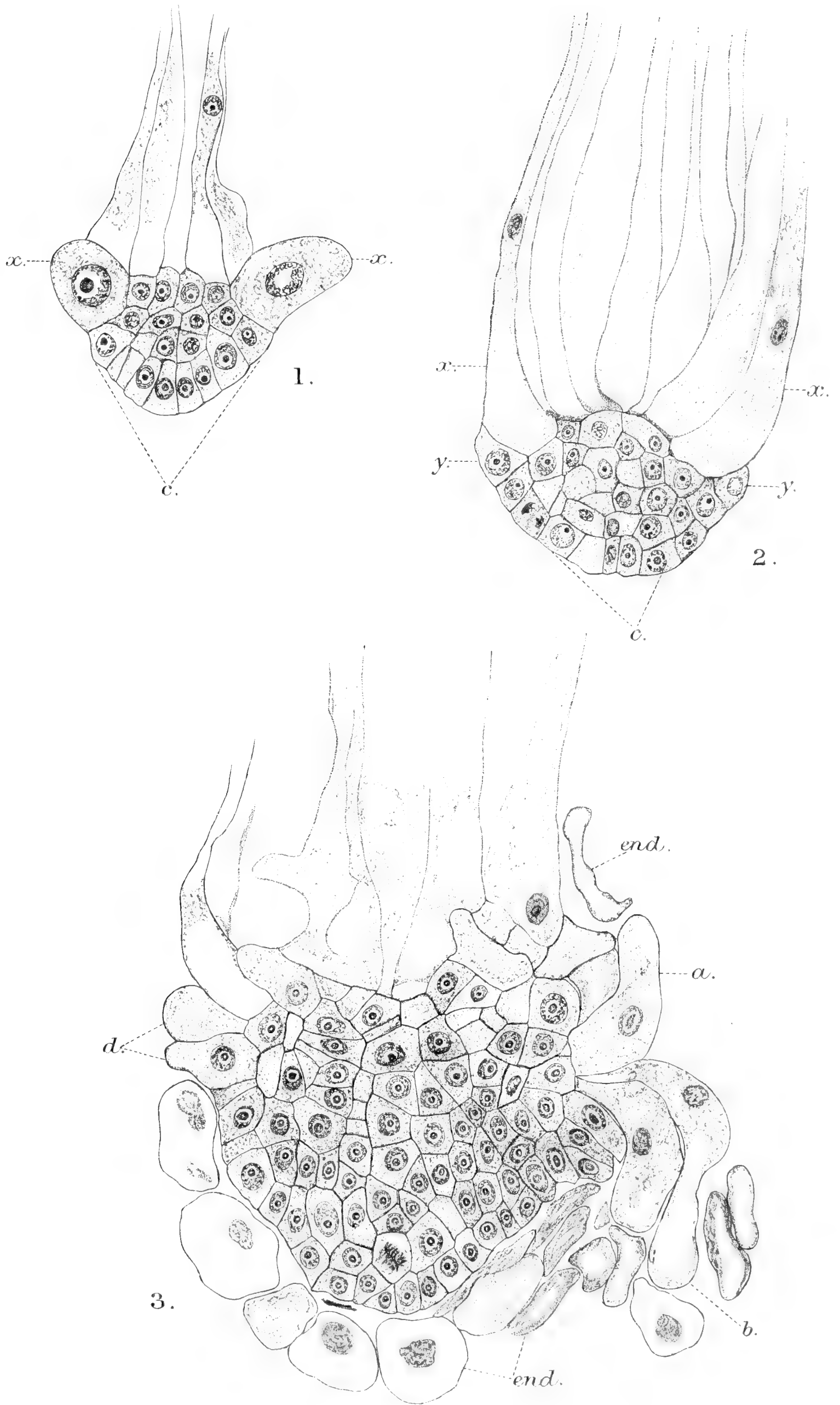
Fig. 5. Embryo of mature seed.  $c$  = cotyledons; *l.c.* = lateral cones; other lettering as in Fig. 4.

Fig. 6. Twin-embryos resulting from bifurcation.

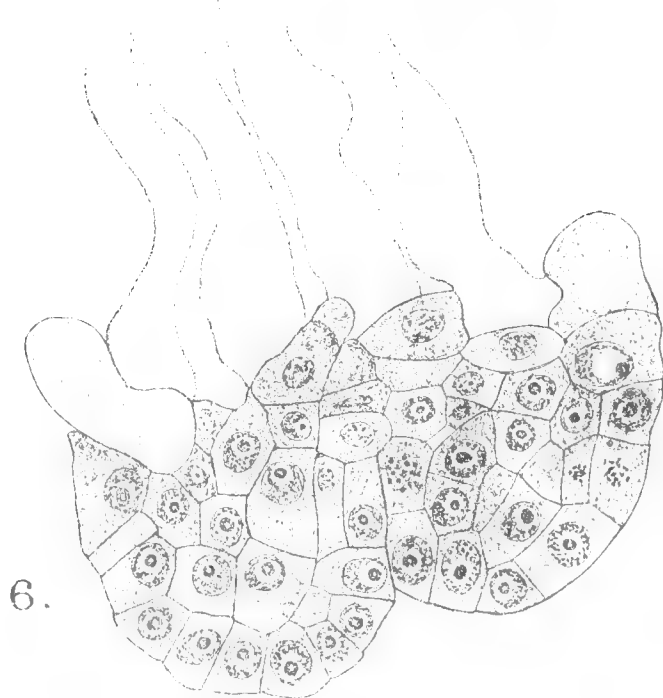
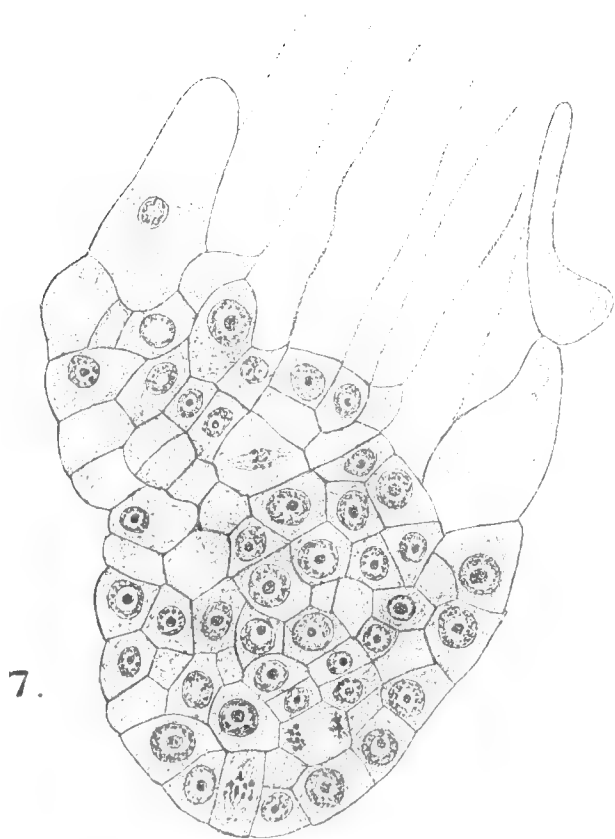
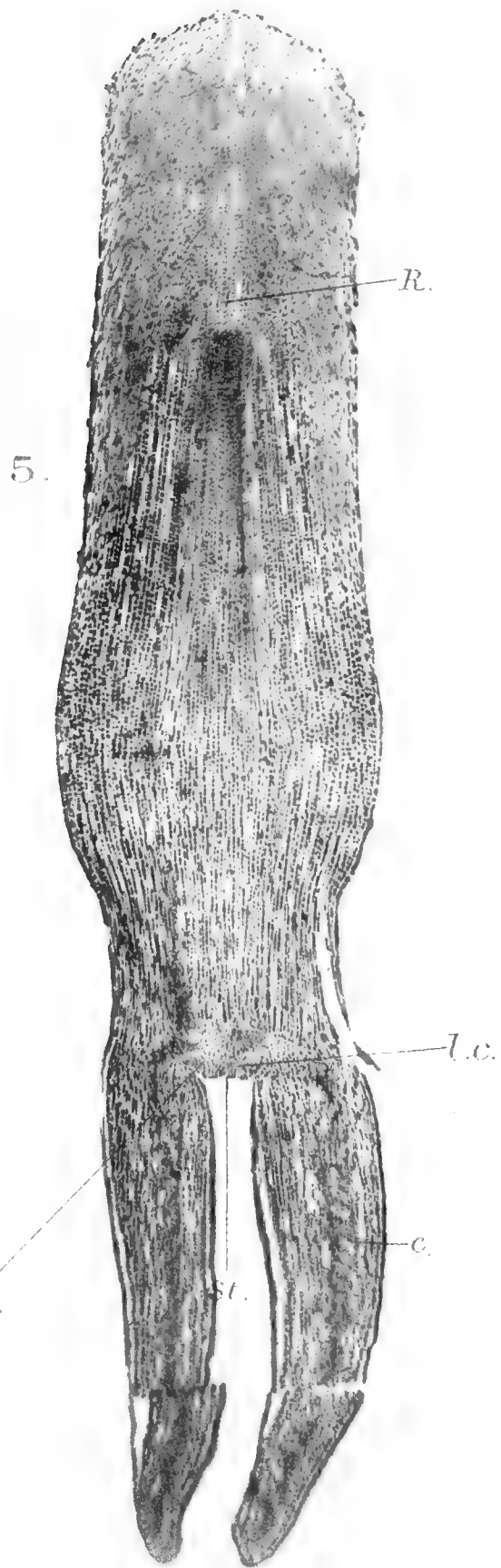
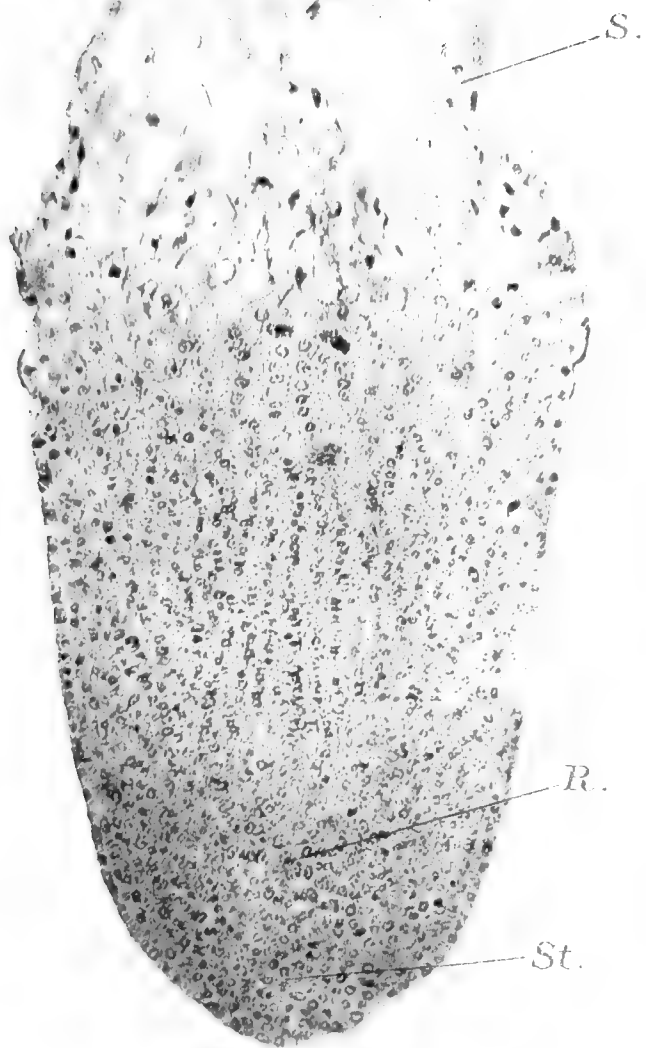
Fig. 7. A young embryo formed as a lateral branch from an older one. One of the initial cells of the latter is in course of division.

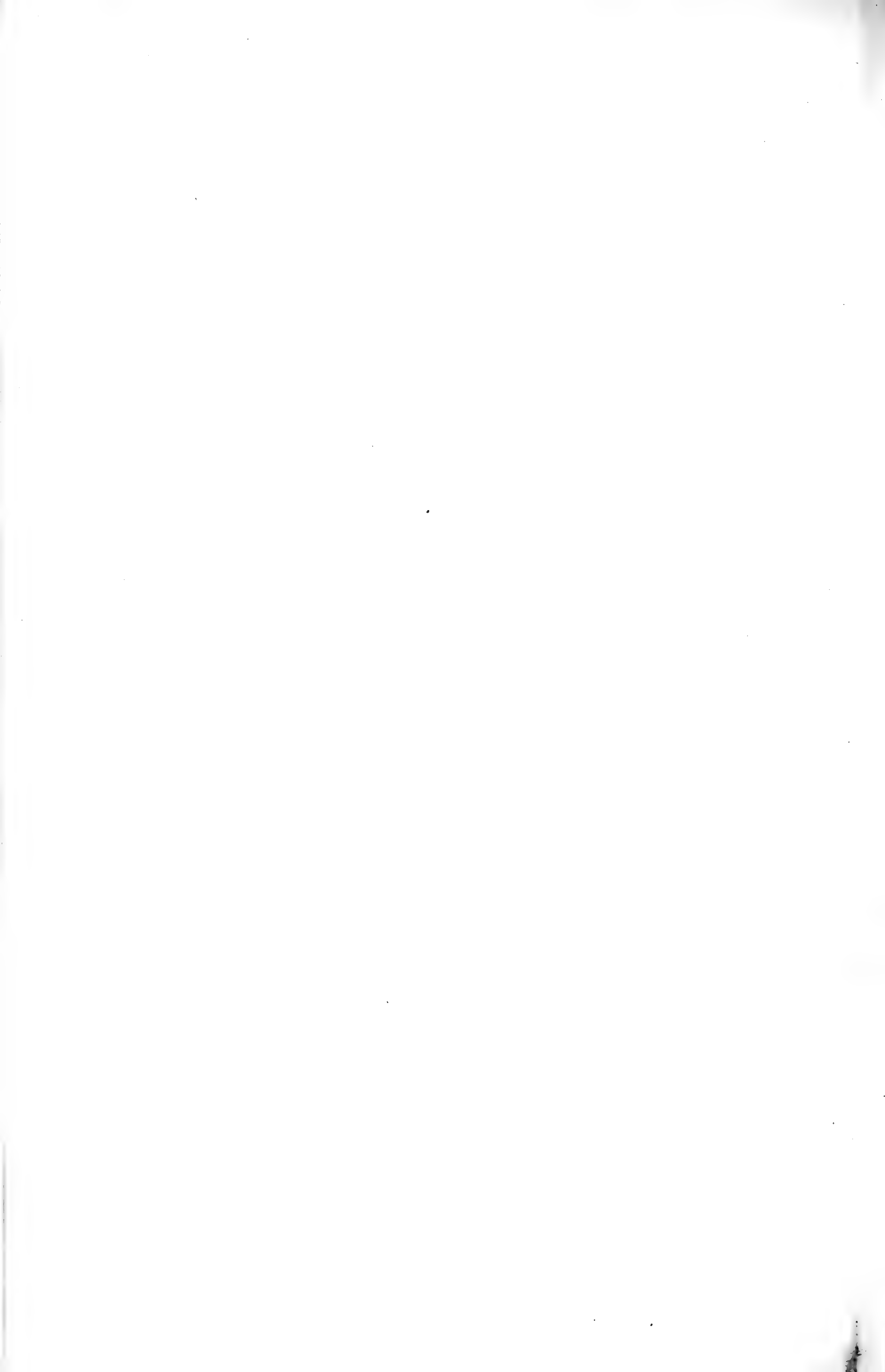






PEARSON.— WELWITSCHIA.





# On the Affinities of the Genus *Yezonia*.<sup>1</sup>

BY

EDWARD C. JEFFREY,

*Professor of Plant Morphology in Harvard University.*

With Plate LXV.

RECENTLY Dr. Marie C. Stopes of the University of Manchester and Professor K. Fujii of the University of Tokio have published an extensive monograph on the Cretaceous plants of Hokkaido, Northern Japan. Among the remains described are species of Pteridophyta, Gymnosperms, and Angiosperms. The present communication has to do with one of the Gymnospermous species published by these authors, viz. *Yezonia vulgaris*, of which they state that 'it is impossible to find any family among the Gymnosperms with which we can satisfactorily include this plant. That it is a Gymnosperm is proved by the character of the wood of the main axis, by the guard cells of the stomates, as well as by a variety of minor characters.' It will be the aim of this article to show that *Yezonia* represents the unrecognized twigs of one of the commonest of the Mesozoic Conifers and that its affinities are with the Araucarineae. This state of affairs would in all probability have been recognized by the Anglo-Japanese authors had they been able to study the external form as well as the internal structure of their material. Unfortunately the condition of the material, which occurs in the form of nodules, and the methods adopted by these investigators made such inference difficult.

It will be well to describe in some detail the internal structure of the Jurassic and Cretaceous genus *Brachyphyllum*, especially in regard to the peculiarities of structure emphasized by Stopes and Fujii as characteristic of their supposed new genus *Yezonia*.

## EXTERNAL FORM.

Figures of the superficial appearance of *Brachyphyllum* are common in all memoirs dealing with Upper Jurassic and Cretaceous plants. The reader may be referred to the recent memoir of Dr. Arthur Hollick and the writer for a representation of a species common in the Cretaceous deposits

<sup>1</sup> Contributions from the Phanerogamic Laboratories of Harvard University, No. 35.

of New Jersey and New York (Memoirs of the New York Botanical Garden, vol. iii, Studies of Cretaceous Coniferous Remains from Kreischerville, New York, Pl. 4, Figs. 12-14). It is obvious from these figures and similar ones published by other authors that in the case of *Brachyphyllum* we have to do with leafy twigs characterized by spiral phyllotaxy of fleshy leaves appressed to the surface of the stem and originating with a broad base. The leaves were free from the surface of the stem only along their extreme margins and were characterized by superficial striations converging towards the apex, marking lines of sclerified hypodermal tissue.

#### GENERAL INTERNAL STRUCTURE.

Fig. 1, Pl. LXV, shows a transverse section of a young specimen of *Brachyphyllum macrocarpum* from the Kreischerville deposit. Indications of the presence of five leaves may be seen on the surface of the stem, two of which are much broader because cut through their bases in the plane of section and are on the left of the figure. Three remaining leaves, cut nearer the apex, appear on the right. The central cylinder can scarcely be seen with the degree of magnification employed, but is composed of separate strands of xylem. Fig. 2, Pl. LXV, shows a somewhat tangential longitudinal section of a similar specimen. Here again a number of leaves occupy the margins of the figure. Several of the fibrovascular strands of the central cylinder appear in the middle of the figure. A bundle can be seen passing into the second leaf apex on the right of the figure. This is the normal mode of fibrovascular supply to the foliar organs of *Brachyphyllum*. Fig. 3, Pl. LXV, shows a tangential section through a leaf of the genus under discussion to illustrate the branching leaf veins, which distinguish this Conifer from the mass of other Mesozoic representatives of the order. The branching of the foliar strands is from above downwards, the figure being oriented in its natural position. To the left of the figure can be seen indications of the presence of stone cells, such as are common in both the cortex and pith of Cretaceous Araucarineae. In the outer portion of the leaf the numerous fibrovascular strands become merged in a broad zone of transfusion tissue. This is well shown in the longitudinal radial view presented in Fig. 4, Pl. LXV, the light stripe down the middle of the figure marking the position of the transfusion tissue.

#### THE STOMATA OF THE LEAF.

Fig. 5, Pl. LXV, illustrates the appearance of the outer and free surface of the leaf. Along the free margin is seen the epidermis thickened by a layer of hypoderma, the latter being interrupted opposite depressions in the surface of the leaf corresponding to the stomata. Fig. 6, Pl. LXV, represents the leaf surface in the vicinity of a stoma highly magnified. The

stomatic guard cells appear as two small elements of equal size in the centre of the figure. Over them impend the accessory cells, the whole stomatic apparatus strongly resembling the similar structures found in the leaf of the living *Pinus*. Above and below the stomatic guard cells and their accessory elements is the epidermis, strengthened by the hypodermal cells mentioned above. Fig. 7, Pl. LXV, shows the surface of the leaf of *Brachyphyllum* in tangential section. Lines of stomata can be clearly seen, in some instances separated by rows of elongated hypodermal fibres. Towards the lower side of the figure the plane of section dips into the mesophyll, while on the sides and above it passes out into the cuticle. Fig. 8, Pl. LXV, shows the epidermal and hypodermal structure somewhat highly magnified. The rows of stomata appear clearly in alternation with stripes composed of intervening hypodermal cells. The degree of enlargement is now sufficient to show that the hypodermal elements have a striated condition of the wall which is an artifact due to fossilization. The guard cells of the stomata appear as dark lens-shaped bodies on either side of the stomatic aperture. On the lower right-hand side, as well as on the upper left, the plane of section is deep enough to engage part of the cavities of the guard cells. Outside the guard cells lie the accessory cells, which are quite generally four in number. Comparison may be made between the two last figures and the one made from much less favourable material by Stopes and Fujii.<sup>1</sup> The magnification is less than that used in our Fig. 8, so that the details of structure stand out less clearly.

#### STRUCTURE OF THE AXIS.

The feature of the anatomical structure of the axis which the Anglo-Japanese authors most strongly emphasize in connexion with their genus *Yezonia* is the occurrence of irregularly alternating zones of thicker and thinner walled wood tracheides, which do not correspond to annual rings. Fig. 9, Pl. LXV, illustrates this peculiarity with particular clearness for *Brachyphyllum*. There are obviously two successive zones of thin-walled and thick-walled tracheides respectively. In Fig. 10, Pl. LXV, the same feature is shown less clearly in another specimen. Fig. 11, Pl. LXV, illustrates the same phenomenon under a higher degree of magnification. These figures may be compared with Text-fig. 9, *A, B, C, D, E*, and Text-fig. 10, *A, B, C, D*, of the memoir of Stopes and Fujii cited above. In our Fig. 10, Pl. LXV, may be distinguished the cortical and medullary sclerotic nests, which equally characterized *Brachyphyllum* and *Yezonia*, Stopes and Fujii. The Anglo-Japanese authors lay considerable stress on the presence of a layer of periderm in the outer region of the stem of their genus *Yezonia*. In the upper region of our Figs. 10 and 11, superior to the

<sup>1</sup> Studies on the Structure and Affinities of Cretaceous Plants. Phil. Trans. Roy. Soc., London, Series B, vol. cci, Pl. 3, Fig. 9.



cavities representing resin canals, may be seen a somewhat meandering zone of periderm. The general topography of this layer is shown more clearly in Figs. 1 and 4, Pl. 11, and Figs. 1 and 2, Pl. 12, of the joint memoir by Dr. Arthur Hollick and the writer, cited above. On the left of Fig. 11 of the present article may be seen with particular clearness the radial rows of periderm cells.

In Fig. 12, Pl. LXV, is shown a longitudinal section of the wall of the woody cylinder under a sufficient degree of magnification to indicate the height and position of the medullary rays of the wood. It is obvious that the latter are from one to two or three cells in altitude, precisely as in the description of the ray structure of *Yezonia*, Stopes and Fujii. The pitting in the tracheides of *Yezonia* also corresponds absolutely with that found in *Brachyphyllum*, for in both the pits occur in a single row on the radial wall of the tracheides. It has been observed in *Brachyphyllum*, especially in older stems, that the pits are flattened by mutual contact, as in the *Araucarineae*. This feature, however, is often not distinguishable in the wood of smaller twigs.

The Anglo-Japanese authors attach considerable importance to certain features of the resin canals in the cortex of their genus *Yezonia*. They found, for instance, that there is frequently a layer of periderm present outside the resin canals, which may in some instances invade the cavity of the canal itself. All these features are paralleled in *Brachyphyllum*, and are illustrated in Figs. 10 and 11 of the present article as well as in those of the large memoir cited above. They also emphasize the absence of any clearly defined epithelial layer around the secretory cavities or resin canals. A similar condition occurs in *Brachyphyllum*, as may be seen by examining the resin canals occurring in our Figs. 10 and 11. That resiniparous epithelial cells were really absent in the living plant is doubtful. It has been pointed out in an article written by Dr. Arthur Hollick and the present author<sup>1</sup> that the resin canals of *Brachyphyllum*, with a strong degree of probability, secreted both resin and mucilage, as is the case with the resin canals of *Araucarian Conifers* of the present epoch. The dark substance surrounding the resin canals in our Figs. 10 and 11, as well as the similarly indistinguishable cellular boundaries of the resin spaces described by Stopes and Fujii for their genus *Yezonia*, is in all probability of a mucilaginous nature or represents a combination of limiting cells and mucilaginous contents. We have found a similar obscurity of the limiting layer of the resin canals in the cone-scales of almost all the *Araucarian Coniferales* from the *Kreischerville* deposits. This condition is shown particularly well in Figs. 1, 2, and 3, Pl. 26, of our large memoir on the *Conifers of Kreischerville*. The feature emphasized by Stopes and Fujii has no special

<sup>1</sup> Affinities of certain Cretaceous Plants commonly referred to the Genera *Dammara* and *Brachyphyllum*. *American Naturalist*, xl, pp. 189-215, 1906.

importance and certainly cannot be interpreted in any way as indicating some affinity between the genus *Yezonia* and the Cycads as they suggest.

The consideration of the facts adduced in the foregoing paragraphs for *Yezonia* and *Brachyphyllum* makes it clear that these two genera agree absolutely in the following points: structure, relative size, phyllotaxy and venation of the leaves, including the organization and arrangement of the stomata in rows between hypodermal bands; anatomical structure of the fibrovascular tissues, including irregular zonal variations in the thickness of the tracheides not in any way corresponding to annual rings, pitting of the tracheides, height of the medullary rays; structure of the pith and cortex, stone cells being present in both; structure of the phloem devoid of the hard bast fibres characteristic of Cupressineous and Taxodineous Conifers; and finally by the presence of a broad transfusion zone in the outer region of the stem corresponding to the lower surface of the appressed leaves.

Or to put the matter in another way, if all the points of agreement between the description of the supposed new genus *Yezonia*, given on p. 32 of the memoir of Stopes and Fujii, and the account of the anatomy and habit of *Brachyphyllum*, given in the present article and in the large memoir of Dr. Hollick and the present author, were italicized, it would be necessary to italicize the whole description.

It is appropriate at this point to refer to another supposed new Gymnospermous genus described by the Anglo-Japanese authors. On pp. 52-7 of their memoir they refer to certain remains, which they designate under the name *Cryptomeriopsis antiqua*. These are characterized as densely leafy twigs, with falcate four-sided foliar organs, which have internally a single fibrovascular bundle flanked by downwardly curving stripes of transfusion tissue. There are three foliar resin canals. The stem possesses a woody cylinder traversed by low uniseriate rays, and the phloem is without the hard bast fibres found in living representatives of the Cupressineae and Taxodineae. In the pith were found evidences of the presence of stone cells. All these details of structure correspond absolutely with the account of the structure of *Geinitzia* (*Sequoia*) *Reichenbachii*, one of the commonest Cretaceous Conifers, given in the joint memoir by Dr. Hollick and the writer (op. cit., pp. 38-41, Pl. 5, Figs. 7-10, Pl. 8, Figs. 3 and 4, Pl. 16, Figs. 2-4, Pl. 17, Figs. 1-4, Pl. 18, Figs. 1-4). There can accordingly be little doubt that the *Cryptomeriopsis antiqua* of Stopes and Fujii is in reality not allied in any way to the Cupressineae and Taxodineae, but represents the leafy twigs of the well-known Cretaceous species *Geinitzia* (*Sequoia*) *Reichenbachii*, which, as has been pointed out by Dr. Hollick and the writer in the memoir so often cited in the present article, is in reality an Araucarian Conifer and has not the slightest affinity with the existing genus *Sequoia*, to which it has been most generally

referred by those who have studied it only in impressions. It is of course possible that the *Cryptomeriopsis antiqua* of Stopes and Fujii may not be specifically identical with *Geinitzia Reichenbachi*. In any case there can scarcely be any doubt as to the generic equivalence, and the species must be very closely allied.

#### CONCLUSIONS.

It will be apparent from the foregoing paragraphs that there is the closest superficial and structural resemblance between the genera *Yezonia* and *Cryptomeriopsis* of Stopes and Fujii and the Cretaceous genera, of world-wide distribution in the Northern Hemisphere, *Brachyphyllum* and *Geinitzia* respectively, so well known from impressions and recently described for their structural features as well. There seems accordingly no reason to maintain these new generic names since the older ones already hold the ground.

This conclusion as to the true affinities of these two supposedly new Cretaceous genera of the Anglo-Japanese authors will have the advantage of connecting the interesting Cretaceous flora of Hokkaido, Northern Japan, with the Cretaceous flora of the rest of the Northern Hemisphere. 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 the similar remains described structurally from the Cretaceous clays of Kreischerville, Staten Island, N.Y.<sup>1</sup> If this general inference is correct, and it seems supported by very strong evidence, 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, including Abietineae and Araucarineae, the latter simulating in their external appearance both vegetative and reproductive genera of the Cupressineae and Sequoiineae (Taxodineae), which in reality did not exist at so early an epoch, and with which they certainly have not the slightest affinity.

#### SUMMARY.

1. *Yezonia*, Stopes and Fujii, is in reality *Brachyphyllum*, and as a consequence should be known, in accordance with accepted principles of nomenclature, only under the earlier generic name.

2. *Cryptomeriopsis*, Stopes and Fujii, is in reality another long known Cretaceous genus, *Geinitzia*, and should likewise be eliminated.

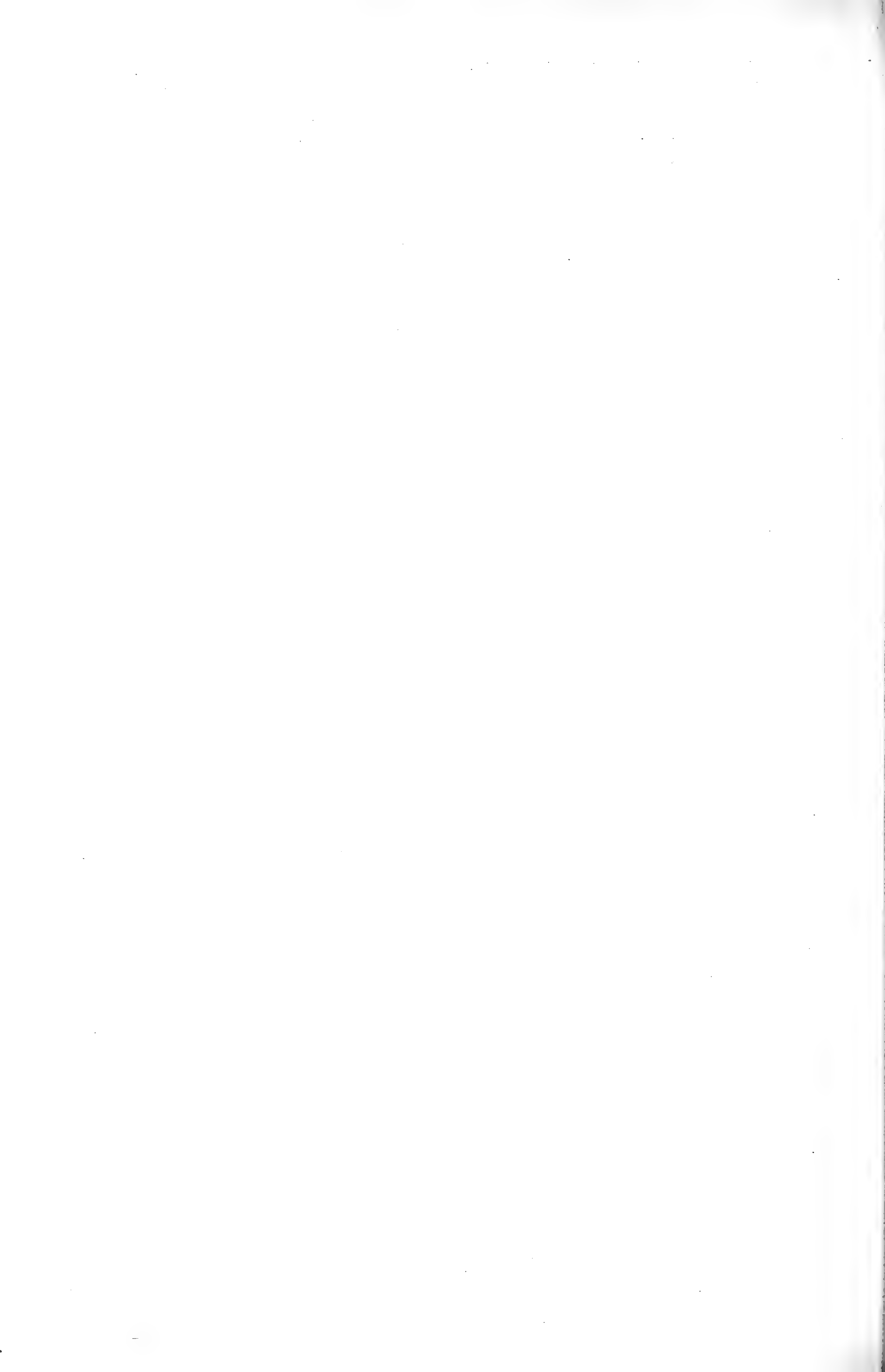
3. The Cretaceous Coniferous flora of Northern Japan had much in common with that described structurally and from impressions from the eastern coast of North America.

<sup>1</sup> Stopes and Kershaw, Ann. Bot., xxiv, No. 94.

DESCRIPTION OF PLATE LXV.

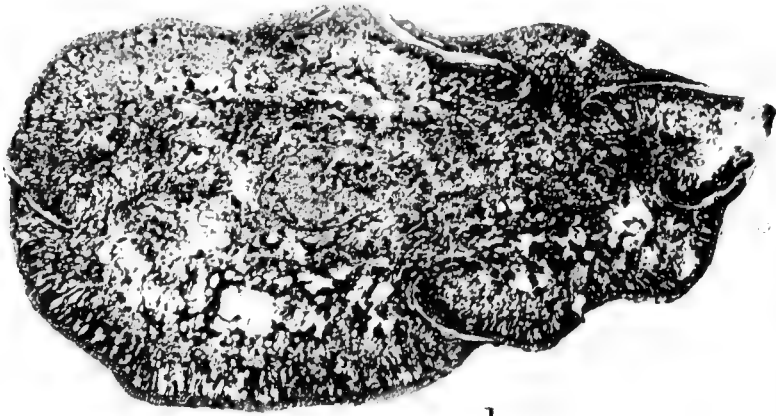
Illustrating Prof. Jeffrey's paper on the genus *Yezonia*.

- Fig. 1. Transverse section of young stem of *Brachyphyllum macrocarpum*.  $\times 10$ .  
Fig. 2. Longitudinal section of a young branch of *B. macrocarpum*.  $\times 10$ .  
Fig. 3. Tangential section of the leaf of *B. macrocarpum*.  $\times 40$ .  
Fig. 4. Longitudinal radial section of older stem of *B. macrocarpum*, showing zone of transfusion tissue.  $\times 40$ .  
Fig. 5. Transverse section through a part of the leaf of *B. macrocarpum*.  $\times 40$ .  
Fig. 6. Section through the stoma of *B. macrocarpum*.  $\times 180$ .  
Fig. 7. Tangential section of surface of leaf of *B. macrocarpum*, showing stripes of hypodermal tissue and rows of stomata.  $\times 80$ .  
Fig. 8. Stomata of the same much more highly magnified.  $\times 180$ .  
Fig. 9. Part of woody cylinder of *B. macrocarpum*, showing the irregularly thickened zones of tracheids.  $\times 80$ .  
Fig. 10. Another specimen, less highly magnified, showing resin canals and periderm above the zone of wood.  $\times 40$ .  
Fig. 11. The same in a different region more highly magnified.  $\times 80$ .  
Fig. 12. Section through the wood of the wall of the fibrovascular cylinder of an older specimen of *Brachyphyllum*.  $\times 40$ .









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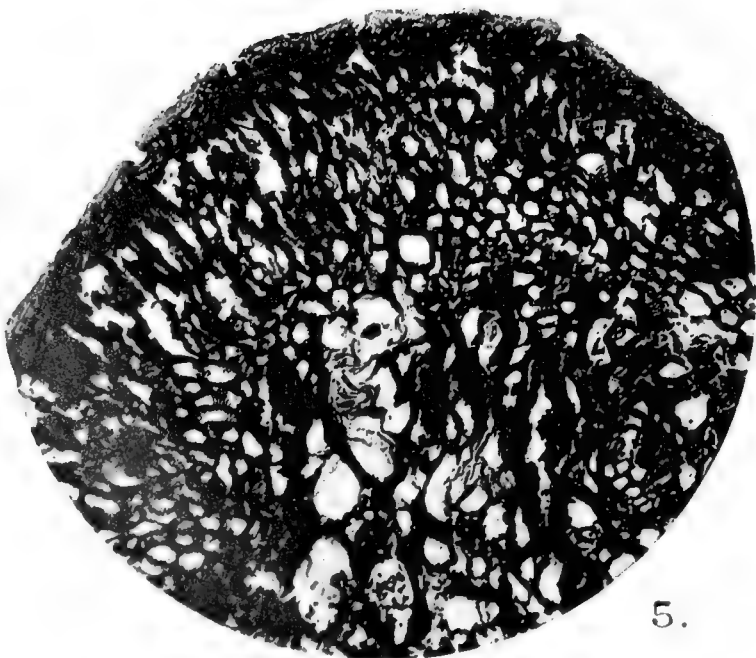
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6.

JEFFREY

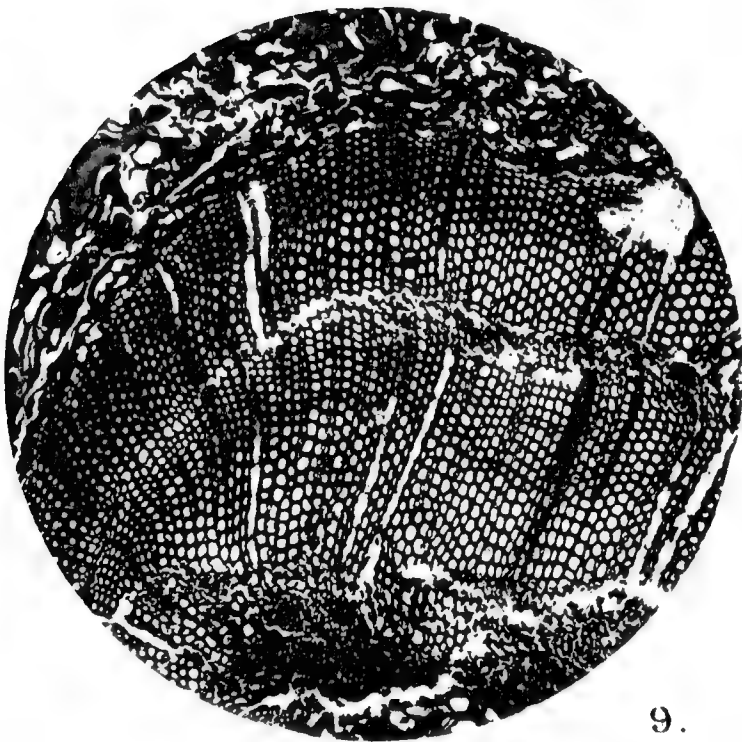
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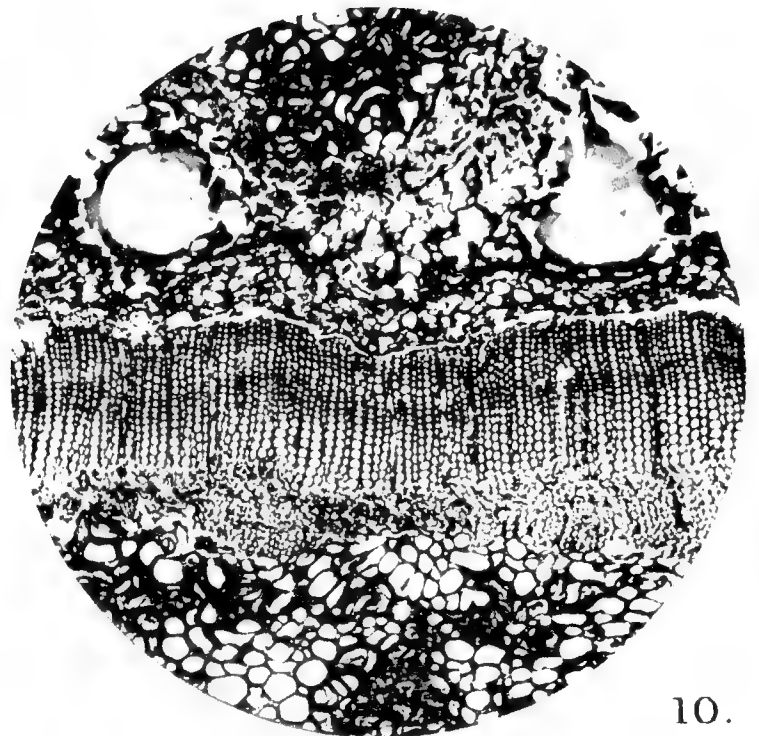
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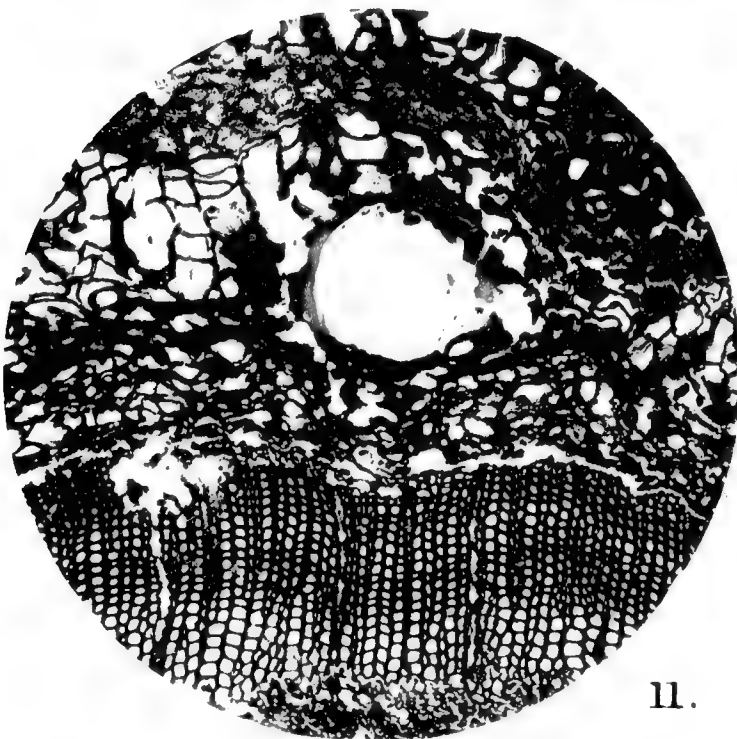
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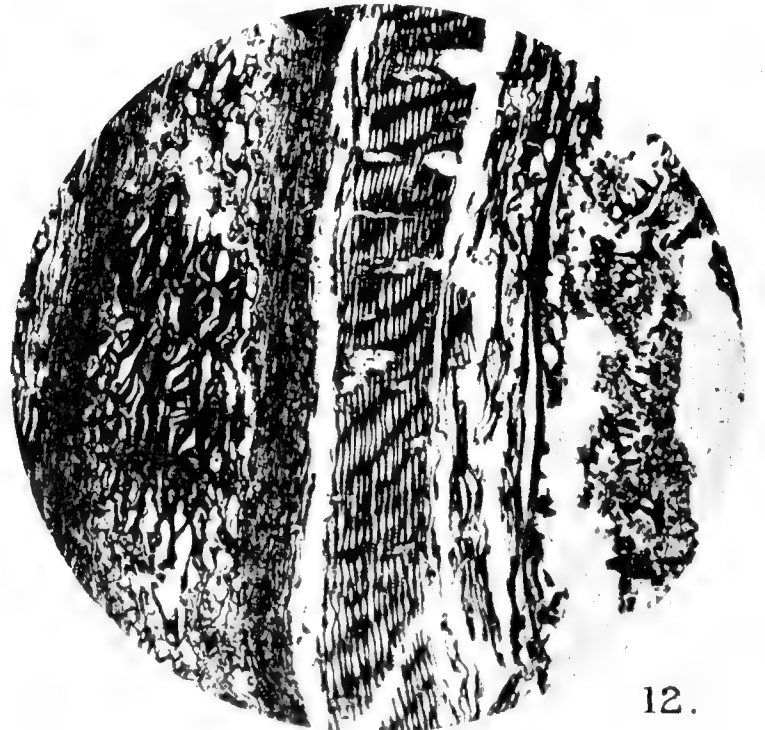
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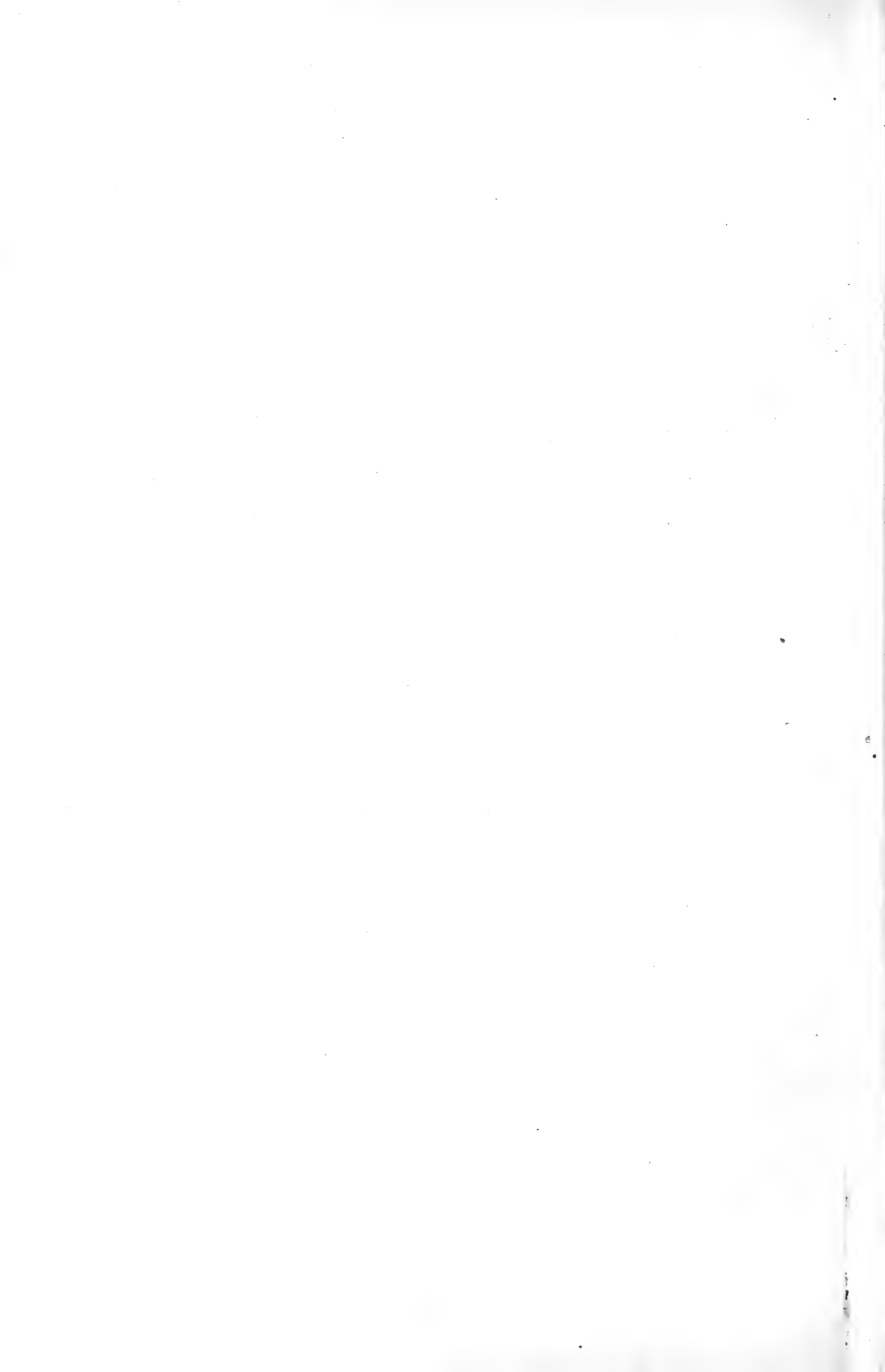
10.



11.



12.



# On the Question of the Occurrence of 'Heterotypical Reduction' in Somatic Cells.

BY

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With Plates LXVI and LXVII.

**D**URING the last few years an increasing interest has centred round the problems presented by the processes which underlie the structural changes of nuclear and cell division. In particular, attention has been directed to the occurrence in some plant and animal tissues of certain deviations from what are held to be the normal character and sequence of events. It has been shown by various observers that such abnormalities as imperfectly separated nuclei, multipolar spindles, and chromosomes of peculiar shape may be induced artificially, as by the application of certain drugs, by sudden changes of temperature, or by mechanical stimulation. Attempts have been made to bring these experimental phenomena into line with those which have been shown to occur in some morbid growths; it is indeed evident that the possibility of so inducing structural modifications, comparable to those occurring naturally in certain pathological conditions, is one of great interest, and presents valuable opportunities of experiment.

From the numerous researches in this field have emerged two chief problems, which are at present giving rise to considerable controversy. These are: (1) the possible occurrence of a phase of amitosis in the cells of the higher plants and animals; and (2) the possible existence in somatic cells of a capacity for heterotypical reduction of their chromosome number. The present paper deals chiefly with the second of these two problems.

The question of amitosis was first brought forward in 1902, by Wasielewski, who stated that, under the influence of a dilute solution of chloral hydrate, a phase of amitotic division occurred in the root-cells of pea, bean, onion, &c., followed by a return of apparently normal mitosis. Wasielewski concluded, and in this conclusion he is supported by Nathansohn, that, contrary to the opinion then generally held, this must be regarded as evidence of the interchangeability of the two processes. In 1902-4, however, his work was repeated by Němec, who disputed the above con-

[*Annals of Botany*, Vol. XXIV. No. XCVI. October, 1910.]



clusion. Němec insisted that the large lobed, or 'bridge' nuclei, which Wasielewski had supposed to be in amitotic division, really arose through arrested mitosis, this arrest resulting in incomplete separation of the two groups of chromosomes, which consequently formed, in late telophase, a lobed nucleus easily mistaken for one undergoing amitotic division. He further stated that the chloral hydrate acted upon the achromatic structures of the cell, attacking and dissolving the fibres normally visible in division, and so arresting the movement of the split chromosomes to their respective poles; also that the two groups of chromosomes tended to fuse together again in one mass, and that the single nucleus so formed contained double the normal amount of chromatin and broke up, on subsequent division, into a number of chromosomes twice that characteristic of the tissue in question.

The interest of Němec's work, however, rests not so much upon his treatment of the problem of amitosis, as upon the theory of the occurrence in somatic cells of heterotypical-reduction, which he brought forward in explanation of the fate of the above tetraploid nuclei. Němec pointed out that after a period of some hours the latter disappear, and suggested three possible ways in which this disappearance might occur. (1) That the large tetraploid cells pass over into the permanent tissue, their nuclei becoming inactive. (2) That should the tetraploid number occur in initial cells, these having lost the power of frequent division through the action of the chloral hydrate, their function as initial cells is taken on by their neighbours. Or (3), that by a sudden automatic process of reduction, very similar to the heterotype reduction occurring in spore mother-cells, the tetraploid number of chromosomes is reduced to the diploid number. Němec himself adopted the third of these hypotheses on the following grounds. He had observed certain curious tetrad-like figures in the pea during recovery from treatment with chloral hydrate, and believed these to be an indication of the occurrence of two divisions in immediate sequence, comparable to the heterotype and homotype divisions seen in sporogenous tissue. Again, he had found that the number of tetraploid figures in roots fixed, for example, after twenty-seven hours' growth subsequent to treatment with the drug, was markedly less than in those fixed at an earlier stage of the experiment, and that at forty-eight hours such figures had entirely disappeared. Finally, he had observed one abnormally large cell, containing, not the tetraploid, as might have been expected from its size, but the normal number of chromosomes; and he held this normal number to have arisen by reduction.

Němec maintained that such a reduction-process, occurring as he believed in somatic tissue, as a result of the action of chloral hydrate, was a reversion to a primitive capacity of reduction, innate in somatic cells, and from which he suggested has been evolved the elaborate reduction-process seen in the sexual cells of the higher plants and animals. 'Man könnte schliessen, dass die Fähigkeit zur Kernverschmelzung und zur

gesetzmässigen Modification der Chromosomen eigentlich allen normal einkernigen Zellen zukomme, dass aber diese Fähigkeit unter normalen Verhältnissen bloss bei der geschlechtlichen Fortpflanzung sich zu äussern Gelegenheit habe.' This statement is one of so much importance, and the evidence brought forward by Němec in its support was apparently so inadequate, that in 1904 his work was repeated by Strasburger with the following result.

Strasburger agreed with Němec's conclusion as to the non-occurrence of amitosis, but stated that although he obtained the majority of Němec's figures, such as binuclear cells, lobed nuclei, and nuclei with apparently double the normal number of chromosomes; yet, that in the large number of preparations which he examined, he found no heterotypical figures either at 20, 27, 35, or 45 hours after treatment with chloral hydrate. He further stated that although his proofs of the non-occurrence of heterotypical reduction were negative in character, yet that their number 'ist so gross, dass ich bestimmt behaupten kann, dass heterotypische Reduktionsteilungen in chloralisierten Erbsenwurzeln nicht vorkommen. Wie wir sahen, war die entgegengesetzte Němec'sche Behauptung nur auf einen Fall gestützt und die Natur dieses Falles ausserdem sehr fraglich. Daher meine ich, dass die Angabe über autoregulative Herabsetzung der Chromosomenzahl in chloralisierten Erbsenwurzeln durch heterotypische Reduktionsteilung endgültig aus der Literatur gestrichen werden darf.' Strasburger further pointed out that pair-like grouping of the chromosomes on the equatorial plate was not peculiar to heterotypical division, but also occurred in normal tissue, and was really only due to a chance grouping of the chromosomes. 'Eine entsprechende Häufung der Beobachtungen lehrt, dass es sich in solchen Fällen wirklich nur um eine zufällige Erscheinung handelt, welche diese Gruppierung veranlasste.' Further, he maintained that no affinities existed between these pairs of chromosomes necessary for the formation of tetrads; and, finally, that the union of two diploid nuclei resulting, as in these experiments, by accident, was a process entirely different from that of the union of two haploid nuclei in sexual fusion and was probably due to the action of the protoplast. 'Im übrigen bekommt man in solchen syndiploiden Kernplatten nur Chromosomenpaare, nicht Doppelpaare zu sehen. Durch die Vereinigung der beiden elterlichen Chromosomen sind augenscheinlich die durch ihre Homologie veranlassten Anziehungen in diploiden Kernen ausgeglichen, und es bleibt keine ungesättigte Affinität übrig, um die homologen Paare von zwei diploiden Kernen zusammenzuführen. Während die Vereinigung der haploiden Kerne im Geschlechtsakte auf chemotaktischen oder sonstigen Wirkungen beruhen mag, die sich zwischen den Chromosomen geltend machen, ist allem Anschein nach eine sich vollziehende Vereinigung von Kernen in einer durch Zufall mehrkernig gewordenen Zelle ein Vorgang anderer Art. Ich möchte meinen, dass diese Verschmelzung, die ja meist erfolgt, wenn auch nicht immer zu



erfolgen braucht, den Kernen aufgezwungen wird durch den Protoplasten, weil dieser normaler Weise nur auf einen Kern zentriert ist.'

In explanation of the disappearance of the tetraploid and binuclear cells, Strasburger suggested that as these were to be seen after many hours' subsequent growth, some of them must be passed over into the permanent tissue; also, that the amitotic-like forms seen in nuclei distant from the root-tip might easily result in complete separation and degeneration of their constituents. He gave figures of binuclear cells alternating with others containing degenerating masses of chromatin, and concluded that the disappearance of the tetraploid cells was probably due to lack of sufficient centripetal force to hold together such an abnormal amount of chromatin as they contained; that consequently their nuclei broke up either at the equatorial plate or diaster stage, or while in rest; and that in this process, a certain amount of the chromatin tended to degenerate, and the normal number of chromosomes was re-established.

Unfortunately, however, Strasburger did not obtain the crucial figures, according to the nature of which and to their occurrence or non-occurrence Němec's theory really stands or falls; and as the figures upon which he based his criticism of the latter did not represent those described by Němec as heterotypical in character, the matter remained still in dispute. In February, 1909, Němec reaffirmed his statement, and based upon it further arguments in support of his view of the non-continuity of the chromosomes as individuals, and of the subsidiary rôle played by them in the matter of heredity. In November of the same year, Strasburger, in defining his position with regard to the theories of 'Pfropfhybriden', again noted that Němec's heterotypical figures did not occur. Finally, as recently as June, 1910, Němec once more insisted upon their occurrence. He further supported his contention with certain results obtained in the lateral roots of radicles which had been several times subjected to the action of chloral hydrate. In these he stated that there were at first to be seen numerous tetraploid cells, but that after a certain time, i. e. in those lateral roots which had attained a considerable length, all such cells had disappeared. He maintained that their disappearance must result from one of three processes: (1) from reduction of the double number of chromosomes and consequent re-establishment of the diploid number; (2) from the degeneration and absorption of the tetraploid cells; or (3) from the cessation of their activity of division.

Among other observers who have investigated this problem there are great differences of opinion. Woycicki, Haecker, and Schiller, working respectively with the pollen mother-cells of *Larix dahurica* and Cyclops eggs, support Němec in his view of the nature of heterotype division. They explain the supposed reversion to the latter, and also the occurrence of multipolar spindles, as being expressions of an 'atavistic tendency', the re-establishment of

a more 'primitive' condition; and suggest that the abnormal occurrence of heterotype reduction-figures may be regarded as a direct reaction to certain classes of stimuli. On the other hand, Laibach, in reviewing the position, strongly supports Strasburger in his view that such a reversion does not occur; and in a recent paper on the action upon mitosis of certain toxic solutions, Stockberger disputes Němec's data, and attributes his results, as also those of some other authors, largely to the action of the distilled water used in the chloral hydrate solution.

The question of the occurrence in somatic cells of heterotype reduction is one which bears upon problems of the widest interest. Among such may be mentioned those of heredity, of the nature of the processes occurring in hybrids, or, again, of those which obtain in pathological growths. The importance therefore attaching to it, together with the uncertainty in which it had been left, seemed to demand a re-examination of the phenomena concerned. The series of experiments here described were consequently undertaken in the hope of obtaining more definite data.

#### METHOD.

The plants used for experiment were cultivated at the Chelsea Physic-garden by Mr. Hales, to whom I am much indebted for a constant supply of suitable material. After some preliminary experiments with pea, bean, hyacinth, onion, and *Galtonia*, three plants were chosen for examination; namely, *Galtonia* or *Hyacinthus candicans*, *Vicia Faba*, and *Pisum sativum*. These were grown under the optimum conditions obtainable, either in damp sawdust or over water, according to the nature of the plant, and only healthy individuals were selected for experiment. All the experiments were carried out with as little as possible disturbance of the roots, and were checked by normal controls which were taken through precisely the same processes of washing, fixing, &c., as were used in the preparation of the experimental tissues. A further series of controls was obtained by fixing some roots direct from the sawdust or water in which they had been cultivated, without any preliminary washings or unnecessary manipulation. By these means it was possible to isolate the specific effects of the drug used in experiment, and to eliminate from consideration those effects resulting from the methods of preparation. The object of the investigation being to ascertain the nature of the heterotypical figures described by Němec, it was decided to follow the method of poisoning adopted by the latter, modifying it in detail, in the event of a wider range of data proving necessary. Young plants of *Galtonia*, bean, and pea were placed with their root-tips immersed up to 2-3 cm. in dilute solutions of chloral hydrate. The times of immersion were varied from  $\frac{1}{2}$  hr.,  $\frac{3}{4}$  hr., 1 hr.,  $1\frac{1}{2}$  hrs. to 2 hrs.; and three different percentages of chloral hydrate were used, namely 1.0%, 0.75%, and 0.5%. Of these alternatives the most favourable for producing

the required conditions was found to be that adopted by Němec, namely immersion in 0.75 % solution for 1 hr. With this a minimum amount of vacuolization and shrinkage of the tissue, together with a maximum specific effect of the drug, was obtained. With the stronger solution greater vacuolization occurred, and at the same time apparently less specific action of the drug. With the weaker solution, although little general disturbance of the tissue was caused, the specific action of the chloral hydrate was not sufficiently pronounced to give decided results. A weak solution and prolonged immersion proved preferable in result to a strong solution and short immersion. In many cases the individuals experimented upon were allowed to grow subsequently, and their vigorous production of stem and leaves indicated that the general health of the plant had not been materially affected. With regard to the double series of normal controls, it was found that a certain amount of shrinkage and vacuolization occurred in those roots which had been subjected to washings previous to fixation, and that these features were not seen, or were inconspicuous, in the roots fixed straight from normal growth. Fixations were made (1) immediately after poisoning, (2) after washing, and (3) after periods of subsequent growth varying from 4 hrs. to 70 hrs. or longer. The fixatives used were strong Flemming, chrom-acetic-acid, and acetic-alcohol. Strong Flemming was found to give the best results, causing less shrinkage of the tissue than occurred with the other fixatives, and at the same time leaving its capacity for staining unimpaired. It was therefore used in the majority of the experiments, the other fixatives being resorted to at need, for comparison. The tissues were evaporated down from 10 % to concentrated glycerine, or taken up from water through gradually increased percentages of alcohol; they were then taken from absolute alcohol through xylol or cedar-wood oil into paraffin of 48° or 52° C. melting-point, the paraffin stage being shortened as much as was compatible with complete penetration, in order to avoid overheating of the tissues. Transverse and longitudinal sections were cut at about 5  $\mu$ , and stained with Heidenhain's haematoxylin and congo-red, or with Flemming's triple stain.

The results obtained by this method are in very close agreement with those described by Němec; and they include, contrary to Strasburger's experience, certain figures in the pea so like those occurring in the heterotype reduction of sexual tissue as to be almost indistinguishable from them. At the same time these figures present some features which appear to prove that they are not reduction-figures at all, but, owing to some peculiar condition of the chromatin, chance to be closely similar to such in appearance. The effect of the chloral hydrate is essentially the same in all the tissues examined, with the exception of the above figures, which occurred in the pea only, and also of certain individual peculiarities which, although perhaps insignificant in themselves, are interesting as throwing light upon the nature

of the processes concerned. It will be best, therefore, to describe in detail typical experiments on the three plants—*Galtonia*, bean, and pea, and then to discuss the evidence obtained as to the nature of the 'heterotypical' figures.

HYACINTHUS CANDICANS.

In working with *Galtonia*, the bulbs were grown over water in ordinary hyacinth glasses, and the experiments carried out as described above. After immersion of their roots in the solution of chloral hydrate, the bulbs were replaced over water, and fixations were made after varying periods of further growth. The periods examined were 3, 5, 16, 20, 40, 68, and 70 hrs., but owing to the occurrence of certain periods of diminished nuclear activity, caused by the drug, some of these fixations presented practically the same features as those preceding them. The roots which afforded most information as to the action of the poison were those fixed at intervals of about 5, 20, and 40 hrs. after replacement over water. Numerous series of experiments were performed upon the root tissue of *Galtonia* for the reason that, owing to the large size of its cells and to its general clearness, it affords good figures for counting and comparing the chromosome number in the normal and pathological cells. It was found that the results of the various series were the same in general character, although some minor points of difference were frequently noticeable between two corresponding fixations in two experiments. This was owing, no doubt, to differences between individual roots, or between the conditions under which the experiments had been performed, such for instance as slight dissimilarities of temperature and light. In making a microscopical examination of the tissues which had been subjected to experiment, two different kinds of effect were observed; namely, the general action of the chloral hydrate upon the entire tissue, affecting nuclei, cytoplasm, and cell walls; and the peculiar structural alterations arising secondarily. The first effect passed off more or less completely, unless the experiment had been of a very severe character, and the tissue as a whole resumed its normal appearance. The second effect, on the contrary, was of a more permanent nature, and tended to persist unless eradicated by some activity of the tissue. This second effect also afforded, at the later periods of the experiment, the only data available for tracing the action of the chloral hydrate upon the root.

In the experiment on *Galtonia* selected for description the roots were fixed:—

1. After 1 hr. in 0.75 % chloral hydrate,
2. „ 1 hour's washing in tap-water,
3. „ 5 hours' subsequent growth over water,
4. „ 22 „ „ „ „ „
5. „ 44 „ „ „ „ „

and therefore show the effect of the poison continuing over a considerable



time. It must be clearly borne in mind that although the moments of fixation are of necessity chosen arbitrarily they do not represent sharply defined stages in the action of the drug, or in the reaction of the tissue to the latter. That action is essentially a continuous one, and its effects must be traced as such through the periods subsequent to the experiment.

*1 and 2. After 1 hr. in 0.75 % chloral hydrate and 1 hour's washing.*

The two first fixations present practically the same features, and may be described together. The poison is seen to have had a marked effect upon the entire tissue, which is diffusely stained and lacking in sharply defined details. This appearance is particularly noticeable in the periblem and outermost cell layers, the denser meristem and rows of cells within the endodermis being rather more normal in appearance. The cell walls are seen to be slightly swollen, and the cytoplasm, especially that of the external layers, is vacuolated and thickly speckled over with small fragments of chromatin (Pl. LXVI, Fig. 1). The number of nuclei in division is very small; the majority being in complete rest, finely granular in appearance, with ill-defined membranes and taking the stain feebly (Fig. 1). The only constituent, indeed, of the resting nuclei which is well stained is the nucleolus. The latter is surrounded by a clear space and often divided into two or more bodies. Very few nuclei in spireme are visible, and these almost all in the internal cell rows. Their coiled threads show conspicuous longitudinal division (Fig. 1). The effect of the poison is seen most strikingly, however, in such nuclei as are in division. These without exception show a marked alteration in character; there is a complete absence of achromatic fibres, and the chromosomes, which have a pronounced longitudinal split, are either crowded together at the centre of the cell, or scattered irregularly across it, and at the equatorial plate stage their split halves show no definite arrangement on a plate (Figs. 4 and 5). In the diasters the two groups of chromosomes, instead of going to the poles, form two irregular masses at a little distance from each other, and in many cases still partly joined together by strands of chromatin. At the telophase, these irregular imperfectly divided masses of chromosomes are becoming vacuolated, preparatory to going into rest in that position, or in those cases where division has been complete, the two masses form two daughter nuclei close together, and flattened on their contiguous sides. In the majority of such binuclear cells there is no sign of a cell-plate between the two nuclei, or at most only a slightly denser plasma (Fig. 1); a few, however, have a fragment of plate at the side or centre of the cell. Other cells again contain, instead of two separate nuclei, one long lobed nucleus, of the kind known as a 'bridge' nucleus, with an unfinished cell-plate in the curve of the bridge. It is evident that these binuclear and 'bridge' nuclear cells result from the various degrees of imperfect division described above. The chromatin of the division figures

presents a peculiar appearance, being granular and somewhat swollen. Although the nuclei at the spireme stage are almost confined to the internal cell-rows, division figures are found here and there throughout the entire root-tip, in external cells, periblem, and plerome, and also at a considerable distance from the growing point. It is important to bear the latter detail in mind in considering, at the later stages of fixation, the binuclear and tetraploid cells which result from the above division figures. The fact of finding such cells far back in the permanent tissue does not necessarily indicate that they have passed over to that position from the meristem.

3. *After five hours' subsequent growth over water.*

Roots fixed at this period still present as a whole a somewhat pathological appearance, and show a considerable degree of inactivity, the majority of the nuclei being in rest. At the same time these resting nuclei stain more sharply than did those seen at the earlier fixations, they are also becoming more coarsely granular and their membranes are better defined. Most of the stain, however, is still taken up by the nucleolus. The latter is very striking, being broken up into two or three deeply staining bodies, which often lie apart in the nucleus and appear in some cases to be thrust out into the cytoplasm. The external cell layers still show the fine fragmentation of chromatin throughout the cytoplasm noted at the earlier fixation, but the cytoplasm itself is now less vacuolated. Although a slight return of fibrillation is visible in some cells, the majority of the division figures are very irregular, and contain no clear fibrillar or cell-plate structures; or at most only a faint fibrillation between the irregular masses of chromosomes which constitute the diaster. The equatorial plates consist of scattered chromosomes with widely split halves. The chromatin, particularly that of the telophases and diasters, is very granular, and appears swollen. Large numbers of cells are to be seen containing two small nuclei lying somewhat close together, in some cases with flattened contiguous sides as in those observed earlier, but in others with a rounded outline.

4. *After twenty-two hours' subsequent growth.*

At the end of twenty-two hours' growth the roots show a considerable return of their staining properties. The cell walls are clearly defined, and the cytoplasm even, and free from the scattered bodies described above. The nuclei stain sharply, and the membrane of those in rest is conspicuous. Division has been resumed to a considerable degree, and is taking place rapidly throughout the root-tip. Many nuclei are to be seen in spireme, in diaster, and in telophase. Few, if any, of the division figures, however, are normal in character, the achromatic fibrillar structures being very scanty, and the majority of the diasters showing imperfectly separated chromosome-groups adhering to each other by strands of chromatin (Figs. 2 and 7).



The latter is rather less granular and swollen in appearance than at the earlier fixations. Binuclear cells are to be seen here and there throughout the root-tip, and present some significant features. In the first place, such cells, particularly those at a little distance from the growing point, now show a distinct increase in size compared with those containing one nucleus. Again, although in the majority of them the two nuclei are still close together and distorted in shape, yet in many they are rounded in outline and lie at a distance from each other in the cytoplasm. Further, one of the nuclei frequently appears to be undergoing degeneration; staining diffusely, and with its nucleolus broken up into numerous small droplets. The last condition is of note, in connexion with the occurrence here and there of empty shrivelled cells containing masses of degenerated chromatin. Many of the binuclear cells show a fragment of cell-plate lying between the two nuclei. There are also cells with long lobed nuclei, often curved round an unfinished plate (Fig. 7). The nucleoli, which are surrounded by a conspicuous clear space, are generally divided into two or three bodies; and, as described above, in those nuclei which appear to be disintegrating, they are completely broken up into small round globules.

The abnormal division figures and binuclear cells are found throughout the root-tip, at some distance from the growing point as well as quite close to the latter.

5. *After forty-four hours' subsequent growth.*

In roots fixed after the lapse of forty-four hours, the general action of the chloral hydrate upon the tissue as a whole has practically disappeared. The cell walls are clearly defined, the achromatic structures again conspicuous, and the constituents of the nuclei sharply stained. Division is also taking place rapidly throughout the root-tip. There are, however, certain striking abnormalities to be seen. Here and there, but chiefly in the closely packed cell layers immediately within the endodermis, are rows of cells containing peculiar lobed or amoeboid nuclei. These cells are unusually large, and their transverse walls frequently oblique in direction. In some cases these oblique walls enclose small portions of nuclear material which appear to have been separated off from the large amoeboid nuclei, and to be in process of disintegration (Fig. 3). Masses of degenerating chromatin, varying in size, are also visible here and there wedged in between the walls of two contiguous cells. The individual cells in these rows may amount to as many as seven or eight in number. Another peculiar feature is to be seen in groups of three cells set obliquely to each other. These occur, not in the extreme tip of the root, where oblique orientation is a normal phenomenon, but further back from the growing point, where such orientation is not normally found. Comparison of these groups with Fig. 8 makes it appear probable that they have arisen by uneven multipolar division of a single

large nucleus, although figures showing the actual process of such division were not observed in *Galtonia*. In some cases one of the three cells of the group is considerably smaller than the others, and contains, instead of a nucleus, a mass of disintegrated chromatin. The most striking feature, however, of the roots fixed at this period is the presence of certain cells of relatively enormous size. These are scattered throughout the breadth of the root-tip, in periblem and plerome, but are more numerous at a distance from, than they are near to, the growing point. They have clear-cut walls, even cytoplasm, and sharply stained nuclei. Some of them contain a single large nucleus at rest or in spireme, and often elongated in shape; others have two nuclei of a normal appearance, lying well apart in the cytoplasm, and with no intervening cell-plate or fibrillar structures (Pl. LXVII, Fig. 17); and others show two nuclei, one of which is well stained and either at rest or in spireme, while the other has taken the stain diffusely, is granular, and contains a nucleolus broken up into numerous small bodies (Figs. 12 and 13). Such of these cells as are in division contain either two separate equatorial plates, diasters, or telophases, often set obliquely to each other (Figs. 18-20), or one large division figure (Figs. 15 and 16). On counting the chromosomes of some of the figures with a camera lucida, it was found that they contained roughly, and in some cases exactly, twice the normal somatic number, i. e. thirty-two instead of sixteen on the equatorial plate, and fifty-seven or more in diaster. Here and there is to be seen a row of three cells in telophase, of which the central is considerably larger than the two outer ones. These have evidently arisen, as may be seen from the intermediate figure (Fig. 21), through fusion of the inner contiguous polar masses of two diasters occurring in one cell. Finally, many of the abnormally large cells, in particular those situated far back towards the permanent tissue, show complete fragmentation and degeneration of their nuclear contents; instead of a definite nucleus, they contain a number of small, round, feebly staining bodies, each with one or more minute nucleoli (Fig. 14).

In some of the roots fixed at this period the number of the binuclear or tetraploid cells is considerably less than at the fixation made after twenty-two hours' subsequent growth over water.

#### SUMMARY OF THE RESULTS OBTAINED WITH THE ROOTS OF *HYACINTHUS CANDICANS*.

It is apparent from the above description that, as shown by Němec, the immediate result of the action of the chloral hydrate upon these roots is a disappearance of the achromatic fibres and an arrest of the cell-plate formation. Consequently the split chromosomes on the equatorial plate fail to pass to the poles, fuse together again, and go into rest as one irregular

mass, which must, from the nature of its formation, contain twice the normal number of chromosomes. With recovery of the tissue from the action of the drug, these tetraploid cells increase considerably in size, and further, divide mitotically and so form two daughter nuclei, also with the tetraploid chromosome number. The fact that the number of these tetraploid nuclei near the growing point is seen to be diminished at the later periods of the experiment, together with that of the occurrence of numerous large multinuclear cells and others with two diaster figures, appears to show that the tetraploid nuclei eventually break up. This they may do either while in rest or during division, and so form smaller nuclei with the normal diploid number of chromosomes, or something approximating to it. That they tend finally to disintegrate entirely is indicated by the fact that a gradual series can be traced from the above multinuclear cells, to some which are completely shrivelled and contain merely distorted masses of chromatin. A difficulty in estimating the effect of the chloral hydrate upon a particular root consists in the fact of the secondary nature of the structural modifications resulting from the action of the drug; which modifications would otherwise afford the readiest means for such an estimation. The number of the binuclear or tetraploid cells visible after partial recovery and further growth of the tissue is no real gauge of the extent of the action, but is dependent upon the number of nuclei in actual process of division during the immersion of the root in the poison. Again, the position of a tetraploid cell after a period of subsequent growth is largely dependent upon that of the dividing cell from which it originated, and therefore is not a very definite datum in determining its history, or at least its rate of division. The real nature of the action of the chloral hydrate upon the tissue remains doubtful. That it affects the chromatin itself appears from the fact that, at the earlier fixations, the whole nucleus looks finely granular and somewhat abnormal, taking the stain feebly; and the chromosomes also are granular and appear to be slightly swollen. It is possible that the disappearance of the achromatic fibres is due not to the action of the poison upon any definite fibrillar structure as such, but to a modification of the chemico-physical relations normally existing between chromatin and cytoplasm; a modification arising secondarily, and as a result of the action of the chloral hydrate upon the chromatin itself.

Examination of Figs. 1 and 17 reveals certain interesting features in the relation to each other of the nuclei contained in the tetraploid cells. In roots examined directly after removal from the poison, or after a short period of subsequent growth, these nuclei, which, as described above, are abnormally granular, with badly defined membranes, are seen to lie close together in the cytoplasm. They do not seem to exert any mutual attraction and their contiguous sides are flattened. They look indeed as if crushed together; a condition ascribed by Strasburger to the action of

the 'protoplast' (Fig. 1). Fig. 17 shows, however, that later, the two nuclei of a tetraploid cell generally lie widely apart in the latter, as though repelling each other, in accordance with Gerassimow's account of the behaviour of two healthy nuclei when occurring in one cell. In connexion with Gerassimow's hypothesis that such repulsion is due to an 'electrical force', and also with the general problem of the nature of nuclear activity, it is of particular interest to find that at certain stages of division two nuclei will fuse together, instead of repelling each other. This is seen in the telophase shown in Fig. 21. Here the contiguous telogroups of two diasters occurring in one cell have amalgamated to form a large central daughter nucleus; there is no crushing together of these two groups and a clear connexion can be traced between their respective networks of linin.

Figs. 17-19 illustrate another point of interest in this connexion, namely the close similarity between the states of activity of the two nuclei in a tetraploid cell. In all cases where these nuclei present a normal appearance, they are at precisely the same stage, their spiremes, diasters, or telophases showing practically identical features. Where any difference is discernible between them, one of the nuclei without exception exhibits signs of degeneration. This similarity of activity seems to suggest that the various phases of rest and division, through which a nucleus passes, are initiated by some stimulus resulting from its interaction with the cytoplasm.

Figs. 1, 12, and 13 show, in agreement with Hertwig's theory of a 'Kernplasmacoefficient', the existence of a certain correlation between the size of a cell and the amount of its contained chromatin. As was noted above, after an interval of some hours the tetraploid cells, at first no larger than those containing one nucleus, show a considerable increase in size. The recovery by the tetraploid nuclei of their normal appearance, and presumably of their healthy activity, seems indeed to be invariably accompanied by marked growth of the cell, which may attain fully twice its usual dimensions.

The behaviour of the nucleoli after treatment of the root with chloral hydrate requires comment. At the earlier stages of the experiment these appear to fragment into innumerable minute particles, which are scattered through nucleus and cytoplasm, a condition which possibly indicates the occurrence of rapid excretion. Later, they very generally divide into two or more bodies (it should be noted that this condition occurs, though to a less extent, in normal roots), and the shape of some of the nuclei containing them, which tends to be of a dumb-bell character, suggests that amitotic division may perhaps take place at this stage, although it probably does not do so earlier, or as a direct response to the action of the poison. Although, however, the occurrence, at this period, of numbers of cells containing two small nuclei of rounded outline might support such a suggestion, the data are insufficient for a decision on this



point. Finally, in such nuclei as are in process of disintegration, the nucleoli break up entirely into small round bodies. In the poisoned roots, the clear space surrounding the nucleolus is more conspicuous than is the case in normal tissue.

With regard to the occurrence of 'heterotype' reduction-figures, nothing at all resembling such is seen in the roots of *Galtonia*.

#### VICIA FABAE.

With the bean, two alternative methods of cultivation were adopted, the roots being started either over water, by pinning the cotyledons to a flat cork, or in damp sawdust. Those grown in sawdust proved to be the more generally satisfactory, and were used in the following experiment. In the latter, which was chosen from among a considerable number, the roots were fixed after 22, 48, and 70 hours' subsequent growth; which periods of fixation may be supplemented by two others, at 65 and 85 hours, taken from a parallel experiment.

1. After 1 hour in a 0.75% solution of chloral hydrate.
2. „ 22 hours' subsequent growth in sawdust.
3. „ 48 „ „ „ „ „
4. „ 65 „ „ „ „ „ „ (from a parallel experiment)
5. „ 70 „ „ „ „ „ „
6. „ 85 „ „ „ „ „ „ (from a parallel experiment)

Although the root tissue of the bean is somewhat dense, and its cells relatively small and closely packed together, and although it consequently presents, as a whole, features less favourable for examination than are those seen in *Galtonia*, yet at the same time it is characterized by certain peculiarities of considerable interest, which will be noted below. These roots appear to be slightly more resistant to the action of the chloral hydrate than are those of *Galtonia*; at the fixation made directly after their removal from the drug they still show many nuclei in division, although the character of these division figures is far from normal.

#### 1. *After 1 hour in 0.75% chloral hydrate.*

In roots fixed straight from the poison, the general effect of the latter is seen to be similar to that described above in *Galtonia*. The cell walls are swollen, the cytoplasm shrunken and vacuolated, and the resting nuclei, particularly those of the external layers, are diffusely stained, and have a granular appearance and badly defined membranes. The nucleoli have taken the stain deeply, and are surrounded by a conspicuous clear space, this last feature being much more marked in the poisoned roots than it is in normal tissue, although it also occurs occasionally in the latter. In the

majority of the nuclei the nucleoli are broken up into two or more bodies. A feature peculiar to the bean is seen in such cells as contain nuclei at the late spireme stage; surrounding the closely coiled thread is a hyaline area of a curious bipolar shape, evidently representing the polar-cap fibres which have disappeared under the influence of the poison. These fibres in the normal bean are particularly conspicuous, and hence their appearance is more noticeable in this plant than in *Galtonia*. There is throughout the tissue a general absence of fibrillar structures, with the result that the dividing nuclei present very irregular figures. At the equatorial plate and diaster stages the chromosomes are scattered throughout the cell, and at the telophase are to be seen going into rest in two masses, still joined together by thick strands of chromatin. The chromatin, as in *Galtonia*, has a peculiar swollen appearance and is finely granular, and the chromosomes are somewhat shorter and thicker than in the normal root. They also show a marked longitudinal split. The division figures are scattered throughout the root-tip, although in greater numbers in the internal than in the external cell layers.

2. *After 22 hours' subsequent growth in sawdust.*

After the lapse of 22 hours the general appearance of the tissue is more normal, although cell walls, cytoplasm, and nuclei still show the effects of the poison to a certain extent, in swelling, vacuolization, and diffuse staining. The chromatin itself also is still granular. The activity of division seems to have returned in full force, and innumerable figures of spireme, diaster, and telophase are to be seen throughout the breadth of the root, and from the extreme tip back to the permanent tissue at some distance from it. Figs. 8, 9, 11 show the striking modifications which have arisen in many of these division figures; and also a second feature characteristic of the bean, namely the frequent occurrence of multipolar divisions. Most of the latter seem to be tripolar; they are, however, extremely irregular in character. The inequality in size of the chromosome masses at the three poles, the direction of the strands of chromatin which in some cases join these masses together, and also the existence of such peculiar figures as that shown in Fig. 8, indicate indeed that the tripolar arrangement must have arisen through the breaking up of the chromatin into unequal groups at the stage of the equatorial plate. These tripolar divisions have in some cases been complete, and are separated at the telophase by well-defined cell walls; in other cases the latter are only partially formed, and strands of chromatin persist here and there between the groups of chromosomes. Evidently resulting from these multipolar divisions are numerous groups of cells, generally three in number and of peculiar shapes. In some of these groups the cells are completely separated from each other, but in others imperfect dividing walls are seen, and the nuclei



are lobed or amoeboid in shape (Fig. 11). One or more of the nuclei of such a group are frequently in process of disintegration and contain a fragmented nucleolus. Fibrillar structures are again visible throughout the root-tip, but vary greatly in their degree of definition, being conspicuous in some nuclei (Fig. 10), whilst in others they are almost lacking. Polar caps are to be seen round the denser spiremes, but are still somewhat irregular and faintly stained. Many binuclear cells are visible, containing two small nuclei, lying close together in the cytoplasm, but quite round in outline and resembling those seen in *Galtonia* in fixations after 22 hours' growth. In other such cells one of the two nuclei is often undergoing degeneration, being feebly stained and full of nucleolar bodies.

3. *After 48 hours' subsequent growth.*

At this period of the experiment the appearance of the tissue as a whole is good, showing well-defined cell walls, unbroken cytoplasm, and nuclei which take up the stain strongly in all their constituents. At the same time there is an even greater number of notable structural peculiarities than at the preceding fixation, showing that the binuclear and tetraploid cells resulting from the action of the chloral hydrate have resumed division, while retaining the modifications due to that action. Scattered throughout the root-tip, and of considerably larger dimensions than their neighbours, these abnormal cells present a striking appearance. They occur most frequently in pairs, but here and there are to be seen in rows of four or five (cf. Fig. 3), the individual cells containing figures at all stages of activity. In some are to be seen equatorial plates or diasters of unusual size; these, on counting their chromosomes with the camera lucida, are found to contain twice the normal somatic number. In others there are two separate division figures at the equatorial plate, diaster, or telophase stage, both of which figures are invariably in identical phases of activity, a point of considerable interest, as was noted above. In a few of the latter, fusion is taking place between the contiguous groups of the two telophases. In such cells as contained earlier a fragment of cell-plate as well as two nuclei, three walls are visible at the termination of the above division; these consist of the thick fragmentary wall of the original arrested mitosis from which arose the binuclear condition of the cell, and the two thinner walls, generally oblique in position, formed during the double mitosis which has just occurred (Figs. 20, 21). Here and there in the permanent tissue is to be seen a much elongated cell with two nuclei; these frequently appear to be lapsing into inactivity. The nucleoli seem to be very active at this period, staining deeply, and being broken up into several bodies and in some cases fragmented into numerous minute globules. Large binuclear cells with their nuclei at rest are conspicuous, and in many of these one of the latter is disintegrating (cf. Figs. 12 and 13). The abnormal figures just described are

found in the extreme tip of the root, as well as throughout periblem and plerome far back to the permanent tissue. Fewer of them, however, occur near the growing point than at a distance from it.

4. *After 65 hours' subsequent growth.*

At the end of 65 hours' growth subsequent to experiment, there is no effect of the poison to be seen other than is shown in structural modifications similar to those which have just been described. The tissue takes up the stain very well in external as well as internal cell rows, showing all details sharply defined. As to the above structural changes, the chief points in which they differ from those seen at the earlier stage are the following: The tetraploid cells, with their double-number diasters, duplicated equatorial plates, and large telophases, now lie rather further back in the tissue than at the preceding fixation, and they are if anything rather more abnormal in size. Many of them also contain as many as three nuclei, all of which may be seen in process of degeneration, staining diffusely and with fragmented nucleoli. Here and there, crushed between two large cells or wedged obliquely between several cells of peculiar shape, are masses of disintegrated chromatin, presumably showing where some cell has lapsed into degeneration. Far back from the growing point, in the permanent tissue, are to be seen long cells emptied of their contents and with two, or in some instances three, nuclei which have dropped against the cell wall and fallen into inactivity.

5. *After 70 hours' subsequent growth.*

After an interval of 70 hours subsequent to the experiment, again the same characteristics are to be observed. The tissue as a whole seems to have recovered its normal condition, all vacuolation of the cytoplasm, swelling of the cell walls, and diffuseness of staining having disappeared. But the above structural peculiarities still persist. These show, however, an increase in two slight but significant changes observed at the preceding fixation. In the first place the tetraploid cells have reached even more abnormal dimensions, catching the eye at once under a low power of magnification. And in the second place there is an increase in the preponderance of the number of these cells found at a distance from the root-tip, over that found near the growing point. It should also be noted that the masses of disintegrated chromatin appear to be more numerous than at the preceding fixation. The nucleoli are still for the most part more broken up than is the case in normal tissue.

6. *After 85 hours of subsequent growth.*

Roots examined after an interval of 85 hours show the same kind of features as those described at the preceding fixation, but the tetraploid

and binuclear cells are fewer in number, and also occur at a greater distance from the growing point. It would seem, therefore, that these cells are either gradually passing over into the permanent tissue, beyond the active region of the root-tip, or are breaking down one by one into masses of disorganized chromatin, which masses are probably then absorbed by the surrounding tissue.

In none of the experiments performed on bean roots were any figures found which could be interpreted as arising through heterotype reduction.

#### SUMMARY OF THE RESULTS OBTAINED WITH *VICIA FABAE*.

It will be seen from the above details that the action of the chloral hydrate is of the same nature in the case of the bean as in that of *Galtonia*. Its immediate effects are observable in swelling of the cell walls, shrinkage and vacuolization of the cytoplasm, disappearance of the achromatic fibres, and a certain alteration in the consistency of the chromatin. These features pass off with recovery of the tissue during subsequent growth; and the net visible result of the action of the poison, apart from enhanced activity of the nucleoli, remains in the structural modifications which have arisen secondarily in certain individual cells, and which persist for a considerable time. These modifications are due to the tetraploid condition induced by the imperfect mitoses which have taken place in such cells, owing to their lack of achromatic fibres. They are seen in figures of nuclei of abnormal shape and size, which, in diaster, are found to contain the tetraploid number of chromosomes, and which occur in cells of unusual dimensions and often peculiar orientation.

As in the case of *Galtonia*, no 'heterotypical' figures are found in the bean. Consequently the evidence obtainable from this plant on the question at issue, namely the occurrence of heterotypical reduction in somatic cells, consists in the data which it affords as to the fate of the tetraploid cells. This evidence will be discussed later; it may however be noted here that the numerous figures of multipolar division, and nuclear fragmentation, described above, seem to be sufficient to account for the disappearance of these cells without resorting to a hypothesis so difficult of proof as is that brought forward by Němec.

The chief points in the results obtained with the bean, which distinguish it from *Galtonia*, are the occurrence of multipolar division figures, and the comparative absence of those showing fragmentation of the large nuclei into numerous smaller ones. The second point is probably partly dependent on the first; fragmentation occurring less frequently in the bean owing to the greater opportunity of otherwise restoring the normal amount of chromatin, afforded by the above multipolar divisions. It may be added that considerable interest attaches to the latter owing to their close similarity to certain types of division seen in pathological growths.

PISUM SATIVUM.

As in the case of the bean, two methods of cultivation were used in starting pea roots for experiment, and again the most satisfactory results were obtained by that of growing them in damp sawdust. In spite of the fact that those started over water had the advantage of less manipulation, and could be poisoned and replaced on the cork without any friction of the root-tip, it was found to be extremely difficult to keep them free from mould.

The pea proved to be the most fruitful in results of the three plants examined; for not only is it particularly sensitive to the action of the chloral hydrate—showing a remarkable variety of abnormal figures after treatment with the latter, but it is also the only one in which occur the all-important 'heterotypical' figures. This is said advisedly, in spite of the statement made by Němec in his most recent paper, to the effect that these figures occur also in the onion. In his detailed description, published in 1904, of the action of chloral hydrate upon the pea, the bean, and the onion, Němec stated that the 'heterotypical' figures were found in the pea only, and since that statement has published no further data in correction of it. As was mentioned above, the onion was one among a number of plants used for preliminary examination before selecting individuals on which to carry out the detailed experiments. In this examination nothing resembling 'heterotypical' figures was observed in the onion, although the other effects of the chloral hydrate were well marked.

With a view to inducing modifications in the figures in question, which might afford data for the determination of their true nature, a considerable number of experiments were performed on the pea with varied strengths of solution and periods of immersion.

For comparison with the results described above in *Galtonia* and the bean, however, it will be best to give first an account of an experiment in which pea roots were immersed for 1 hour in a 0.75% solution of chloral hydrate, and to set aside for the moment the question of the nature of the 'heterotypical' figures.

In the experiment here described, the roots after immersion in a 0.75% solution of the poison were fixed at the following intervals:—

1. After 1 hour's washing in tap water.
2. „ 22 hours' subsequent growth in sawdust.
3. „ 45 „ „ „ „ „
4. „ 60 „ „ „ „ „ „

(1) *After 1 hour's washing.*

The appearance presented by roots fixed directly after washing is extremely abnormal, and the tissue is so diffusely stained that it is somewhat



difficult to distinguish clearly the various details; with the aid of transverse and longitudinal sections, however, the following features can be made out. The cell walls throughout the breadth of the root are swollen and distorted, and the cytoplasm, particularly that of the periblem, is greatly vacuolated. Considerable activity is still visible, although the scarcity of nuclei in spireme, even in the inner cell rows, shows that it has suffered an arrest. The majority of the division figures occur in the densely packed cells lying immediately inside the endodermis. No achromatic fibres remain, and consequently these figures show all degrees of irregularity, the chromosomes at the diaster and equatorial plate stages being either crushed together or scattered through the cell. Here and there they are seen to be of the peculiar thick X-like shape which characterizes those of reproductive tissue (Fig. 23). The resting nuclei are granular and diffusely stained, and have an ill-defined membrane; but the nucleoli contained in them have taken the stain intensely and are in great activity, broken up into several bodies, or fragmented into numerous small particles which are scattered through the cytoplasm (cf. Fig. 1). The droplets often to be seen in the nucleoli of normal roots are so pronounced as to form a conspicuous vacuolization. A peculiar feature is seen here and there throughout the root-tip, but occurring most frequently in the plerome, in cells with striking deeply stained globules of what appears to be nucleolar material. These globules seem to have been extruded from the nucleus, and are generally aggregated in one corner of the cell. The chromatin is granular and swollen in the spireme figures as well as in the diasters, and in telophase shows very imperfect separation of the two chromosome groups. Round such spiremes as are massed together preparatory to breaking up into individual chromosomes is visible the hyaline space so noticeable in the bean, showing where dissolution of the polar-cap fibres has taken place. A few binuclear cells are to be seen containing two nuclei of a distorted shape lying close together in the cytoplasm.

(2) *After 22 hours' subsequent growth in sawdust.*

A period of 22 hours was allowed to elapse before the next fixation, in order that the tissue as a whole might have recovered sufficiently to offer figures suitable for drawing. It should be remarked, however, that in a fixation made after a lapse of 3 hours only, in a parallel experiment, the abnormalities observed were of the same nature as those to be described below, but the general condition of the root was bad. At the end of 22 hours of subsequent growth the tissue presents a clearer appearance, but there is still much swelling of the cell walls and vacuolization of the cytoplasm, and the outer cell rows are diffusely stained. The activity of division is very considerable, however, and many cells in mitosis are visible, in greatest numbers in meristem and plerome. The achromatic fibres are very imperfect, with the result that the above figures show much irregularity, and

every degree of incomplete separation of the groups of chromosomes. Here and there also occur tripolar divisions of peculiar shapes, often having a large mass of chromatin at one pole and a smaller mass at each of the others. In a few of these multipolar figures division has been complete, but in the majority strands of chromatin persist between the respective chromosome groups. The chromatin itself is granular, and when broken up into chromosomes shows the 'heterotypical' character to a marked degree. Groups of cells in rest are seen, of curious shape and oblique orientation, and containing nuclei of various size, lobed or amoeboid, and frequently with connexions remaining through the cell walls. Such groups of cells have evidently arisen through the imperfect multipolar divisions just described. Other cells contain nuclei of the familiar 'bridge' shape, and generally also a fragment of unfinished cell-plate. Others, again, have two nuclei at rest or going into division, and showing the duplication of equatorial plates, diasters, and telophases already seen in *Galtonia* and the bean. These tetraploid cells have now increased in size. They occur throughout the root-tip, and rows of three or four are to be found near the growing point as well as further back. The round, deeply stained bodies noticed at the preceding fixation are extremely marked, in some cases almost obscuring the nucleus. Here and there are seen already, at some distance from the growing point, cells in which fragmentation of the nucleus itself has taken place.

(3) *After 45 hours of subsequent growth.*

After an interval of 45 hours the tissue has recovered its staining properties and presents a more normal appearance generally, the cell walls being better defined, and the cytoplasm less vacuolated. At the same time the number of structural abnormalities has if anything increased, these being the more evident from the great rapidity with which division is now taking place throughout the root-tip. Although the achromatic fibres are more definite than at the preceding fixation, the division figures are still very irregular in character. The equatorial plates tend to be curved or split into a Y-shape, and multipolar division figures of great variety are to be seen. In some telophases a third small mass of chromatin is visible at one extremity of the cell-plate which divides two large masses. This has the appearance of having dropped out of the zone of activity during mitosis, rather than that of having arisen through tripolar division. Tetraploid cells are seen, containing two nuclei at all stages of rest and activity. Others show one equatorial plate or diaster of unusual size, which, on counting, is found to contain the tetraploid chromosome number, i. e. 28 instead of the 14 normally found in the somatic tissue of the pea. The above cells, of greatly increased dimensions, are now found principally at a considerable distance from the growing point, and occur in rows of two



or three. At the extreme tip of the root they are fewer in number than at the preceding fixation. Far back from the growing point, in the permanent tissue, occur much elongated cells, with two nuclei dropping against the wall and relapsing into inactivity. In this part of the root also are visible long cells, of which the nucleus is broken up into small round bodies, feebly stained, and evidently in process of disintegration. Degenerated masses of chromatin and shrivelled cell walls are to be seen here and there wedged in between groups of cells which are themselves frequently of peculiar shape; these probably represent the disintegration of one member of a tripolar, or a part of a tetraploid mitosis. Fragmentation of the nucleoli is very noticeable. 'Heterotypical' figures are still conspicuous in many cells, but in others this character appears to be diminishing, and intermediate forms between it and the normal occur here and there.

(4) *After 60 hours' subsequent growth.*

At the end of 60 hours of subsequent growth, the features presented by the roots are essentially the same as those described at the preceding fixation. Certain points of difference which require note, however, are the following. Although the condition of the tissue as a whole seems to have improved, the nuclei staining sharply, and cell walls and cytoplasm appearing more normal, and although many nuclei in spireme are visible, yet at the same time the percentage of disintegration figures has considerably increased, and the nucleoli also are for the most part broken up into numberless small bodies and scattered through the entire cell. These figures suggest the inference that the tetraploid cells are going to the wall with some rapidity. Another point of importance is found in the appearance of the 'heterotypical' chromosomes. The gradual transition, observed at the preceding fixation, from the marked 'heterotypical' character of these to one more nearly resembling that of normal chromosomes is increasingly evident. Numerous cells are to be seen, best shown in transverse section, in which the chromosomes, although amounting to twenty-eight in number, are yet approximately normal in shape (Fig. 25).

As remarked above, in the hope of inducing modifications of the 'heterotypical' figures, by which it might be possible to detect their real nature, a considerable variety of experiments were carried out upon pea-roots. Different strengths of solution of the chloral hydrate were used, and longer or shorter periods of immersion. The latter varied between  $\frac{1}{2}$  hr., 1 hr.,  $1\frac{1}{2}$  hrs., 2 hrs., and two percentages of chloral hydrate were tested besides that in the above experiments, namely 1% and 0.5%. It was found that the effect of the poison upon the tissue as a whole varied in degree only, not in nature. With the 1% solution the action was very violent, and great distortion of the nuclei and shrinkage of the cytoplasm resulted.

This was the case after immersion for  $\frac{1}{2}$  hour, and after more prolonged immersion these features were more pronounced. With this strength of solution a very marked 'heterotypical' character was seen in the chromosomes, but there appeared to be less characteristic action of the poison upon the tissue, and fewer structural abnormalities resulted from it. With a 0.5% solution of chloral hydrate on the other hand, much better effects were obtained, but prolonged immersion proved necessary in order to get the characteristic action of the drug at all marked. Very little shrinkage and distortion were seen with this percentage. The heterotypical appearance of the chromosomes was also less marked. The point of importance in these results consisted in the apparent possibility of modifying the degree of the heterotypical character of the chromosomes.

SUMMARY OF THE RESULTS OBTAINED IN THE ROOTS OF  
PISUM SATIVUM.

It is evident, from the results described above, that in the pea, as in *Galtonia* and the bean, the gross effect of the action of the chloral hydrate upon the root tissue consists in the disappearance of the achromatic fibres and consequent production of tetraploid cells. At the earlier stages of fixation there is the same arrest of the movement of the split chromosomes to the poles, and re-fusion to form one mass; later, the same growth to unusual size of the cells containing such masses; and finally, the same multipolar division or degeneration of the abnormally large nuclei, both the latter processes being very well shown.

The point of particular interest in the pea, however, lies in the occurrence of Němec's 'heterotypical' figures, which may now be discussed at length. These figures are very striking (Figs. 23 and 26), and occur not only in roots fixed after a period of growth subsequent to immersion in the chloral hydrate, but also in those fixed directly after removal from the latter. After an interval of twenty-two hours the majority of division figures seem either to consist entirely of such 'heterotypical' chromosomes, or to contain a certain number of them scattered amongst others more normal in shape. They are seen after forty-four hours' subsequent growth, and also at sixty hours, but at these later stages there are fewer of pronounced heterotypical appearance, many showing a character intermediate between this and that of the normal pea chromosome.

Since, then, the occurrence of the heterotypical figures cannot be doubted, the question resolves itself into that of determining whether they really arise in connexion with the process of reduction, and are comparable to those seen in reproductive tissue, or whether their shape is caused by some process of a different nature.

The evidence in favour of their being true reduction-figures is as follows:—

They are found in tissue known to contain nuclei with double the normal number of chromosomes, which nuclei moreover finally disappear.

They are singularly like the heterotype reduction-figures of sporogenous cells, showing the X, Y, and tetrad shapes characteristic of the latter.

They do not, obviously, occur in normal tissue.

On the other hand, the evidence against their true reduction nature is as follows:—

They occur in the pea alone of a considerable variety of plants, and it is significant that the normal pea has chromosomes shorter and thicker in shape than are those of *Galtonia* and the bean, &c. The pea chromosomes would consequently show any modification of shape due, for instance, to an alteration in their chemico-physical state, more conspicuously than would those of the latter plants; should such an alteration take the form of swelling and massing together of their chromatin, a very slight change in that direction would give them the stumpy, peculiar appearance of heterotypical figures. The normal pea has further a curious break at one end of its longitudinally split chromosomes (Fig. 22), and the latter therefore present a shape which, with the above modification, might even resemble an X.

Again, the chromosomes in question are more strikingly heterotypical in character soon after poisoning, than they are after a longer period of subsequent growth. At the same time tetraploid cells are still numerous at the later stages, and therefore there would appear to be as much cause for heterotypical reduction then as earlier. This seems an indication that their shape is due to a chemical or physical change of variable amount; an inference which is borne out by the fact that different strengths of poison produce different degrees of alteration in shape.

Again, their occurrence in the pea alone would lead to the supposition that if their function is to reduce the amount of chromatin in the tetraploid cells, there would naturally be, in these roots, fewer or none of the multipolar and degeneration figures found in those of the other plants examined. In the pea these methods would be superfluous in presence of the more perfect process of automatic reduction. Actually, however, there are as many multipolar divisions in this plant as in the bean, and the degeneration figures are particularly numerous.

Again, 'heterotype' figures do occur, though very rarely, and not in their extreme form, in normal tissue; and then generally in the external cells, and particularly in roots fixed in acetic-alcohol, a fixative of which the action is certainly more violent than is that of Flemming (Fig. 24).

Further, it has been impossible to show clearly a double cleavage of the chromosomes, such as would be present if they were really heterotypical in nature. All instances found of 'tetrad' figures were open to the interpretation of being 'dyads' doubled on themselves, and so pre-

senting their four ends to view in the same plane; or else to that of chance apposition of two pairs of chromosomes, an arrangement seen frequently on normal equatorial plates, as was pointed out by Strasburger (Fig. 28). The marked longitudinal fission of the chromosomes seen also as early as the spireme thread stage—noted above as characteristic of chloralized tissue—is not really comparable to the condition seen in the tetrads of sporogenous tissue. It is frequently conspicuous in diasters containing twice the somatic chromosome number. But though it must in such figures represent a premature fission of the chromosomes of the daughter nuclei, yet no previous heterotype pairing of chromosomes is entailed by it, as is obvious from the number of the latter found on counting.

Finally, the 'heterotype' figures are found in cells which still contain the tetraploid number of chromosomes. This last point appears to be conclusive evidence of their non-reducing character. In the cell shown in Fig. 26, each of the twenty-eight double chromosomes is markedly heterotypical in character; yet it is scarcely to be supposed that the cell in question can have contained more than twenty-eight pairs of chromosomes, which number already is twice that in the normal somatic nucleus. Since, then, there has been no reduction, the figures must owe their heterotypical character to some other factor than that of the pairing of chromosomes.

Consideration of the above points of evidence suggests that this factor is a chemico-physical one, and that the stumpy heterotypical figures in the pea are to be explained as due to the occurrence of swelling and massing together of its normally short, thick chromosomes under influence of the chloral hydrate. It has been noted above that, as in the pea, so also in *Galtonia* and the bean, there is, after treatment with chloral hydrate, an evident alteration in the consistency of the chromatin, which becomes swollen-looking, granular, and massed together. The chromosomes of these plants are so long and thin, however, that a very considerable degree of contraction would be necessary to make them resemble heterotypical figures, whereas in the pea very little would effect this. Fig. 27 is of note as showing the emergence of the 'heterotypical' chromosomes directly from the coiled spireme; it should be remarked in connexion with it, that the entire absence in the root tissue of anything like a synaptic stage is another point of evidence against the true heterotypical nature of these chromosomes.

Great importance is attached by Němec, in his arguments for the occurrence in root tissue of heterotype reduction, to the disappearance of the tetraploid cells from the chloralized root-tips. He particularly emphasizes this point in his most recent paper. In this he states that complete disappearance of these cells occurs in lateral roots, which, if examined at an earlier fixation, are seen to contain them in considerable numbers. It has not been possible to repeat these last experiments in detail. The disappearance of the tetraploid cells from the roots in question would seem,



however, to be due merely to a slightly more rapid occurrence of the processes which cause that disappearance in the young tap-roots used in the experiments described above. Also in a few lateral roots which were examined, although no tetraploid figures were to be seen in the extreme tip, in one instance, at any rate, such a cell was found at the point of exit from the tap-root. It seems probable, therefore, that search at this point would reveal a certain number of the tetraploid cells, relapsed into comparative inactivity and therefore inconspicuous and likely to escape notice.

It was pointed out earlier that there is a certain difficulty in tracing the fate of the tetraploid cells, owing to the variety of their points of origin and to the difficulty of ascertaining their rate of division. Although such cells are seen near the growing point after a comparatively prolonged period of regrowth, yet their number at that point does slowly diminish. The hypothesis of heterotype reduction of the chromatin would seem to have been sufficiently disproved above. Therefore, either the activity of division of the tetraploid cells is so much lessened that they pass out of the zone of active growth, without giving rise to the rows of cells of like tetraploid nature, which they would normally produce, or they break down rapidly and are absorbed by the neighbouring cells. Probably both processes are in action. It has been stated by Gerassimow that the increase in size of a cell is correlated with a decrease in its rate of division. In apparent accordance with this statement, large tetraploid cells are found, generally singly, sometimes in twos or threes, but never in extensive rows, at the growing point. Further, not many disintegration figures are to be seen at the latter, these occurring generally further back. Multipolar division occurs to a considerable extent throughout the entire root-tip.

From the occurrence then of the multipolar divisions, fragmentation and disintegration figures described, and also of certain abnormally large cells far back from the growing point, it may be inferred that the tetraploid cells either break up into several smaller individuals with something approaching the normal amount of chromatin, or disintegrate, or pass over into the permanent tissue in accordance with Němec's second hypothesis. It seems clear that, at any rate in the root tissues of the plants examined in these experiments, heterotype reduction does not play a part in the disappearance of the tetraploid cells; that on the contrary, their nuclei, instead of reverting to any such automatic process, tend to break up by one or other of the above methods. From the nature of the figures observed, it would appear that this breaking up is due ultimately, as suggested by Strasburger, to a lack of sufficient centripetal force to hold together so large a mass of chromatin as is contained in these nuclei.

SUMMARY.

1. By treatment with a dilute solution of chloral hydrate the occurrence of tetraploid cells may be induced in the young root-tips of pea, bean, and *Galtonia*.

2. This condition arises secondarily, and as the result of the disappearance of the achromatic fibres of the cell, under the influence of the chloral hydrate. The movement of the split chromosomes to the poles is arrested and a single large nucleus arises by their re-fusion to form one mass.

3. Three types of tetraploid cell may result. Such a cell may contain (1) two nuclei, (2) an amoeboid or 'bridge' nucleus, (3) a single nucleus of normal shape but abnormal size.

4. With recovery by the tetraploid nuclei of their normal appearance, the cells containing them increase in size till they attain fully twice their usual dimensions.

5. In division, the tetraploid cells give rise (1) to single large figures in which it is possible to count twice the normal number of chromosomes, (2) to two separate division figures in one cell, (3) to multipolar divisions.

6. The tetraploid cells gradually disappear from the root-tip. This disappearance is probably due to their division into several smaller cells; their fragmentation and absorption, of which process many figures occur; or to their passing over into the permanent tissue and relapsing into inactivity.

7. Certain figures occur in the pea remarkably like the heterotype reduction-figures seen in sporogenous tissue. These, however, are shown to be merely peculiar forms, which result probably from some chemico-physical effect of the chloral hydrate upon the chromatin, and which, though they resemble the figures characteristic of reproductive cells, have in reality no connexion with the process of normal reduction.

In conclusion, my hearty thanks are due to Professor J. Bretland Farmer, under whose direction this work has been carried out.

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## EXPLANATION OF PLATES LXVI AND LXVII.

Illustrating Miss Kemp's paper on 'Heterotypical Reduction' in Somatic Cells.

All drawings were made with the camera lucida, under a 2 mm. apochr. homo. imm. Zeiss N. M. 1.40 with comp. oc. 18.  $\times 2,250$ . Figs. 1, 2, and 3 were drawn under a  $\frac{1}{v}$  Leitz Wetzl., with 12 oc. Approx.  $\times 1,199$ .

### PLATE LXVI.

Fig. 1. Periblem cells of *Galtonia* root after 1 hr. in 0.75 per cent. chloral hydrate and 1 hour's washing in tap-water. Shows fragmentation and scattering of the chromatin throughout the cell, and effect of the poison on nuclei at rest and in division.

Fig. 2. Section of *Galtonia* root after 22 hours' growth subsequent to experiment. One nucleus is seen in imperfect division; another amoeboid in shape; a third broken up into three bodies, of which two are in process of degeneration.

Fig. 3. Row of cells from *Galtonia* root after 44 hours' growth subsequent to experiment. These are of unusual size and contain amoeboid nuclei, from which small portions tend to be cut off.

Figs. 4 and 5. Irregular equatorial plates from *Galtonia* root after 5 hours' growth subsequent to experiment: in Fig. 4 the chromosomes are clustered together at the centre of the cell; in Fig. 5 they are scattered across it.

Figs. 6 and 7 show conditions arising from Figs. 4 and 5; the partially separated groups of chromosomes are going into rest; an imperfect cell-plate is seen.

Fig. 8 shows cell from root of *Vicia Faba*; this contains an irregular tripolar division consisting of three masses of chromatin of unequal size; one cell-plate divides the large from the two smaller masses.

Figs. 9 and 10 show transverse and longitudinal views of further stage of tripolar division; the masses of chromatin and cell-plates are again somewhat irregular.

Fig. 11 shows a group of three cells from root of *Vicia Faba*, evidently arising from tripolar divisions similar to the above; a connexion between two of the cells persists through the dividing wall.

Figs. 12 and 13. Tetraploid cells from *Galtonia* root after 44 hours' growth subsequent to experiment; these have attained a great size, as may be seen from the dividing walls of the neighbouring cells visible; they contain two nuclei, one of which is at rest (in Fig. 12), or in spireme (in Fig. 13); the other in process of degeneration.

Fig. 14. Cell from root of *Vicia Faba* at distance from growing point, in permanent tissue; the nucleus has fragmented into several bodies, and is in process of disintegration.

PLATE LXVII

Figs. 15 and 16. Tetraploid cells from *Galtonia* root, containing diasters of greatly over the normal number of chromosomes: 47 in Fig. 15; 56 in Fig. 16.

Figs. 17, 18, and 19 show tetraploid cells, Figs. 17 and 19 from *Galtonia*, Fig. 18 from *Vicia Faba*, containing two nuclei at same moment of activity; spireme, equatorial plate, or diaster.

Figs. 20 and 21. Two large tetraploid cells from root of *Vicia Faba*, illustrating the fusion of the two contiguous groups of chromatin; in Fig. 21 the actual connexion between the linin network of the two groups is seen.

Fig. 22. Transverse section of cell from normal root of *Pisum sativum*; shows break at one end of the split chromosomes.

Fig. 23. Transverse section of cell from chloralized root of *Pisum sativum* after 22 hours' growth subsequent to an immersion for  $1\frac{1}{2}$  hrs. in 0.75 per cent. chloral hydrate. The chromosomes are of a marked 'heterotypical' character.

Fig. 24. Transverse section of cell from normal root of *Pisum sativum*, fixed in acetic-alcohol; shows intermediate shape between that of Fig. 22 and Fig. 23.

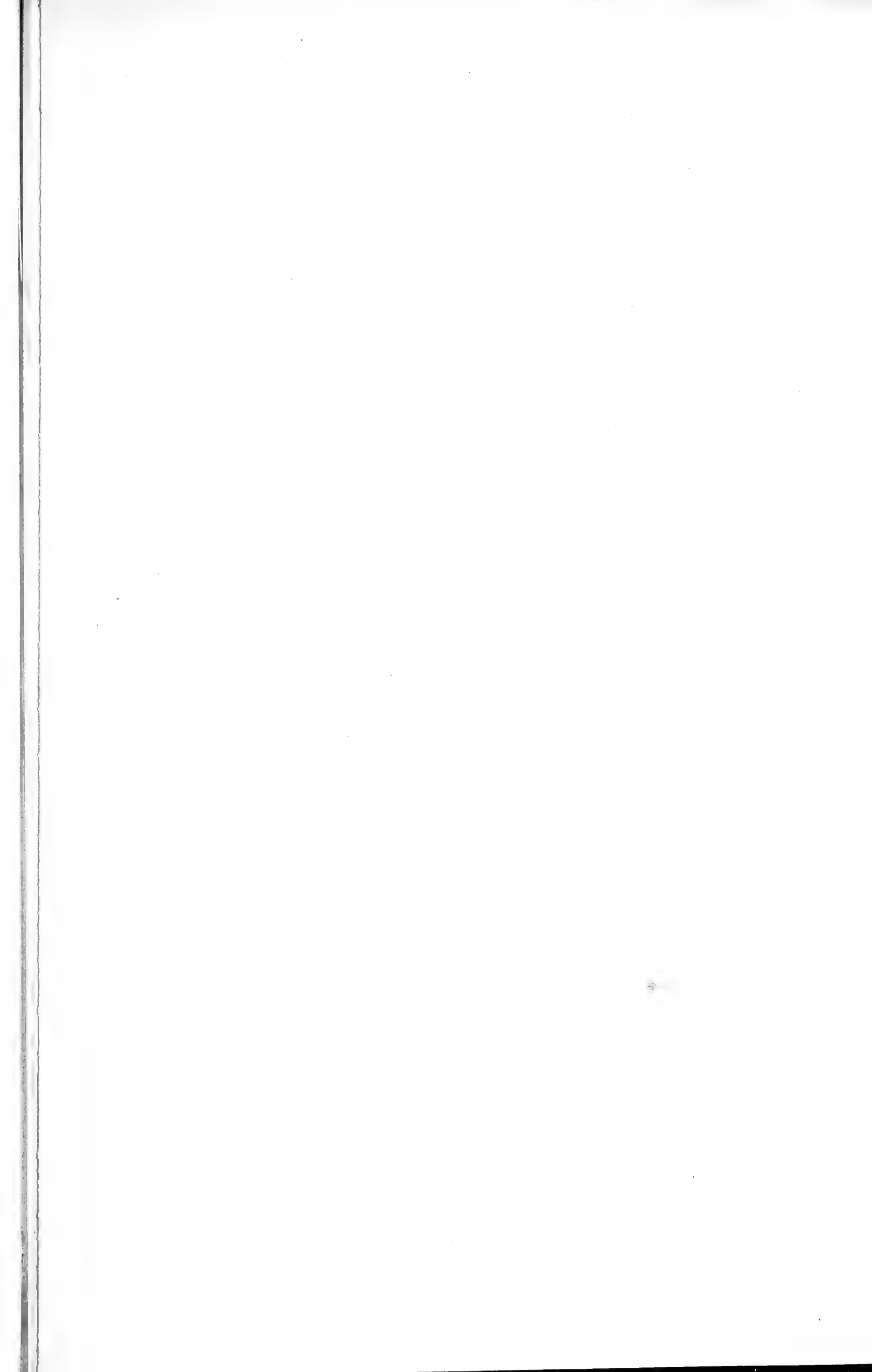
Fig. 25. Transverse section of tetraploid cell from chloralized root of *Pisum sativum* after 48 hours' growth subsequent to immersion for  $1\frac{1}{2}$  hrs. in 0.75 per cent. chloral hydrate; shows 28 chromosomes of approximately normal shape.

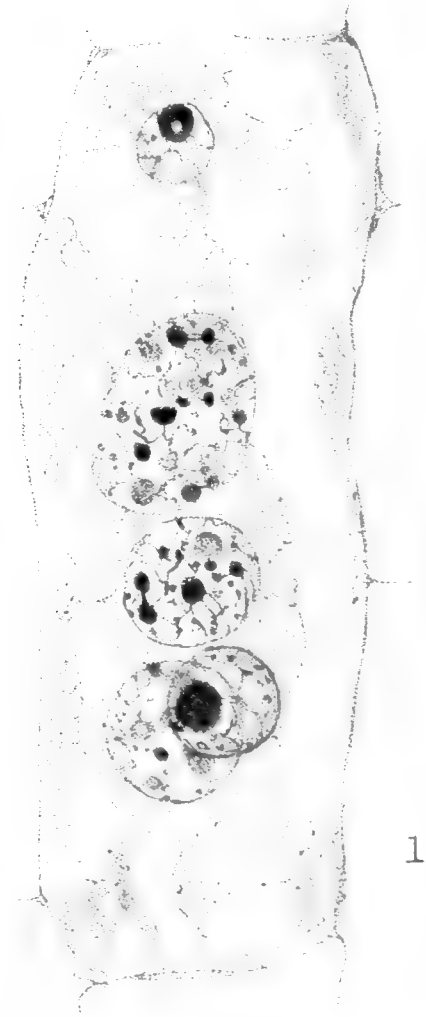
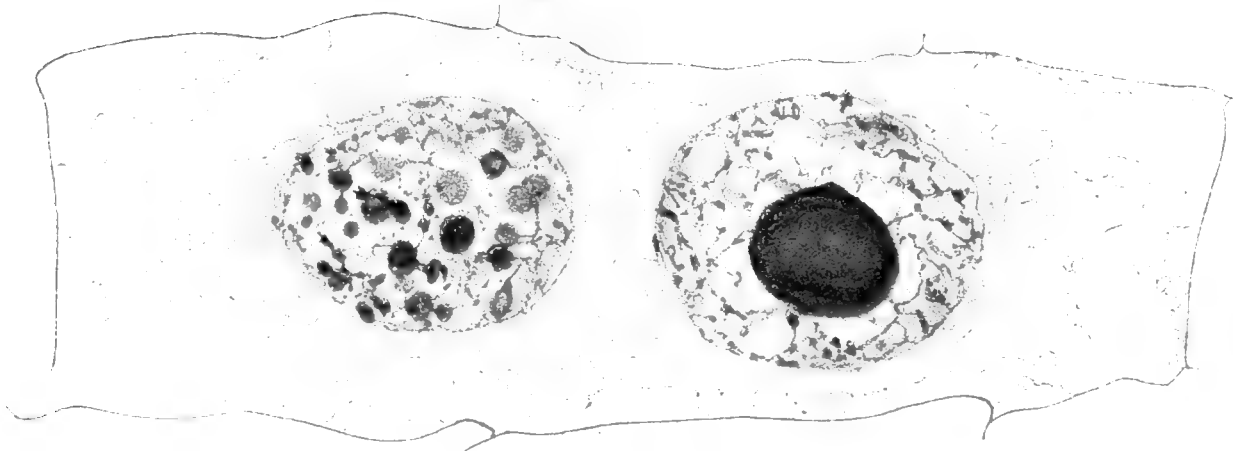
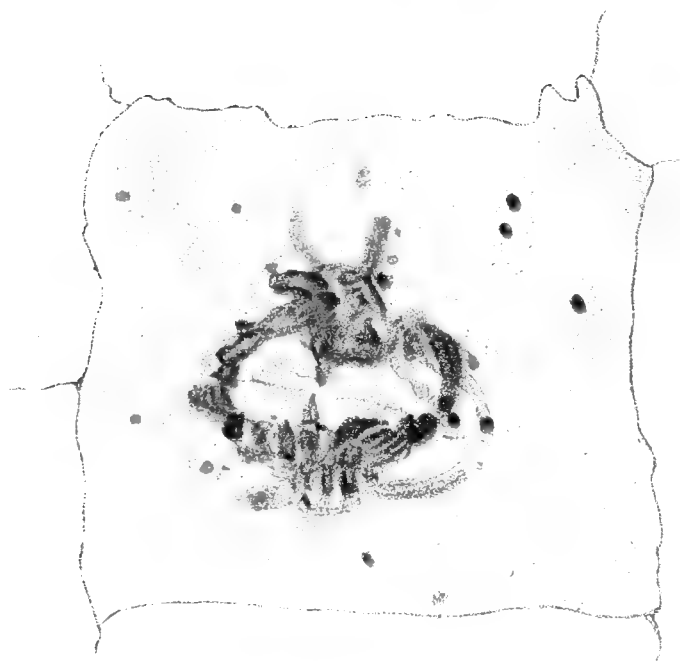
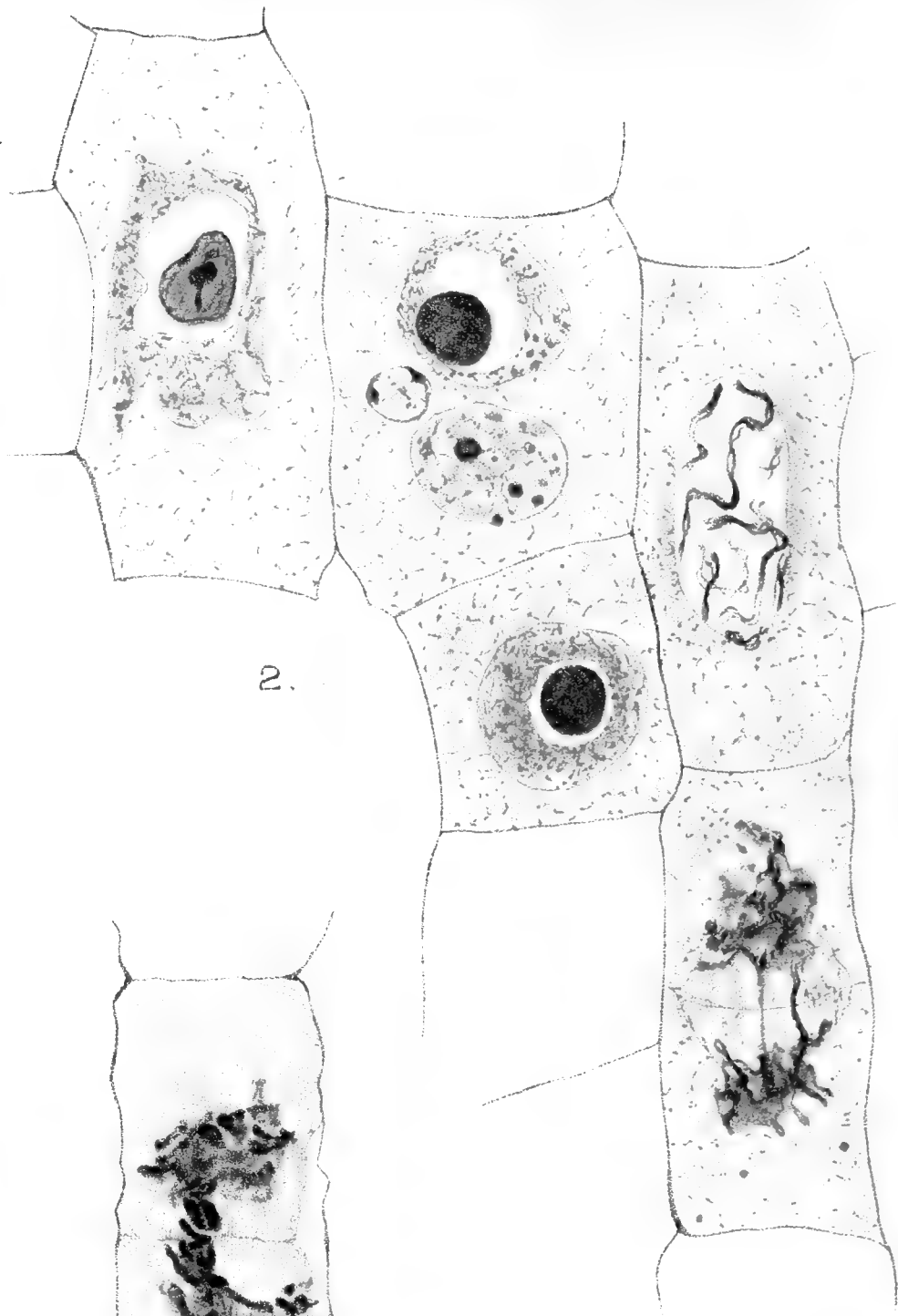
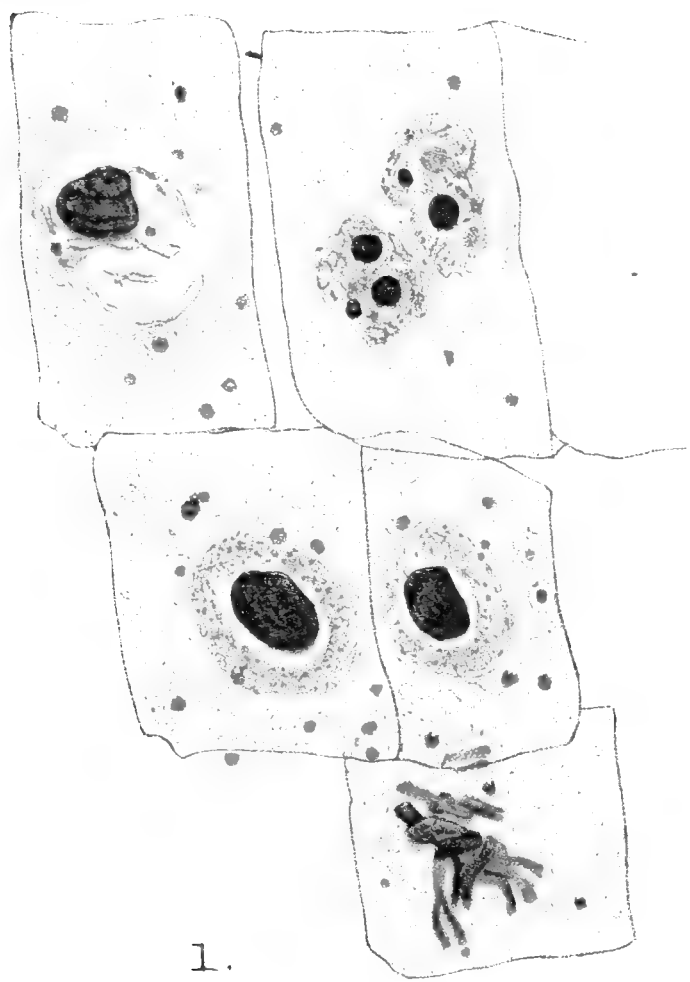
Fig. 26. Longitudinal section of tetraploid cell from root of *Pisum sativum* after 22 hours' growth subsequent to  $1\frac{1}{2}$  hours' immersion in 0.75 per cent. chloral hydrate; shows 28 chromosomes of pronounced 'heterotypical' shape.

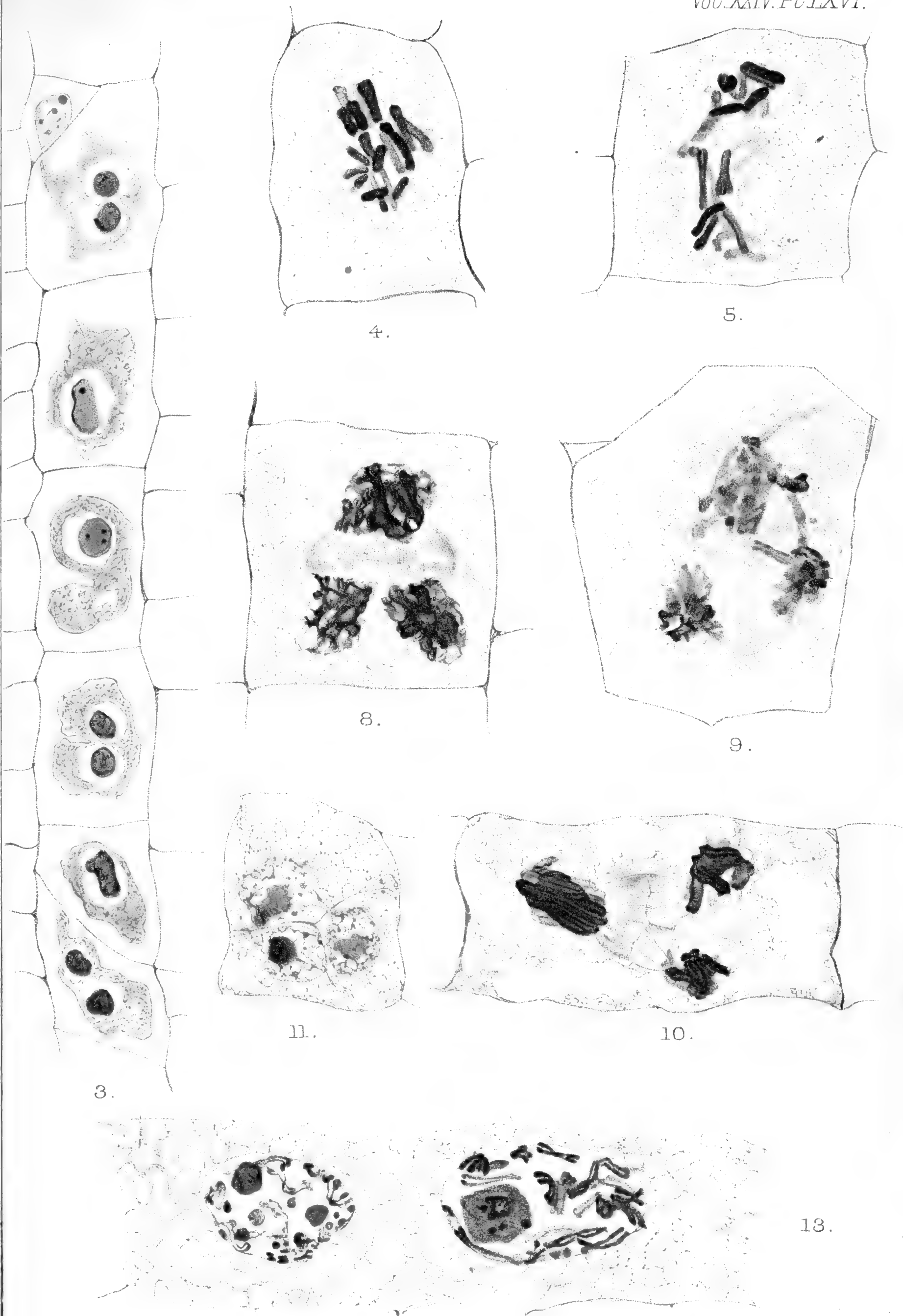
Fig. 27. Cell from longitudinal section of root of *Pisum sativum*; shows 'heterotypical' chromosomes coming out of the spireme stage.

Fig. 28. Cell from normal *Pisum sativum*; shows parallel arrangement of chromosomes on equatorial plate.







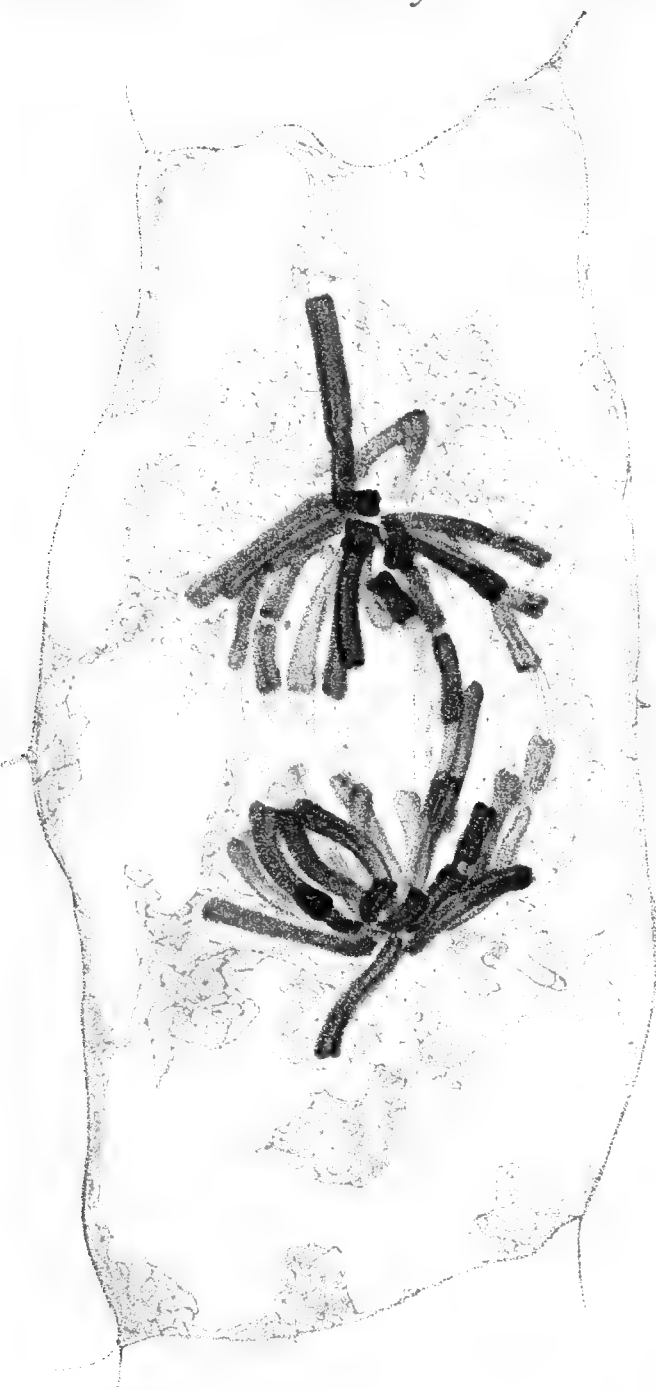


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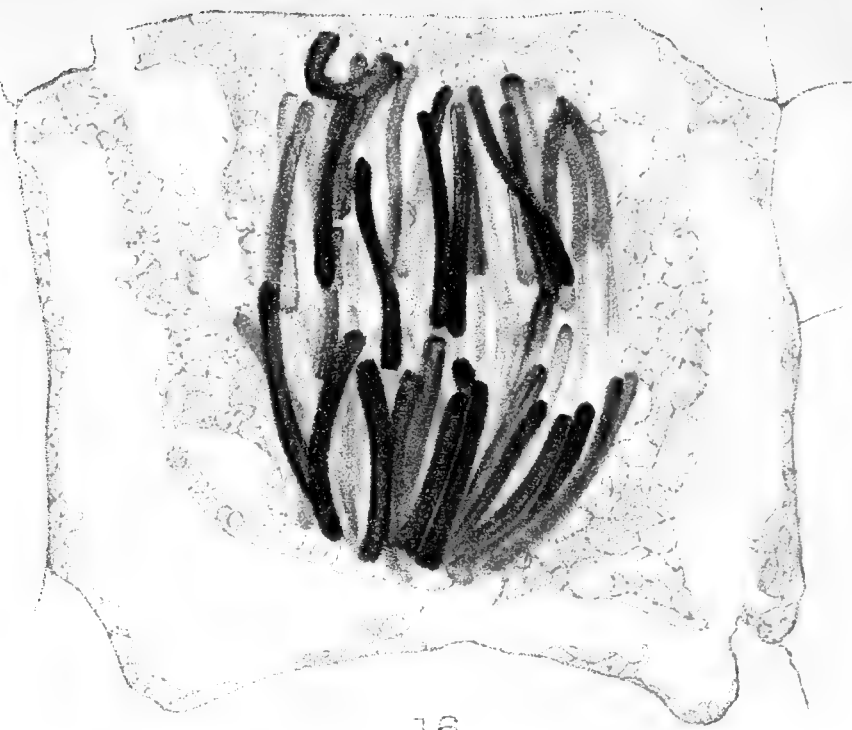




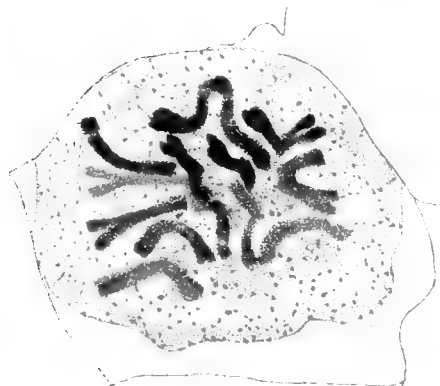




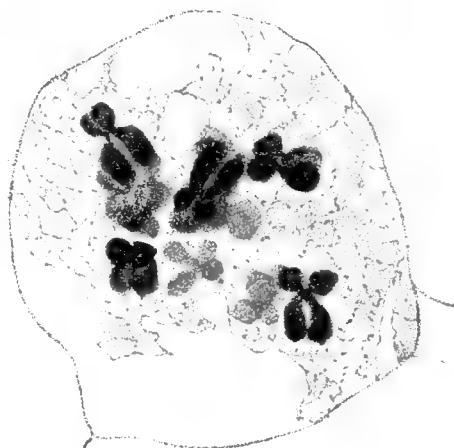
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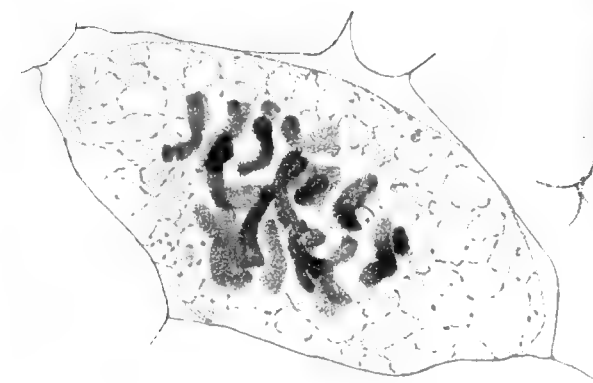
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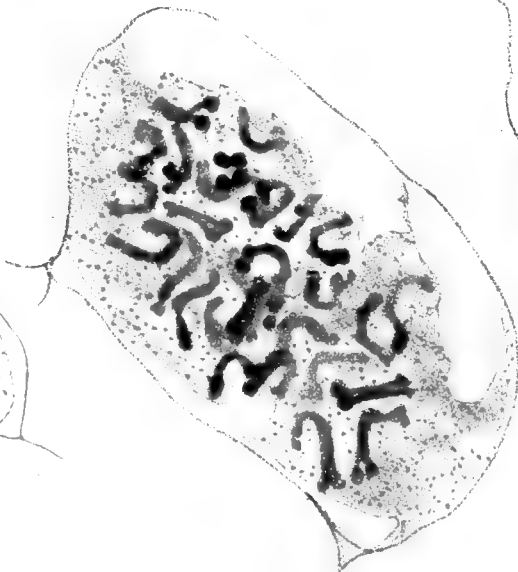
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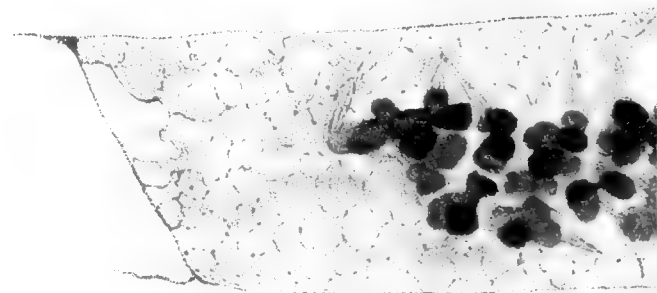
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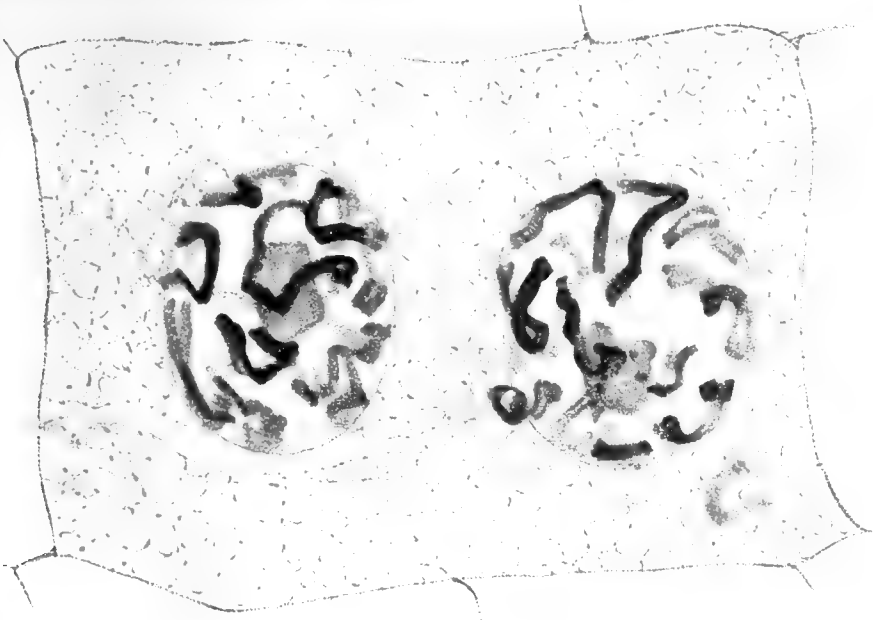


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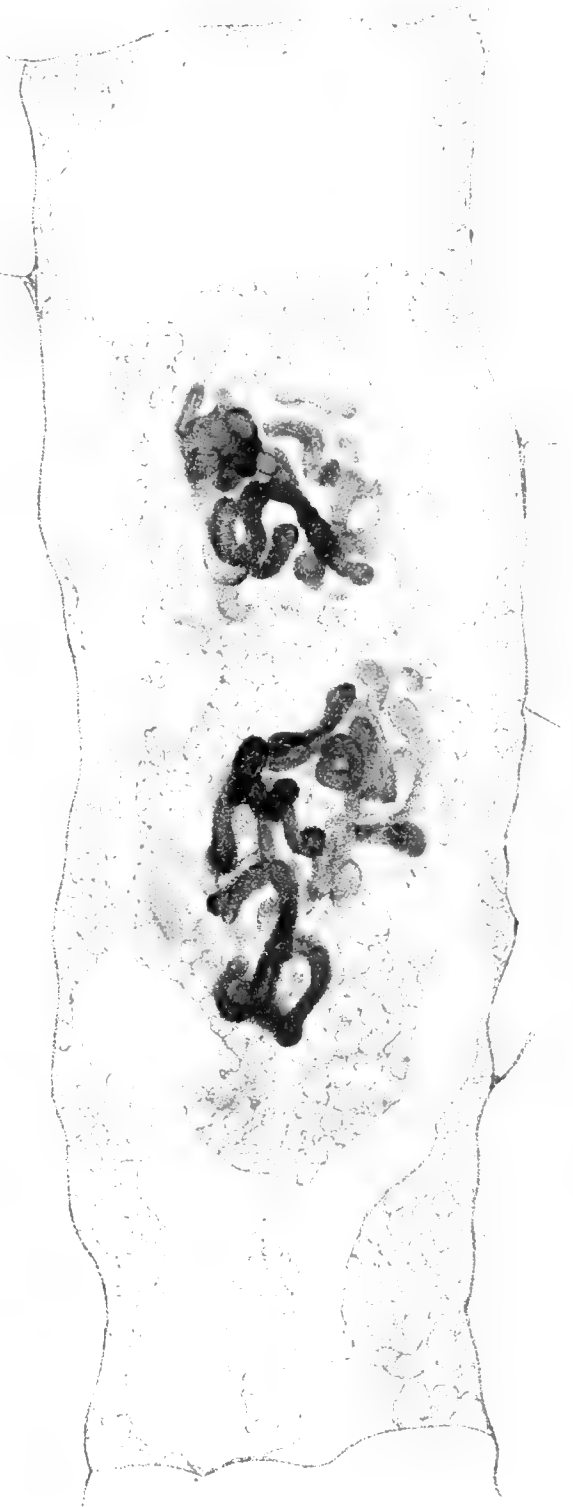


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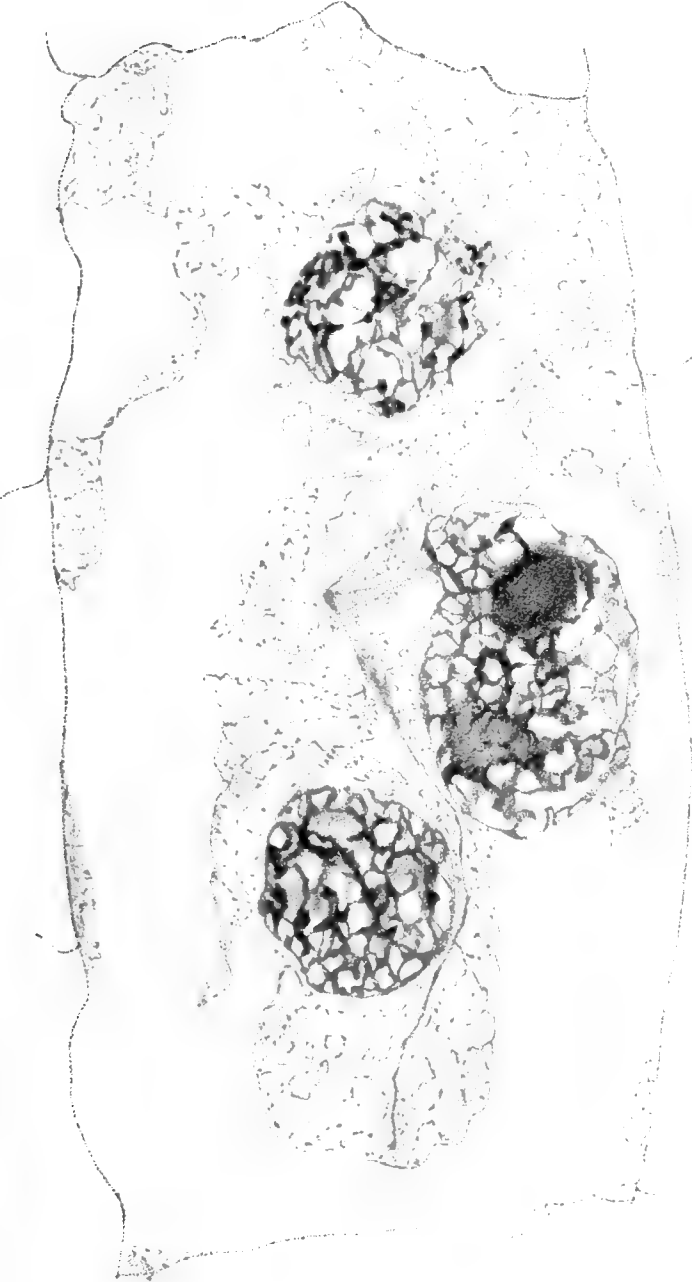
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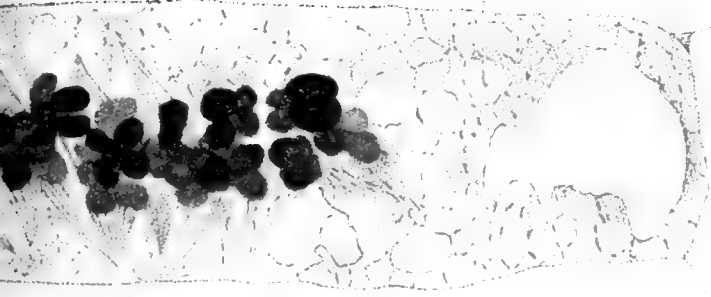
21.



27.



28.



26.



# The Beginning of Photosynthesis and the Development of Chlorophyll.<sup>1</sup>

BY

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*Newnham College, Cambridge.*

With ten Figures in the Text.

THIS work deals with the question of how soon the power of photosynthesis attains an appreciable magnitude when young leaves are developing in light, and when leaves that have been etiolated in the dark are exposed to light and turn green.

Several investigators have attributed to etiolated chloroplasts the power of assimilating  $\text{CO}_2$  in the light, before they develop detectable amounts of chlorophyll. The evidence that was brought forward in support of this has been in all cases indirect, and based almost entirely upon the reaction of bacteria in the Engelmann method.

At Dr. F. F. Blackman's suggestion I have carried out direct gasometric investigations on this point. The method adopted has been to measure the output of  $\text{CO}_2$  in the respiration of etiolated or greening shoots alternately in dark and light. The first dawning of the power of photosynthesis should thus be made evident by the amount of respiratory  $\text{CO}_2$  in the light being consistently smaller than that in the dark by just that fraction which the shoot was capable of assimilating.

The power of photosynthesis was expected to augment as the shoot gradually became greener, and even if the yellow tissues should show no detectable assimilation of  $\text{CO}_2$ , the green should do so, and one would have been able to say at what tint of greenness the function reached an appreciable magnitude.

<sup>1</sup> This paper constitutes Part VII of 'Experimental Researches on Vegetable Assimilation and Respiration'. The earlier papers of this series carried out at Cambridge under the general direction of Dr. F. F. Blackman are:—I and II, Blackman, *Phil. Trans. Roy. Soc. B*, 1895; III, Matthaei, *Phil. Trans. B*, 1904; IV, Blackman and Matthaei, *Roy. Soc. Proc. B*, vol. 76; V and VI, Thoday, *Roy. Soc. Proc. B*, vol. 82.



It was thought that in such a case the whole assimilatory apparatus might be efficiently developed, except the green pigment, and as this increased by degrees, so the power of photosynthesis would increase. Thus the amount of chlorophyll present would then be the limiting factor for assimilation, and interesting data might be looked for relating the amount of the pigment present to the amount of photosynthesis that could be affected.

Contrary to expectation it was found that not only etiolated shoots of a deep orange yellow possessed no measurable power of assimilation, but shoots which had developed quite a considerable depth of green had not yet attained this power.

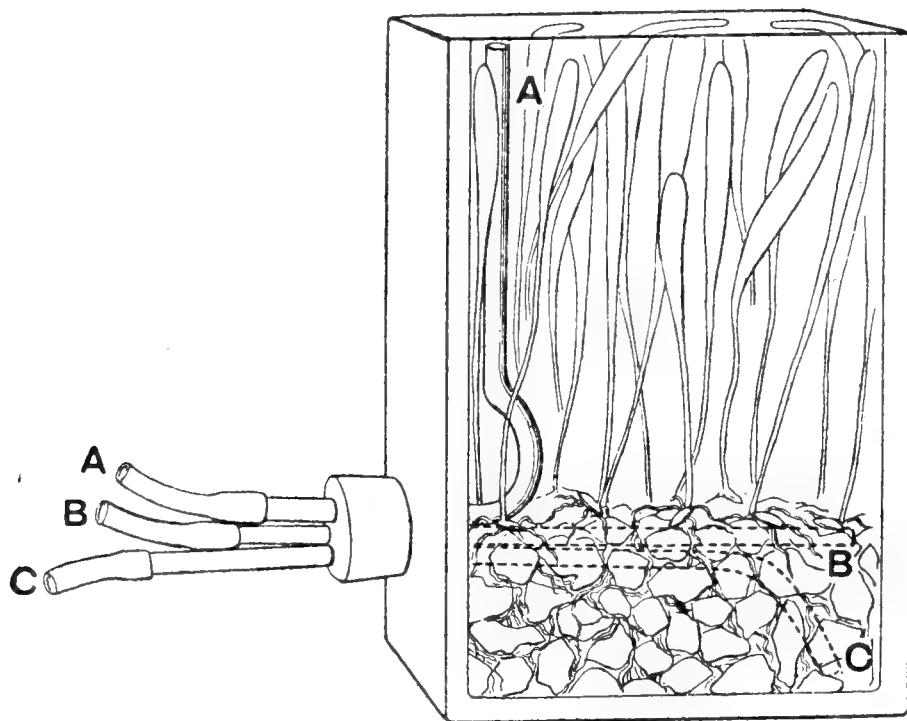


FIG. 1.

Only when a full grass-green colour was attained was the assimilation of  $\text{CO}_2$  sufficient to cause the amount of  $\text{CO}_2$  given out in the light to be unmistakably less than the amount given out in the dark.

In searching for the first faint signs of assimilation it is unnecessary to add  $\text{CO}_2$  to the air passing over the plant; the  $\text{CO}_2$  supplied by the plant's own respiration will serve as material for its photosynthetic activity. This source of  $\text{CO}_2$  has the further advantage of being produced within the plant itself, so that there are no difficulties of diffusion to be overcome, such as might arise with a cuticularized organ in which the stomata were insufficiently developed to freely admit the passage of  $\text{CO}_2$  from the air surrounding the plant. The experiments carried out with whole seedlings of Barley may first be described and then those more critical ones done with cut shoots of Barley and *Vicia Faba*.

For these first experiments Barley was germinated in a glass cell through which a current of air could be drawn and the output of  $\text{CO}_2$  measured continuously. The arrangement is shown in Fig. 1. The glass

cell measures  $7\frac{1}{2}$  inches by  $4\frac{1}{2}$  inches by  $2\frac{1}{2}$  inches, and the lower part contains a stratum of broken pot on which the grains (100) are germinated. A cork at one side admits three tubes, and the cell can be closed above by a thin glass plate waxed down air-tight. The air current enters at B and leaves the chamber by A. During the early stages of growth the jar, not yet sealed at the top, is kept completely darkened. Every other day the stagnant water is drawn off by C and fresh water is added from above, C being now closed by a clamp. When the seedlings have reached the desired age, the lid is sealed on, and the jar brought out of the dark room and placed within one inch of a north window in the laboratory, with a white paper screen behind it. It had previously been ascertained that the diffuse light in this position is strong enough to enable green seedlings to assimilate practically the whole of their own respiratory  $\text{CO}_2$ .

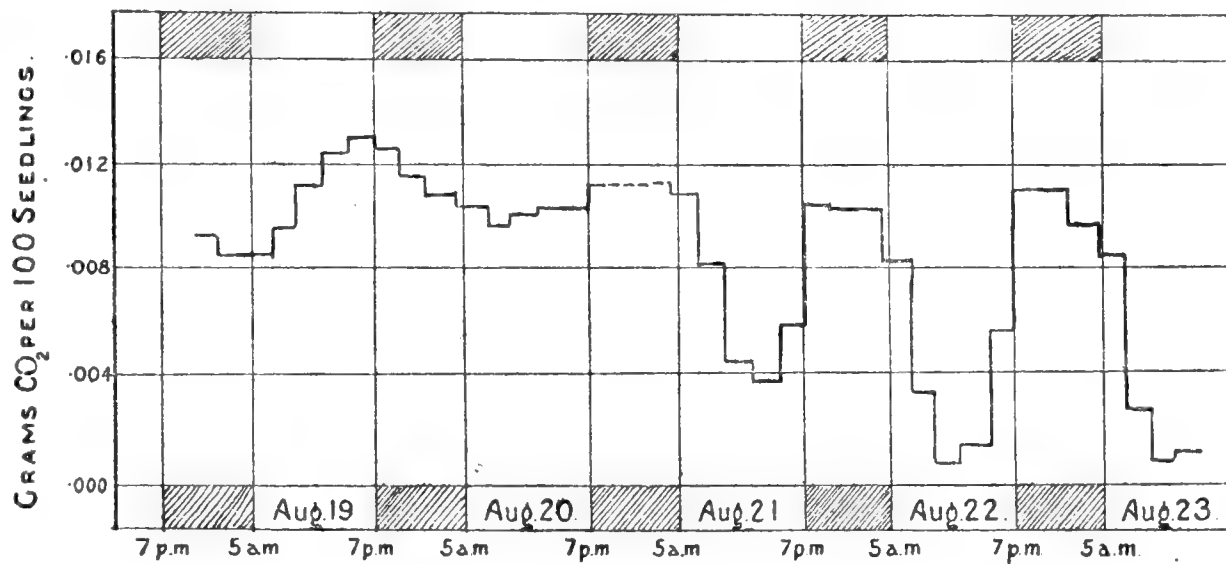


FIG. 2.

In Exp. I the jar was set up in the window five days after sowing the seeds, when most of the plants showed the first colourless sheathing leaf-base one inch tall, and none of the functional leaves had yet burst through the tip of the sheath. The intention was to follow the development with the natural alternation of day and night, and see how soon the power of photosynthesis would be equal to assimilating the bulk of the respiratory  $\text{CO}_2$ .

Fig. 2 shows the magnitude of the carbon-dioxide output measured continuously for five days. The periods of natural darkness 7 p. m.—5 a. m. each day are shaded in the diagram. The stepped curve shows the respiration in grams per hour for the 100 seedlings, estimations being taken every three hours by the procedure mentioned on p. 809. The days were very dull throughout and the temperature in this spot was between  $18^\circ$  and  $20^\circ$  C. by day, falling to  $15^\circ$  or  $16^\circ$  C. by night. The readings began at 10 p. m. on August 18. On August 19 there were still no leaves through the sheath, and by day respiration rose with the temperature, falling again

at night. On August 20 the leaf-tips of bright green protruded about half an inch through the sheath, but there was no sign of appreciable reduction of the respiration by photosynthesis. On August 21 the green leaves were  $1\frac{1}{4}$  inches long and the photosynthesis was slight but certain, the  $\text{CO}_2$  falling to 0.004 gram in the middle of the day. On August 25 the leaves were 3 inches long and could assimilate about  $\frac{1}{11}$  of the respiration. This was the maximum activity that this particular arrangement of apparatus allows, and on August 25, when the leaves were 5 inches high, some reaching to the glass roof, the photosynthesis was not found to be any greater.

The general teaching of the experiment is that photosynthetic power lags behind greenness in natural development more than might have been expected, only catching up the respiration on the third day after the green leaf begins to show. In the bacterial test for photosynthesis it is this moment, when carbon-dioxide fixation overpowers carbon-dioxide pro-

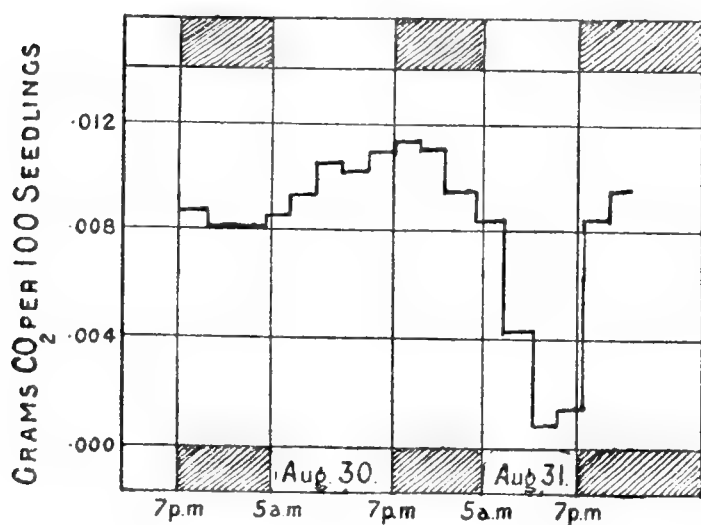


FIG. 3.

The barley seedlings were therefore allowed to develop in complete darkness until the protruded leaves were about 5 inches long, reaching nearly to the top of the cell. The cell was closed, placed in position in the window, and estimations started at 5 p. m. on August 29, being, however, completely darkened by a cloth till night had set in. On August 30, the first day of illumination, the bright yellow etiolated leaves developed a considerable tint of green except at the leaf-tips, but Fig. 3 shows that there was no diminution of the respiratory output of  $\text{CO}_2$  until the subsequent day, when the photosynthetic power quickly became equal to assimilating  $\frac{1}{11}$  of the respiration.

It appears, therefore, that there is the same lag of photosynthetic activity behind the development of chlorophyll in the illuminated etiolated leaves as in the younger leaves developing in natural illumination. The only previous analytical data on this subject with which I am acquainted are the measurements made by Wiesner<sup>1</sup> thirty-three years ago. He seems to have

<sup>1</sup> Wiesner: Die Entstehung des Chlorophylls. Wien, 1877, pp. 107-111.

duction, that brings on the motile response of the bacterium, but in the present experiments the chloroplast activity has to contend with the carbon-dioxide production of the whole plant, not of its particular mesophyll cell only.

The second experiment of this type was designed to see if there would be a similar lag in photosynthetic activity when leaves that are fully protruded but etiolated in darkness are turning green in diffuse light.

obtained quite contrary results to those here arrived at, and he concludes that  $\text{CO}_2$  somehow is concerned in the development of chlorophyll from etiolin, because he finds that less  $\text{CO}_2$  escapes during illumination in the early stages of greening than in a period of darkness. The differences that he observed were not great and not always in the same direction, but it is not easy to explain the general lack of agreement with the present work, especially as Wiesner also used Barley seedlings.

In order to examine this question more critically similar experiments were made with cut tips of etiolated shoots of *Vicia Faba* and with cut etiolated leaves of Barley, exposed to a constant artificial light. In cut shoots there is no longer the respiration of roots, seeds, and colourless parts to be contended with, and the faintest beginnings of photosynthesis should be easily detected.

The cut shoots were placed in a different type of chamber, shown in Fig. 4,<sup>1</sup> which has a much smaller internal volume. It is of the same pattern as the chambers described by Blackman and Matthaei in the fourth paper of this series. The air current enters at D and leaves at E, having passed through a calcium chloride tube, G, to prevent the water evaporated from the wet chamber being subsequently deposited and blocking the tubes. Tube F is for supplying water.

The chamber is supported vertically on a brass stand and is submerged in a large cubical copper bath kept at a constant temperature.

On the side of the bath in front of the chamber is a large glass window, through which the etiolated shoots can be illuminated by a pair of Keith high-pressure incandescent gas-burners, kept at a standard distance from the chamber.

A continuous current of air freed from  $\text{CO}_2$  is sucked by an aspirator through the submerged chamber. On leaving at E it passes to a set of parallel Pettenkofer tubes each containing a known quantity of standard

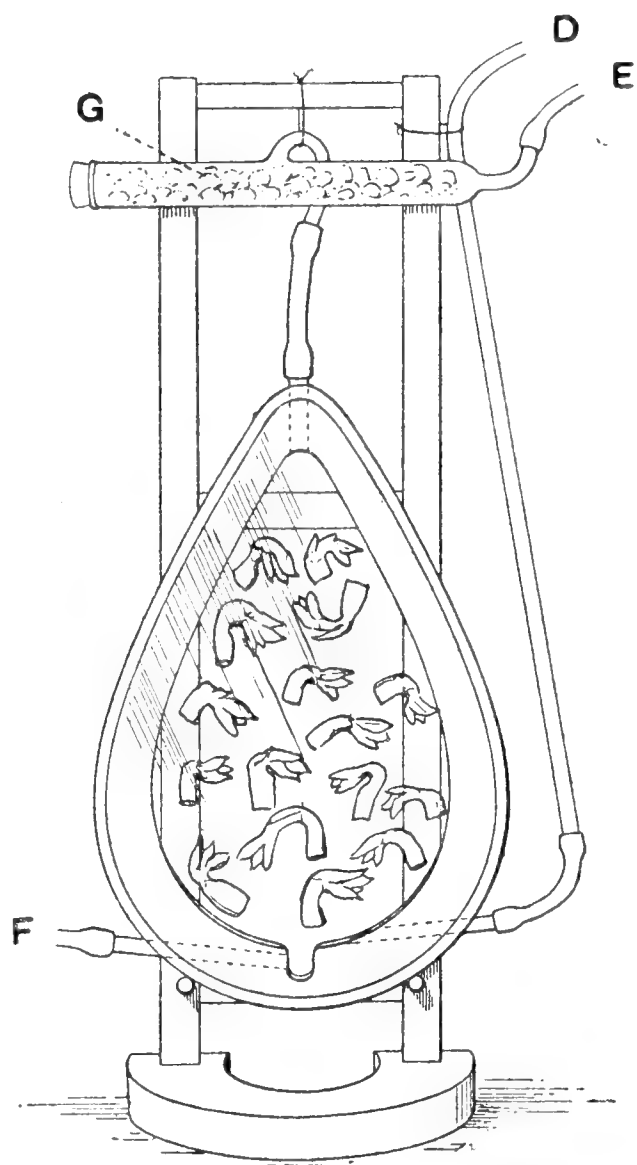


FIG. 4.

<sup>1</sup> The etiolated bean shoots shown in the figure are held lightly in position by pressure between the glass front and the glass back of the chamber.

baryta solution. The current was moved consecutively from one tube to another by clockwork at intervals of two hours.

The tubes which had been used were removed, titrated, and refilled, at convenient times.

The method used for titration was to wash out the contents of the tubes with a constant amount of boiled distilled water into a beaker and titrate with  $N/10$  HCl, using phenolphthalein as an indicator. The tubes were then refilled with baryta solution, and replaced in the stand. Contamination of  $CO_2$  from the atmosphere during washing, titrating, and refilling was allowed for by a small 'washing factor' obtained from a number of controls carried out for that purpose.

As the temperatures of all the experiments, with one exception, were approximately constant the readings are directly comparable.

Before proceeding to experimental data it is necessary to consider what would happen to the respiration of a shoot or leaf, if it were cut off from its source of food material, and kept continuously in the dark.

In such a starved condition there is always a considerable fluctuation in the successive respiration readings when these are of such short periods as one or two hours, however constant the temperature may be kept; more especially is this the case when the organ is at first placed under the experimental conditions. Nevertheless, there is a general broad regularity underlying these fluctuations, according to which the respiration remains at a uniform *average* level for the first few hours (the exact time depending on the temperature), and then begins to fall in a regular curve, at first rather rapidly, and then slower and slower towards a lessened uniform value.

The different experiments all illustrate this, as well as the general truth that, the higher the temperature, the steeper is this curve of declining respiration.

### *Experiment III.*

We may take first an experiment made with etiolated Barley seedlings. The plants were germinated in the dark, and when the first foliage shoots were about 6 inches high, the shoot was cut off at the level of the soil. Enough of these shoots were placed vertically upright in the chamber to make a continuous layer of deep yellow etiolated leaf material. They were kept in position by being lightly held by the two glass plates of the chamber, there being water in the bottom of the chamber to moisten the air.

The chamber was set up on its stand and placed in the bath, regulated to a temperature of  $25.2^\circ$  C. facing the window, and the current of  $CO_2$ -free air started through it, with the bath carefully darkened.

After a preliminary of two hours a continuous series of twenty-one two-hour estimations of the respiration was carried out. Table I records the results; and in the last column are inserted notes of the colour that was



TABLE I.  
ETIOLATED LEAVES OF BARLEY.

No. of Reading.	Grams of CO <sub>2</sub> given out.	Illumination.	Colour of Leaves.
1	.0035	Dark	Orange yellow
2	.0032	"	
3	.0036	Light	Traces of green
4	.0035	"	
5	.0030	"	Dark
6	.0028	Dark	
7	.0029	"	"
8	.0024	"	
9	.0028	"	Light
10	.0026	Light	
11	.0024	"	Pale green
12	.0020	"	
13	.0022	Dark	"
14	.0020	"	
15	.0021	"	"
16	.0019	"	
17	.0018	"	Light
18	.0018	Light	
19	.0020	"	Grass green
20	.0021	Dark	
21	.0020	"	

attained by the leaves, due to the cumulative effect of the separate periods of illumination.

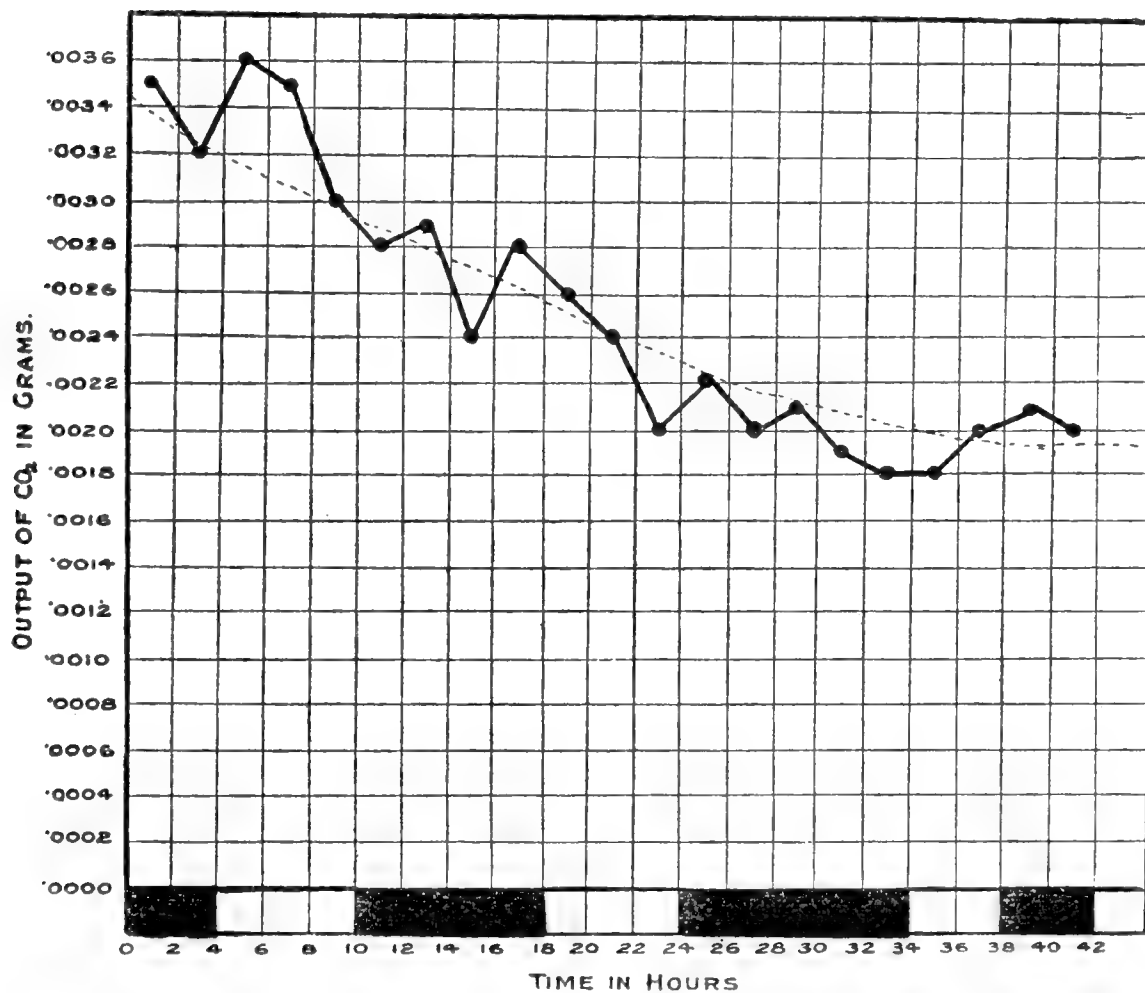


FIG. 5.



The respiration fluctuates considerably, but obviously there is no marked lowering of the output of  $\text{CO}_2$  during the light periods. Fig. 5 enables us to compare respiration in the dark and light more precisely, and the dark periods are indicated by the black bands along the bottom of the graphic.

The dotted line indicates what would be the ideal form of the curve of respiration in continuous darkness, and it is clear that the fluctuations *below* this do not preponderate in the light periods any more than those *above* preponderate in the dark.

As their distribution was entirely random there is not the slightest evidence of photosynthetic activity in the light, although at the third period of illumination the leaves were grass green.

#### *Experiment IV.*

The rest of the experiments were made with a very different material, etiolated shoots of *Vicia Faba*. The seeds were germinated in the dark, and

TABLE II.  
ETIOLATED SHOOT-TIPS OF *Vicia Faba*.

<i>No. of Reading.</i>	<i>Grams of <math>\text{CO}_2</math> given out.</i>	<i>Illumination.</i>	<i>Colour of Leaves.</i>
1	.0025	Dark	Orange yellow
2	.0040	"	
3	.0033	"	
4	.0029	"	
5	.0024	Light	Traces of green
6	.0024	"	
7	.0022	Dark	
8	.0021	"	
9	.0019	"	
10	.0018	"	
11	.0017	"	
12	.0015	"	
13	.0015	"	
14	.0014	"	
15	.0015	"	
16	.0019	Light	Very pale green
17	.0019	"	
18	.0018	"	
19	.0021	Dark	
20	.0020	"	
21	.0018	"	
22	.0017	"	
23	.0016	"	
24	.0018	"	
25	.0019	Light	Grass green
26	.0020	"	
27	.0015	"	
28	.0015	"	
29	.0019	"	
30	.0019	"	



FIG. 6.

TABLE III.

ETIOLATED SHOOT-TIPS OF *Vicia Faba*.

No. of Reading.	Grams of CO <sub>2</sub> given out.	Illumination.	Colour of Leaves.
1	.0021	Dark	Orange yellow
2	.0017	"	
3	.0015	"	
4	.0014	"	
5	.0014	Light	
6	.0015	"	Traces of green
7	.0015	"	
8	.0016	Dark	
9	.0015	"	
10	.0015	"	
11	.0016	"	
12	.0014	"	
13	.0017	Light	
14	.0014	"	
15	.0014	"	
16	.0013	"	Pale green
17	.0012	"	
18	.0012	Dark	
19	.0012	"	
20	.0015	"	
21	.0015	Light	
22	.0013	"	
23	.0015	"	
24	.0011	"	Grass green
25	.0011	"	
26	.0012	Dark	
27	.0011	"	

when the shoots were some 4 inches high the tops which bore the undeveloped leaves folded together were cut off about  $1\frac{1}{2}$  inches long. About twenty of these, weighing about 14 grams, were used for each experiment.

In this experiment the shoots were of a deep orange colour at the beginning, and a series of thirty two-hour estimations of the respiration was started, in the course of which there were three periods of illumination as shown in Table II. The temperature varied between  $22.6^{\circ}$  C. and  $23^{\circ}$  C.

On inspection of Fig. 6 it will be seen that readings 5 and 6 in the light are lower than any of the four readings that precede them, but this is clearly not evidence of photosynthesis but only the result of the general fall of the respiration that accompanies starvation. In the second light period the readings are on the contrary higher than those just preceding them. While in the third light period the average output of  $\text{CO}_2$  is about the same as in the dark. Therefore these are merely chance variations.

#### Experiment V.

This was an experiment with similar material, i. e. etiolated shoots of *Vicia Faba*, but it was carried out at a much lower temperature,  $12^{\circ}$ – $12.6^{\circ}$  C.

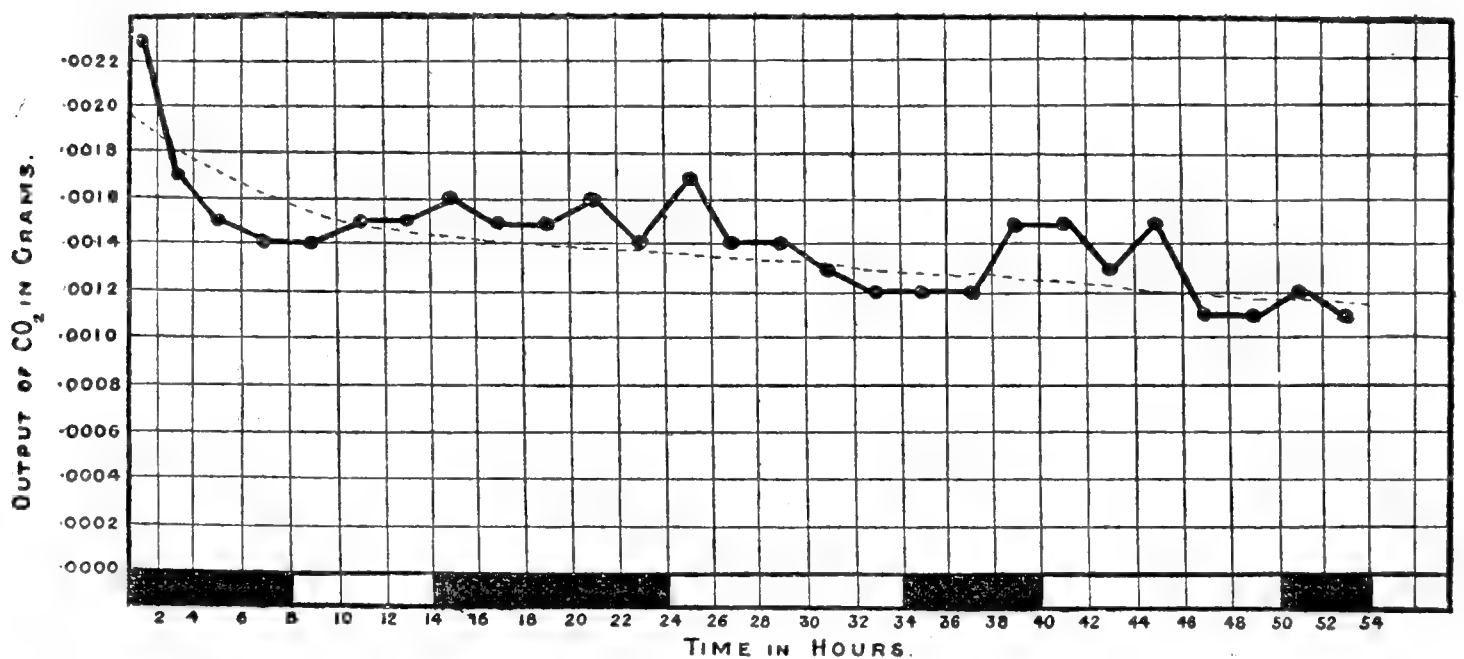


FIG. 7.

The general decline in the respiration (see Table III) is therefore very much less, and, as shown in Fig. 7, the average  $\text{CO}_2$  production in the light is the same as that in the dark throughout the experiment.

#### Experiment VI.

The early part of this experiment proceeded just as in the previous ones, and there was no sign of photosynthesis up to the twenty-fourth hour, by which time the shoots had become grass green (Table IV). Then the chamber was taken out of the bath, and with the shoots still in it, was placed in an upright position close to a north window. Here it was left

from Saturday mid-day to early Tuesday morning. This exposure for two successive days to diffuse light caused the leaves to become almost the deep green colour of the normal bean plant, though the stems remained pale.

TABLE IV.

ETIOLATED SHOOT-TIPS OF *Vicia Faba*.

No. of Reading.	Grams of CO <sub>2</sub> given out.	Illumination.	Colour of Leaves.
1	.0021	Dark	Distinct pale green
2	.0021	"	
3	.0017	Light	
4	.0016	"	
5	.0015	"	
6	.0016	"	
7	.0016	"	
8	.0015	Dark	
9	.0016	"	
10	.0015	Light	
11	.0019	"	
12	.0018	"	
Chamber containing shoots in north window for 60 hours including 34 hours of daylight.			
13	.0001	Light	Almost normal green
14	.0001	"	green
15	.0000	"	Normal green

At last the power of photosynthesis was developed by this prolonged illumination, and upon replacing the chamber in the bath and determining

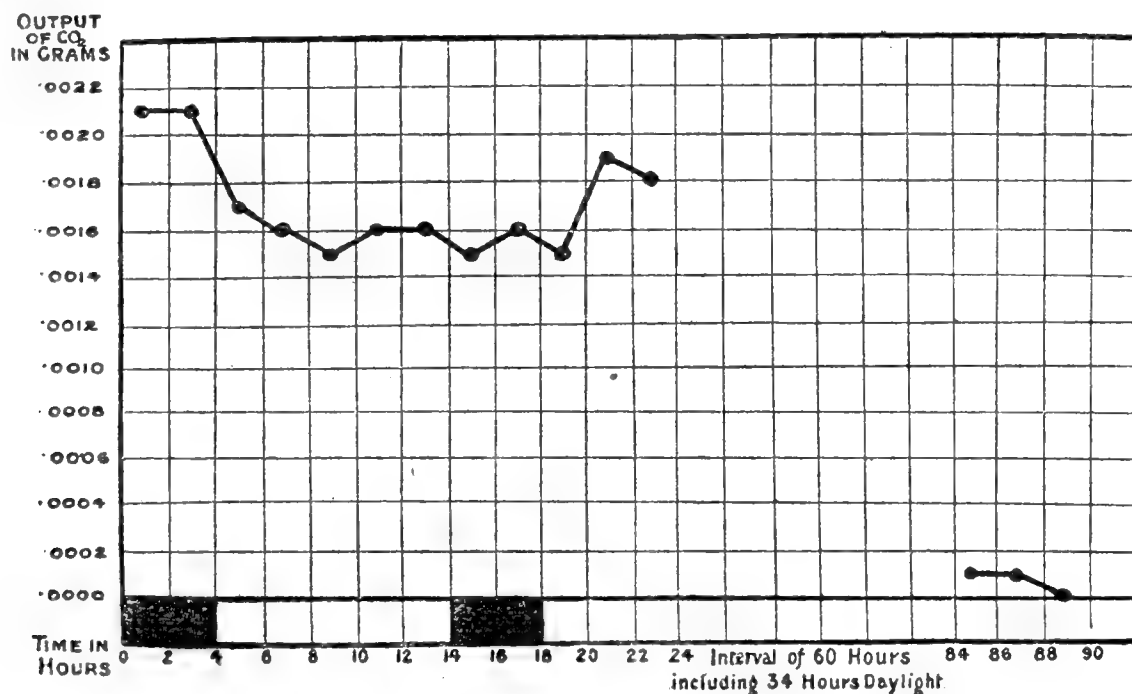


FIG. 8.

the output of CO<sub>2</sub> in the light, it was found that the whole of the CO<sub>2</sub> of respiration was assimilated, and none escaped into the baryta tubes (Fig. 8).

TABLE V.

ETIOLATED SHOOT-TIPS OF *Vicia Faba*.

Exposed to daylight before the beginning of the experiment.

No. of Reading.	Grams of CO <sub>2</sub> given out.	Illumination.	Colour of Leaves.
1	.0021	Dark	Pale green
2	.0017	"	
3	.0015	"	
4	.0016	Light	
5	.0022	"	
6	.0020	"	Grass green
7	.0019	Dark	
8	.0012	"	
9	.0011	"	
10	.0012	"	
11	.0011	"	
12	.0010	"	
13	.0009	Light	
14	.0009	"	
15	.0007	"	Darker green, but not yet normal green
16	.0009	Dark	
17	.0007	"	
18	.0008	"	
19	.0008	"	
20	.0009	"	

*Experiment VII.*

In this experiment the etiolated *Vicia Faba* seedlings, while still growing in pots, were placed in the light (4 p.m.—10.30 a.m.) till they turned pale green. They were then cut and experimented upon as before (see Table V);

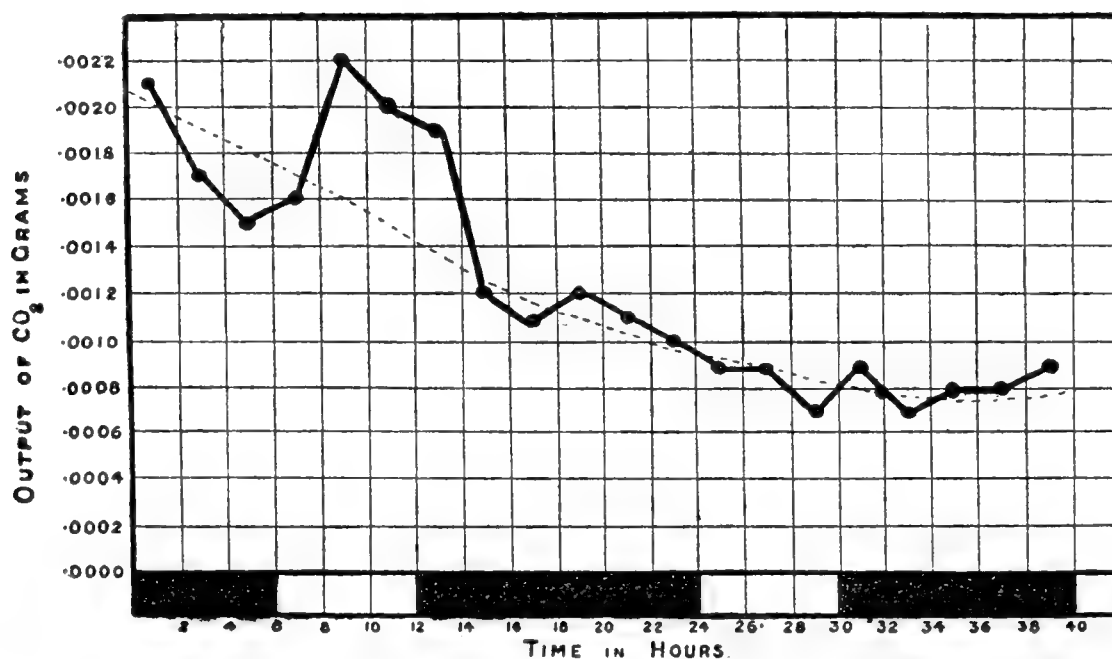


FIG. 9.

the temperature was 25° C. Here also there was no sign of diminished CO<sub>2</sub> output in the light, although at the second period of illumination the leaves

were quite deep green. On the contrary in the first light period in Fig. 9, it will be seen that there was a marked rise in the curve, though this was probably a big chance fluctuation in the level first part of the starvation curve of respiration.

TABLE VI.

SHOOT-TIPS OF *Vicia Faba*.

Developed in dark but exposed, growing, to daylight until their normal green colour was attained.

No. of Reading.	Grams of CO <sub>2</sub> given out.	Illumination.	Colour of Leaves.
1	.0027	Dark	Leaves normal dark green
2	.0022	"	
3	.0021	"	
4	.0010	Light	
5	.0007	"	
6	.0000	"	
7	.0015	Dark	
8	.0016	"	
9	.0015	"	
10	.0014	"	
11	.0010	"	

Experiment VIII.

The last experiment to be recorded completes the series by showing that etiolated shoots of *Vicia* seedlings, which have been kept growing in daylight longer than those of Exp. VII, until they turned their normal dark green colour, were able to assimilate the whole of the CO<sub>2</sub> of respiration.

The detailed results are given in Table VI and Fig. 10.

A series of eleven two-hour readings was made. As soon as the respiration had settled down the chamber was illuminated and there was at once a big drop in the CO<sub>2</sub> output, which declined rapidly to nothing in reading 6.

No doubt the photosynthetic power was by that time sufficiently developed to enable the shoot to utilize additional CO<sub>2</sub> from the outer air had any been provided. On darkening, the respiration rose again to continue the ordinary curve of slowly decreasing values, indicated by the broken line.

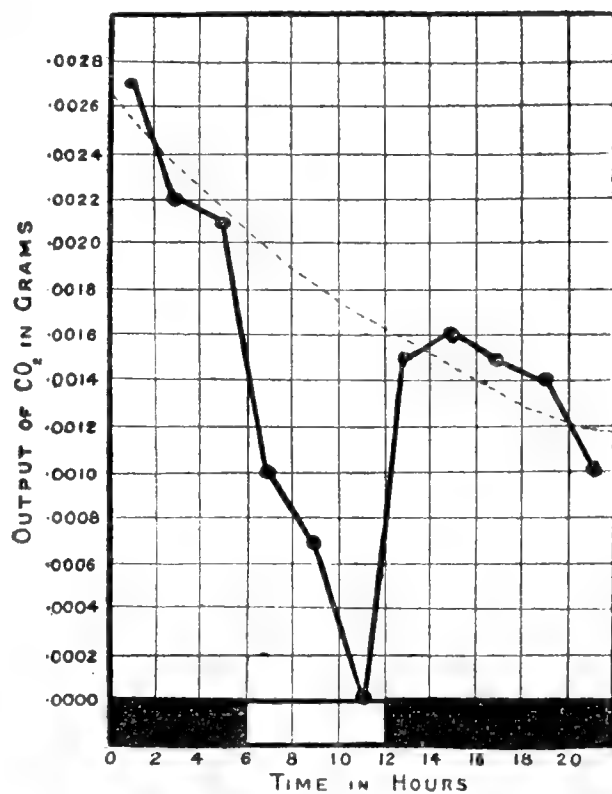


FIG. 10.



In many of these experiments with cut shoots the development of chlorophyll is very slow. This is no doubt to be attributed to the lack of carbohydrate reserves in these growing shoots, as Palladin<sup>1</sup> has shown that the rate of greening of etiolated shoots is closely correlated with the presence of sugar.

There seems to be no reason to regard this slow greening as detrimental to the validity of the conclusions drawn here.

#### CONCLUSION.

It is clearly established by the foregoing experiments that etiolated leaves do not possess any appreciable power of carrying out photosynthesis of CO<sub>2</sub>, either when they are orange yellow, or when they have developed a large part of their green chlorophyll.

It is possible that there may be present a minute potentiality of photosynthesis at this stage, too small to be detected gasometrically as even a lowering of the respiration, but it is clear that this does not amount to one-tenth part of the respiration, and certainly not 1 per cent. of the activity subsequently developed.

When the power of photosynthesis does appear, after the leaves have attained almost a full green colour, it develops very rapidly.

We are forced to conclude that the first development of this function is not in any relation to the amount of chlorophyll produced, and that the amount of chlorophyll present is never a limiting factor to assimilation in these early stages of the assimilating organs.

If this is so, then it must be some other component part of the photosynthetic machinery which controls the beginning of complete functional activity. This part is not developed by illumination so quickly as the green pigment is developed, and therefore the pigment, and other parts of the total machinery, lie idle at the stage we have examined awaiting the development of the last factor.

Finally, I would like to express my thanks to Dr. Blackman for his help in the experimental part and his kind interest throughout the work.

<sup>1</sup> Palladin : Ergrünen u. Wachsthum. Ber. deut. Bot. Ges., ix, 1891.

## NOTES.

**SPORANGIA ATTRIBUTED TO BOTRYOPTERIS ANTIQUA, KIDSTON.**—*Botryopteris antiqua*, the most ancient and simplest species of the genus, was first described by Mr. R. Kidston, F.R.S., in 1908,<sup>1</sup> from the well-known plant-bearing bed, of Lower Carboniferous age, at Pettycur, near Burntisland. The petioles and smaller branches of the rachis are common in the Pettycur material, though the stems are not often met with. M. F. Pelourde has recently identified the species in the 'Culm' of Esnost, near Autun.<sup>2</sup> Associated with the petioles we commonly find small sporangia, quite similar to those which so usually occur with the Coal-measure species *B. hirsuta* and *B. ramosa*,<sup>3</sup> but somewhat smaller. The sporangia are of an approximately spherical form; the wall is one cell thick and provided with a broad plate of enlarged cells resembling the areola or false annulus of the Osmundaceae. Sporangia of this type have often been observed in petrifications of Carboniferous age; they have recently been described and figured by M. Pelourde, from the same block in which he found the Autun specimens of *Botryopteris antiqua*.<sup>4</sup> He does not venture to draw any conclusion as to the plant to which the sporangia belonged, though the probability of their connexion with the *Botryopteris* was evidently present to his mind. Neither has there been, as yet, any decisive ground, apart from analogy, for specially connecting the Pettycur sporangia with the associated *Botryopteris*.

In examining a section, cut by Mr. W. Hemingway from a block of my Pettycur material, I observed a group of the sporangia in question in such close association with a rachis of *Botryopteris antiqua* as to strongly suggest a connexion between them. The sporangia are four in number and are ranged in a quadrangular group, as shown in the sketch, immediately opposite a small *Botryopteris* rachis, the upper surface of which is turned towards them. The whole arrangement is exceedingly definite and appears unlikely to be accidental. The sporangia are well preserved; in each of them the characteristic multiseriate annulus is conspicuous (see Figure). They are full of spores, many of which have a marked triangular form, probably exaggerated by contraction, just as is the case in the spores of the sporangia associated with *B. ramosa*.<sup>5</sup> The flat plate of cells (marked *i* in the Figure) lying between two of the sporangia suggests a kind of indusium; it will be remembered that an elaborate

<sup>1</sup> On a new species of *Dineuron* and of *Botryopteris* from Pettycur, Fife. Trans. Roy. Soc., Edinburgh, vol. xlvi, Part II, No. 16, 1908.

<sup>2</sup> Observations sur quelques végétaux fossiles de l'Autunois. Ann. Sci. Nat., Bot., 9<sup>e</sup> série, t. xi, 1910.

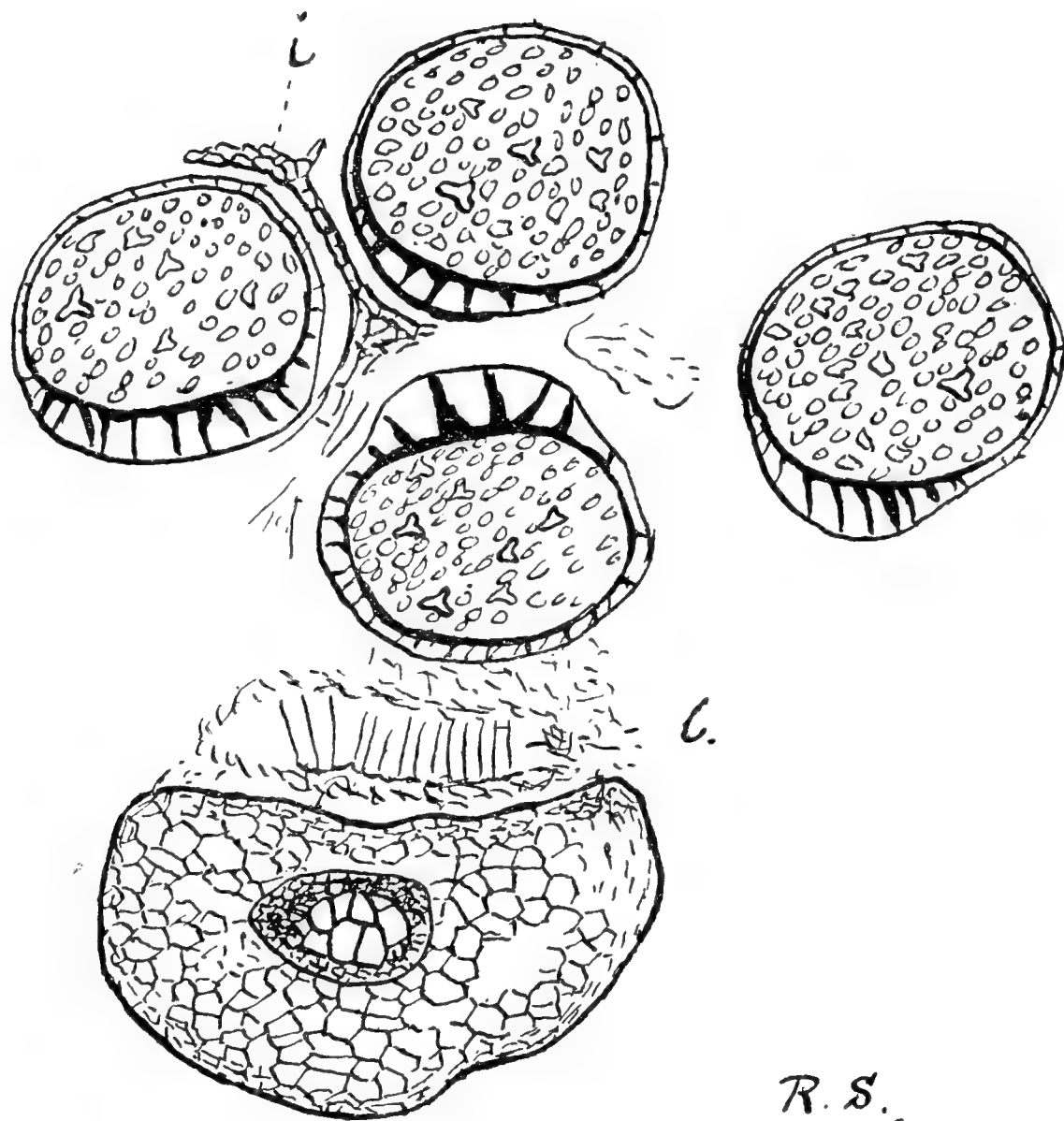
<sup>3</sup> D. H. Scott, Studies in Fossil Botany, p. 332, Figs. 124 and 125, 2nd ed., 1908.

<sup>4</sup> Pelourde, l. c., p. 367, Figs. 6 and 7.

<sup>5</sup> Scott, l. c., Fig. 125.

indusium was described by Renault in *B. forensis*,<sup>1</sup> but the subject is one that requires further investigation before any conclusion can be drawn.

The diameter of the sporangia in the Pettycur specimen is from  $280\ \mu$  to  $240\ \mu$ ; that of the spores about  $25\ \mu$ , and that of the annulus cells from  $50\ \mu$  to  $60\ \mu$ .



Group of four annulate sporangia in close association with a rachis of *Botryopteris antiqua*. *i*, indusium-like structure between two sporangia. (The palisade tissue at *l* is a foreign body, being a fragment of the sporangium-wall of *Lepidostrobus Veltheimianus*.) Drawn by Mrs. D. H. Scott, F.L.S.  $\times$  about 100. (Scott Coll. 2496.)

The specimen just described appears to heighten the probability that the sporangia of an Osmundaceous type associated with *Botryopteris antiqua* really belonged to that plant; it thus seems worth placing on record, though evidence of actual continuity is still lacking.

D. H. SCOTT.

OAKLEY, HANTS.

<sup>1</sup> Bassin Houiller et Permien d'Autun et d'Épinac. Flore Fossile, 2<sup>e</sup> partie, p. 54, Figs. 22 and 23, 1896.

**NOTE ON THE PROTHALLUS OF LEPIDODENDRON VELTHEIMIANUM.**—As the prothalli of Palaeozoic Lycopodineae are not as a rule well preserved, we know exceedingly little about the gametophyte generation of the members of this group. In the case, however, of the megaspores of *Lepidodendron Veltheimianum*, which occur in abundance in the Pettycur Limestone, the prothallus is never shed from the spore and occasionally its tissue is more or less perfectly preserved. In 1908<sup>1</sup> I drew attention to a specimen of this megaspore which was almost completely preserved, and in the same year Dr. Scott figured another example.<sup>2</sup> The prothallus in my specimen showed a certain amount of differentiation into a cap of small-celled parenchyma at the apex of a larger-celled tissue. This cap was situated below the three ear-like projections of the spore coat which characterize this megaspore. As the spore coat did not show any trace of rupture (although the lines of rupture were indicated) I concluded that the specimen was immature. There were no traces of archegonia present in this example.

The megaspore figured by Dr. Scott also shows traces of this differentiation, the smaller-celled parenchyma occurring where the spore wall is ruptured; but part of the prothallus seems to have been extruded from the spore.

Recently I have obtained a specimen which, though less perfectly preserved than either of the above, throws considerable light on the development of the archegonia. The spore coat in this example also is ruptured (the splitting probably occurring between two adjacent ear-like projections), and below this gap there is a very distinct archegonium. The only cells of the prothallus which are preserved are round the archegonium, and they are similar in size to the cells of the cap in the examples mentioned above. In the accompanying figures, for which I am indebted to Mrs. Scott, this archegonium is exceedingly well shown. Fig. 1 *a* represents the whole spore with the external surface coated by knobbed hairs and the prothallus inside the spore coat. On the surface of this prothallus (which is shown more highly magnified in Fig. 1 *b*) a distinct papilla is seen. This papilla consists of the neck cells of the archegonium (*n. c.*) and immediately below the neck there is a dark mass (*A*) which probably represents the *central cell*. Separating the lower neck cells on the one side from those on the other is a narrow dark mass which in life would be the *neck canal cell* (*n. c. c.*).

As far then as we can judge from this specimen, the development of the archegonium in *Lepidodendron Veltheimianum* was essentially similar to that in *Selaginella*. The archegonium mother-cell appears to have been one of the superficial prothallial cells which has divided into an upper and a lower cell, as in *Selaginella*. By the division of the upper cell into four and the subsequent division of each of these into three, the twelve neck cells have been produced. The whole neck consists of three tiers of four cells and there is no indication that it was ever bent over. On the right-hand side of Fig. 1 *a* there appear to be four neck cells in one row, but this is due no doubt to the wall between the two adjacent cells being oblique. The lower cell then appears to have divided into two, the upper forcing its way between the lower neck cells and

<sup>1</sup> Trans. Bot. Soc., Edinburgh, vol. xxiii, 1908.

<sup>2</sup> Scott, Studies in Fossil Botany, 2nd ed., Part I, p. 188, Fig. 77, London, 1908.

forming the *neck canal cell* (*n. c. c.*) of the archegonium, while the lower forms the *central cell*. The whole archegonium seems to have been almost ripe, but it had not yet opened.

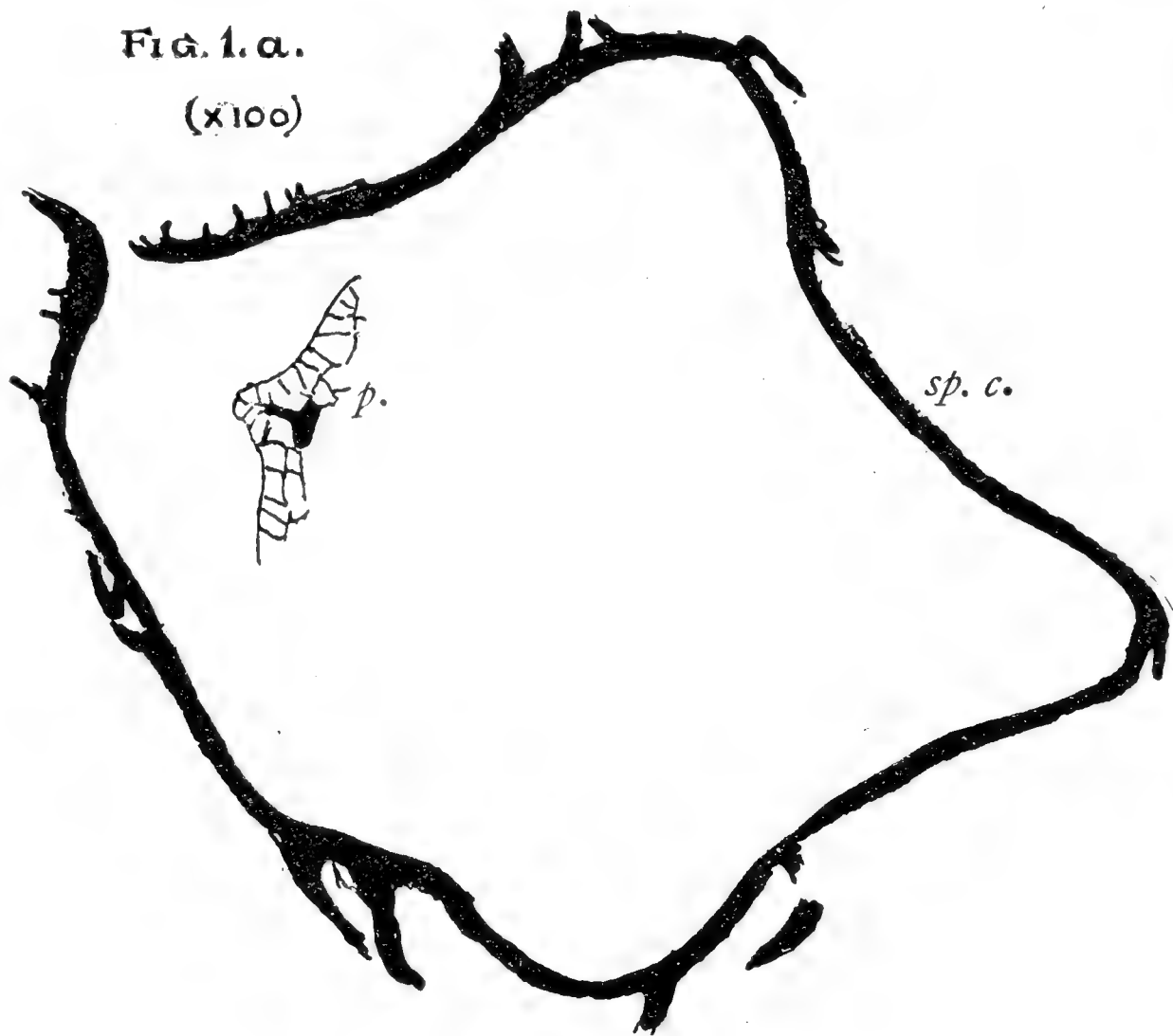


FIG. 1b.  
(x275)

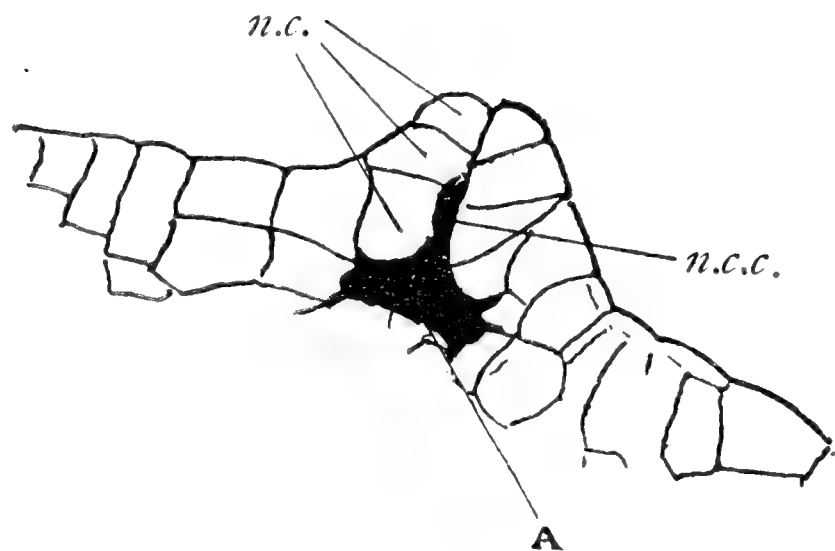


FIG. 1 a. Megaspore of *L. Veltheimianum* with prothallus. *sp. c.* = spore coat; *p.* = prothallus.  
Slide G. C. 1215. x 100.

FIG. 1 b. Prothallus of Fig. 1 a. *A* = central cell; *n. c.* = neck cells; *n. c. c.* = neck canal cell. x 275.

From drawings by Mrs. D. H. Scott.

The discovery of the archegonium in *Lepidodendron Veltheimianum* still further accentuates the similarity between the gametophyte generation of this plant (and probably of other species) and that of the living *Selaginella*.

W. T. GORDON.

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